hydroxyethyl)piperazine-N'-(4-butanesulfonic acid), N-[tris(hydroxymethyl)methyl]-3-aminopropanesulfonic acid, N-tris(Hydroxymethyl)methyl-4-aminobutanesulfonic acid, N-(1,1-dimethyl-2-hydroxyethyl)-3-amino-2-hydroxypropanesulfonic acid, 2-(cyclohexylamino)ethanesulfonic acid, 3-(cyclohexylamino)-2-hydroxy-1-propanesulfonic acid, 3-(cyclohexylamino)-1-propanesulfonic acid, *N*-(2-acetamido)iminodiacetic acid, 4-(cyclohexylamino)-1-butanesulfonic acid, *N*-[tris(hydroxymethyl)methyl]glycine, 2-amino-2-(hydroxymethyl)-1,3-propanediol, and trometamol.

The anti-CTLA4 antibody used in the methods of the invention can be incorporated into pharmaceutical compositions suitable for administration to a subject. Typically, the pharmaceutical composition comprises the antibody and a pharmaceutically acceptable carrier. As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. Examples of pharmaceutically acceptable carriers include one or more of water, saline, phosphate buffered saline, dextrose, glycerol, ethanol and the like, as well as combinations thereof. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Pharmaceutically acceptable substances such as wetting or minor amounts of auxiliary substances such as wetting or emulsifying agents, preservatives or buffers, which enhance the shelf life or effectiveness of the antibody or antibody portion.

The antibodies and therapeutic agents used in the present invention may be in a variety of forms. These include, for example, liquid, semi solid and solid dosage forms, such as liquid solutions (*e.g.*, injectable and infusible solutions), dispersions or suspensions, tablets, pills, powders, liposomes and suppositories. The preferred form depends on the therapeutic agent, the intended mode of administration and the therapeutic application. Typical preferred compositions are in the form of injectable or infusible solutions, such as compositions similar to those used for passive immunization of humans with other antibodies. The preferred mode of administration is parenteral (*e.g.*, intravenous, subcutaneous, intraperitoneal, intramuscular). In a preferred embodiment, the antibody is administered by intravenous infusion or injection. In another preferred embodiment, the antibody is administered by intramuscular or subcutaneous injection.

Therapeutic compositions typically must be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, dispersion, liposome, or other ordered structure suitable to high drug concentration. Sterile injectable solutions can be prepared by incorporating the antibody in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active

compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile filtered solution thereof. The proper fluidity of a solution can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prolonged absorption of injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin.

The antibodies can be administered by a variety of methods known in the art, including, without limitation, oral, parenteral, mucosal, by-inhalation, topical, buccal, nasal, and rectal. For many therapeutic applications, the preferred route/mode of administration is subcutaneous, intramuscular, intravenous or infusion. Non-needle injection may be employed, if desired. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results.

In certain embodiments, the antibody may be prepared with a carrier that will protect the compound against rapid release, such as a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Many methods for the preparation of such formulations are patented or generally known to those skilled in the art. *See, e.g., Sustained and Controlled Release Drug Delivery Systems*, J. R. Robinson, ed., Marcel Dekker, Inc., New York (1978).

Transdermal delivery of biological molecules, including antibodies, cytokines, and the like, includes microneedles to create micron-sized temporary transport pathways in the skin through which large molecules can be transported. *See, e.g.*, Banga, *In: Therapeutic peptides and proteins: Formulation, processing and delivery systems*, 2nd ed., Taylor & Francis (Eds.), 2006; Martanto et al., *J. Controlled Release* **112**:357-361 (2006). In one embodiment, microneedles include those composed of silicon, metal or a polymer. In another embodiment, maltose microneedles which dissolve in the skin may be used to administered the compositions of the invention, including anti-CTLA4 antibody, anti-CD40, anti-IGF-1R antibody, rituximab, trastuzumab, IL-15, and the like.

In yet another embodiment, nucleic acid, including nucleic acids encoding a protein, preferably, anti-CTLA4 antibody (*e.g.*, CP-675,206, ipilimumab, and the like), or an ODN, can be delivered transdermally using biolistics to provide expression of the antibody or delivery of the ODN in a micromilieu where antigen presenting cells (*e.g.*, dendritic cells) and/or T

regulatory cells are present (*e.g.*, Tregs in lymph nodes). Gold microparticles comprising nucleic acids encoding anti-CTLA4 antibody and/or comprising an ODN or nucleic acids encoding a protein of interest, are propelled through the skin using, for example, a "gene gun" (PMED Device, *e.g.*, model ND-10, PowderMed Ltd., Oxford, UK). *See, e.g.,* US Patent Nos. 6,194,389; 6,168,587; 5,478,744. In one embodiment, a synthetic oligodeoxynucleotide (ODN) toll-like receptor 9 agonist (*e.g.*, PF03512676) may be administered using biolistics such that a microparticle comprising the nucleic acid encoding the antibody and a microparticle comprising the ODN, or a microparticle comprising both, are both delivered transdermally. In another embodiment, a nucleic acid encoding a TAA (*e.g.,* gp100, tyrosinase, MAGE, MART-1, NY-ESO-1, and the like) is delivered transdermally using biolistics such that the TAA is expressed in and presented by APCs transfected with the nucleic acid. In yet another embodiment, various combinations of an antigen (*e.g.*, TAA), an antibody (anti-CTLA4, anti-CD40, anti-CD20, anti-HER2, etc.), a cytokine (e.g., GM-CSF, IL-15, TNFα, among others), an ODN (e.g., PF03512676), a costimulatory molecule (e.g., PD-1, OX40, 4-1BB, etc.), and the like, may be delivered using biolistics to induce, enhance or prolong and anti-tumor response in a patient.

Dosage regimens may be adjusted to provide the optimum desired response. For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the antibody and the particular therapeutic or prophylactic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

It is to be noted that dosage values may vary with the type and severity of the condition to be alleviated, and may include single or multiple doses. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that dosage ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition.

In one embodiment, the antibody is administered in a formulation as a sterile aqueous solution having a pH that ranges from about 5.0 to about 6.5 and comprising from about 1 mg/ml to about 200 mg/ml of antibody, from about 1 millimolar to about 100 millimolar of

histidine buffer, from about 0.01 mg/ml to about 10 mg/ml of polysorbate 80, from about 100 millimolar to about 400 millimolar of trehalose, and from about 0.01 millimolar to about 1.0 millimolar of disodium EDTA dihydrate. Exemplary formulations encompass, but are not limited to, such formulations described in PCT/US2006/007551 filed March 2, 2006, now published as WO 2006/096488 on Sept. 14, 2006, and PCT/US2006/007555 filed March 2, 2006, now published as WO 2006/096491 on Sept. 14, 2006.

In another embodiment, the antibody is administered in an intravenous formulation as a sterile aqueous solution containing 5 mg/m, or more preferably, about 10 mg/ml, or yet more preferably, about 15 mg/ml, or even more preferably, about 20 mg/ml of antibody, with sodium acetate, polysorbate 80, and sodium chloride at a pH ranging from about 5 to 6. Preferably, the intravenous formulation is a sterile aqueous solution containing 5 or 10 mg/ml of antibody, with 20 mM sodium acetate, 0.2 mg/ml polysorbate 80, and 140 mM sodium chloride at pH 5.5. Further, a solution comprising an anti-CTLA4 antibody can comprise, among many other compounds, histidine, mannitol, sucrose, trehalose, glycine, poly(ethylene) glycol, EDTA, methionine, and any combination thereof, and many other compounds known in the relevant art.

The compositions of the present invention optionally may further comprise a pharmaceutically acceptable antioxidant in addition to a chelating agent. Suitable antioxidants include, but are not limited to, methionine, sodium thiosulfate, catalase, and platinum. For example, the composition may contain methionine in a concentration that ranges from 1 mM to about 100 mM, and in particular, is about 27 mM.

In one embodiment, part of the dose is administered by an intraveneous bolus and the rest by infusion of the antibody formulation. For example, a 0.01 mg/kg intravenous injection of the antibody may be given as a bolus, and the rest of a predetermined antibody dose may be administered by intravenous injection. A predetermined dose of the antibody may be administered, for example, over a period of an hour and a half to two hours to five hours.

With regard to a therapeutic agent, where the agent is, *e.g.*, a small molecule, it can be present in a pharmaceutical composition in the form of a physiologically acceptable ester or salt, such as in combination with a physiologically acceptable cation or anion, as is well known in the art.

The formulations of the pharmaceutical compositions described herein may be prepared by any method known or hereafter developed in the art of pharmacology. In general, such preparatory methods include the step of bringing the active ingredient into association with a carrier or one or more other accessory ingredients, and then, if necessary or desirable, shaping or packaging the product into a desired single- or multi-dose unit.

Pharmaceutical compositions that are useful in the methods of the invention may be prepared, packaged, or sold in formulations suitable for oral, rectal, vaginal, parenteral, topical, pulmonary, intranasal, buccal, ophthalmic, or another route of administration. Other contemplated formulations include projected nanoparticles, liposomal preparations, resealed erythrocytes containing the active ingredient, and immunologically-based formulations.

A pharmaceutical composition of the invention may be prepared, packaged, or sold in bulk, as a single unit dose, or as a plurality of single unit doses. As used herein, a "unit dose" is discrete amount of the pharmaceutical composition comprising a predetermined amount of the active ingredient. The amount of the active ingredient is generally equal to the dosage of the active ingredient which would be administered to a subject or a convenient fraction of such a dosage such as, for example, one-half or one-third of such a dosage.

The relative amounts of the active ingredient, the pharmaceutically acceptable carrier, and any additional ingredients in a pharmaceutical composition of the invention will vary, depending upon the identity, size, and condition of the subject treated and further depending upon the route by which the composition is to be administered. By way of example, the composition may comprise between 0.1% and 100% (w/w) active ingredient.

In addition to the active ingredient, a pharmaceutical composition of the invention may further comprise one or more additional pharmaceutically active therapeutic agents. Particularly contemplated additional agents include anti-emetics, anti-diarrheals, chemotherapeutic agents, cytokines, and the like.

Controlled- or sustained-release formulations of a pharmaceutical composition of the invention may be made using conventional technology.

A formulation of a pharmaceutical composition of the invention suitable for oral administration may be prepared, packaged, or sold in the form of a discrete solid dose unit including, but not limited to, a tablet, a hard or soft capsule, a cachet, a troche, or a lozenge, each containing a predetermined amount of the active ingredient. Other formulations suitable for oral administration include, but are not limited to, a powdered or granular formulation, an aqueous or oily suspension, an aqueous or oily solution, or an emulsion.

As used herein, an "oily" liquid is one which comprises a carbon-containing liquid molecule and which exhibits a less polar character than water.

A tablet comprising the active ingredient may, for example, be made by compressing or molding the active ingredient, optionally with one or more additional ingredients. Compressed tablets may be prepared by compressing, in a suitable device, the active ingredient in a free-flowing form such as a powder or granular preparation, optionally mixed with one or more of a binder, a lubricant, an excipient, a surface active agent, and a dispersing agent. Molded tablets may be made by molding, in a suitable device, a mixture of

the active ingredient, a pharmaceutically acceptable carrier, and at least sufficient liquid to moisten the mixture.

Pharmaceutically acceptable excipients used in the manufacture of tablets include, but are not limited to, inert diluents, granulating and disintegrating agents, binding agents, and lubricating agents. Known dispersing agents include, but are not limited to, potato starch and sodium starch glycolate. Known surface active agents include, but are not limited to, sodium lauryl sulphate. Known diluents include, but are not limited to, calcium carbonate, sodium carbonate, lactose, microcrystalline cellulose, calcium phosphate, calcium hydrogen phosphate, and sodium phosphate. Known granulating and disintegrating agents include, but are not limited to, corn starch and alginic acid. Known binding agents include, but are not limited to, gelatin, acacia, pre-gelatinized maize starch, polyvinylpyrrolidone, and hydroxypropyl methylcellulose. Known lubricating agents include, but are not limited to, magnesium stearate, stearic acid, silica, and talc.

Tablets may be non-coated or they may be coated using known methods to achieve delayed disintegration in the gastrointestinal tract of a subject, thereby providing sustained release and absorption of the active ingredient. By way of example, a material such as glyceryl monostearate or glyceryl distearate may be used to coat tablets. Further by way of example, tablets may be coated using methods described in U.S. Patents numbers 4,256,108; 4,160,452; and 4,265,874 to form osmotically-controlled release tablets. Tablets may further comprise a sweetening agent, a flavoring agent, a coloring agent, a preservative, or some combination of these in order to provide pharmaceutically elegant and palatable preparation.

Hard capsules comprising the active ingredient may be made using a physiologically degradable composition, such as gelatin. Such hard capsules comprise the active ingredient, and may further comprise additional ingredients including, for example, an inert solid diluent such as calcium carbonate, calcium phosphate, or kaolin.

Soft gelatin capsules comprising the active ingredient may be made using a physiologically degradable composition, such as gelatin. Such soft capsules comprise the active ingredient, which may be mixed with water or an oil medium such as peanut oil, liquid paraffin, or olive oil.

Liquid formulations of a pharmaceutical composition of the invention which are suitable for oral administration may be prepared, packaged, and sold either in liquid form or in the form of a dry product intended for reconstitution with water or another suitable vehicle prior to use.

Liquid suspensions may be prepared using conventional methods to achieve suspension of the active ingredient in an aqueous or oily vehicle. Aqueous vehicles include,

for example, water and isotonic saline. Oily vehicles include, for example, almond oil, oily esters, ethyl alcohol, vegetable oils such as arachis, olive, sesame, or coconut oil, fractionated vegetable oils, and mineral oils such as liquid paraffin. Liquid suspensions may further comprise one or more additional ingredients including, but not limited to, suspending agents, dispersing or wetting agents, emulsifying agents, demulcents, preservatives, buffers, salts, flavorings, coloring agents, and sweetening agents. Oily suspensions may further comprise a thickening agent. Known suspending agents include, but are not limited to, sorbitol syrup, hydrogenated edible fats, sodium alginate, polyvinylpyrrolidone, gum tragacanth, gum acacia, and cellulose derivatives such as sodium carboxymethylcellulose, methylcellulose, hydroxypropyl methylcellulose. Known dispersing or wetting agents include, but are not limited to, naturally-occurring phosphatides such as lecithin, condensation products of an alkylene oxide with a fatty acid, with a long chain aliphatic alcohol, with a partial ester derived from a fatty acid and a hexitol, or with a partial ester derived from a fatty acid and a hexitol anhydride (*e.g.*, polyoxyethylene stearate, heptadecaethyleneoxycetanol, polyoxyethylene sorbitol monooleate, and polyoxyethylene sorbitan monooleate, respectively). Known emulsifying agents include, but are not limited to, lecithin and acacia. Known preservatives include, but are not limited to, methyl, ethyl, or n-propyl-para-hydroxybenzoates, ascorbic acid, and sorbic acid. Known sweetening agents include, for example, glycerol, propylene glycol, sorbitol, sucrose, and saccharin. Known thickening agents for oily suspensions include, for example, beeswax, hard paraffin, and cetyl alcohol.

Liquid solutions of the active ingredient in aqueous or oily solvents may be prepared in substantially the same manner as liquid suspensions, the primary difference being that the active ingredient is dissolved, rather than suspended in the solvent. Liquid solutions of the pharmaceutical composition of the invention may comprise each of the components described with regard to liquid suspensions, it being understood that suspending agents will not necessarily aid dissolution of the active ingredient in the solvent. Aqueous solvents include, for example, water and isotonic saline. Oily solvents include, for example, almond oil, oily esters, ethyl alcohol, vegetable oils such as arachis, olive, sesame, or coconut oil, fractionated vegetable oils, and mineral oils such as liquid paraffin.

Powdered and granular formulations of a pharmaceutical preparation of the invention may be prepared using known methods. Such formulations may be administered directly to a subject, used, for example, to form tablets, to fill capsules, or to prepare an aqueous or oily suspension or solution by addition of an aqueous or oily vehicle thereto. Each of these formulations may further comprise one or more of dispersing or wetting agent, a suspending

agent, and a preservative. Additional excipients, such as fillers and sweetening, flavoring, or coloring agents, may also be included in these formulations.

A pharmaceutical composition of the invention may also be prepared, packaged, or sold in the form of oil-in-water emulsion or a water-in-oil emulsion. The oily phase may be a vegetable oil such as olive or arachis oil, a mineral oil such as liquid paraffin, or a combination of these. Such compositions may further comprise one or more emulsifying agents such as naturally occurring gums such as gum acacia or gum tragacanth, naturally-occurring phosphatides such as soybean or lecithin phosphatide, esters or partial esters derived from combinations of fatty acids and hexitol anhydrides such as sorbitan monooleate, and condensation products of such partial esters with ethylene oxide such as polyoxyethylene sorbitan monooleate. These emulsions may also contain additional ingredients including, for example, sweetening or flavoring agents.

A pharmaceutical composition of the invention may be prepared, packaged, or sold in a formulation suitable for rectal administration. Such a composition may be in the form of, for example, a suppository, a retention enema preparation, and a solution for rectal or colonic irrigation.

Suppository formulations may be made by combining the active ingredient with a non-irritating pharmaceutically acceptable excipient which is solid at ordinary room temperature (*i.e.*, about 20°C) and which is liquid at the rectal temperature of the subject (*i.e.*, about 37°C in a healthy human). Suitable pharmaceutically acceptable excipients include, but are not limited to, cocoa butter, polyethylene glycols, and various glycerides. Suppository formulations may further comprise various additional ingredients including, but not limited to, antioxidants and preservatives.

Retention enema preparations or solutions for rectal or colonic irrigation may be made by combining the active ingredient with a pharmaceutically acceptable liquid carrier. As is well known in the art, enema preparations may be administered using, and may be packaged within, a delivery device adapted to the rectal anatomy of the subject. Enema preparations may further comprise various additional ingredients including, but not limited to, antioxidants and preservatives.

A pharmaceutical composition of the invention may be prepared, packaged, or sold in a formulation suitable for vaginal administration. Such a composition may be in the form of, for example, a suppository, an impregnated or coated vaginally-insertable material such as a tampon, a douche preparation, or gel or cream or a solution for vaginal irrigation.

Methods for impregnating or coating a material with a chemical composition are known in the art, and include, but are not limited to methods of depositing or binding a chemical composition onto a surface, methods of incorporating a chemical composition into

the structure of a material during the synthesis of the material (i.e. such as with a physiologically degradable material), and methods of absorbing an aqueous or oily solution or suspension into an absorbent material, with or without subsequent drying.

Douche preparations or solutions for vaginal irrigation may be made by combining the active ingredient with a pharmaceutically acceptable liquid carrier. As is well known in the art, douche preparations may be administered using, and may be packaged within, a delivery device adapted to the vaginal anatomy of the subject. Douche preparations may further comprise various additional ingredients including, but not limited to, antioxidants, antibiotics, antifungal agents, and preservatives.

As used herein, "parenteral administration" of a pharmaceutical composition includes any route of administration characterized by physical breaching of a tissue of a subject and administration of the pharmaceutical composition through the breach in the tissue. Parenteral administration thus includes, but is not limited to, administration of a pharmaceutical composition by injection of the composition, by application of the composition through a surgical incision, by application of the composition through a tissue-penetrating non-surgical wound, and the like. In particular, parenteral administration is contemplated to include, but is not limited to, subcutaneous, intraperitoneal, intramuscular, intrasternal injection, and kidney dialytic infusion techniques.

Formulations of a pharmaceutical composition suitable for parenteral administration comprise the active ingredient combined with a pharmaceutically acceptable carrier, such as sterile water or sterile isotonic saline. Such formulations may be prepared, packaged, or sold in a form suitable for bolus administration or for continuous administration. Injectable formulations may be prepared, packaged, or sold in unit dosage form, such as in ampules or in multi-dose containers containing a preservative. Formulations for parenteral administration include, but are not limited to, suspensions, solutions, emulsions in oily or aqueous vehicles, pastes, and implantable sustained-release or biodegradable formulations as discussed below. Such formulations may further comprise one or more additional ingredients including, but not limited to, suspending, stabilizing, or dispersing agents. In one embodiment of a formulation for parenteral administration, the active ingredient is provided in dry (i.e. powder or granular) form for reconstitution with a suitable vehicle (*e.g.*, sterile pyrogen-free water) prior to parenteral administration of the reconstituted composition.

A composition of the present invention can be administered by a variety of methods known in the art. The route and/or mode of administration vary depending upon the desired results. The active compounds can be prepared with carriers that protect the compound against rapid release, such as a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible

polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Many methods for the preparation of such formulations are described by *e.g.*, *Sustained and Controlled Release Drug Delivery Systems*, J. R. Robinson, ed., Marcel Dekker, Inc., New York, (1978). Pharmaceutical compositions are preferably manufactured under GMP conditions.

The pharmaceutical compositions may be prepared, packaged, or sold in the form of a sterile injectable aqueous or oily suspension or solution. This suspension or solution may be formulated according to the known art, and may comprise, in addition to the active ingredient, additional ingredients such as the dispersing agents, wetting agents, or suspending agents described herein. Such sterile injectable formulations may be prepared using a non-toxic parenterally-acceptable diluent or solvent, such as water or 1,3-butane diol, for example. Other acceptable diluents and solvents include, but are not limited to, Ringer's solution, isotonic sodium chloride solution, and fixed oils such as synthetic mono- or di-glycerides. Other parentally-administrable formulations which are useful include those which comprise the active ingredient in microcrystalline form, in a liposomal preparation, or as a component of a biodegradable polymer systems. Compositions for sustained release or implantation may comprise pharmaceutically acceptable polymeric or hydrophobic materials such as an emulsion, an ion exchange resin, a sparingly soluble polymer, or a sparingly soluble salt.

Formulations suitable for topical administration include, but are not limited to, liquid or semi-liquid preparations such as liniments, lotions, oil-in-water or water-in-oil emulsions such as creams, ointments or pastes, and solutions or suspensions. Topically-administrable formulations may, for example, comprise from about 1% to about 10% (w/w) active ingredient, although the concentration of the active ingredient may be as high as the solubility limit of the active ingredient in the solvent. Formulations for topical administration may further comprise one or more of the additional ingredients described herein.

A pharmaceutical composition of the invention may be prepared, packaged, or sold in a formulation suitable for pulmonary administration via the buccal cavity. Such a formulation may comprise dry particles which comprise the active ingredient and which have a diameter in the range from about 0.5 to about 7 nanometers, and preferably from about 1 to about 6 nanometers. Such compositions are conveniently in the form of dry powders for administration using a device comprising a dry powder reservoir to which a stream of propellant may be directed to disperse the powder or using a self-propelling solvent/powder-dispensing container such as a device comprising the active ingredient dissolved or suspended in a low-boiling propellant in a sealed container. Preferably, such powders comprise particles wherein at least 98% of the particles by weight have a diameter greater than 0.5 nanometers and at least 95% of the particles by number have a diameter

less than 7 nanometers. More preferably, at least 95% of the particles by weight have a diameter greater than 1 nanometer and at least 90% of the particles by number have a diameter less than 6 nanometers. Dry powder compositions preferably include a solid fine powder diluent such as sugar and are conveniently provided in a unit dose form.

Low boiling propellants generally include liquid propellants having a boiling point of below 65°F at atmospheric pressure. Generally the propellant may constitute 50 to 99.9% (w/w) of the composition, and the active ingredient may constitute 0.1 to 20% (w/w) of the composition. The propellant may further comprise additional ingredients such as a liquid non-ionic or solid anionic surfactant or a solid diluent (preferably having a particle size of the same order as particles comprising the active ingredient).

Pharmaceutical compositions of the invention formulated for pulmonary delivery may also provide the active ingredient in the form of droplets of a solution or suspension. Such formulations may be prepared, packaged, or sold as aqueous or dilute alcoholic solutions or suspensions, optionally sterile, comprising the active ingredient, and may conveniently be administered using any nebulization or atomization device. Such formulations may further comprise one or more additional ingredients including, but not limited to, a flavoring agent such as saccharin sodium, a volatile oil, a buffering agent, a surface active agent, or a preservative such as methylhydroxybenzoate. The droplets provided by this route of administration preferably have an average diameter in the range from about 0.1 to about 200 nanometers.

The formulations described herein as being useful for pulmonary delivery are also useful for intranasal delivery of a pharmaceutical composition of the invention.

Another formulation suitable for intranasal administration is a coarse powder comprising the active ingredient and having an average particle from about 0.2 to 500 micrometers. Such a formulation is administered in the manner in which snuff is taken, i.e., by rapid inhalation through the nasal passage from a container of the powder held close to the nares.

Formulations suitable for nasal administration may, for example, comprise from about as little as 0.1% (w/w) and as much as 100% (w/w) of the active ingredient, and may further comprise one or more of the additional ingredients described herein.

A pharmaceutical composition of the invention may be prepared, packaged, or sold in a formulation suitable for buccal administration. Such formulations may, for example, be in the form of tablets or lozenges made using conventional methods, and may, for example, 0.1 to 20% (w/w) active ingredient, the balance comprising an orally dissolvable or degradable composition and, optionally, one or more of the additional ingredients described herein. Alternately, formulations suitable for buccal administration may comprise a powder or an

aerosolized or atomized solution or suspension comprising the active ingredient. Such powdered, aerosolized, or aerosolized formulations, when dispersed, preferably have an average particle or droplet size in the range from about 0.1 to about 200 nanometers, and may further comprise one or more of the additional ingredients described herein.

A pharmaceutical composition of the invention may be prepared, packaged, or sold in a formulation suitable for ophthalmic administration. Such formulations may, for example, be in the form of eye drops including, for example, a 0.1-1.0% (w/w) solution or suspension of the active ingredient in an aqueous or oily liquid carrier. Such drops may further comprise buffering agents, salts, or one or more other of the additional ingredients described herein. Other ophthalmalmically-administrable formulations which are useful include those which comprise the active ingredient in microcrystalline form or in a liposomal preparation.

As used herein, "additional ingredients" include, but are not limited to, one or more of the following: excipients; surface active agents; dispersing agents; inert diluents; granulating and disintegrating agents; binding agents; lubricating agents; sweetening agents; flavoring agents; coloring agents; preservatives; physiologically degradable compositions such as gelatin; aqueous vehicles and solvents; oily vehicles and solvents; suspending agents; dispersing or wetting agents; emulsifying agents, demulcents; buffers; salts; thickening agents; fillers; emulsifying agents; antioxidants; antibiotics; antifungal agents; stabilizing agents; and pharmaceutically acceptable polymeric or hydrophobic materials. Other "additional ingredients" which may be included in the pharmaceutical compositions of the invention are known in the art and described, for example in *Remington's Pharmaceutical Sciences*, Genaro, ed., Mack Publishing Co., Easton, PA (1985), which is incorporated herein by reference.

In one embodiment of the invention, the composition comprising CP-675,206 comprises a sterile solution comprising 20 mM histidine buffer, pH 5.5, 84 mg/ml trehalose dehydrate, 0.2 mg/ml polysorbate 80, and 0.1 mg/ml disodium EDTA hydrate. In one aspect, CP-675,206 is packaged in clear glass vials with a rubber stopper and an aluminum seal. In another aspect, the vial contains about 20 mg/ml of CP-675,206 with a nominal fill of about 400 mg per vial.

The anti-CTLA4/therapeutic agent active ingredient combination of the invention, can be administered to an animal, preferably a human. The precise dosage of each active ingredient administered will vary depending upon any number of factors, including but not limited to, the type of animal and type of disease state being treated, the age of the animal and the route of administration.

The anti-CTLA4 antibody may be administered to an animal as frequently as several times daily, or it may be administered less frequently, such as once a day, once a week, once

every two weeks, once a month, or even less frequently, such as once every several months or even once a year or less. The frequency of the dose will be readily apparent to the skilled artisan and will depend upon any number of factors, such as, but not limited to, the type and severity of the disease being treated, the type and age of the animal, etc.

The therapeutic agent, preferably, a chemotherapeutic agent, may be administered to an animal as frequently as several times daily, or it may be administered less frequently, such as once a day, once a week, once every two weeks, once a month, or even less frequently, such as once every several months or even once a year or less. The frequency of the dose will be readily apparent to the skilled artisan and will depend upon any number of factors, such as, but not limited to, the type and severity of the disease being treated, the type and age of the animal, etc.

The antibody and therapeutic agent can be co-administered in that they can be administered separately, on different dates or at different times of the day, as well as simultaneously or on the same date. Co-administration thus encompasses any temporal combination of administration of the antibody and the therapeutic agent such that administration of the two agents mediates a therapeutic benefit to the patient that is detectably greater than administration of either agent in the absence of the other.

An antibody-therapeutic agent combination of the invention may be co-administered with numerous other compounds (other antihormonal therapy agents, cytokines, chemotherapeutic and/or antiviral drugs, among many others). Alternatively, the compound(s) may be administered an hour, a day, a week, a month, or even more, in advance of the antibody-therapeutic agent combination, or any permutation thereof. Further, the compound(s) may be administered an hour, a day, a week, or even more preferably, after administration of radiation, stem cell transplant, or administration of any therapeutic agent (*e.g.*, cytokine, chemotherapeutic compound, and the like), or any permutation thereof. The frequency and administration regimen will be readily apparent to the skilled artisan and will depend upon any number of factors such as, but not limited to, the type and severity of the disease being treated, the age and health status of the animal, the identity of the compound or compounds being administered, the route of administration of the various compounds, and the like.

VI. Kits

The invention includes various kits which comprise a therapeutically effective amount of a human anti-CTLA4 antibody of the invention and a therapeutically effective amount of a therapeutic agent, along with an applicator and instructional materials which describe use of the combination to perform the methods of the invention. Although exemplary kits are

described below, the contents of other useful kits will be apparent to the skilled artisan in light of the present disclosure. Each of these kits is included within the invention.

The invention includes a kit for treatment of breast cancer in a patient in need thereof. The kit includes a human anti-CTLA4 antibody of the invention and at least one therapeutic agent. The inhibitor encompasses, but is not limited to, a chemotherapeutic, an antibody, a cytokine, a vaccine, an immunomodulator, among many others. The kit further comprises an applicator, including, but not limited to, a syringe, for administration of the components of the kit to a patient. Further, the kit comprises an instructional material setting forth the pertinent information for the use of the kit to treat breast cancer in the patient.

More preferably, the kit comprises at least one anti-CTLA4 antibody selected from an antibody having the heavy and light chain amino acid sequence of antibody 4.1.1, 4.8.1, 4.10.2, 4.13.1, 4.14.3, 6.1.1, 11.2.1 (CP-675,206), 11.6.1, 11.7.1., 12.3.1.1, 12.9.1.1, and ipilimumab, even more preferably, the antibody is an antibody having the heavy and light chain amino acid sequence of antibody 4.13.1, 11.2.1 (CP-675,206), and ipilimumab (MDX-010). Preferably, the antibody is an antibody having the heavy and light sequence of 11.2.1 (CP-675,206). Even more preferably, the anti-CTLA4 antibody is CP-675,206 or ipilimumab. Most preferably, the antibody is CP-675,206.

The kit can comprise any number of additional therapeutic agents for treatment of cancer. Such agents are set forth previously and include chemotherapeutic compounds, cancer vaccines, signal transduction inhibitors, agents useful in treating abnormal cell growth or cancer, antibodies or other ligands that inhibit tumor growth by binding to IGF-1R, and cytokines, among many others.

The invention also relates to an article of manufacture (*e.g.*, dosage form adapted for i.v. administration) comprising a human anti-CTLA4 antibody in the amount effective to treat cancer (*e.g.*, more than 10 mg/kg, at least 15 mg/kg, or 15 mg/kg) and a therapeutically effective amount of a second therapeutic agent. In certain embodiments, the article of manufacture comprises a container or containers comprising a human anti-CTLA4 antibody, the therapeutic agent, and a label and/or instructions for use to treat cancer.

In one embodiment, the therapeutic agent is at least one agent selected from a hormonal therapy agent, a taxane, a heat shock protein, a topoisomerase inhibitor, a hormonal therapy agent (*e.g.,* leuprolide), a chemotherapeutic compounds, a vinca alkaloid, a platinum compound, a cancer vaccine, a tumor-specific antigen, an angiogenesis inhibitor, a signal transduction inhibitor, agents useful in treating abnormal cell growth or cancer, antibodies or other ligands that inhibit tumor growth by binding to IGF-1R, and cytokines, among others.

The kit can comprise any number of additional therapeutic agents for treatment of cancer. Such agents are set forth previously and include chemotherapeutic compounds, cancer vaccines, signal transduction inhibitors, agents useful in treating abnormal cell growth or cancer, antibodies or other ligands that inhibit tumor growth by binding to IGF-1R, and cytokines, and palliative care agents (*e.g.,* anti-diarrheals, anti-emetics, etc.) among many others.

In another embodiment of the invention, the kit is for treatment of breast cancer. The kit comprises an anti-CTLA4 antibody and a taxane. In one aspect, the taxane is docetaxel or paclitaxel. In another aspect, the treatment is first line therapy for the treatment of patients with locally advanced or metastatic triple receptor negative breast cancer.

In one embodiment, the kit is for treatment of ovarian cancer. In one aspect, the kit comprises an anti-CTLA4 antibody and a therapeutic agent, where the therapeutic agent is paclitaxel. In another aspect, the kit further comprises carboplatin. In yet another aspect, the kit is for first-line treatment of advanced carcinoma of the ovary. In another aspect, the kit is for second line treatment of a patient who has progressed following prior paclitaxel-based therapy.

In an embodiment of the invention, the kit is for treatment of non-small cell lung cancer. In one embodiment, the kit comprises an anti-CTLA4 antibody and a chemotherapeutic agent, wherein the therapeutic agent is selected from the group consisting of a platin (*e.g.,* carboplatin (PARAPLATIN), paclitaxel (TAXOL)), docetaxel (TAXOTERE), sunitinib (SU11248), erlotinib (TARCEVA), bevacizumab (AVASTIN), pemetrexed (ALIMTA), and PF03512676 (CpG-7909).

In one embodiment, kit is for first line therapy of locally advanced Stage IIIb or metastatic Stage IV NSCLC where the kit comprises an anti-CTLA4 antibody and a platin. In another embodiment, the kit comprises carboplatin and paclitaxel. In another embodiment, the kit comprises the antibody and bevacizumab. In a further embodiment, the kit comprises the antibody and PF03512676. In another embodiment of the invention, the kit comprises the antibody and sunitinib.

In one embodiment, the kit is for second line treatment of Stage IIIb or metastatic Stage IV NSCLC after failure of platinum-based therapy. In one embodiment, the kit comprises an anti-CTLA4 antibody and docetaxel. In another embodiment, the kit comprises the antibody and erlotinib. In yet another embodiment, the kit comprises the antibody and pemetrexed. In yet another embodiment, the kit comprises an anti-CTLA4 antibody.

In another embodiment of the invention, the kit is for third line treatment of locally advanced Stage IIIb or metastatic Stage IV NSCLC. The kit comprises an effective amount of

an anti-CTLA4 antibody. In one aspect, the kit is for treatment of cancer after failure of prior platinum-based chemotherapy and EGFR inhibition-based therapy.

In one embodiment, the kit is for treatment of pancreatic cancer. The kit comprises an anti-CTLA4 antibody and a therapeutic agent, wherein the agent is gemcitabine. In one aspect, the treatment is first line therapy for locally advanced, non-resectable Stage II or Stage III, or metastatic Stage IV adenocarcinoma of the pancreas.

In one embodiment, the kit is for treatment of melanoma and comprises an effective amount of an anti-CTLA4 antibody and an effective amount of IFNα. In one aspect, the treatment is adjuvant therapy for Stage II/III melanoma.

In an embodiment of the present invention, the kit is for treatment of colorectal carcinoma. In one aspect, the kit comprises an effective amount of an anti-CTLA4 antibody and an effective amount of each of fluorouracil, leucovorin and oxaliplatin (FOLFOX). In another aspect, the kit comprises an effective amount of an anti-CTLA4 antibody, fluorouracil, leucovorin and irinotecan (FOLFIRI). In one aspect, the treatment is first line therapy for metastatic CRC. In another aspect, the therapy is adjuvant therapy for Stage III colon cancer in a patient who has undergone resection of a primary tumor.

In another embodiment, the kit is for treatment of CRC and comprises an effective amount of an anti-CTLA4 antibody and an effective amount of capecitabine (XELODA). In one aspect, the treatment is first line therapy for a patient intolerant of therapy comprising oxaliplatin (ELOXATIN) or irinotecan (CAMPTOSAR).

The invention is further described in detail by reference to the following experimental examples. These examples are provided for purposes of illustration only, and are not intended to be limiting unless otherwise specified. Thus, the invention should in no way be construed as being limited to the following examples, but rather, should be construed to encompass any and all variations which become evident as a result of the teaching provided herein.

## EXAMPLES

### EXAMPLE 1:

### CP-675,206 in Combination with rituximab for First-line Treatment of Indolent Non-Hodgkin's Lymphoma

Patients having indolent non-Hodgkin's lymphoma are treated with CP-675,206 and rituximab. Indolent Non-Hodgkin's Lymphomas (NHLs) are slow growing forms of lymphoma and encompass what were previously referred to as low grade and some categories of intermediate grade NHL in the Working Formulation. Non-Hodgkin's lymphoma (NHL) with at

least one lesion that can be accurately measured in two dimensions and whose size is ≥ 2 cm x 1 cm by conventional CT scan or ≥ 1 cm x 1 cm by spiral CT scan are given standard rituximab (RITUXAN) antibody therapy per established protocols.

The patient is further administered a single IV infusion (100 mL/hr) of CP-675,206 at a dose of about 3 mg/kg. Prophylactic anti-emetics and anti-diarrheals are given as appropriate. The treatment is repeated after 28 days with escalation of CP-675,206 dose, that is, 6 mg/kg, 10 mg/kg and 15 mg/kg, every 28 days thereafter for maximum of 12 cycles in the absence of intolerable toxicity or disease progression. Preferably, CP-675,206 is administered at at least about 10 mg/kg every three weeks for four cycles and then every three months thereafter.

Rituximab is administered via intravenous infusion at about 375 mg/m$^2$ once weekly for about four or eight doses. More preferably, rituximab is administered on day 1 of each cycle of chemotherapy.

Preferably, the patient is premedicated with antihistamine (H1) at least one half hour prior to infusion of either antibody. Premedication is recommended but not required.

Doses are escalated using an accelerated titration design utilizing a dose-doubling schema with 3-6 subjects per cohort. Within each new cohort there is no required waiting period between subjects. Subsequent cohorts may not be opened until the first subject at the current dose level has been observed for 21 days and subsequent subjects have been observed for 14 days.

Rituximab and anti-CTLA4 antibody are administered either sequentially or simultaneously either once, or repeatedly, as determined.

Following combination treatment with rituximab and anti-CTLA4 antibody, anti-CTLA4 antibody is administered as single agent (SA) therapy as described *supra* for combination therapy.

CP-675,206 is provided in 20 ml clear glass vials with a rubber stopper and an aluminum seal. Each vial contains 20 mg/ml (with a nominal fill of 400 mg/vial) of CP-675,206, in a sterile aqueous solution comprising 20 mM histidine buffer, PH 5.5, 84 mg/ml trehalose dehydrate, 0.2 mg/ml polysorbate 80, and 0.1 mg/ml disodium EDTA dehydrate.

For all patients, Eastern Cooperative Oncology Group performance status (ECOG PS), vital signs, and body weight are assessed pre-dose, and vital signs can be repeated post-dose, as clinically indicated. A physical examination (including ophthalmologic assessment and signs of autoimmunity) is performed on Day 1. Samples for hematology panel (hematocrit, RBC count, WBC count, differential), chemistry (Alkaline Phosphatase, calcium, chloride, GGT, LDH, magnesium, phosphorus, random glucose, sodium, urea, uric acid), urinalysis (blood, protein), others (activated partial thromboplastin time [APTT],

prothrombin time (PT), autoantibody panel, C reactive protein, TSH, T3, T4, amylase, lipase, serum C3, C4, serum Ig level), are obtained.

Baseline human anti-human antibody (HAHA) titer is determined and pharmacokinetic (PK) specimen is obtained pre-dose.

The following endpoints are measured: PK parameters, HAHA, response rate and time to progression. Time to progression and overall survival are calculated using the Kaplan-Meier product limit method.

EXAMPLE 2:

CP-675,206 in Combination with rituximab for Second-line Treatment of Aggressive Non-Hodgkin's Lymphoma

Patients having aggressive, CHOP-R refractory NHL with at least one lesion that can be accurately measured in two dimensions and whose size is ≥ 2 cm x 1 cm by conventional CT scan or ≥ 1 cm x 1 cm by spiral CT scan are given standard rituximab antibody therapy per established protocols.

The patient is further administered a single IV infusion (100 mL/hr) of CP-675,206 as described herein at a dose of about 3 mg/kg. Prophylactic anti-emetics and anti-diarrheals are given as appropriate. The treatment is repeated after 28 days with escalation of the anti-CTLA4 antibody dose, that is, 6 mg/kg, 10 mg/kg and 15 mg/kg, every 28 days thereafter for maximum of 12 cycles in the absence of intolerable toxicity or disease progression. Preferably, CP-675,206 is administered at at least about 10 mg/kg every three weeks for four cycles and then every three months thereafter. The patient is administered rituximab according to an art-recognized dosing regimen.

Preferably, the patient is premedicated with antihistamine (H1) at least one half hour prior to infusion of anti-CTLA4. Premedication is recommended but not required.

Doses are escalated using an accelerated titration design utilizing a dose-doubling schema with 3-6 subjects per cohort. Within each new cohort there is no required waiting period between subjects. Subsequent cohorts may not be opened until the first subject at the current dose level has been observed for 21 days and subsequent subjects have been observed for 14 days.

Rituximab (*e.g.*, RITUXAN) and the anti-CTLA4 antibody are administered either sequentially or simultaneously either once, or repeatedly, as determined.

Following combination treatment with RITUXAN and the anti-CTLA4 antibody, the CTLA4 antibody is administered as described *supra*.

CP-675,206 is provided in 20 ml clear glass vials with a rubber stopper and an aluminum seal. Each vial contains 20 mg/ml (with a nominal fill of 400 mg/vial) of CP-

675,206, in a sterile aqueous solution comprising 20 mM histidine buffer, PH 5.5, 84 mg/ml trehalose dehydrate, 0.2 mg/ml polysorbate 80, and 0.1 mg/ml disodium EDTA dehydrate.

For all patients, ECOG performance status, vital signs, and body weight are assessed pre-dose, and vital signs can be repeated post-dose, as clinically indicated. A physical examination (including ophthalmologic assessment and signs of autoimmunity) is performed on Day 1. Samples for hematology panel (hematocrit, RBC count, WBC count, differential), chemistry (Alkaline Phosphatase, calcium, chloride, GGT, LDH, magnesium, phosphorus, random glucose, sodium, urea, uric acid), urinalysis (blood, protein), others (activated partial thromboplastin time [APTT], prothrombin time (PT), autoantibody panel, C reactive protein, TSH, T3, T4, amylase, lipase, serum C3, C4, serum Ig level), are obtained.

Baseline human anti-human antibody (HAHA) titer is determined and pharmacokinetic (PK) specimen is obtained pre-dose.

The following endpoints are measured: PK parameters, HAHA, response rate and time to progression. Time to progression and overall survival are calculated using the Kaplan-Meier product limit method.

EXAMPLE 3:

CP-675,206 in Combination with 5-fluorouracil or capecitabine for Adjuvant or First-line Treatment of Colorectal Carcinoma

Patients having colorectal carcinoma (CRC) and intolerant of oxaliplatin (ELOXATIN) or irinotecan (CAMPTOSAR) with at least one lesion that can be accurately measured in two dimensions and whose size is ≥ 2 cm x 1 cm by conventional CT scan or ≥ 1 cm x 1 cm by spiral CT scan are given standard chemotherapy using 5FU (fluorouracil) or XELODA (capecitabine) per established protocols.

The patient is further administered a single IV infusion (100 mL/hr) of CP-675,206 as described herein at a dose of about 3 mg/kg. Prophylactic anti-emetics and anti-diarrheals are given as appropriate. The treatment is repeated after 28 days with escalation of the anti-CTLA4 antibody dose, that is, 6 mg/kg, 10 mg/kg and 15 mg/kg, every 28 days thereafter for maximum of 12 cycles in the absence of intolerable toxicity or disease progression. Preferably, CP-675,206 is administered at at least about 10 mg/kg every three weeks for four cycles and then every three months thereafter.

Preferably, the patient is premedicated with antihistamine (H1) at least one half hour prior to infusion of anti-CTLA4. Premedication is recommended but not required.

Doses are escalated using an accelerated titration design utilizing a dose-doubling schema with 3-6 subjects per cohort. Within each new cohort there is no required waiting period between subjects. Subsequent cohorts may not be opened until the first subject at the

current dose level has been observed for 21 days and subsequent subjects have been observed for 14 days.

Capecitabine for second line therapy or 5-fluorouracil for adjuvant therapy is administered sequentially or simultaneously with CP-675,206 either once, or repeatedly, as determined. Capecitabine and fluorouracil are administered according to well-known protocols, for examples, those disclosed in the FDA-approved label for each compound.

CP-675,206 is provided in 20 ml clear glass vials with a rubber stopper and an aluminum seal. Each vial contains 20 mg/ml (with a nominal fill of 400 mg/vial) of CP-675,206, in a sterile aqueous solution comprising 20 mM histidine buffer, PH 5.5, 84 mg/ml trehalose dehydrate, 0.2 mg/ml polysorbate 80, and 0.1 mg/ml disodium EDTA dehydrate.

For all patients, ECOG performance status, vital signs, and body weight are assessed pre-dose, and vital signs can be repeated post-dose, as clinically indicated. A physical examination (including ophthalmologic assessment and signs of autoimmunity) is performed on Day 1. Samples for hematology panel (hematocrit, RBC count, WBC count, differential), chemistry (Alkaline Phosphatase, calcium, chloride, GGT, LDH, magnesium, phosphorus, random glucose, sodium, urea, uric acid), urinalysis (blood, protein), others (activated partial thromboplastin time [APTT], prothrombin time (PT), autoantibody panel, C reactive protein, TSH, T3, T4, amylase, lipase, serum C3, C4, serum Ig level), are obtained.

Baseline human anti-human antibody (HAHA) titer is determined and pharmacokinetic (PK) specimen is obtained pre-dose.

The following endpoints are measured: PK parameters, HAHA, response rate and time to progression. Time to progression and overall survival are calculated using the Kaplan-Meier product limit method.

EXAMPLE 4:

CP-675,206 in Combination with FOLFOX for Adjuvant or First Line Treatment of Colorectal Carcinoma

Following surgery/radiotherapy patients having colorectal carcinoma (CRC) with at least one lesion that can be accurately measured in two dimensions and whose size is ≥ 2 cm x 1 cm by conventional CT scan or ≥ 1 cm x 1 cm by spiral CT scan are given standard chemotherapy using FOLFOX (fluorouracil, leucovorin and oxaliplatin) per established protocols.

The patient is further administered a single IV infusion (100 mL/hr) of anti-CTLA4 antibodies as described herein at a dose of about 3 mg/kg. Prophylactic anti-emetics and anti-diarrheals are given as appropriate. The treatment is repeated after 28 days with escalation of the anti-CTLA4 antibody dose, that is, 6 mg/kg, 10 mg/kg and 15 mg/kg, every 28 days thereafter for maximum of 12 cycles in the absence of intolerable toxicity or disease

progression. Preferably, CP-675,206 is administered at at least about 10 mg/kg every three weeks for four cycles and then every three months thereafter.

FOLFOX or FOLFIRI therapy is administered according to standard protocols. Briefly, for FOLFOX4, each chemotherapy cycle comprises a two hour infusion of 85 mg/m$^2$ oxaliplatin (ELOXATIN, Sanofi) on day 1 followed by a two hour infusion of 200 mg/m$^2$ leucovorin on days 1 and 2, followed by a bolus of 400 mg/m$^2$ of fluorouracil on days 1 and 2 and then a twenty-two hour infusion of 600 mg/m$^2$ of fluorouracil administered over two consecutive days (referred to as the De Gramont schedule). Chemotherapy is repeated every two weeks.

In an exemplary regimen, FOLFIRI is administered as a ninety minute infusion of 180 mg/m$^2$ irinotecan on day 1, a two hour infusion of folinic acid (leucovorin) at 400 mg/m$^2$ on day 1, and 400-500 mg/m$^2$ fluorouracil IV bolus after leucovorin on day 1, followed by fluorouracil at 2400-3000 mg/m$^2$ by continuous i.v. over forty-six hours starting on day one. The chemotherapy cycle is repeated every two weeks.

Preferably, the patient is premedicated with antihistamine (H1) at least one half hour prior to infusion of anti-CTLA4. Premedication is recommended but not required.

Doses are escalated using an accelerated titration design utilizing a dose-doubling schema with 3-6 subjects per cohort. Within each new cohort there is no required waiting period between subjects. Subsequent cohorts may not be opened until the first subject at the current dose level has been observed for 21 days and subsequent subjects have been observed for 14 days.

FOLFOX therapy is administered sequentially or simultaneously with anti-CTLA4 antibody either once, or repeatedly, as determined.

CP-675,206 is provided in 20 ml clear glass vials with a rubber stopper and an aluminum seal. Each vial contains 20 mg/ml (with a nominal fill of 400 mg/vial) of CP-675,206, in a sterile aqueous solution comprising 20 mM histidine buffer, PH 5.5, 84 mg/ml trehalose dehydrate, 0.2 mg/ml polysorbate 80, and 0.1 mg/ml disodium EDTA dehydrate.

For all patients, ECOG performance status, vital signs, and body weight are assessed pre-dose, and vital signs can be repeated post-dose, as clinically indicated. A physical examination (including ophthalmologic assessment and signs of autoimmunity) is performed on Day 1. Samples for hematology panel (hematocrit, RBC count, WBC count, differential), chemistry (Alkaline Phosphatase, calcium, chloride, GGT, LDH, magnesium, phosphorus, random glucose, sodium, urea, uric acid), urinalysis (blood, protein), others (activated partial thromboplastin time [APTT], prothrombin time (PT), autoantibody panel, C reactive protein, TSH, T3, T4, amylase, lipase, serum C3, C4, serum Ig level), are obtained.

Baseline human anti-human antibody (HAHA) titer is determined and pharmacokinetic (PK) specimen is obtained pre-dose.

The following endpoints are measured: PK parameters, HAHA, response rate and time to progression. Time to progression and overall survival are calculated using the Kaplan-Meier product limit method.

EXAMPLE 5:

CP-675,206 and Gemcitabine for Treatment of Pancreatic Cancer

Following surgery/radiotherapy, if any, patients having pancreatic cancer with at least one lesion that can be accurately measured in two dimensions and whose size is ≥ 2 cm x 1 cm by conventional CT scan or ≥ 1 cm x 1 cm by spiral CT scan are given standard chemotherapy comprising gemcitabine (*e.g.*, GEMZAR) per established protocols.

The patient is further administered a single IV infusion of anti-CTLA4 antibodies as described herein at a dose of at least about 10 mg/kg, preferably, at about 15 mg/kg. Prophylactic anti-emetics and anti-diarrheals are given as appropriate. The treatment is repeated after 28 days with escalation of the anti-CTLA4 antibody dose, if desired, every 28 days thereafter for maximum of 12 cycles in the absence of intolerable toxicity or disease progression. Preferably, CP-675,206 is administered at at least about 10 mg/kg every three weeks for four cycles and then every three months thereafter.

Gemcitabine is administered according to standard protocols. More particularly, gemcitabine (GEMZAR, Lilly) is administered by intravenous infusion at a dose of 1000 $mg/m^2$ over 30 minutes once weekly for up to 7 weeks (or until toxicity necessitates reducing or holding a dose) followed by a week of rest from treatment. Subsequent cycles should consist of infusions once weekly for 3 consecutive weeks out of every 4 weeks . CP-675,206 is administered after the initial gemcitabine cycle (seven weeks on and one week rest) and once every three weeks thereafter.

Preferably, the patient is premedicated with antihistamine (H1) at least one half hour prior to infusion of anti-CTLA4. Premedication is recommended but not required.

Where appropriate, antibody dose is escalated using an accelerated titration design utilizing a dose-doubling schema with 3-6 subjects per cohort. Within each new cohort there is no required waiting period between subjects. Subsequent cohorts may not be opened until the first subject at the current dose level has been observed for 21 days and subsequent subjects have been observed for 14 days.

CP-675,206 is provided in 20 ml clear glass vials with a rubber stopper and an aluminum seal. Each vial contains 20 mg/ml (with a nominal fill of 400 mg/vial) of CP-675,206, in a sterile aqueous solution comprising 20 mM histidine buffer, PH 5.5, 84 mg/ml trehalose dehydrate, 0.2 mg/ml polysorbate 80, and 0.1 mg/ml disodium EDTA dehydrate.

For all patients, ECOG performance status, vital signs, and body weight are assessed pre-dose, and vital signs can be repeated post-dose, as clinically indicated. A physical examination (including ophthalmologic assessment and signs of autoimmunity) is performed on Day 1. Samples for hematology panel (hematocrit, RBC count, WBC count, differential), chemistry (Alkaline Phosphatase, calcium, chloride, GGT, LDH, magnesium, phosphorus, random glucose, sodium, urea, uric acid), urinalysis (blood, protein), others (activated partial thromboplastin time [APTT], prothrombin time (PT), autoantibody panel, C reactive protein, TSH, T3, T4, amylase, lipase, serum C3, C4, serum Ig level), are obtained.

Baseline human anti-human antibody (HAHA) titer is determined and pharmacokinetic (PK) specimen is obtained pre-dose.

The following endpoints are measured: PK parameters, HAHA, response rate and time to progression. Time to progression and overall survival are calculated using the Kaplan-Meier product limit method.

EXAMPLE 6:

CP-675,206 in Combination with imatinib mesylate for First-line Treatment of Chronic Myeloid Leukemia

Patients having chronic myeloid leukemia (CML) are given standard chemotherapy using imatinib mesylate (GLEEVEC) per established protocols.

The patient is further administered a single IV infusion (100 mL/hr) of anti-CTLA4 antibodies as described herein at a dose of about 3 mg/kg. Prophylactic anti-emetics and anti-diarrheals are given as appropriate. The treatment is repeated after 28 days with escalation of the anti-CTLA4 antibody dose, that is, 6 mg/kg, 10 mg/kg and 15 mg/kg, every 28 days thereafter for maximum of 12 cycles in the absence of intolerable toxicity or disease progression. Preferably, CP-675,206 is administered at at least about 10 mg/kg every three weeks for four cycles and then every three months thereafter.

Imatinib mesylate (GLEEVEC, Novartis) is administered daily at about 400 mg/day for patients in chronic phase CML and 600 mg/day for adult patients in accelerated phase or blast crisis. Imatinib treatment may be continued as long as there is no evidence of progressive disease or unacceptable toxicity.

Preferably, the patient is premedicated with antihistamine (H1) at least one half hour prior to infusion of anti-CTLA4. Premedication is recommended but not required.

Doses are escalated using an accelerated titration design utilizing a dose-doubling schema with 3-6 subjects per cohort. Within each new cohort there is no required waiting period between subjects. Subsequent cohorts may not be opened until the first subject at the current dose level has been observed for 21 days and subsequent subjects have been observed for 14 days.

Imatinib mesylate is administered sequentially or simultaneously with anti-CTLA4 antibody either once, or repeatedly, as determined.

CP-675,206 is provided in 20 ml clear glass vials with a rubber stopper and an aluminum seal. Each vial contains 20 mg/ml (with a nominal fill of 400 mg/vial) of CP-675,206, in a sterile aqueous solution comprising 20 mM histidine buffer, PH 5.5, 84 mg/ml trehalose dehydrate, 0.2 mg/ml polysorbate 80, and 0.1 mg/ml disodium EDTA dehydrate.

For all patients, ECOG performance status, vital signs, and body weight are assessed pre-dose, and vital signs can be repeated post-dose, as clinically indicated. A physical examination (including ophthalmologic assessment and signs of autoimmunity) is performed on Day 1. Samples for hematology panel (hematocrit, RBC count, WBC count, differential), chemistry (Alkaline Phosphatase, calcium, chloride, GGT, LDH, magnesium, phosphorus, random glucose, sodium, urea, uric acid), urinalysis (blood, protein), others (activated partial thromboplastin time [APTT], prothrombin time (PT), autoantibody panel, C reactive protein, TSH, T3, T4, amylase, lipase, serum C3, C4, serum Ig level), are obtained.

Baseline human anti-human antibody (HAHA) titer is determined and pharmacokinetic (PK) specimen is obtained pre-dose.

The following endpoints are measured: PK parameters, HAHA, response rate and time to progression. Time to progression and overall survival are calculated using the Kaplan-Meier product limit method.

EXAMPLE 7:

CP-675,206 in Combination with Imatinib Mesylate (GLEEVEC) for First-line Treatment of Chronic Lymphocytic Leukemia

Patients having chronic lymphocytic leukemia (CLL) are given standard chemotherapy using imatinib mesylate (GLEEVEC) per established protocols.

The patient is further administered a single IV infusion (100 mL/hr) of anti-CTLA4 antibodies as described herein at a dose of about 3 mg/kg. Prophylactic anti-emetics and anti-diarrheals are given as appropriate. The treatment is repeated after 28 days with escalation of the anti-CTLA4 antibody dose, that is, 6 mg/kg, 10 mg/kg and 15 mg/kg, every 28 days thereafter for maximum of 12 cycles in the absence of intolerable toxicity or disease progression. Preferably, CP-675,206 is administered at at least about 10 mg/kg every three weeks for four cycles and then every three months thereafter. Imatinib mesylate (GLEEVEC, Novartis) is administered daily at about 400 mg/day or 600 mg/day, and may be continued as long as there is no evidence of progressive disease or unacceptable toxicity.

Preferably, the patient is premedicated with antihistamine (H1) at least one half hour prior to infusion of anti-CTLA4. Premedication is recommended but not required.

Doses are escalated using an accelerated titration design utilizing a dose-doubling schema with 3-6 subjects per cohort. Within each new cohort there is no required waiting period between subjects. Subsequent cohorts may not be opened until the first subject at the current dose level has been observed for 21 days and subsequent subjects have been observed for 14 days.

GLEEVEC is administered sequentially or simultaneously with anti-CTLA4 antibody either once, or repeatedly, as determined.

CP-675,206 is provided in 20 ml clear glass vials with a rubber stopper and an aluminum seal. Each vial contains 20 mg/ml (with a nominal fill of 400 mg/vial) of CP-675,206, in a sterile aqueous solution comprising 20 mM histidine buffer, PH 5.5, 84 mg/ml trehalose dehydrate, 0.2 mg/ml polysorbate 80, and 0.1 mg/ml disodium EDTA dehydrate.

For all patients, ECOG performance status, vital signs, and body weight are assessed pre-dose, and vital signs can be repeated post-dose, as clinically indicated. A physical examination (including ophthalmologic assessment and signs of autoimmunity) is performed on Day 1. Samples for hematology panel (hematocrit, RBC count, WBC count, differential), chemistry (Alkaline Phosphatase, calcium, chloride, GGT, LDH, magnesium, phosphorus, random glucose, sodium, urea, uric acid), urinalysis (blood, protein), others (activated partial thromboplastin time [APTT], prothrombin time (PT), autoantibody panel, C reactive protein, TSH, T3, T4, amylase, lipase, serum C3, C4, serum Ig level), are obtained.

Baseline human anti-human antibody (HAHA) titer is determined and pharmacokinetic (PK) specimen is obtained pre-dose.

The following endpoints are measured: PK parameters, HAHA, response rate and time to progression. Time to progression and overall survival are calculated using the Kaplan-Meier product limit method.

## EXAMPLE 8:

### CP-675,206 in Combination with carboplatin and paclitaxel for First-line Treatment of Advanced Ovarian Carcinoma

Patients having advanced carcinoma of the ovary are given standard chemotherapy using carboplatin and paclitaxel per established protocols.

The patient is further administered a single IV infusion (100 mL/hr) of anti-CTLA4 antibodies as described herein at a dose of about 3 mg/kg. Prophylactic anti-emetics and anti-diarrheals are given as appropriate. The treatment is repeated after 28 days with escalation of the anti-CTLA4 antibody dose, that is, 6 mg/kg, 10 mg/kg and 15 mg/kg, every 28 days thereafter for maximum of 12 cycles in the absence of intolerable toxicity or disease progression. Preferably, CP-675,206 is administered at at least about 10 mg/kg every three weeks for four cycles and then every three months thereafter.

In an exemplary regimen, paclitaxel is administered every three weeks at 175 mg/m$^2$ by i.v. infusion over ninety minutes followed by carboplatin (area under the time-concentration curve [AUC] of 5) administered i.v. over thirty to sixty minutes.

Preferably, the patient is premedicated with antihistamine (H1) at least one half hour prior to infusion of anti-CTLA4. Premedication is recommended but not required.

Doses are escalated using an accelerated titration design utilizing a dose-doubling schema with 3-6 subjects per cohort. Within each new cohort there is no required waiting period between subjects. Subsequent cohorts may not be opened until the first subject at the current dose level has been observed for 21 days and subsequent subjects have been observed for 14 days.

Carboplatin and paclitaxel combination is administered sequentially or simultaneously with anti-CTLA4 antibody either once, or repeatedly, as determined.

CP-675,206 is provided in 20 ml clear glass vials with a rubber stopper and an aluminum seal. Each vial contains 20 mg/ml (with a nominal fill of 400 mg/vial) of CP-675,206, in a sterile aqueous solution comprising 20 mM histidine buffer, PH 5.5, 84 mg/ml trehalose dehydrate, 0.2 mg/ml polysorbate 80, and 0.1 mg/ml disodium EDTA dehydrate.

For all patients, ECOG performance status, vital signs, and body weight are assessed pre-dose, and vital signs can be repeated post-dose, as clinically indicated. A physical examination (including ophthalmologic assessment and signs of autoimmunity) is performed on Day 1. Samples for hematology panel (hematocrit, RBC count, WBC count, differential), chemistry (Alkaline Phosphatase, calcium, chloride, GGT, LDH, magnesium, phosphorus, random glucose, sodium, urea, uric acid), urinalysis (blood, protein), others (activated partial thromboplastin time [APTT], prothrombin time (PT), autoantibody panel, C reactive protein, TSH, T3, T4, amylase, lipase, serum C3, C4, serum Ig level), are obtained.

Baseline human anti-human antibody (HAHA) titer is determined and pharmacokinetic (PK) specimen is obtained pre-dose.

The following endpoints are measured: PK parameters, HAHA, response rate and time to progression. Time to progression and overall survival are calculated using the Kaplan-Meier product limit method.

EXAMPLE 9:

CP-675,206 in Combination with carboplatin and paclitaxel and either bevacizumab, PF03512676, or sunitinib, for First-line Treatment of Non-Small Cell Lung Cancer

Patients having locally advanced Stage IIIb or mestatatic Stage IV NSCLC are given standard chemotherapy using carboplatin and paclitaxel per established protocols.

The patient is further administered a single IV infusion (100 mL/hr) of anti-CTLA4 antibodies as described herein at a dose of about 15 mg/kg. Prophylactic anti-emetics and anti-diarrheals are given as appropriate. The treatment is repeated after 28 days for approximately 12 cycles in the absence of intolerable toxicity or disease progression. Preferably, CP-675,206 is administered at at least about 10 mg/kg every three weeks for four cycles and then every three months thereafter.

The patient is further administered either bevacizumab (AVASTIN), PF03512676, or sunitinib per established protocols. Bevacizumab may be administered at about 5 mg/kg every fourteen days by i.v. infusion until disease progression is detected. Sunitinib may be administered as one 50 mg oral dose taken daily, on a schedule of four weeks on treatment followed by two weeks off. Sunitinib may be taken with or without food. Dose increase or reduction of 12.5 mg increments is recommended based on individual safety and tolerability. An exemplary carboplatin and paclitaxel dosing regimen is as provided *supra.*

Preferably, the patient is premedicated with antihistamine (H1) at least one half hour prior to infusion of anti-CTLA4. Premedication is recommended but not required.

Carboplatin and paclitaxel combination is administered sequentially or simultaneously with anti-CTLA4 antibody either once, or repeatedly, as determined. Similarly, bevacizumab, sunitinib, or PF03512676 is administered sequentially or simultaneously with anti-CTLA4-carboplatin-paclitaxel, either once, or repeatedly, as indicated.

CP-675,206 is provided in 20 ml clear glass vials with a rubber stopper and an aluminum seal. Each vial contains 20 mg/ml (with a nominal fill of 400 mg/vial) of CP-675,206, in a sterile aqueous solution comprising 20 mM histidine buffer, PH 5.5, 84 mg/ml trehalose dehydrate, 0.2 mg/ml polysorbate 80, and 0.1 mg/ml disodium EDTA dehydrate.

For all patients, ECOG performance status, vital signs, and body weight are assessed pre-dose, and vital signs can be repeated post-dose, as clinically indicated. A physical examination (including ophthalmologic assessment and signs of autoimmunity) is performed on Day 1. Samples for hematology panel (hematocrit, RBC count, WBC count, differential), chemistry (Alkaline Phosphatase, calcium, chloride, GGT, LDH, magnesium, phosphorus, random glucose, sodium, urea, uric acid), urinalysis (blood, protein), others (activated partial thromboplastin time [APTT], prothrombin time (PT), autoantibody panel, C reactive protein, TSH, T3, T4, amylase, lipase, serum C3, C4, serum Ig level), are obtained.

Baseline human anti-human antibody (HAHA) titer is determined and pharmacokinetic (PK) specimen is obtained pre-dose.

The following endpoints are measured: PK parameters, HAHA, response rate and time to progression. Time to progression and overall survival are calculated using the Kaplan-Meier product limit method.

EXAMPLE 10:

CP-675,206 in Combination with either docetaxel, erlotinib or pemetrexed, for Second Line Treatment of Non-Small Cell Lung Cancer

Patients having locally advanced Stage IIIb or mestatatic Stage IV NSCLC, and which have previously failed platinum-based chemotherapy, are given standard chemotherapy using one of either docetaxel (TAXOTERE), erlotinib (TARCEVA), or pemetrexed (ALIMTA) per established protocols.

The patient is further administered a single IV infusion (100 mL/hr) of anti-CTLA4 antibodies as described herein at a dose of about 15 mg/kg. Prophylactic anti-emetics and anti-diarrheals are given as appropriate. The treatment is repeated after 28 days for approximately 12 cycles in the absence of intolerable toxicity or disease progression. Preferably, CP-675,206 is administered at at least about 10 mg/kg every three weeks for four cycles and then every three months thereafter.

Docetaxel (TAXOTERE, Sanofi-Aventis) is administered at about 75 $mg/m^2$ by i.v. infusion over one hour every three weeks. Erlotinib (TARCEVA, OSI Pharms.) is administered as a daily dose of about 150 mg taken at least one hour or two hours after ingestion of food. Treatment should continue until disease progression or unacceptable toxicity occurs. Pemetrexed (ALIMTA, Lilly) is administered at a dose of about 500 $mg/m^2$ administered as an i.v. infusion over 10 minutes on day 1 of each 21-day cycle.

Preferably, the patient is premedicated with antihistamine (H1) at least one half hour prior to infusion of anti-CTLA4. Premedication is recommended but not required.

Docetaxel, erlotinib or pemetrexed is administered sequentially or simultaneously with anti-CTLA4 antibody either once, or repeatedly, as determined.

CP-675,206 is provided in 20 ml clear glass vials with a rubber stopper and an aluminum seal. Each vial contains 20 mg/ml (with a nominal fill of 400 mg/vial) of CP-675,206, in a sterile aqueous solution comprising 20 mM histidine buffer, PH 5.5, 84 mg/ml trehalose dehydrate, 0.2 mg/ml polysorbate 80, and 0.1 mg/ml disodium EDTA dehydrate.

For all patients, ECOG performance status, vital signs, and body weight are assessed pre-dose, and vital signs can be repeated post-dose, as clinically indicated. A physical examination (including ophthalmologic assessment and signs of autoimmunity) is performed on Day 1. Samples for hematology panel (hematocrit, RBC count, WBC count, differential), chemistry (Alkaline Phosphatase, calcium, chloride, GGT, LDH, magnesium, phosphorus, random glucose, sodium, urea, uric acid), urinalysis (blood, protein), others (activated partial thromboplastin time [APTT], prothrombin time (PT), autoantibody panel, C reactive protein, TSH, T3, T4, amylase, lipase, serum C3, C4, serum Ig level), are obtained.

Baseline human anti-human antibody (HAHA) titer is determined and pharmacokinetic (PK) specimen is obtained pre-dose.

The following endpoints are measured: PK parameters, HAHA, response rate and time to progression. Time to progression and overall survival are calculated using the Kaplan-Meier product limit method.

EXAMPLE 11:

CP-675,206 in Combination with platinum-based chemotherapy for Treatment of Non-Small Cell Lung Cancer

Patients having advanced Stage IIIb (with effusion) or mestatatic Stage IV NSCLC, and which have previously responded or remained stable after a platinum-based chemotherapy first line regimen, are given CP-675,206 at least about three weeks but not more than about six weeks after the lose of first line platinum-based therapy.

The patient is further administered a single IV infusion (100 mL/hr) of anti-CTLA4 antibodies as described herein at a dose of about 15 mg/kg. Prophylactic anti-emetics and anti-diarrheals are given as appropriate. The treatment is repeated after 28 days for approximately 12 cycles in the absence of intolerable toxicity or disease progression. More preferably, CP-675,206 is administered at at least about 10 mg/kg every three weeks for four cycles and then every three months thereafter.

Preferably, the patient is premedicated with antihistamine (H1) at least one half hour prior to infusion of anti-CTLA4. Premedication is recommended but not required.

Docetaxel, erlotinib or pemetrexed is administered sequentially or simultaneously with anti-CTLA4 antibody either once, or repeatedly, as determined.

CP-675,206 is provided in 20 ml clear glass vials with a rubber stopper and an aluminum seal. Each vial contains 20 mg/ml (with a nominal fill of 400 mg/vial) of CP-675,206, in a sterile aqueous solution comprising 20 mM histidine buffer, PH 5.5, 84 mg/ml trehalose dehydrate, 0.2 mg/ml polysorbate 80, and 0.1 mg/ml disodium EDTA dehydrate.

For all patients, ECOG performance status, vital signs, and body weight are assessed pre-dose, and vital signs can be repeated post-dose, as clinically indicated. A physical examination (including ophthalmologic assessment and signs of autoimmunity) is performed on Day 1. Samples for hematology panel (hematocrit, RBC count, WBC count, differential), chemistry (Alkaline Phosphatase, calcium, chloride, GGT, LDH, magnesium, phosphorus, random glucose, sodium, urea, uric acid), urinalysis (blood, protein), others (activated partial thromboplastin time [APTT], prothrombin time (PT), autoantibody panel, C reactive protein, TSH, T3, T4, amylase, lipase, serum C3, C4, serum Ig level), are obtained.

Baseline human anti-human antibody (HAHA) titer is determined and pharmacokinetic (PK) specimen is obtained pre-dose.

The following endpoints are measured: PK parameters, HAHA, response rate and time to progression. Time to progression and overall survival are calculated using the Kaplan-Meier product limit method.

EXAMPLE 12:

Natural history of diarrhea associated with CTLA4 blockade by anti-CTLA4 antibody

Diarrhea resulting from immune activation has been associated with CTLA4 blockade. For example in patients with stage IV melanoma receiving ipilimumab (MDX-010), a number of patients developed grade 3/4 autoimmune enterocolitis and severe diarrhea (Attia et al., 2005). In a single-dose phase I trial of CP-675,206 at doses up to 15 mg/kg in patients with solid tumors (n = 39), 9 instances of diarrhea were reported including 3 grade 3 events (Ribas et al., 2005). The incidence and severity of diarrhea was assessed in patients receiving CP-675,206 in a large phase I/II study.

An open-label phase I/II trial of CP-675,206 was conducted in patients with stage III (unresectable) or stage IV melanoma and an ECOG PS ≤ 1. Diarrhea was assessed in patients treated at the phase II doses: 10 mg/kg once per month (Q1M) in phase I (n = 22), or 10 mg/kg Q1M (n = 44) or 15 mg/kg once every 3 months (Q3M) (n = 45) in phase II.

Medians of 3.5 doses (range, 1 to 18) at 10 mg/kg Q1M (10Q1M) in phase I, 3 doses (range, 1 to 26) at 10 mg/kg Q1M in phase II, and 1 dose (range, 1 to 9) at 15 mg/kg Q3M (15Q3M) were administered with 100% dose compliance. Treatment-related diarrhea was reported by 43 (39%) of 111 patients, and grade 3 diarrhea occurred in 14 (13%) patients. One patient had grade 4 colitis resulting in a colectomy. Diarrhea (all grades) occurred with similar frequency in each dose group; however, grade 3 treatment-related diarrhea occurred in 8% of patients treated with 15 mg/kg Q3M compared with 18% of patients treated with 10 mg/kg Q1M in phase I and 14% of patients treated with 10 mg/kg Q1M in phase II. Among 9 patients with an objective response, 8 experienced diarrhea (3 of which were grade 3). The majority of cases (65%) were mild to moderate in severity with a median time to onset of 51 days (range, 1 to 583 days) and resolution of 8 days (range, 1 to 182 days). More than half of patients who reported serious events of diarrhea were treated with steroids.

Diarrhea associated with CP-675,206 was primarily mild to moderate in severity, transient, and manageable. In addition, 15 mg/kg Q3M may be better tolerated than 10 mg/kg Q1M.

EXAMPLE 13:

Survival of patients with metastatic melanoma treated with anti-CTLA4 monoclonal antibody CP-675,206 in a phase I/II study

CP-675,206 has demonstrated clinical activity in patients with metastatic melanoma. Prolonged survival was observed in a prior single-dose phase I study, even in patients who did not achieve objective tumor responses.

A multidose phase I/II trial was conducted in patients (N = 119) with histologically confirmed stage IIIc (unresectable) or stage IV recurrent metastatic melanoma and ECOG PS ≤ 1. The study consisted of a phase I, open-label, multidose study (3, 6, and 10 mg/kg) and a phase I expansion cohort for HLA-A2.1+ patients (10 mg/kg monthly [10Q1M]), followed by a phase II open-label study of 2 dosing regimens: 10 mg/kg Q1M and 15 mg/kg every 3 months (15Q3M). The primary endpoint was safety in phase I, immune monitoring in the expansion cohort, and response in phase II. Survival was analyzed as a secondary endpoint.

In the phase I study, Kaplan-Meier estimates of median overall survival were 17.6 months for all dose groups combined (n = 28). In the phase II study, median survival was 10.3 months in the 10 mg/kg arm and 11.0 months in the 15 mg/kg arm. Survival outcomes were favorable, compared with historical median survival of 7 months, independent of whether patients achieved an objective response.

| Regimen | Enrolled, n | Died, n (%) | Kaplan-Meier estimate* of median survival, months (95% CI) |
|---|---|---|---|
| Phase I | 28 | 18 (64.3) | 17.6 (8, 25.2) |
| 3 mg/kg | 3 | 3 (100.0) | 8.0 (4.4, 17.8) |
| 6 mg/kg | 3 | 3 (100.0) | 7.5 (4.4, 19.5) |
| 10 mg/kg | 8 | 6 (75.0) | 19.3 (6.01, NE) |
| 10 mg/kg expansion | 14 | 6 (42.9) | 25.1 (9.9, NE) |
| Phase II | | | |
| 10 mg/kg, Q1M | 44 | 29 (65.8) | 10.3 (6.6, 13.2) |
| 15 mg/kg, Q3M | 45 | 32 (71.1) | 11.0 (8.1, 16.7) |

*Patients censored when last known to be alive.

NE = Cannot be estimated.

Patients participating in a multiple dose study of CP-675,206 showed a survival time that was greater than expected on historic controls.

EXAMPLE 14:

Comparison of dose and dosing schedule of CP-675,206 anti-CTLA4 monoclonal antibody in a Phase II clinical trial in patients with advanced melanoma

A two-stage, two-arm phase II trial was conducted to assess the optimal dosing regimen for pivotal clinical trial testing of CP-675,206.

Eligible patients had measurable melanoma (stage IIIc or IV) progressing on or after prior therapy with ECOG PS ≤ 1. In stage 1, 18 patients per arm were randomized to either 10 mg/kg once per month (10Q1M) or 15 mg/kg every 3 months (15Q3M). If 3 or more patients in either arm had complete response (CR) or partial response (PR), then 25 more patients were entered to that arm. Primary endpoint was objective tumor response (OR), and secondary endpoints were safety and survival.

Eighty-nine (89) patients received at least 1 dose (44 at 10Q1M, 45 at 15Q3M), with both study arms moving to stage 2. Ninety-six percent of patients had stage IV disease, and 57% had elevated LDH. There were no significant differences in age, sex, stage, or baseline LDH levels between study groups. A median of 3 doses (range, 1 to 26) at 10Q1M and 1 dose (range, 1 to 9) at 15Q3M were administered with 100% compliance. Dose delays occurred in 30% of patients treated at 10Q1M and 16% at 15Q3M. Two (2) patients at 10 Q1M and five (5) patients at 15 Q3M continued on study beyond 12 months.

Six patients at 10Q1M were discontinued due to the following toxicity: three due to diarrhea/colitis (one patient requiring colectomy); one due to Grave's ophthalmopathy; one due to pancreatitis; and one due to hypersensitivity reaction. Two patients at 15Q3M were discontinued: one due to colitis and pancreatitis, and the other due to diarrhea ($P$ = 0.14). There were no toxic deaths. The dose of 15Q3M was associated with lower incidence of grade 3 or 4 adverse events (AEs), that is, 31% compared with 41% at 10 Q1M; $P$ = 0.42.

Responses by investigator assessment were 1 CR and 3 PRs at 10 Q1M, and 1 CR and 2 PRs at 15 Q3M, including responses in skin, lymph nodes (LN), bone, liver, lung, and adrenal glands. Only 1 patient with PR at 10Q1M relapsed, and the remaining responses were ongoing (18+ to 28+ months). Median survival was 10.3 months at 10Q1M and 11.0 months at 15Q3M ($P$ = NS).

The 15 mg/kg every three months (15Q3M) regimen was chosen for further clinical testing based on comparable antitumor efficacy and a trend to improved feasibility and safety compared with 10 mg/kg every month (10Q1M).

EXAMPLE 15:

Increased anti-virus titers in combination immunization of Rhesus monkeys

Combination therapy comprising administration of a vaccine and anti-CTLA4 antibody mediated an increased antiviral immune response.

Rhesus macaques were immunized with FLUZONE subvirion 1998-99 formula (NDC 49281-362-11 Influenza Virus Vaccine, Trivalent Types A and B; Aventis Pasteur Inc. (includes Connaught Labs., Pasteur Merieux and Pasteur Merieux Connaught)). The 1998-99 vaccine contained hemagglutinin A (HA) from three strains: A/Beijing/262/95 (H1N1), A/Sydney/5/97 (H3N2), and B/Harbin/07/94 (a B/Beijing/184/93-like strain). The monkeys were immunized intramuscularly (IM) with FLUZONE on day 0 (week 0) and again 4 weeks later (week 4). Antibody was administered according to the following schedule: Group 1 was administered anti-CTLA4 antibody CP-642,570 (5 mg/kg) intravenously (IV) on week 0; Group 2 was administered an irrelevant control antibody (anti-KLH antibody; 5 mg/kg IV) on week 0; Group 3 was immunized IM with FLUZONE on week 0 and reimmunized IM with FLUZONE on week 4 and administered anti-CTLA4 antibody (5 mg/kg IV) on week 4; Group 4 was immunized IM with FLUZONE on week 0 and reimmunized IM with FLUZONE on week 4 and administered an irrelevant control antibody (anti-KLH antibody at 5 mg/kg IV) on week 4; and Group 5 was immunized IM with FLUZONE on week 0 and reimmunized IM with FLUZONE on week 4 but no antibody was administered. The animals were followed for eight weeks after the initial immunization with FLUZONE.

The anti-CTLA4 antibody administered was human anti-human CTLA4 designated CP-642,570 (also referred to herein as antibody 4.1.1).

Serum was collected at 1 hour and 24 hours after anti-CTLA4 administration and every two weeks thereafter. The following parameters were assessed: anti-CTLA4 serum levels, IgG titers to FLUZONE, level of Neopterin, and level of 2-5 adenylate synthetase.

Plasma levels of anti-CTLA4 antibody confirmed exposure, *i.e.*, plasma levels were approximately 75 µg/ml at 1 hour, 40-50 µg/ml at 24 hours and, for the most part, not detectable by two weeks (Figure 4). One animal in Group 3 had undetectable levels of antibody, possibly due to a missed injection or a switched tube.

The animals were assessed for various markers of immune activation. Neopterin levels were variable (Figure 5). That is, an initial spike in value was detected in the anti-CTLA4 group two weeks post-immunization and treatment. Four weeks after immunization, the basal levels dropped and no differences were observed between the groups, including groups 3 and 4.

Whole blood was obtained and cell pellets were collected and assayed for 2-5 adenylate synthetase. Groups 1 and 2 were compared (Figure 6). The data indicate that

there was a detectable increase in the anti-CTLA4 group (Group 1) compared with the control group.

An increase in anti-FLUZONE IgG level was detected at six weeks in animals treated with anti-CTLA4 at the time of the second immunization (Group 3) (Figure 7). No effect on anti-FLUZONE titer was observed in any of the other groups of animals. The anti-FLUZONE IgG titers for each individual animal is depicted in Figure 8 for prebleed (week -1) (+), week 0 (open triangle), week 2 (dark triangle), week 4 (open square), week 6 (shaded circle), and week 8 (open circle). The data points are grouped according to test group, *i.e.*, Group 1, 2, 3, 4, and 5. Values shown in boxes indicate repeat titers from the same sample.

Figure 9 shows the anti-FLUZONE serum IgG titers at week 6 in Rhesus monkeys. Individual animal results were grouped according to test group and indicate that Group 3 animals (administered anti-CTLA4 antibody on week 4) demonstrated increased anti-FLUZONE IgG (flu-IgG) titers compared with the other animals. One animal exhibited an increased flu-IgG titer but anti-CTLA4 exposure (by plasma level assay) could not be confirmed. While some spikes in anti-flu antibody titers were observed in control groups, the titers were lower in magnitude.

In summary, an increase in flu-IgG and 2-5 adenylate synthetase was observed in animals administered anti-CTLA4 antibody suggesting that anti-CTLA4 administered to virally infected patients, to patients immunized with viral antigens, or a portion thereof, and/or when combined with anti-viral treatment enhanced therapeutic benefit.

The disclosures of each and every patent, patent application, and publication cited herein are hereby incorporated herein by reference in their entirety.

While the invention has been disclosed with reference to specific embodiments, it is apparent that other embodiments and variations of this invention may be devised by others skilled in the art without departing from the true spirit and scope of the invention. The appended claims are intended to be construed to include all such embodiments and equivalent variations.

CLAIMS

1. A method for the treatment of cancer in a patient in need of such treatment, said method comprising administering to said patient a therapeutically effective amount of CP-675,206 anti-CTLA4 antibody in combination with a therapeutically effective amount of at least one therapeutic agent, wherein said cancer and said agent are selected from the group consisting of:

(a) the cancer is non-Hodgkin's lymphoma (NHL) and wherein the agent is rituximab;

(b) the cancer is NHL and the agent is cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP);

(c) the cancer is NHL and the agent is cyclophosphamide, doxorubicin, vincristine, prednisone and rituximab (CHOP-R);

(d) the cancer is lung cancer and the agent is bevacizumab;

(e) the cancer is non-small cell lung cancer (NSCLC) and the agent is gefitinib;

(f) the cancer is NSCLC and the agent is bevacizumab;

(g) the cancer is NSCLC and the agent is a taxane and gemcitabine, and further wherein the taxane is selected from the group consisting of docetaxel and paclitaxel;

(h) the cancer is NSCLC and the agent is a taxane and a platinum compound;

(i) the cancer is NSCLC and the agent is docetaxel;

(j) the cancer is NSCLC and the agent is erlotinib;

(k) the cancer is NSCLC and the agent is pemetrexed;

(l) the cancer is NSCLC and the agent is a platinum compound;

(m) the cancer is gastric cancer and the agent is irinotecan;

(n) the cancer is gastric cancer and the agent is fluorouracil and leucovorin;

(o) the cancer is liver cancer and the agent is doxorubicin, ifosfamide and vincristine;

(p) the cancer is liver cancer and the agent is doxorubicin and vincristine;

(q) the cancer is colorectal carcinoma (CRC) and the agent is fluorouracil;

(r) the cancer is CRC and the agent is capecitabine;

(s) the cancer is CRC and the agent is fluorouracil, leucovorin, and oxaliplatin (FOLFOX);

(t) the cancer is CRC and the agent is fluorouracil, leucovorin, and irinotecan (FOLFIRI);

(u) the cancer is CRC and the agent is cetuximab;

(v) the cancer is chronic myeloid leukemia (CML) and the agent is imatinib mesylate;

(w) the cancer is chronic lymphocytic leukemia (CLL) and the agent is imatinib mesylate;

(x) the cancer is pancreatic cancer and the agent is gemcitabine;

(y) the cancer is breast cancer and the agent is a taxane;

(z) the cancer is breast cancer and the agent is cyclophosphamide, doxorubicin and a taxane;

(aa) the cancer is breast cancer and the agent is selected from the group consisting of tamoxifen, anastrazole, letrozole, and fulvestrant;

(bb) the cancer is breast cancer and the agent is trastuzumab;

(cc) the cancer is breast cancer and the agent is bevacizumab;

(dd) the cancer is breast cancer and the agent is cetuximab;

(ee) the cancer is breast cancer and the agent is axitinib;

(ff) the cancer is bladder cancer and the agent is Bacillus Calmette-Guerin (BCG);

(gg) the cancer is bladder cancer and the agent is gemcitabine and cisplatin;

(hh) the cancer is melanoma and the agent is interferon alpha;

(ii) the cancer is multiple myeloma and the agent is bortezomib;

(jj) the cancer is multiple myeloma and the agent is dexamethasone and thalidomide; and

(kk) the cancer is ovarian cancer and the agent is carboplatin and paclitaxel.

2. The method of claim 1, wherein said treatment is selected from the group consisting of neoadjuvant therapy, adjuvant therapy, first-line therapy, second-line therapy, and third-line therapy.

3. The method of claim 1, wherein said agent is administered sequentially or contemporaneously with said antibody.

4. The method of claim 1 according to subparagraph (i), wherein said taxane is paclitaxel and wherein said platinum compound is carboplatin.

5. The method of claim 4, wherein said method further comprises administering at least one agent selected from the group consisting of bevacizumab, PF03512676, and sunitinib.

6. The method of claim 1(i), further comprising administering at least one agent selected from the group consisting of erlotinib and pemetrexed, wherein said treatment comprises second line therapy.

7. The method of claim 1, wherein said therapeutically effective amount of said antibody, or portion thereof, ranges from about 1 mg/kg to 40 mg/kg.

8. The method of claim 7, wherein said amount ranges from about 3 mg/kg to 15 mg/kg.

9. The method of claim 1 according to subparagraph (s), wherein said treatment is selected from the group consisting of first line therapy and adjuvant therapy following surgical resection of a primary colon tumor.

10. The method of claim 1 according to subparagraph (x), wherein said pancreatic cancer is selected from the group consisting of non-resectable Stage II, locally advanced Stage III, and metastatic Stage IV and wherein said treatment comprises first line therapy.

11. The method of claim 1 according to subparagraph (x), wherein said antibody is administered after administration of the last dose of gemcitabine.

12. The method of claim 17, wherein said antibody is administered at least about three weeks after and within at least six weeks of administration of the last dose of gemcitabine.

13. The method of claim 1 according to subparagraph (y), wherein said treatment comprises first line therapy.

14. The method of claim 1 according to subparagraph (kk), wherein said treatment comprises first line therapy.

15. The method of claim 1 according to subparagraph (l), wherein said NSCLC responded or remained stable after about six cycles of administration of said platinum compound and further wherein said antibody is administered after said platinum-based therapy.

16. A method for preventing or treating infection by HIV or for preventing, treating or delaying the onset of AIDS in a patient in need thereof, said method comprising administering to said patient a therapeutically effective amount of anti-CTLA4 antibody CP-675,206 and further comprising administering a therapeutically effective amount of at least one antiviral agent selected from the group consisting of an HIV protease inhibitor, a non-nucleoside reverse transcriptase inhibitor, a nucleoside/nucleotide reverse transcriptase inhibitor, a CCR5 antagonist, an inhibitor of gp120 interaction with CD4, an HIV fusion inhibitor, a HIV integrase inhibitor, an RNaseH inhibitor, a prenylation inhibitor, and a maturation inhibitor.

17. The method of claim 16, wherein said CCR5 antagonist is maraviroc.

18. The method of claim 17, said method further comprising assessing the co-receptor tropism of said HIV.

19. A method for preventing or treating infection by HIV or for preventing, treating or delaying the onset of AIDS in a patient in need thereof, said method comprising administering to said patient a therapeutically effective amount of an anti-CTLA4 antibody and maraviroc.

20. The method of claim 19, wherein said anti-CTLA4 antibody is selected from the group consisting of CP-675,206 and ipilimumab.

# FIGURE 1 4.1.1

## FIGURE 1A 4.1.1 Heavy Chain cDNA (SEQ ID NO:1)

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atggagtttg ggctgagctg ggttttcctc gttgctcttt taagaggtgt ccagtgtcag     60
gtgcagctgg tggagtctgg gggaggcgtg gtccagcctg ggaggtccct gagactctcc    120
tgtgtagcgt ctggattcac cttcagtagc catggcatgc actgggtccg ccaggctcca    180
ggcaaggggc tggagtgggt ggcagttata tggtatgatg gaagaaataa atactatgca    240
gactccgtga agggccgatt caccatctcc agagacaatt ccaagaacac gctgtttctg    300
caaatgaaca gcctgagagc cgaggacacg gctgtgtatt actgtgcgag aggaggtcac    360
ttcggtcctt ttgactactg gggccaggga accctggtca ccgtctcctc agcctccacc    420
aagggcccat cggtcttccc cctggcgccc tgctccagga gcacctccga gagcacagcg    480
gccctgggct gcctggtcaa ggactacttc cccgaaccgg tgacggtgtc gtggaactca    540
ggcgctctga ccagcggcgt gcacaccttc ccagctgtcc tacagtcctc aggactctac    600
tccctcagca gcgtggtgac cgtgccctcc agcaacttcg gcacccagac ctacacctgc    660
aacgtagatc acaagcccag caacaccaag gtggacaaga cagttgagcg caaatgttgt    720
gtcgagtgcc caccgtgccc agcaccacct gtggcaggac cgtcagtctt cctcttcccc    780
ccaaaaccca aggacaccct catgatctcc cggacccctg aggtcacgtg cgtggtggtg    840
gacgtgagcc acgaagaccc cgaggtccag ttcaactggt acgtggacgg cgtggaggtg    900
cataatgcca agacaaagcc acgggaggag cagttcaaca gcacgttccg tgtggtcagc    960
gtcctcaccg ttgtgcacca ggactggctg aacggcaagg agtacaagtg caaggtctcc   1020
aacaaaggcc tcccagcccc catcgagaaa accatctcca aaaccaaagg gcagccccga   1080
gaaccacagg tgtacaccct gccccatcc cgggaggaga tgaccaagaa ccaggtcagc    1140
ctgacctgcc tggtcaaagg cttctacccc agcgacatcg ccgtggagtg ggagagcaat   1200
gggcagccgg agaacaacta caagaccaca cctcccatgc tggactccga cggctccttc   1260
ttcctctaca gcaagctcac cgtggacaag agcaggtggc agcaggggaa cgtcttctca   1320
tgctccgtga tgcatgaggc tctgcacaac cactacacgc agaagagcct ctccctgtct   1380
```

## FIGURE 1B 4.1.1 Heavy Chain Protein (SEQ ID NO:2)

```
[QVQLVESGGG VVQPGRSLRL SCVASGFTFS SHGMHWVRQA PGKGLEWVAV IWYDGRNKYY    60
ADSVKGRFTI SRDNSKNTLF LQMNSLRAED TAVYYCARGG HFGPFDYWGQ GTLVTVSS]AS   120
TKGPSVFPLA PCSRSTSEST AALGCLVKDY FPEPVTVSWN SGALTSGVHT FPAVLQSSGL   180
YSLSSVVTVP SSNFGTQTYT CNVDHKPSNT KVDKTVERKC CVECPPCPAP PVAGPSVFLF   240

PPKPKDTLMI SRTPEVTCVV VDVSHEDPEV QFNWYVDGVE VHNAKTKPRE EQFNSTFRVV   300
SVLTVVHQDW LNGKEYKCKV SNKGLPAPIE KTISKTKGQP REPQVYTLPP SREEMTKNQV   360
SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPMLDSDGS FFLYSKLTVD KSRWQQGNVF   420
SCSVMHEALH NHYTQKSLSL SPGK                                          444
```

FIGURE 1C 4.1.1 Light Chain cDNA (SEQ ID NO:7)

```
atggaaaccc cagcgcagct tctcttcctc ctgctactct ggctcccaga taccaccgga     60
gaaattgtgt tgacgcagtc tccaggcacc ctgtctttgt ctccagggga aagagccacc    120
ctctcctgca gggccagtca gagtattagc agcagcttct tagcctggta ccagcagaga    180
cctggccagg ctcccaggct cctcatctat ggtgcatcca gcagggccac tggcatccca    240
gacaggttca gtggcagtgg gtctgggaca gacttcactc tcaccatcag cagactggag    300
cctgaagatt ttgcagtgta ttactgtcag cagtatggta cctcaccctg gacgttcggc    360
caagggacca aggtggaaat caaacgaact gtggctgcac catctgtctt catcttcccg    420
ccatctgatg agcagttgaa atctggaact gcctctgttg tgtgcctgct gaataacttc    480
tatcccagag aggccaaagt acagtggaag gtggataacg ccctccaatc gggtaactcc    540
caggagagtg tcacagagca ggacagcaag gacagcacct acagcctcag cagcaccctg    600
acgctgagca aagcagacta cgagaaacac aaagtctacg cctgcgaagt cacccatcag    660
ggcctgagct cgcccgtcac aaagagcttc aacaggggag agtgttag                 708
```

FIGURE 1D 4.1.1 Light Chain Protein (SEQ ID NO:8)

```
[EIVLTQSPGT LSLSPGERAT LSCRASQSIS SSFLAWYQQR PGQAPRLLIY GASSRATGIP    60
DRFSGSGSGT  DFTLTISRLE PEDFAVYYCQ QYGTSPWTFG QGTKVEIK]RT VAAPSVFIFP   120

PSDEQLKSGT  ASVVCLLNNF YPREAKVQWK VDNALQSGNS QESVTEQDSK DSTYSLSSTL   180
TLSKADYEKH  KVYACEVTHQ GLSSPVTKSF NRGEC                              215
```

FIGURE 2A 4.13.1 Heavy Chain DNA (SEQ ID NO:13)

```
caggtgcagc tggtggagtc tgggggaggc gtggtccagc ctgggaggtc cctgagactc    60
tcctgtgcag cgtctggatt caccttcagt agtcatggca tccactgggt ccgccaggct   120
ccaggcaagg ggctggagtg ggtggcagtt atatggtatg atggaagaaa taaagactat   180
gcagactccg tgaagggccg attcaccatc tccagagaca attccaagaa cacgctgtat   240
ttgcaaatga acagcctgag agccgaggac acggctgtgt attactgtgc gagagtggcc   300
ccactggggc cacttgacta ctggggccag ggaaccctgg tcaccgtctc ctcagcctcc   360
accaagggcc catcggtctt ccccctggcg ccctgctcca ggagcacctc cgagagcaca   420
gcggccctgg gctgcctggt caaggactac ttccccgaac cggtgacggt gtcgtggaac   480
tcaggcgctc tgaccagcgg cgtgcacacc ttcccagctg tcctacagtc ctcaggactc   540
tactccctca gcagcgtggt gaccgtgccc tccagcaact tcggcaccca gacctacacc   600
tgcaacgtag atcacaagcc cagcaacacc aaggtggaca agacagttga gcgcaaatgt   660
tgtgtcgagt gcccaccgtg cccagcacca cctgtggcag gaccgtcagt cttcctcttc   720
cccccaaaac ccaaggacac cctcatgatc tcccggaccc ctgaggtcac gtgcgtggtg   780
gtggacgtga gccacgaaga ccccgaggtc cagttcaact ggtacgtgga cggcgtggag   840
gtgcataatg ccaagacaaa gccacgggag gagcagttca acagcacgtt ccgtgtggtc   900
agcgtcctca ccgttgtgca ccaggactgg ctgaacggca aggagtacaa gtgcaaggtc   960
tccaacaaag gcctcccagc ccccatcgag aaaaccatct ccaaaaccaa agggcagccc  1020
cgagaaccac aggtgtacac cctgccccca tcccgggagg agatgaccaa gaaccaggtc  1080
agcctgacct gcctggtcaa aggcttctac cccagcgaca tcgccgtgga gtgggagagc  1140
aatgggcagc cggagaacaa ctacaagacc acacctccca tgctggactc cgacggctcc  1200
ttcttcctct acagcaagct caccgtggac aagagcaggt ggcagcaggg gaacgtcttc  1260
tcatgctccg tgatgcatga ggctctgcac aaccactaca cgcagaagag cctctccctg  1320
tctccgggta aatga                                                   1335
```

FIGURE 2B 4.13.1 Heavy Chain Protein (SEQ ID NO:14)

```
[QVQLVESGGG VVQPGRSLRL SCAASGFTFS SHGIHWVRQA PGKGLEWVAV IWYDGRNKDY    60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARVA PLGPLDYWGQ GTLVTVSS]AS   120
TKGPSVFPLA PCSRSTSEST AALGCLVKDY FPEPVTVSWN SGALTSGVHT FPAVLQSSGL   180
YSLSSVVTVP SSNFGTQTYT CNVDHKPSNT KVDKTVERKC CVECPPCPAP PVAGPSVFLF   240
PPKPKDTLMI SRTPEVTCVV VDVSHEDPEV QFNWYVDGVE VHNAKTKPRE EQFNSTFRVV   300
SVLTVVHQDW LNGKEYKCKV SNKGLPAPIE KTISKTKGQP REPQVYTLPP SREEMTKNQV   360
SLTCLVKGFY PSDIAVEWES NGQPENNYKT TPPMLDSDGS FFLYSKLTVD KSRWQQGNVF   420
SCSVMHEALH NHYTQKSLSL SPGK                                          444
```

FIGURE 2C 4.13.1 Light Chain DNA (SEQ ID NO:19)

```
gaaattgtgt tgacgcagtc tccaggcacc ctgtctttgt ctccagggga aagagccacc    60
ctctcctgca gggccagtca gagtgtcagc agctacttag cctggtacca gcagaaacct   120
ggccaggctc ccaggctcct catctatggt gcatccagca gggccactgg catcccagac   180
aggttcagtg gcagtgggtc tgggacagac ttcactctca ccatcagcag actggagcct   240
gaggattttg cagtgtatta ctgtcaacag tatggtaggt caccattcac tttcggccct   300
gggaccaaag tagatatcaa gcgaactgtg gctgcaccat ctgtcttcat cttcccgcca   360
tctgatgagc agttgaaatc tggaactgcc tctgttgtgt gcctgctgaa taacttctat   420
cccagagagg ccaaagtaca gtggaaggtg gataacgccc tccaatcggg taactcccag   480
gagagtgtca cagagcagga cagcaaggac agcacctaca gcctcagcag caccctgacg   540
ctgagcaaag cagactacga gaaacacaaa gtctacgcct gcgaagtcac ccatcagggc   600
ctgagctcgc ccgtcacaaa gagcttcaac aggggagagt gttag                   645
```

FIGURE 2D 4.13.1 Light Chain Protein (SEQ ID NO:20)

```
[EIVLTQSPGT LSLSPGERAT LSCRASQSVS SYLAWYQQKP GQAPRLLIYG ASSRATGIPD   60
RFSGSGSGTD FTLTISRLEP EDFAVYYCQQ YGRSPFTFGP GTKVDIK]RTV AAPSVFIFPP  120
SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSTLT  180
LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC                              214
```

FIGURE 3A CP-675,206 Heavy Chain DNA (SEQ ID NO:25)

```
atggagtttg ggctgagctg ggttttcctc gttgctcttt taagaggtgt ccagtgtcag    60
gtgcagctgg tggagtctgg gggaggcgtg gtccagcctg ggaggtccct gagactctcc   120
tgtgcagcgt ctggattcac cttcagtagc tatggcatgc actgggtccg ccaggctcca   180
ggcaaggggc tggagtgggt ggcagttata tggtatgatg gaagtaataa atactatgca   240
gactccgtga agggccgatt caccatctcc agagacaatt ccaagaacac gctgtatctg   300
caaatgaaca gcctgagagc cgaggacacg gctgtgtatt actgtgcgag agatccgagg   360
ggagctaccc tttactacta ctactacggt atggacgtct ggggccaagg gaccacggtc   420
accgtctcct cagcctccac caagggccca tcggtcttcc ccctggcgcc ctgctccagg   480
agcacctccg agagcacagc ggccctgggc tgcctggtca aggactactt ccccgaaccg   540
gtgacggtgt cgtggaactc aggcgctctg accagcggcg tgcacacctt cccagctgtc   600
ctacagtcct caggactcta ctccctcagc agcgtggtga ccgtgccctc cagcaacttc   660
ggcacccaga cctacacctg caacgtagat cacaagccca gcaacaccaa ggtggacaag   720
acagttgagc gcaaatgttg tgtcgagtgc ccaccgtgcc cagcaccacc tgtggcagga   780
ccgtcagtct tcctcttccc cccaaaaccc aaggacaccc tcatgatctc ccggaccccc   840
gaggtcacgt gcgtggtggt ggacgtgagc cacgaagacc ccgaggtcca gttcaactgg   900
tacgtggacg gcgtggaggt gcataatgcc aagacaaagc cacgggagga gcagttcaac   960
agcacgttcc gtgtggtcag cgtcctcacc gttgtgcacc aggactggct gaacggcaag  1020
gagtacaagt gcaaggtctc caacaaaggc ctcccagccc ccatcgagaa aaccatctcc  1080
aaaaccaaag ggcagccccg agaaccacag gtgtacaccc tgccccatc ccgggaggag  1140
atgaccaaga accaggtcag cctgacctgc ctggtcaaag gcttctaccc cagcgacatc  1200
gccgtggagt gggagagcaa tgggcagccg gagaacaact acaagaccac acctcccatg  1260
ctggactccg acggctcctt cttcctctac agcaagctca ccgtggacaa gagcaggtgg  1320
cagcagggga acgtcttctc atgctccgtg atgcatgagg ctctgcacaa ccactacacg  1380
cagaagagcc tctccctgtc tccgggtaaa tga                               1413
```

FIGURE 3B CP-675,206 (11.2.1) Heavy Protein (SEQ ID NO:26)

```
[QVQLVESGGG VVQPGRSLRL SCAASGFTFS SYGMHWVRQA PGKGLEWVAV IWYDGSNKYY    60
ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARDP RGATLYYYYY GMDVWGQGTT   120
VTVSS]ASTKG PSVFPLAPCS RSTSESTAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA   180
VLQSSGLYSL SSVVTVPSSN FGTQTYTCNV DHKPSNTKVD KTVERKCCVE CPPCPAPPVA   240
GPSVFLFPPK PKDTLMISRT PEVTCVVVDV SHEDPEVQFN WYVDGVEVHN AKTKPREEQF   300
NSTFRVVSVL TVVHQDWLNG KEYKCKVSNK GLPAPIEKTI SKTKGQPREP QVYTLPPSRE   360
EMTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTTPP MLDSDGSFFL YSKLTVDKSR   420
WQQGNVFSCS VMHEALHNHY TQKSLSLSPG K                                  451
```

FIGURE 3C CP-675,206 Light Chain DNA (SEQ ID NO:31)

```
atggacatga gggtccccgc tcagctcctg gggctcctgc tactctggct ccgaggtgcc   60
agatgtgaca tccagatgac ccagtctcca tcctccctgt ctgcatctgt aggagacaga   120
gtcaccatca cttgccgggc aagtcagagc attaacagct atttagattg gtatcagcag   180
aaaccaggga aagcccctaa actcctgatc tatgctgcat ccagtttgca aagtggggtc   240
ccatcaaggt tcagtggcag tggatctggg acagatttca ctctcaccat cagcagtctg   300
caacctgaag attttgcaac ttactactgt caacagtatt acagtactcc attcactttc   360
ggccctggga ccaaagtgga aatcaaacga actgtggctg caccatctgt cttcatcttc   420
ccgccatctg atgagcagtt gaaatctgga actgcctctg ttgtgtgcct gctgaataac   480
ttctatccca gagaggccaa agtacagtgg aaggtggata acgccctcca atcgggtaac   540
tcccaggaga gtgtcacaga gcaggacagc aaggacagca cctacagcct cagcagcacc   600
ctgacgctga gcaaagcaga ctacgagaaa cacaaagtct acgcctgcga agtcacccat   660
cagggcctga gctcgcccgt cacaaagagc ttcaacaggg gagagtgtta gtga          714
```

FIGURE 3D CP-675,206 (11.2.1) Light Chain Protein SEQ ID NO:32

```
[DIQMTQSPSS LSASVGDRVT ITCRASQSIN SYLDWYQQKP GKAPKLLIYA ASSLQSGVPS   60
RFSGSGSGTD FTLTISSLQP EDFATYYCQQ YYSTPFTFGP GTKVEIK]RTV AAPSVFIFPP  120
SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSTLT  180
LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC                              214
```

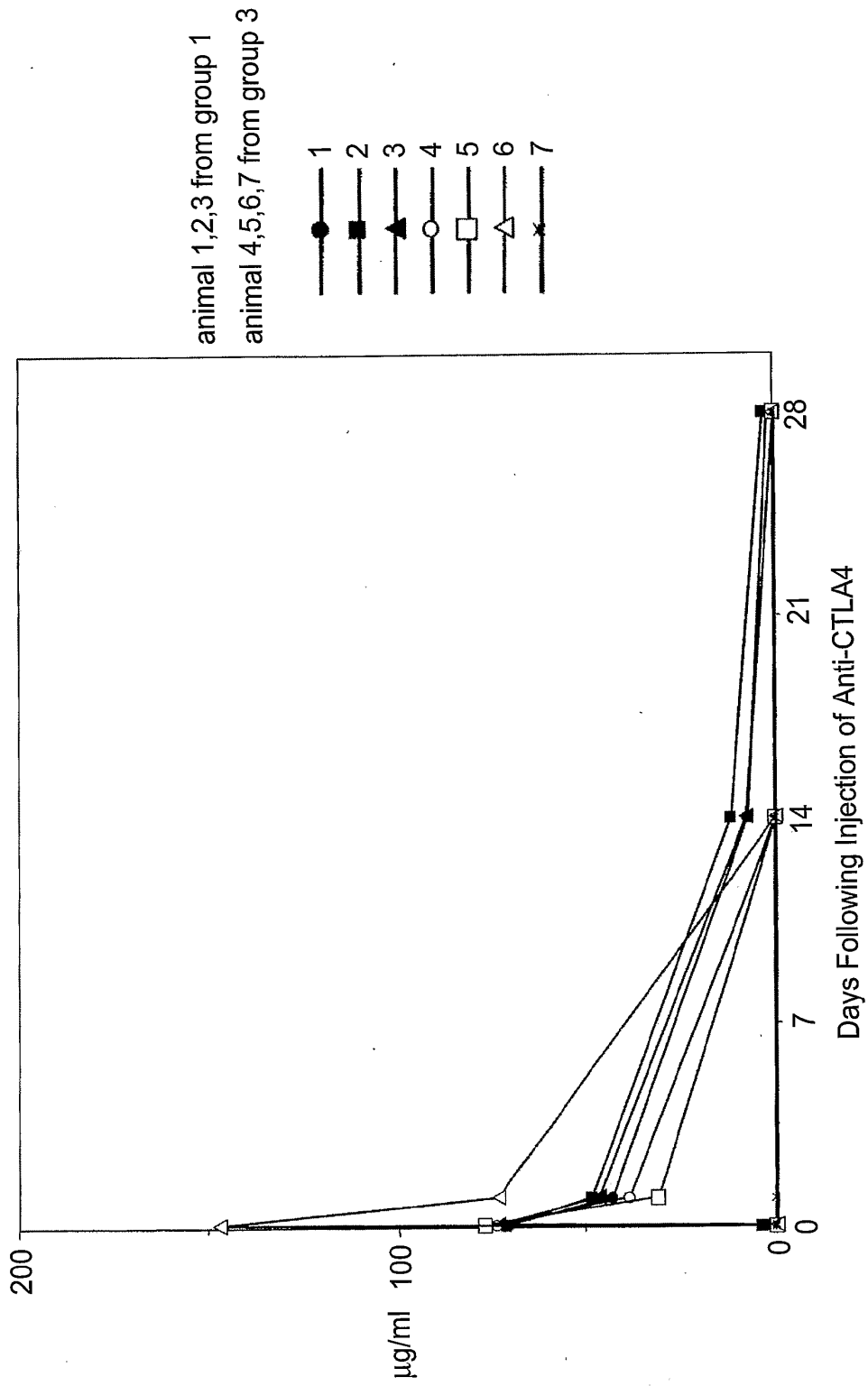



FIG. 4

EX1002 - Page 962

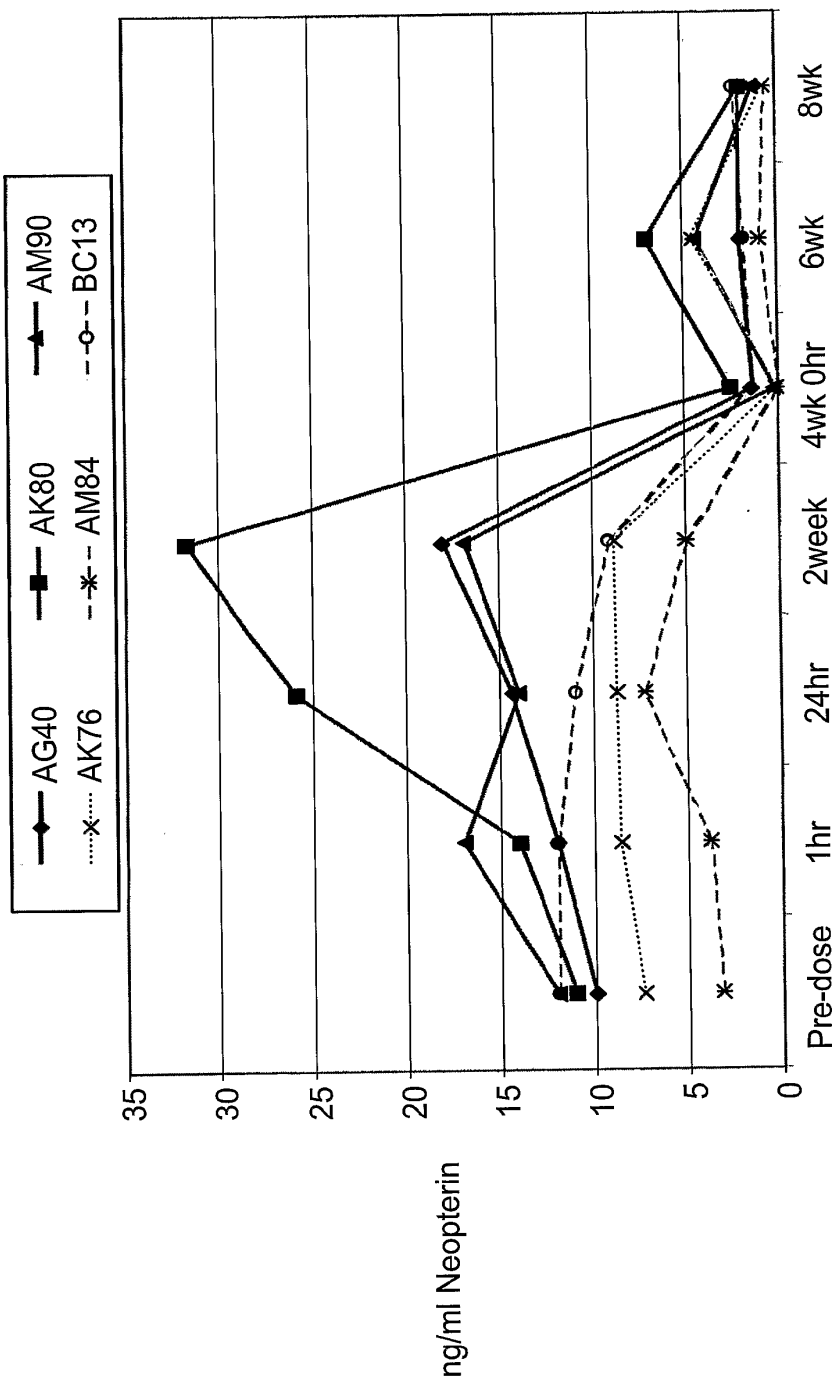

AG40
AK80
AM90
AK76
AM84
BC13
ng/ml Neopterin
35
30
25
20
15
10
5
0
Pre-dose
1hr
24hr
2week
4wk 0hr
6wk
8wk

FIG. 5

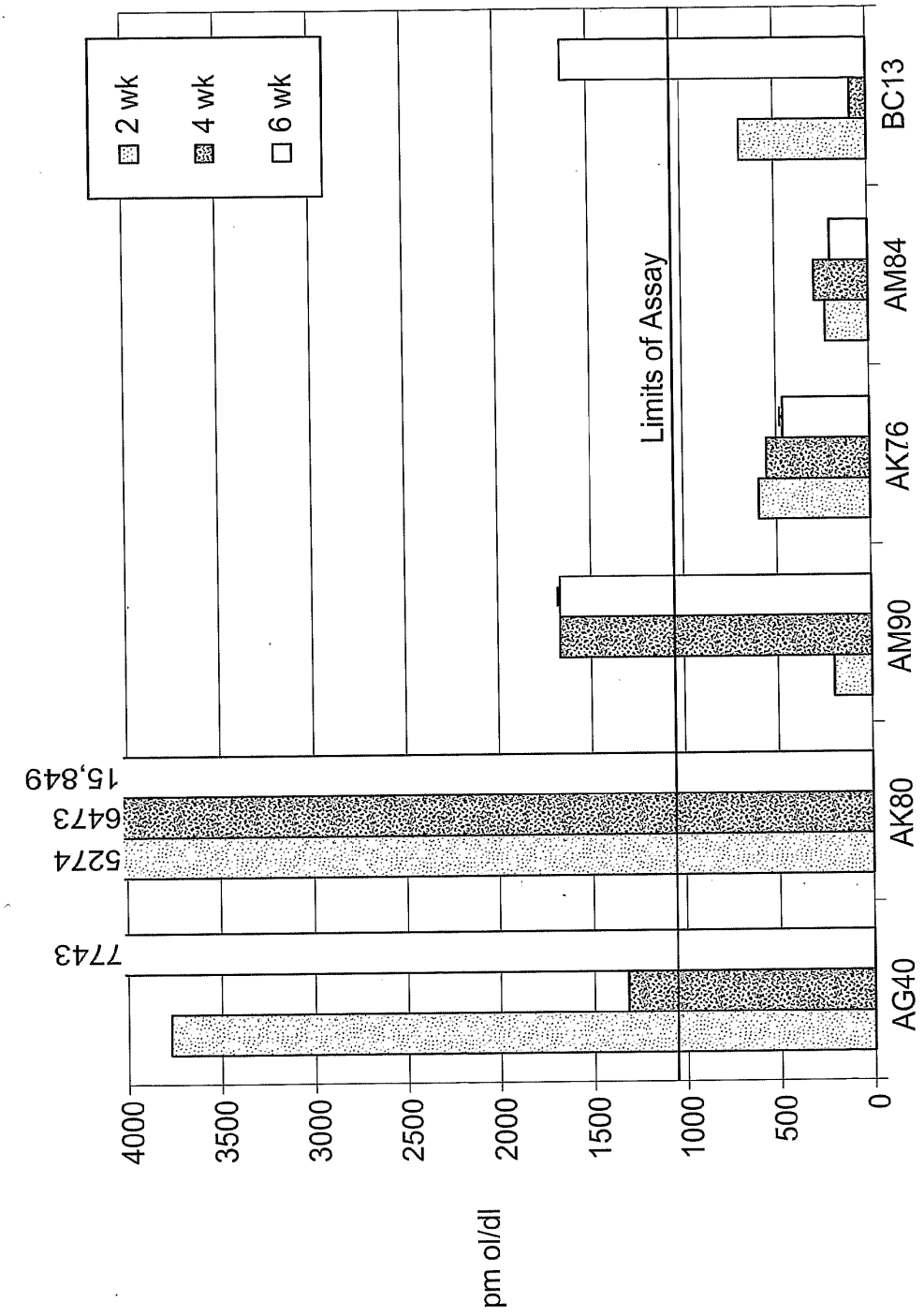



FIG. 6

EX1002 - Page 964

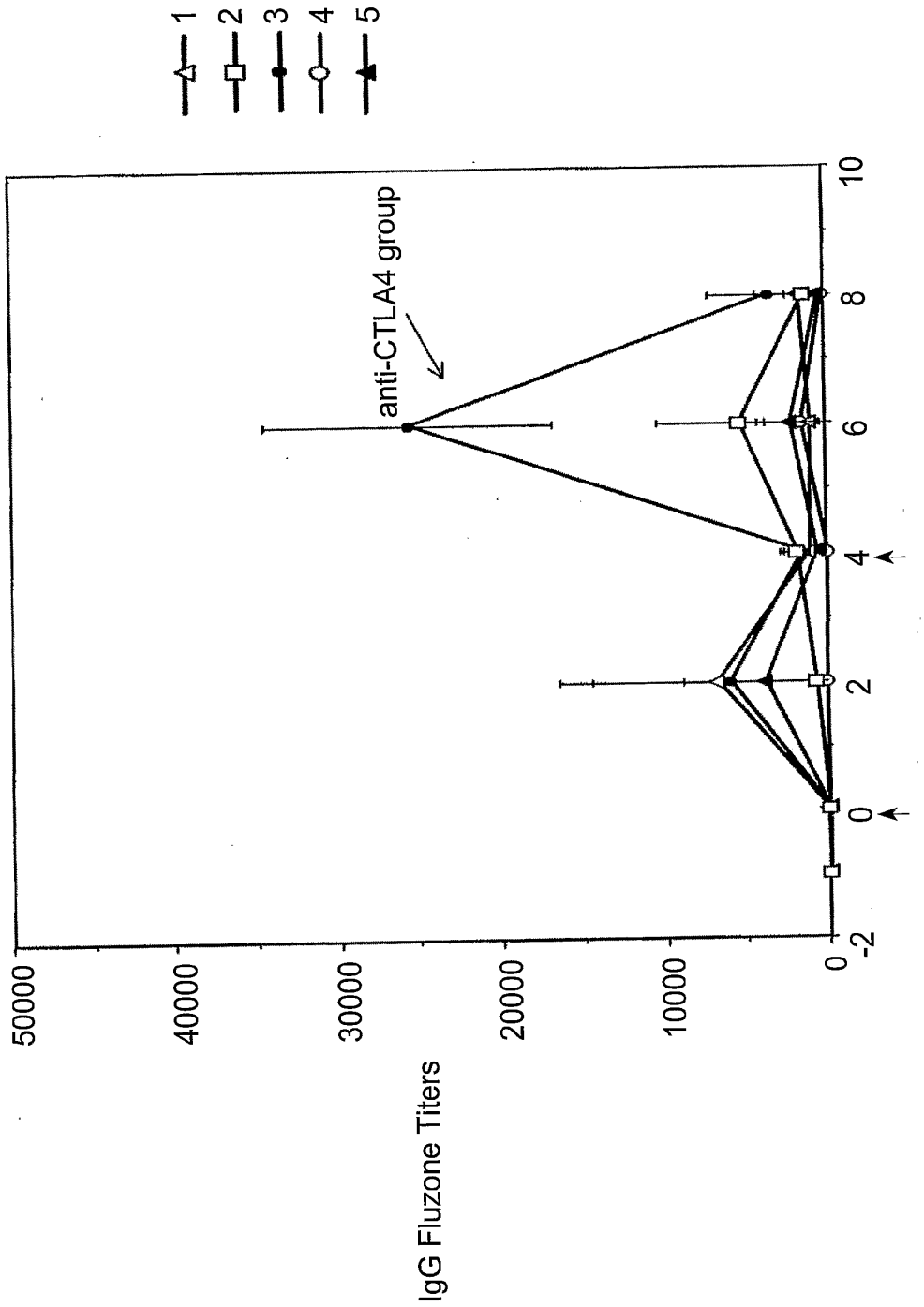

1
2
3
4
5
anti-CTLA4 group
IgG Fluzone Titers
50000
40000
30000
20000
10000
0
-2
0
2
4
6
8
10
Time in Weeks

FIG. 7

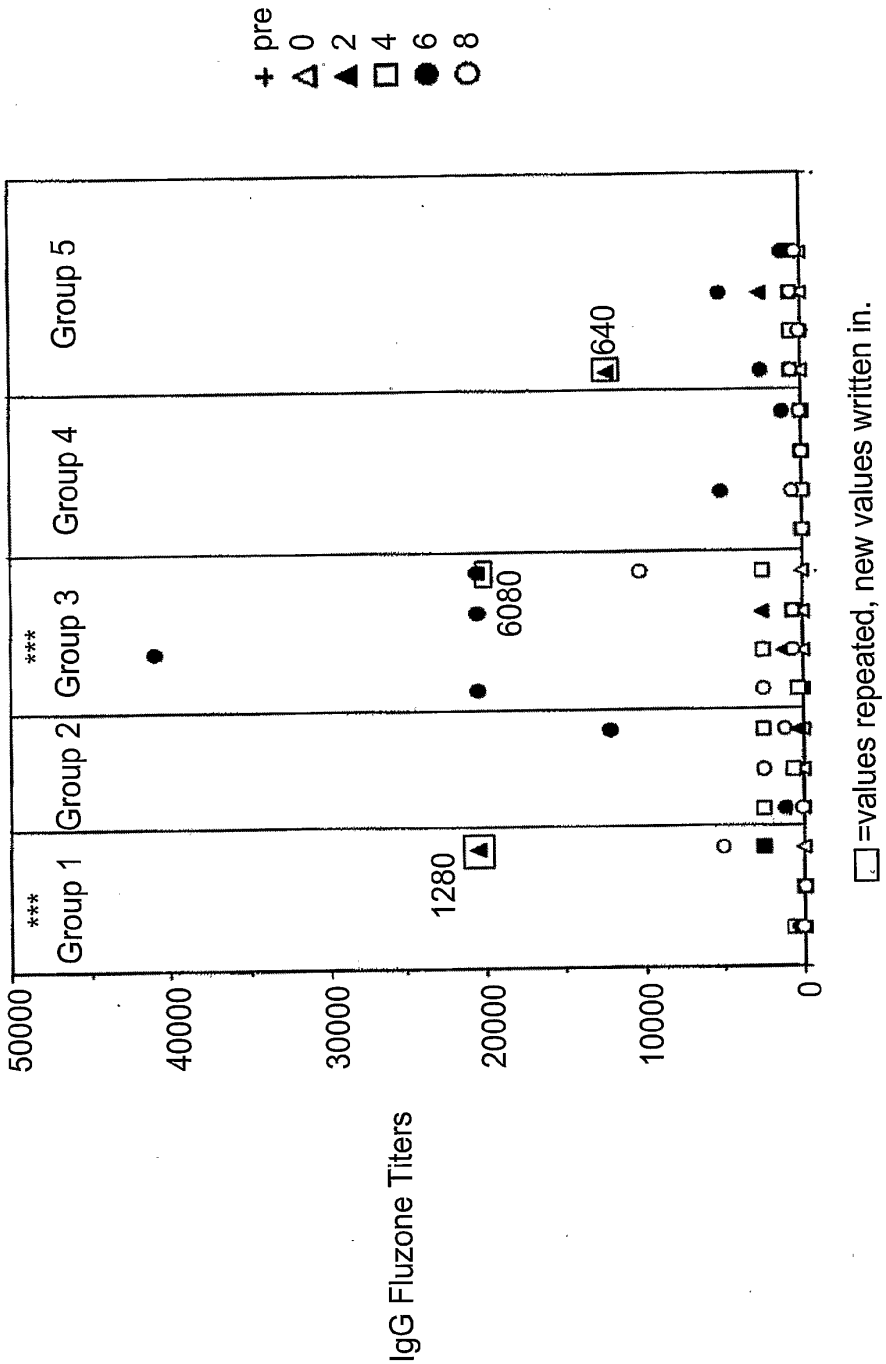

+ pre
0
2
4
6
8
Group 1
Group 2
Group 3
Group 4
Group 5
***
***
1280
6080
640
50000
40000
30000
20000
10000
0
IgG Fluzone Titers
=values repeated, new values written in.

FIG. 8

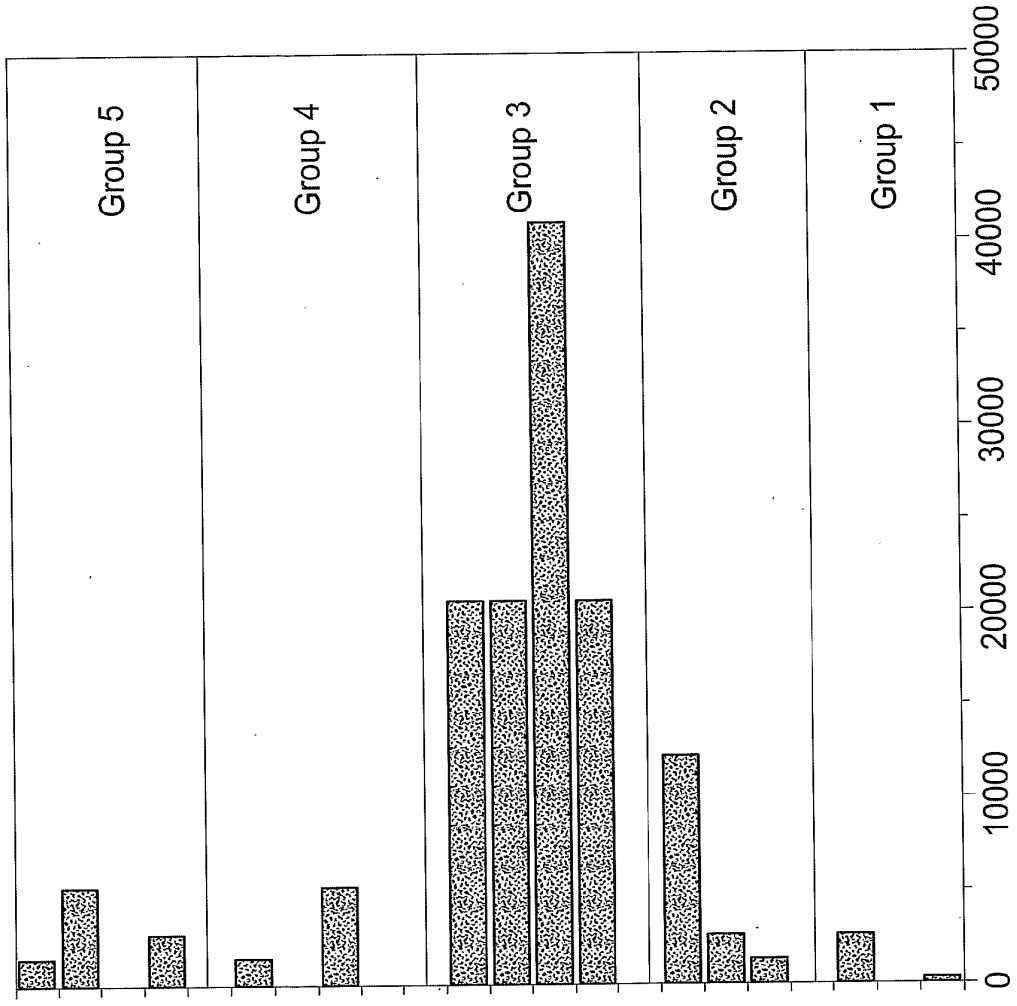

Group 5
Group 4
Group 3
Group 2
Group 1
0
10000
20000
30000
40000
50000
Serum Viral Titer

FIG. 9

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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(54) Title: COMBINATION OF LOCAL AND SYSTEMIC IMMUNOMODULATIVE THERAPIES FOR ENHANCED TREATMENT OF CANCER

(57) Abstract: A method for the treatment of cancer comprising administration of a therapeutically effective amount of an intralesional chemoablative pharmaceutical composition, or variant of said composition, in combination with a therapeutically effective amount of a systemic immunomodulatory anticancer agent. A further method for the treatment of cancer comprising administration of a therapeutically effective amount of an intralesional chemoablative pharmaceutical composition, or variant of said composition, in combination with a therapeutically effective amount of a systemic targeted anticancer agent. The present invention is further directed to pharmaceutical compositions for treatment of cancer. The intralesional chemoablative pharmaceutical composition can comprise an IL chemoablative agent comprising primarily a halogenated xanthene.

# Combination of local and systemic immunomodulative therapies for enhanced treatment of cancer

## Field of the Invention

This invention relates to the fields of oncology and improved therapeutic regimens therefore.

## Background of the Invention

[0001] Pharmacologic approaches for treating cancer have traditionally relied on the use of various single agent systemic therapies (monotherapies). An archetypical example is chemotherapy, which utilizes broadly cytotoxic drugs that target rapidly dividing cells, including alkylating agents like dacarbazine (DTIC) or temozolomide (TMZ), or mitotic inhibitors like paclitaxel, to inhibit or kill the rapidly growing cells typical of cancer. Tumors may not be completely responsive to such monotherapy, either due to their high collateral systemic toxicity necessitating lower, even sub-therapeutic doses or development of tumor resistance that circumvents the activity of the monotherapy agent. More advanced chemotherapy strategies have been developed that are predicated on use of multiple agents in a combination therapy that simultaneously attack the tumor along multiple of biochemical pathways. Many of these regimens, such as the combination of doxorubicin, bleomycin, viblastine and DTIC for Hodgkin's lymphoma, have been developed through empirical testing. Because of the inherent limitations of their individual pharmacologic components, such approaches remain relatively non-specific with high morbidity, allowing considerable room for improvement in terms of efficacy and safety.

[0002] Targeting cancers based on their selective overexpression of certain cell-surface receptors or reliance on specific signaling or metabolic pathways, in particular aberrant pathways present in certain cancers, provides another point of attack. For instance, it has been found that some cancers harbor mutations in certain protein kinases, such as those encoded by the serine/threonine-protein kinase B-Raf gene (BRAF), that are involved in cell signaling and hyperproliferative growth, thereby serving an oncogene role. Targeting these pathways through the use of inhibitors has proven attractive, at least initially, in controlling cancers by staving off the oncolytic signaling. A similar approach based on targeting overexpression of certain receptors, such as epidermal growth factor receptor (EGFR) or vascular endothelial growth factor (VEGF), provides the basis for damping the oncolytic activity of these receptors, for instance by use of antibodies to the targeted receptors (or by use of agents that inhibit the signaling stimulated by these receptors). Unfortunately, as in the case of conventional chemotherapy, these receptors and pathways may play important physiologic roles peripheral to the tumor, leading to toxicity upon their targeting, while the targeted cells also may develop resistance by harnessing alternate biochemical processes or proliferating via selection of resistant clonal subpopulations of tumor cells. Thus, the challenges posed by these types of targeted therapies are substantially similar to those posed by conventional chemotherapy.

[0003] In a growing number of oncology indications it is now clear that cancerous tumors employ various methods to evade detection as aberrant tissue and to reduce immune system competency, thereby avoiding potential identification and destruction by the patient's immune system. As a consequence, a number of approaches have been developed to enhance the capability of the patient's immune system to detect and destroy cancers. For example, the anti-CTLA-4 (cytotoxic T lymphocyte-associated antigen 4) antibodies ipilimumab and tremelimumab are designed to counter downregulation of the immune system by blocking CTLA-4 activity and thus augmenting T-cell response against cancer. Alternate approaches may utilize agents that stimulate certain components of the immune system (i.e., upregulation), including administering non-specific cytokines (such as interleukin 1, 2, or 6, "IL-1", "IL-2" or "IL-6"; interferon-alpha or gamma, "IFN-α" and "IFN-γ"; and granulocyte macrophage colony stimulating factor, "GM-

CSF"), or that attempt to provoke a tumor-specific immune response to certain tumor antigens, such as dendritic cell vaccines and antibodies against specific tumor antigens and even adoptive T-cell therapy. Additional approaches have attempted to elicit systemic response following repeated inoculation of tumors with certain immunostimulatory agents, such as an intralesional vaccine containing an oncolytic herpes virus encoding GM-CSF or a plasmid encoding human leukocyte antigen-B7 and beta-2 microglobulin agent designed to express allogeneic major histocompatibility complex (MHC) class I antigens. For various reasons including, but not limited to, potential systemic toxicity of these immunomodulating agents, differential expression of the targeted moieties or responsiveness of clonal subpopulations, increase of tumor burden during therapy induction, and development of resistance against the selected mode of attack, current regimens may not result in as robust an immune response as desired, again allowing considerable room for improvement in terms of efficacy and safety.

[0004] Combination of systemic immunomodulatory agents with systemic chemotherapy agents or kinase inhibitors has been proposed, for example by Jure-Kunkel and Lee (WO 2010/014784), however there is limited data to determine whether such an approach will be clinically significant. In principal, this approach combines the features of targeted therapy (using chemotherapy or a metabolic inhibitor) with immunomodulation in a combination therapy, and as is the case with standard chemotherapeutic combination therapy it provides a means to attack cancer simultaneously via several different paths, thereby increasing potency while reducing likelihood of resistance. Since the immunologic consequences of chemotherapy may at least partially counteract the activity of the immunomodulator, and their respective systemic adverse effects may be additive or synergistic, such a combination of modalities has significant potential shortcomings. While not the topic of Jure-Kunkel and Lee, targeted therapies when combined with immunomodulatory agents can also have these negative effects. Perhaps most importantly, these potential combinations don't appear to afford additive or synergistic tumoricidal potency in terms of immunologic benefit since neither chemotherapy nor metabolic or aberrant gene targeting can be expected to significantly activate an

antitumor immune response, while the proposed anti-CTLA-4 targeting is similarly unlikely to increase sensitivity of tumor cells to the companion chemotherapy or tumor specific approach. The possibility of increased tumor burden during induction of immunomodulatory therapy further complicates the picture, raising the possibility that the disease may progress to an unacceptably advanced state during the early phases of the regimen.

[0005] Further complicating the therapeutic challenge, tumors that shrink gradually over a long period of time and slowly release immunoreactive tumor materials in response to any of these conventional systemic therapies may fail to trigger a potent protective response and can instead facilitate reduced antitumor immunity. This phenomenon is similar to that underlying low dose therapies for allergies whereby the host is repeatedly exposed to low doses of antigenic material over a prolonged period, eliciting tolerance by causing the immune system to identify these persistent "background" antigens as "self" (i.e., a normal part of the host). In a similar fashion, the slow, low dose release of tumor antigens to the immune system in response to many systemic therapies may deceive the immune system into tolerance toward tumor antigens thereby reducing or negating possible antitumor response, potentially prolonging tumor survival, and allowing continued metastatic spread.

[0006] An alternate class of therapies is predicated on physical restriction of delivery of the therapeutic modality to diseased tissue. These localized therapies attempt to maximize potency of the therapy within tumor tissue while reducing systemic exposure. Approaches include physical or chemical disruption of tumors using intralesional methods, such as percutaneous ethanol injection therapy (PEIT) and radiofrequency (RF) ablation, and locoregional delivery of potent cytotoxic agents, such as isolated limb perfusion (ILP), isolated limb infusion (ILI) or percutaneous hepatic perfusion (PHP), with melphalan (an alkylating agent) or similar agents. While these approaches are often quite effective in maximizing pharmacologic activity against the treated tumor, they have generally exhibited many of the same limitations of systemic therapies due to the inherent shortcomings of the underlying therapeutic modality, including limited specificity for the

targeted cancer with significant locoregional toxicity, and minimal impact on systemic disease, particularly for those approaches having no mechanism for immune stimulation against the treated tumor.

[0007] The use of cancer-specific cytotoxic agents delivered via an intralesional (IL) route (i.e., IL chemoablation) is a novel hybrid approach that has been described by one or more of the present inventors (for example in US 7,648,695, USSN 11/951,800 and USSN 12/315,781, which are incorporated herein in their entirety). This approach maximizes local efficacy against injected tumors while minimizing systemic exposure of the patient to the injected agent and resultant potential for systemic adverse effects. One or more of the present inventors have shown that IL use of a certain specific class of agent (for example certain formulations of certain halogenated xanthenes, exemplified by a 10% (w/v) solution of rose bengal disodium in saline, termed "PV-10" and undergoing clinical testing for treatment of metastatic melanoma, breast carcinoma and hepatocellular carcinoma) can elicit not only highly specific ablation of the injected lesion but also an antitumor immune response ("bystander effect") that can augment local efficacy in the injected tumor and lead to spontaneous regression of uninjected tumors. Nonclinical evidence indicates that high levels of granulocytes (such as basophils, eosinophils and mast cells) may be expressed in the tissue surrounding tumors, indicating that the host is attempting to mount a non-specific immune response to tumor tissue. Treatment of tumors with PV-10 can lead to modulation of this response to one that is more specific and effective (for example, by recruiting mononuclear tumor-infiltrating lymphocytes, TILs, or macrophages into and around the tumor). It is likely that acute tumor disruption resulting from IL chemoablation with PV-10 releases sequestered, intact tumor antigens to local antigen-presenting cells (APCs), facilitating modulation of the immune response and presentation of appropriate antigenic targets to T and B-cells. Collateral destruction of granulocytes surrounding the tumor may precipitate chemokine release and local inflammation, and may serve an adjuvant role in promoting specific antitumor response. In situ destruction of the injected tumor assures presentation of tumor antigens in their natural context, thereby maximizing potential response of the immune system to the treated tumor and to tumors bearing the same immunologic signature. Since

immune response is proportional to the intensity and duration of the insult to the host, the acute exposure achieved through IL chemoablation is immunologically advantageous relative to the lesser intensity insult produced by a systemic therapy that is spread out over a long duration, and this acute exposure potentially vaccinates the patient against the treated tumor.

[0008] Acute ablation of the injected tumor also quickly reduces tumor burden, which may be augmented by injecting all or a substantial fraction of a patient's tumors, either in a single treatment session or a series of treatments fractionated over a period of days or weeks. This may reduce the level of immune suppression exerted by the patient's tumor mass, leading to improved ability of their immune system to mount a successful attack against remaining tumor tissue. The inherent suitability of IL chemoablation for use against large or multiple cancerous lesions, when present, may further enhance outcome by facilitating in situ inoculation against potentially distinct clonal subpopulations in different tumors (or even within individual tumors) that may arise during tumor growth and metastasis.

[0009] While IL chemoablation overcomes many of the shortcomings of prior therapeutic modalities (for example by achieving rapid reduction in tumor burden, maximizing acute exposure to intact tumor antigens in an appropriate context, and affording minimal potential for systemic adverse effects) one or more of the present inventors have found that it may not be ideal for all cancer cases, particularly certain advanced cases having rapidly proliferating tumors, those with widely disseminated disease and those that present in forms that are difficult to fully infiltrate with the IL agent. Accordingly, additional advancements are needed in the fields of oncology and improved therapeutic regimens therefore.

**Summary of the Invention**

[0010] The present invention is directed to a method for the treatment of cancer, said method comprising administration of a therapeutically effective amount of an intralesional chemoablative pharmaceutical composition, or variant of said composition, in combination with a therapeutically effective amount of a systemic immunomodulatory anticancer agent.

[0011] The present invention is also directed to a pharmaceutical composition for the treatment of cancer comprising: a therapeutically effective amount of an intralesional chemoablative pharmaceutical composition; and a therapeutically effective amount of a systemic immunomodulatory anticancer agent.

[0012] In an embodiment of the above method and pharmaceutical composition, the systemic immunomodulatory anticancer agent comprises anti-CTLA-4 antibodies including ipilimumab and tremelimumab.

[0013] In an embodiment of the above method and pharmaceutical composition, the systemic immunomodulatory anticancer agent is selected from the group consisting of non-specific cytokines, such as interleukin-1, interleukin-2, or interleukin-6 (IL-1, IL-2 or IL-6) and aldesleukin; interferon-alpha or interferon-gamma (IFN-α and IFN-γ), interferon alfa-2b and pegylated interferon (including pegylated interferon alfa-2a and pegylated interferon alfa-2b); granulocyte macrophage colony stimulating factor (GM-CSF, molgramostim or sargramostim); dendritic cell vaccines and other allogeneic or autologous therapeutic cancer vaccines, including intralesional vaccines containing an oncolytic herpes virus encoding GM-CSF (OncoVex®) or a plasmid encoding human leukocyte antigen-B7 and beta-2 microglobulin agent designed to express allogeneic MHC class I antigens (Allovectin-7®); and antibodies against specific tumor antigens.

[0014] The present invention is also directed to a method for the treatment of cancer, said method comprising administration of a therapeutically effective amount of an intralesional chemoablative pharmaceutical composition, or variant of said composition,

in combination with a therapeutically effective amount of a systemic targeted anticancer agent.

[0015] The present invention is also directed to a pharmaceutical composition for the treatment of cancer comprising: a therapeutically effective amount of an intralesional chemoablative pharmaceutical composition; and a therapeutically effective amount of a systemic targeted anticancer agent.

[0016] In an embodiment of the above method and pharmaceutical composition, the systemic targeted anticancer agent is selected from the group consisting of drugs that target protein kinases and the receptors that activate them, including afatinib (BIBW 2992), bevacizumab, cetuximab, dasatinib, E7080, erlotinib, gefitinib, imatinib, lapatinib, nilotinib, panitumumab, pazopanib, pegaptanib, ranibizumab, sorafenib, sunitinib, trastuzumab and vandetanib; serine/threonine-selective protein kinase inhibitors, including those targeting the B-Raf/MEK/ERK pathway, such as vemurafenib (also known as PLX4032, RG7204 or RO5185426), GSK2118436 and GSK1120212; aromatase inhibitors, including aminoglutethimide, anastrozole, exemestane, fadrozole, formestane, letrozole, testolactone and vorozole; estrogen receptor antagonists, including lasofoxifene, raloxifene, tamoxifen and toremifene; COX-2 inhibitors, including celecoxib, valdecoxib and rofecoxib; angiogenesis blockers, including IFN-α, IL-12, suramin, and thrombospondin (including thrombospondin 1, ABT-510 and ABT-898); and immune cell therapy, including adoptive T-cell transfer and autologous immune cell therapy.

[0017] In a further embodiment of all of the above methods and pharmaceutical compositions, the intralesional chemoablative pharmaceutical composition comprises an IL chemoablative agent comprising primarily a halogenated xanthene in an appropriate pharmaceutical composition, including a 0.1% (w/v)or higher concentration aqueous solution of the halogenated xanthene or mixtures thereof, or a physiologically acceptable salt of the halogenated xanthene.

[0018] In a further embodiment of all of the above methods and pharmaceutical compositions, the halogenated xanthene is rose bengal (4,5,6,7-tetrachloro-2',4',5',7'-tetraiodofluorescein).

[0019] In a further embodiment of all of the above methods and pharmaceutical compositions, the halogenated xanthene is rose bengal disodium.

[0020] In a further embodiment of all of the above methods and pharmaceutical compositions, the halogenated xanthene is selected from the group consisting of erythrosin B, phloxine B, 4,5,6,7-tetrabromo-2',4',5',7'-tetraiodofluorescein, 2',4,5,6,7-pentachloro-4',5',7'-triiodofluorescein, 4,4',5,6,7-pentachloro-2',5',7'-triiodofluorescein, 2',4,5,6,7,7'-hexachloro-4',5'-diiodofluorescein, 4,4',5,5',6,7-hexachloro-2',7'-diiodofluorescein, 2',4,5,5',6,7 -hexachloro-4',7'-diiodofluorescein, 4,5,6,7-tetrachloro-2',4',5'-triiodofluorescein, 4,5,6,7-tetrachloro-2',4',7'-triiodofluorescein, 4,5,6,7-tetrabromo-2',4',5'-triiodofluorescein, and 4,5,6,7-tetrabromo-2',4',7'-triiodofluorescein.

[0021] In a further embodiment of all of the above methods and pharmaceutical compositions,the halogenated xanthene has a concentration of about 0.1% (w/v) up to about 20% (w/v), and that the pharmaceutical composition includes an electrolyte comprising at least one cation selected from the group consisting of sodium, potassium, calcium and magnesium and at least one anion selected from the group consisting of chloride, phosphate and nitrate, wherein the electrolyte is at a concentration of between about 0.1% (w/v) and about 2% (w/v).

[0022] In a further embodiment of all of the above methods and pharmaceutical compositions, the concentration of said electrolyte in the IL chemoablative pharmaceutical composition is between 0.5 to 1.5% (w/v).

[0023] In a further embodiment of all of the above methods and pharmaceutical compositions, the chemoablative pharmaceutical composition has an osmolality of the composition of greater than about 100 mOsm/kg.

[0024] In a further embodiment of all of the above methods and pharmaceutical compositions, the electrolyte is sodium chloride.

[0025] In a further embodiment of all of the above methods and pharmaceutical compositions, the pharmaceutical composition comprises a hydrophilic vehicle.

[0026] In a further embodiment of all of the above methods and pharmaceutical compositions, the pharmaceutical composition has a pH in the range of between about 4 to about 10.

[0027] In a further embodiment of all of the above methods and pharmaceutical compositions, the pharmaceutical composition has a pH in the range of between about 5 to about 7.

[0028] In a further embodiment of all of the above methods and pharmaceutical compositions, the methods and pharmaceutical compositions are for the treatment of cancers selected from melanoma, breast cancer, primary and metastatic liver cancer, prostate cancer and small cell and non small cell lung cancer.

**Detailed Description of the Presently Preferred Embodiments**

[0029] One aspect of the present invention is the result of unanticipated synergy resulting upon combination of certain local therapeutic modalities, and in particular certain local immunomodulative therapies such as for example IL chemoablation with PV-10 or another halogenated xanthene agent, with certain systemic therapeutic modalities. This combination can boost the therapeutic activity of both therapeutic modalities with the potential for no significant increase, or even an overall decrease, in morbidity relative to that typically achieved using the component therapies separately.

[0030] One or more of the present inventors have shown that IL chemoablation can lead to rapid reduction in a patient's tumor burden, reducing potential for tumor-induced immune suppression, extent and severity of the disease, and continued drag on the patient's immune and other physiologic functions. The resultant acute exposure of the patient's immune system to intact tumor antigens in proper biological context is markedly different from that achieved using systemic chemotherapy, targeted systemic therapies, or other local therapeutic modalities, each of which generally produce at best a gradual, low level chronic exposure of the immune system to tumor antigens, often in an inappropriate context. Chemoablation of entire tumors or substantially the entirety of tumors, and especially chemoablation of multiple tumors, enhances exposure of the patient's immune system to any distinct clonal subpopulations of tumor cells that may be present, maximizing overall response to the in situ antitumor vaccination. Hence, the immunomodulatory effects achieved may be superior in breadth and potency to those achieved using prior therapeutic approaches.

[0031] However, for cases where disease is rapidly proliferating, or is widely disseminated, or presents in a form difficult to fully infiltrate with the IL chemoablative agent, use of complementary therapeutic modalities offers additive or synergistic benefit, particularly when they contribute immunologic stimulation (i.e., immunodulation) that complements that afforded through IL chemoablation. The use of such complementary immunomodulative therapies may have further advantage in terms of additive or synergistic immunologic interactions that allow one or both therapies to be used at reduced doses (relative to that needed when used individually as monotherapies) while retaining high efficacy, thereby reducing undesirable adverse effects.

[0032] In particular, the use of a potent local immunomodulative therapy, such as IL chemoablation with, for example, PV-10 or another halogenated xanthene agent, in conjunction with one or more systemic immunomodulative therapies (especially those that elicit immune system upregulation or counter tumor-induced immune system down regulation) is highly attractive since this combination yields a uniquely salubrious combination: exposure of the patient's potentiated immune system to the intense

antigenic "insult" produced upon IL chemoablation. The effects of such combination may be heightened by potentiation of the immune system at the time of chemoablation or subsequent to chemoablation. Since IL chemoablation is well suited to repeat treatment, continued potentiation of the patient's immune system, for example by continued administration of the systemic immunomodulatory therapy, while IL chemoablation is administered one or more times, is a preferred embodiment. As an alternate embodiment, IL chemoablation may be followed by commencement of systemic immunomodulatory therapy, for example after a delay of several weeks or more when a reduction in local inflammation or other non-specific immunologic effects is desirable.

[0033] The potential of benefits of combining local immunomodulatory therapy with a systemic immunomodulatory therapy regimen may make otherwise undesirable systemic immunomodulatory therapies viable: due to the resultant augmentation in potency of the systemic component of the combination therapy, reduced systemic dose regimens may be possible with commensurate reduction in adverse effects from the systemic therapy. Further, since the adverse effect profile of the local immunomodulatory therapy (i.e., IL chemoablation) is orthogonal to that of most systemic immunomodulatory therapies, a combined local and systemic immunomodulatory therapy is inherently safer and more attractive compared with prior combinations that can produce undesirable additive or synergistic adverse effects.

[0034] The combination of massive exposure to tumor antigens coupled with reduced tumor burden that results from IL chemoablation is particularly attractive in this context, since it maximizes potential immune activation while diminishing potential immune downregulation and physiologic demand from the tumor mass. When combined with a systemic therapy that further enhances immunologic upregulation or reduces downregulation, the effects on antitumor immunity, both at the ablated lesion and at uninjected sites, including those proximal and distant to the injection sites, will be additive or synergistic.

[0035] Many of the advantages accrued upon combining local immunomodulatory therapy with a systemic immunomodulatory therapy may be achieved through similar combination of local immunomodulatory therapy with a systemic targeted therapy, such as IL chemoablation combined with a targeted kinase inhibitor. Since IL chemoablation has a uniquely disruptive effect on tumor tissue, combination of this modality with an approach that targets tumor viability via an orthogonal path, such as those that target aberrant growth signaling or overexpression of receptors involved in tumor hyperproliferation, can yield enhanced efficacy in the treated tumor. For example, by using a systemic targeted therapy to increase stress on the tumor or reduce tumor viability in the wake of IL chemoablation, the cytotoxicity of the IL treatment may be enhanced at the time of IL treatment; response of any remaining tumor tissue may also be increased to immunologic activation resulting from the IL treatment since the systemic therapy will counter proliferation of residual tumor tissue without interfering with development of the immune response from chemoablation. The rapid reduction in tumor burden resulting from IL chemoablation further augments these advantages by reducing immune suppression and physiologic demands from the tumor tissue. Since the systemic targeted therapy is not required to achieve complete control or eradication of substantial tumor masses in this context, but rather serves to augment the activity of the local immunomodulatory therapy, it may be possible to administer the systemic therapy at a reduced dose, thereby minimizing potential adverse effects and making the combined therapy safer and more attractive compared with prior systemic combinations. Addition of the immunologic response resulting from the local immunomodulatory therapy component provides a means to counter resistance problems that have plagued many targeted systemic therapies, such as the BRAF inhibitors, particularly when continuous systemic therapies are required to maintain long term control of the disease, since long term control will result from the immune response rather than perpetual reliance on the targeted systemic therapy.

[0036] In some cases it may be desirable to commence systemic targeted therapy prior to local immunomodulatory therapy, for instance when disease burden is very high or widespread, or when the disease is rapidly proliferating, potentially making effective

administration of the local immunomodulatory therapy difficult or less effective. In this manner, the systemic targeted therapy may be used to control or reduce tumor burden prior to administration of local immunomodulatory therapy in order to enhance responsiveness of the disease to the local immunomodulatory therapy. Such an approach is tantamount to "down staging" disease status prior to commencement of local immunomodulatory therapy. For example, certain BRAF inhibiting drugs have proven effective at temporarily reducing disease burden in advanced stage metastatic melanoma, but resistance often develops within a period of months, negating long term outcome. Treatment of residual disease with local immunomodulatory therapy, such as IL chemotherapy, while it remains under control of the targeted therapy provides a means for elimination of residual tumor burden while stimulating long term immunity to recurrence, thereby improving ultimate outcome.

[0037] Problems affecting attempts to develop and utilize therapeutic cancer vaccines may also be similarly mitigated or resolved through combination of such vaccines with local immunomodulatory therapy. Specifically, lack of survival benefit observed in clinical trials of some such vaccines in advanced stage cancer, such as Canvaxin for stage III or IV melanoma, appears to be due in part to failure of vaccination to overcome existing tumor burden present in patients at the time of vaccination and continued increase in their disease level during the induction interval necessary for development of an immune response from vaccination. As in the case with systemic targeted therapy, the rapid reduction in tumor burden resulting from IL chemoablation can mitigate suppression of the immune system by the patient's disease burden while providing critical time for onset of the immune response from vaccination, thereby maximizing potential local and systemic antitumor effects through the combined action of the local immunomodulatory therapy and systemic vaccination.

[0038] Examples of combination therapies and method of treatment within the present invention include but are not limited to the following:

[0039] Local immunomodulative therapy combined with one or more systemic inhibitor of immune system down regulation, such as anti-CTLA-4 antibodies including but not limited to ipilimumab and tremelimumab.

[0040] Local immunomodulative therapy combined with one or more systemic immune upregulating agent, including: non-specific cytokines, such as interleukin-1, -2, or -6 (IL-1, IL-2 or IL-6) and aldesleukin; interferon-alpha or gamma (IFN-α and IFN-γ), interferon alfa-2b and pegylated interferon (including pegylated interferon alfa-2a and pegylated interferon alfa-2b); granulocyte macrophage colony stimulating factor (GM-CSF, molgramostim or sargramostim); dendritic cell vaccines and other allogeneic or autologous therapeutic cancer vaccines, including intralesional vaccines containing an oncolytic herpes virus encoding GM-CSF (OncoVex®)or a plasmid encoding human leukocyte antigen-B7 and beta-2 microglobulin agent designed to express allogeneic MHC class I antigens (Allovectin-7®); and antibodies against specific tumor antigens.

[0041] Local immunomodulative therapy combined with one or more systemic targeted therapy agent, including: drugs that target protein kinases and the receptors that activate them, including but not limited to afatinib (BIBW 2992), bevacizumab, cetuximab, dasatinib, E7080, erlotinib, gefitinib, imatinib, lapatinib, nilotinib, panitumumab, pazopanib, pegaptanib, ranibizumab, sorafenib, sunitinib, trastuzumab and vandetanib; serine/threonine-selective protein kinase inhibitors, including but not limited to those targeting the B-Raf/MEK/ERK pathway, such as vemurafenib (also known as PLX4032, RG7204 or RO5185426), GSK2118436 and GSK1120212; aromatase inhibitors, including but not limited to aminoglutethimide, anastrozole, exemestane, fadrozole, formestane, letrozole, testolactone and vorozole; estrogen receptor antagonists, including but not limited to lasofoxifene, raloxifene, tamoxifen and toremifene; COX-2 inhibitors, including but not limited to celecoxib, valdecoxib and rofecoxib; angiogenesis blockers, including IFN-α, IL-12, suramin, and thrombospondin (including thrombospondin 1, ABT-510 and ABT-898); and immune cell therapy, including but not limited to adoptive T-cell transfer and autologous immune cell therapy.

[0042] Typically, monotherapy dose schedules are set by determining the maximum tolerated dose (MTD) in early-stage clinical trials. The MTD (or a close variation thereon) is then promulgated to later-stage clinical trials for assessment efficacy and more detailed assessment of safety. These MTDs frequently become the established therapeutic dose upon completion of clinical testing. Example dosing schedules for a number of systemic agents that may be combined in the present invention with local immunomodulative therapy are provided in Table 1.

Table 1. Example systemic immunomodulatory or targeted anticancer agents

| Systemic Agent | Typical Dose Schedule |
|---|---|
| Ipilimumab | 3 mg/kg q21d for 4 treatments |
| Tremelimumab | 15 mg/kg q3m |
| Aldesleukin | 600,000 IU/kg q8h (up to 14 doses before 9 day rest and repeat; rest at least 7 wks before repeat of course) |
| interferon alfa-2b | 20 million IU/$m^2$ 5 times per week for 4 weeks (induction phase) followed by 10 million IU/$m^2$ three times per week (maintenance phase) |
| pegylated interferon | 6 μg/kg qwk for eight weeks (induction phase) followed by 3 μg/kg qwk (maintenance phase) |
| Oncovex® | 4 mL IL at $10^8$ pfu/mL |
| GM-CSF | 125 μg/$m^2$ daily for 14 wks followed by 14 days rest |
| Allovectin-7® | 2 mg IL qwk for 6 wks |
| Afatinib | 20-50 mg daily |
| Bevacizumab | 5-15 mg/kg q14d-q21d |
| Cetuximab | 400 mg/$m^2$ followed by weekly maintenance at 250 mg/$m^2$ |
| Dasatinib | 100 mg daily |
| Erlotinib | 100-150 mg daily |
| Gefitinib | 250 mg daily |
| Imatinib | 400-600 mg daily (increased to twice daily if well |

| | |
|---|---|
| | tolerated or disease progresses) |
| Lapatinib | 1250 mg daily for 21 day cycle |
| Nilotinib | 400 mg twice daily |
| Panitumumab | 6 mg/kg q14d |
| Pazopanib | 800 mg daily |
| Pegaptanib | 0.3 mg q6wks |
| Ranibizumab | 0.5 mg q4wks |
| Sorafenib | 400 mg twice daily |
| Sunitinib | 50 mg daily for 4 weeks followed by 2 week recovery |
| Trastuzumab | 4 mg/kg followed by weekly maintenance at 2 mg/kg |
| Vandetanib | 200-300 mg daily |
| Vemurafenib (PLX4032) | 960 mg twice daily |
| GSK2118436 [a] | 150 mg twice daily |
| GSK1120212 [a] | 2 mg daily |
| aminoglutethimide | 250 mg q6h |
| Anastrozole | 1 mg daily |
| Exemestane | 25 mg daily |
| Fadrozole | 1 mg twice daily |
| Formestane | 250 mg daily |
| Letrozole | 2.5 mg daily |
| Vorozole | 2.5 mg daily |
| Raloxifene | 60 mg daily |
| Tamoxifen | 20-40 mg daily |
| Toremifene | 60 mg daily |
| Celecoxib | 200-400 mg twice daily |
| Rofecoxib | 20-25 mg daily |
| Suramin | 1 g qwk |
| thrombospondin (ABT-510 [a]) | 20 mg daily to 100 mg twice daily |

[a] Proprietary code name for drug under development for which no nonproprietary name is currently available.

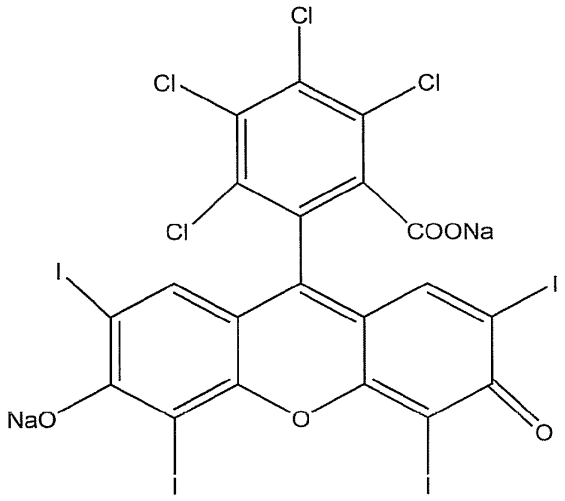
[0043] Because of additive or synergistic effects, the combination therapies and method of treatment of the present invention will generally allow use of the systemic agent at a level at or below the typical dose schedule for the systemic agent, such as those described in Table 1, when used with a local immunomodulative therapy, such as that described infra.

[0044] Local immunomodulative therapy includes but is not limited to intralesional chemoablation using an IL chemoablative agent consisting primarily of rose bengal (4,5,6,7-tetrachloro-2',4',5',7'-tetraiodofluorescein ) or another halogenated xanthene, including erythrosin B, phloxine B, 4,5,6,7-tetrabromo-2',4',5',7'-tetraiodofluorescein, 2',4,5,6,7-pentachloro-4',5',7'-triiodofluorescein, 4,4',5,6,7-pentachloro-2',5',7'-triiodofluorescein, 2',4,5,6,7,7'-hexachloro-4',5'-diiodofluorescein, 4,4',5,5',6,7-hexachloro-2',7'-diiodofluorescein, 2',4,5,5',6,7 -hexachloro-4',7'-diiodofluorescein, 4,5,6,7-tetrachloro-2',4',5'-triiodofluorescein, 4,5,6,7-tetrachloro-2',4',7'-triiodofluorescein, 4,5,6,7-tetrabromo-2',4',5'-triiodofluorescein, and 4,5,6,7-tetrabromo-2',4',7'-triiodofluorescein in an appropriate pharmaceutical composition, including a 0.1% (w/v) or higher concentration aqueous solution of rose bengal (i.e., PV-10) or equivalent solution of another halogenated xanthene or mixtures thereof. A physiologically acceptable salt of the halogenated xanthene may be used in this composition.

[0045] The present invention includes immunotherapeutic procedures wherein large amounts of tumor antigen are exposed to a patient's immune system, for example upon intralesional delivery of an immunomodulator, including but not limited to intralesional rose bengal, in combination with one or more systemic immunomodulator, to enhance the immune-mediated antitumor response.

[0046] About the preferred IL chemoablative agents:

[0047] Local immunomodulative therapy includes, as a preferred embodiment, intralesional chemoablation using rose bengal or another halogenated xanthene. A preferred form, rose bengal disodium, has the following formula:




[0048] Certain details of this preferred embodiment for the local immunomodulative composition are described in Applicants' co-pending application USSN 12/315,781, which is incorporated herein in its entirety. This preferred embodiment of the present invention is described here with particular relevance to melanoma. However, the present invention may also find application for the treatment of other hyperproliferative diseases including, but not limited to, cancers, such as for example, breast cancer, primary and metastatic liver cancer, prostate cancer and small cell and non small cell lung cancer, and no limitation is intended thereby.

[0049] Malignant melanoma is the most serious form of skin cancer and accounts for 80% of skin cancer deaths.

[0050] The extent of spread of a disease is described by stages. Stage 0 melanoma is a very early stage disease known as melanoma in situ. Patients with melanoma in situ are classified as Tis (tumor in situ). The tumor is limited to the epidermis with no invasion of surrounding tissues, lymph nodes, or distant sites. Melanoma in situ is considered to be

very low risk for disease recurrence or spread to lymph nodes or distant sites. Treatment is by surgical excision with a margin of healthy skin.

[0051] In stage I melanoma, the tumor has penetrated in to the skin by less than 1mm but has not spread. Treatment is by wide local excision and the probability of disease free survival in five years is between 90 to 95%.

[0052] Stage II melanoma describes a tumor that has penetrated more than 1mm into the skin but has not spread. Wide local excision is the preferred treatment. However, excision at this stage carries a much higher risk and less favorable prognosis than excision of a Stage I tumor.

[0053] Stage III melanoma is characterized by the existence of one or more nodal, in-transit or satellite metastasis but has not spread to distant or visceral sites. In-transit metastases are distant from the primary tumor but not reaching the draining nodal basin. Satellite metastases are intralymphatic extensions of the primary tumor and are typically found closer to the primary tumor than in-transit metastasis. Five year survival for stage III patients ranges from approximately 24% (gross nodal disease) to 80% (microscopic nodal disease).

[0054] Stage IV melanoma is when the disease has spread to distant sites. Survival of stage IV melanoma drops to approximately 10%.

[0055] Similar staging systems exist for all major cancers, and are generally based on the clinical presentation and histopathologic details of the disease and how these factors have been shown to impact survival.

[0056] Standard treatment for easily removable Stage III tumors is wide area excision together with removal of lymph nodes. Adjunct treatment such as radiotherapy and chemotherapy and for regional limb metastases, regional infusion of melphalan or other chemotherapeutic agents may also be given. However, in some cases, surgery is

contraindicated due to the number and/or location of tumors and other treatment options must be considered. Unfortunately, response levels for these other options are not high. For example, melanoma is largely resistant to radiation therapy. Systemic chemotherapy also has modest response rates against melanoma. The most effective chemotherapy regimen to-date is single-agent dacarbazine, which is only successful in 10-15% of cases. Two combination chemotherapy regimens commonly used in the treatment of patients with advanced-stage melanoma are the cisplatin, vinblastine and DTIC (CVD) regimen and the Dartmouth regimen, which is a combination of cisplatin, DTIC, carmustine and tamoxifen.

[0057] When melanoma occurs in the extremities, chemotherapy agents may be delivered via hyperthermic isolated limb perfusion (ILP). With this technique, blood vessels are accessed surgically, the blood flow to and from the limb is stopped using a tourniquet, and a warmed solution of chemotherapy drug is administered directly into the blood of the limb, allowing higher doses of drugs to be dispensed than with systemic treatment. A less invasive regional therapy is isolated limb infusion (ILI) whereby vascular access is gained via a percutaneous route in the groin.

[0058] Another treatment option is intralesional therapy in which a chemotherapeutic agent is injected directly into the tumor. Bacille Calmette Guerin (BCG) was one of the earliest reagents used for IL therapy. A review of data from 15 trials found 19% complete response (CR) and 26% partial response (PR) with extended survival in 13% of stage III patients.

[0059] IL interferons (IFN) have yielded mixed results ranging from a report of 45% objective response rate (ORR, 31% CR + 14% PR) for IFN-$\alpha$ to either no result or transient response with IFN-$\gamma$. Both regimes produced significant toxicity and side effects.

[0060] IL interleukin-2 appears to be the most promising IL therapy to date with an ORR in 83% of patients (62%CR + 21% PR) receiving 2-3 weekly IL treatments. Some

patients reported flu like symptoms and some authors noted that although new lesions appeared during the course of treatment, some patients experienced a marked slowing of the appearance of new cutaneous lesions.

[0061] IL therapy with cisplatin or IL cisplatin with electroporation has yielded results ranging from 38%ORR (19%CR + 19%PR) to 53% ORR (47%CR + 7% PR). However, the ORR reported for lesions with a median diameter of 0.6cm of 53% decreased to 44% for lesions having a median diameter of 3.0cm.

[0062] Substantial efficacy has been reported upon a single electrochemotherapy treatment with IL bleomycin. However, as with cisplatin, response was generally reduced in larger tumors.

[0063] It may be appreciated that there remains a need for alternative methods for the treatment of hyperproliferative diseases and in particular stage III and IV melanoma.

[0064] According to a preferred embodiment of the present invention, there is provided a method for the treatment of cancer in a patient, such as metastatic melanoma, the method comprising treatment of the cancer patient with a local immunomodulative therapy combined with one or more systemic immunomodulatory therapy or systemic targeted therapy, wherein said local immunomodulatory therapy comprises intralesional administration of a chemoablative pharmaceutical composition comprising a hydrophilic vehicle containing 4,5,6,7-Tetrachloro-2′,4′,5′,7′-tetraiodofluorescein (i.e. rose bengal), or certain other halogenated xanthene, or a physiologically acceptable salt thereof. It is preferred that the halogenated xanthene be present in this pharmaceutical composition at a concentration of about 0.1% (w/v) up to about 20% (w/v), and that the pharmaceutical composition include an electrolyte comprising at least one cation selected from the group consisting of sodium, potassium, calcium and magnesium and at least one anion selected from the group consisting of chloride, phosphate and nitrate, wherein the electrolyte is at a concentration of between about 0.1% (w/v) and about 2% (w/v). It is also preferred that the pH of the pharmaceutical composition be between about 4 to about 10.

[0065] The term "physiologically acceptable salt" refers to any non-toxic alkali metal, alkaline earth metal, and ammonium salt commonly used in the pharmaceutical industry, including the sodium, potassium, lithium, calcium, magnesium, barium, ammonium and protamine zinc salts, which can be prepared by methods known in the art. Preferably, the salts are sodium, potassium, calcium and ammonium in either the mono or dibasic salt form.

[0066] Especially preferred in this IL chemoablative pharmaceutical composition is the disodium salt of rose bengal. Previous work by one or more of the present inventors (WO 02/05812) reported their discovery that rose bengal exhibits preferential uptake into cancer cells but is essentially excluded from normal cells.

[0067] One or more of the present inventors have also reported their discovery that the nature of the vehicle in which the halogenated xanthene, or a physiologically acceptable salt thereof, is administered can significantly influence the degree of partitioning into tumor cells. In particular, one or more of the present inventors have surprisingly discovered that at an electrolyte concentration of between about 0.1% (w/v) and about 2.0% (w/v), partitioning into tumor cells may rapidly be increased.

[0068] An approximation of an agent's potential for tissue accumulation can be estimated based upon the partition coefficient $K_p$. This *in vitro* parameter is purported to have predictive values relating to *in vitro* delivery at the cellular level. In particular, a value greater than unity is considered to indicate agents capable of localizing in tissue, and thereby being capable of exhibiting enhanced chemotherapeutic efficacy in such tissue. One or more of the present inventors surmise that values much greater than approximately 50-100 may indicate excess lipophilicity (tendency to accumulate in organic environments) that may compromise delivery of an agent in a desirable aqueous (i.e., hydrophilic) formulation. $K_p$ is determined by measuring the ratio of equilibrium concentrations of an agent in a lipophilic phase (*n*-octanol) contacted with an aqueous phase.

[0069] One or more of the present inventors have also reported their discovery that it is preferred that the pH of the IL chemoablative pharmaceutical composition is in the range of between about 4 to about 10, and more preferably between about 5 to about 9, to yield maximum solubility of the halogenated xanthene in an aqueous vehicle and assure compatibility with biological tissue. A particularly preferred pH is between about 4 to about 7, preferably between about 5 to about 7, more preferably between about 6 to about 7. At these pH values, the halogenated xanthenes generally remain in dibasic form, rather than the water insoluble lactone that forms at low pH.

[0070] The pH of the IL chemoablative pharmaceutical composition may be regulated or adjusted by any suitable means known to those of skill in the art. The composition may be buffered or the pH adjusted by addition of acid or base or the like. As the halogenated xanthenes, or physiologically acceptable salts thereof, are weak acids, depending upon halogenated xanthene concentration and/or electrolyte concentration, the pH of the composition may not require the use of a buffer and/or pH modifying agent. It is especially preferred, however, that the composition does not contain any buffer, allowing it to conform to the biological environment once administered.

[0071] One or more of the present inventors have also reported their discovery that $K_p$ is also dependent upon electrolyte concentration with the $K_p$ value increasing with electrolyte concentration. Particularly preferred concentrations of electrolyte in the IL chemoablative pharmaceutical composition are between 0.5 to 1.5% (w/v), and even more preferably at a concentration of about 0.8 to 1.2% (w/v) and most preferably at a concentration of about 0.9% (w/v), this latter concentration being especially preferred since it corresponds to an approximately isotonic solution.

[0072] In a further preferred embodiment of the present invention, the electrolyte in the IL chemoablative pharmaceutical composition is sodium chloride.

[0073] Electrolytes at such levels increase the osmolality of the IL chemoablative pharmaceutical composition. Thus, as an alternative to specifying a range of electrolyte concentrations, osmolality may be used to characterize, in part, the electrolyte level of the composition. It is preferred that the osmolality of the composition be greater than about 100 mOsm/kg, and more preferably that the osmolality of the composition be greater than about 250 mOsm/kg and most preferably that it is about 300 – 500 mOsm/kg.

[0074] One or more of the present inventors have found that the preferred concentration of halogenated xanthene and/or dose of IL chemoablative pharmaceutical composition will be dependent upon factors including, but not limited to, tumor size, number and location. For visceral or other internal lesions, such as cancers of the liver, intralesional administration may be by percutaneous or intraoperative administration.

[0075] One or more of the present inventors have also found that halogenated xanthene concentrations in the IL chemoablative pharmaceutical composition above about 1% (w/v) to 3% (w/v) are particularly useful for chemoablative use, since lower concentrations are generally insufficient to directly elicit destruction of target tissues. Thus, in a preferred embodiment, the concentration of halogenated xanthene is in the range of from about 3% (w/v) to about 20% (w/v). In another embodiment, the concentration of halogenated xanthene is from about 3% (w/v) to about 10% (w/v). In another embodiment, the concentration of halogenated xanthene is from about 10% (w/v) to about 20% (w/v). In still another embodiment, the concentration of halogenated xanthene is about 10% (w/v). One or more of the present inventors have surprisingly found that at these concentrations, not only can an efficient therapeutic response be obtained, but the solution is also highly stable and can be readily handled both in manufacture and use. These preferred concentrations may be expressed in weight to volume (w/v), however, concentration in weight to weight (w/w) is substantially equivalent.

[0076] Typical dosages of the IL chemoablative pharmaceutical composition administered by IL administration range from between 0.1 mL/cc lesion volume to about

2 mL/cc lesion volume, most preferably between about 0.25 mL/cc to about 0.75 mL/cc lesion volume. Such doses typically correspond to a patient dose of between about 10 mg to about 1500 mg of halogenated xanthene (which are significantly higher than those doses used for diagnostic liver tests).

[0077] Since the pharmaceutical composition is for IL administration, which is an intracorporeal route, it is further preferred that it be sterile, such as required for conformance to U. S. Pharmacopeia (USP) test <71>, and further that it contains negligible levels of pyrogenic material, such that it conforms to USP<85> (limulus amebocyte lysate assay) or to USP <151> (rabbit pyrogen test), or to substantially equivalent requirements, at a pyrogen or endotoxin level equivalent to not more that (NMT) 10 endotoxin units (EU) per mL. Moreover, the pharmaceutical composition should conform to requirements limiting content of particulate matter as defined in USP <788> (i.e., NMT 3000 particulates greater than 10 microns in size, and NMT 300 particulates greater than 25 microns in size, per container) or substantially equivalent requirements. Each of these references from the USP is incorporated herein by reference.

[0078] Still further, one or more of the present inventors have found that a hydrophilic vehicle is preferred for the pharmaceutical composition to maximize preference of the halogenated xanthene for partitioning into cancerous tissue. Accordingly, it is preferred that the pharmaceutical composition contains a minimum of non-hydrophilic components that might interfere with such partitioning. It is preferred that the hydrophilic vehicle is water, and it is most preferred that this pharmaceutical composition consists substantially of water.

[0079] One or more of the present inventors have found that such pharmaceutical compositions as described herein are optimally packaged in glass vials having a capacity of approximately 1 to 10 mL, and more preferably approximately 5 mL. Such capacities are well suited as unidose forms (i.e., single use packages) for IL treatments.

[0080] In a preferred embodiment, the formulation of the pharmaceutical composition is not buffered. In this case, it is preferred that packaging containers be made of the USP Type I (low extractable or chemically resistant borosiciliate) or USP Type II (low-extractable soda lime) glass and that the inside surface of such glass containers be surface treated to reduce surface alkalinity of the container that could adversely affect pH or long-term stability. Typical surface treatment applicable to such containers is described in USP<661>. The inside of such surface-treated glass containers should be rinsed with a suitable solvent, such as distilled water one or more times prior to filling in order to remove any residue of such surface treatment. The containers should also be depyrogenated prior to filling, for example, by heating to 250°C or higher for several hours or more, and should be sterile or sterilized prior to filling using methods common in the field. If is further preferred that such containers have a minimum neck size, for example, of less than 10 mm and more preferably 5 mm or less, to reduce surface area of the closures of the containers (and hence exposure of the medicament to such closures).

[0081] One or more of the present inventors have further found that a septum-type closure, composed preferably of a pharmaceutical grade elastomeric material with a Teflon or similar inner coating, is particularly suitable for use with the IL chemoablative pharmaceutical composition since it facilitates insertion of a needle into the container for withdrawal of a dose of medicament while exhibiting minimal potential for interaction with the container contents.

[0082] It is also preferred that the pharmaceutical composition does not include any preservatives. One or more of the present inventors have found that it is generally preferable to avoid use of preservatives, many of which may deleteriously interfere with the pharmaceutical composition or formulation thereof, or may complex or otherwise interact with or interfere with the delivery of the halogenated xanthene active component. To the extent that a preservative may be used, one or more of the present inventors have found that imidurea is preferred as it does not interact with halogenated xanthenes, either in the pharmaceutical composition or upon administration.

[0083] This description has been offered for illustrative purposes only and is not intended to limit the invention of this application.

[0084] What is claimed as new and desired to be protected by Letters Patent is set forth in the appended claims.

Claims:

1. A method of treatment of cancer comprising administering a therapeutically effective amount of an intralesional chemoablative pharmaceutical composition in combination with a therapeutically effective amount of a systemic immunomodulatory anticancer agent.

2. The method of Claim 1 wherein said systemic immunomodulatory anticancer agent comprises anti-CTLA-4 antibodies including ipilimumab and tremelimumab.

3. The method of Claim 1 wherein said systemic immunomodulatory anticancer agent is selected from the group consisting of non-specific cytokines, such as interleukin-1, interleukin-2, or interleukin-6 (IL-1, IL-2 or IL-6) and aldesleukin; interferon-alpha or interferon-gamma (IFN-α and IFN-γ), interferon alfa-2b and pegylated interferon (including pegylated interferon alfa-2a and pegylated interferon alfa-2b); granulocyte macrophage colony stimulating factor (GM-CSF, molgramostim or sargramostim); dendritic cell vaccines and other allogeneic or autologous therapeutic cancer vaccines, including intralesional vaccines containing an oncolytic herpes virus encoding GM-CSF (OncoVex®) or a plasmid encoding human leukocyte antigen-B7 and beta-2 microglobulin agent designed to express allogeneic MHC class I antigens (Allovectin-7®); and antibodies against specific tumor antigens.

4. The method of Claim 1 wherein said intralesional chemoablative pharmaceutical composition comprises an IL chemoablative agent comprising primarily a halogenated xanthene in an appropriate pharmaceutical composition, including a 0.1% (w/v) or higher concentration aqueous solution of the halogenated xanthene or mixtures thereof, or a physiologically acceptable salt of the halogenated xanthene.

5. The method of Claim 4 wherein the halogenated xanthene is rose bengal (4,5,6,7-tetrachloro-2',4',5',7'-tetraiodofluorescein).

6. The method of Claim 5 wherein the halogenated xanthene is rose bengal disodium.

7. The method of Claim 4 wherein the halogenated xanthene is selected from the group consisting of erythrosin B, phloxine B, 4,5,6,7-tetrabromo-2',4',5',7'-tetraiodofluorescein, 2',4,5,6,7-pentachloro-4',5',7'-triiodofluorescein, 4,4',5,6,7-pentachloro-2',5',7'-triiodofluorescein, 2',4,5,6,7,7'-hexachloro-4',5'-diiodofluorescein, 4,4',5,5',6,7-hexachloro-2',7'-diiodofluorescein, 2',4,5,5',6,7 -hexachloro-4',7'-diiodofluorescein, 4,5,6,7-tetrachloro-2',4',5'-triiodofluorescein, 4,5,6,7-tetrachloro-2',4',7'-triiodofluorescein, 4,5,6,7-tetrabromo-2',4',5'-triiodofluorescein, and 4,5,6,7-tetrabromo-2',4',7'-triiodofluorescein.

8. The method of Claim 4 wherein said halogenated xanthene has a concentration of about 0.1% (w/v) up to about 20% (w/v), and that the pharmaceutical composition includes an electrolyte comprising at least one cation selected from the group consisting of sodium, potassium, calcium and magnesium and at least one anion selected from the group consisting of chloride, phosphate and nitrate, wherein the electrolyte is at a concentration of between about 0.1% (w/v) and about 2% (w/v).

9. The method of Claim 8 wherein the concentration of said electrolyte in the IL chemoablative pharmaceutical composition is between 0.5 to 1.5% (w/v).

10. The method of Claim 4 wherein said chemoablative pharmaceutical composition has an osmolality of the composition of greater than about 100 mOsm/kg.

11. The method of Claim 8 wherein said electrolyte is sodium chloride.

12. The method of Claim 4 wherein said pharmaceutical composition comprises a hydrophilic vehicle.

13. The method of Claim 4 wherein said pharmaceutical composition has a pH in the range of between about 4 to about 10.

14. The method of Claim 13 wherein said pharmaceutical composition has a pH in the range of between about 5 to about 7.

15. The method of Claim 1 wherein said method is for the treatment of cancers selected from melanoma, breast cancer, primary and metastatic liver cancer, prostate cancer and small cell and non small cell lung cancer.

16. A method of treatment of cancer comprising administering a therapeutically effective amount of an intralesional chemoablative pharmaceutical composition in combination with a therapeutically effective amount of a systemic targeted anticancer agent.

17. The method of Claim 16 wherein said systemic targeted anticancer agent is selected from the group consisting of drugs that target protein kinases and the receptors that activate them, including afatinib (BIBW 2992), bevacizumab, cetuximab, dasatinib, E7080, erlotinib, gefitinib, imatinib, lapatinib, nilotinib, panitumumab, pazopanib, pegaptanib, ranibizumab, sorafenib, sunitinib, trastuzumab and vandetanib; serine/threonine-selective protein kinase inhibitors, including those targeting the B-Raf/MEK/ERK pathway, such as vemurafenib (also known as PLX4032, RG7204 or RO5185426), GSK2118436 and GSK1120212; aromatase inhibitors, including aminoglutethimide, anastrozole, exemestane, fadrozole, formestane, letrozole, testolactone and vorozole; estrogen receptor antagonists, including lasofoxifene, raloxifene, tamoxifen and toremifene; COX-2 inhibitors, including celecoxib, valdecoxib and rofecoxib; angiogenesis blockers, including IFN-α, IL-12, suramin, and thrombospondin (including thrombospondin 1, ABT-510 and ABT-898); and immune cell therapy, including adoptive T-cell transfer and autologous immune cell therapy.

18. The method of Claim 16 wherein said intralesional chemoablative pharmaceutical composition comprises an IL chemoablative agent comprising primarily a halogenated xanthene in an appropriate pharmaceutical composition, including a 0.1% (w/v) or higher concentration aqueous solution of the halogenated xanthene or mixtures thereof, or a physiologically acceptable salt of the halogenated xanthene.

19. The method of Claim 18 wherein the halogenated xanthene is rose bengal (4,5,6,7-tetrachloro-2',4',5',7'-tetraiodofluorescein).

20. The method of Claim 19 wherein the halogenated xanthene is rose bengal disodium.

21. The method of Claim 18 wherein the halogenated xanthene is selected from the group consisting of erythrosin B, phloxine B, 4,5,6,7-tetrabromo-2',4',5',7'-tetraiodofluorescein, 2',4,5,6,7-pentachloro-4',5',7'-triiodofluorescein, 4,4',5,6,7-pentachloro-2',5',7'-triiodofluorescein, 2',4,5,6,7,7'-hexachloro-4',5'-diiodofluorescein, 4,4',5,5',6,7-hexachloro-2',7'-diiodofluorescein, 2',4,5,5',6,7 -hexachloro-4',7'-diiodofluorescein, 4,5,6,7-tetrachloro-2',4',5'-triiodofluorescein, 4,5,6,7-tetrachloro-2',4',7'-triiodofluorescein, 4,5,6,7-tetrabromo-2',4',5'-triiodofluorescein, and 4,5,6,7-tetrabromo-2',4',7'-triiodofluorescein.

22. The method of Claim 18 wherein said halogenated xanthene has a concentration of about 0.1% (w/v) up to about 20% (w/v), and that the pharmaceutical composition includes an electrolyte comprising at least one cation selected from the group consisting of sodium, potassium, calcium and magnesium and at least one anion selected from the group consisting of chloride, phosphate and nitrate, wherein the electrolyte is at a concentration of between about 0.1% (w/v) and about 2% (w/v).

23. The method of Claim 22 wherein the concentration of said electrolyte in the IL chemoablative pharmaceutical composition is between 0.5 to 1.5% (w/v).

24. The method of Claim 18 wherein said chemoablative pharmaceutical composition has an osmolality of the composition of greater than about 100 mOsm/kg.

25. The method of Claim 22 wherein said electrolyte is sodium chloride.

26. The method of Claim 18 wherein said pharmaceutical composition comprises a hydrophilic vehicle.

27. The method of Claim 18 wherein said pharmaceutical composition has a pH in the range of between about 4 to about 10.

28. The method of Claim 27 wherein said pharmaceutical composition has a pH in the range of between about 5 to about 7.

29. The method of Claim 16 wherein said method is for the treatment of cancers selected from melanoma, breast cancer, primary and metastatic liver cancer, prostate cancer and small cell and non small cell lung cancer.

30. A pharmaceutical composition for the treatment of cancer comprising:

a therapeutically effective amount of an intralesional chemoablative pharmaceutical composition; and

a therapeutically effective amount of a systemic immunomodulatory anticancer agent.

31. The pharmaceutical composition of Claim 30 wherein said systemic immunomodulatory anticancer agent comprises anti-CTLA-4 antibodies including ipilimumab and tremelimumab.

32. The pharmaceutical composition of Claim 30 wherein said systemic immunomodulatory anticancer agent is selected from the group consisting of non-specific cytokines, such as interleukin-1, interleukin-2, or interleukin-6 (IL-1, IL-2 or IL-6) and

aldesleukin; interferon-alpha or interferon-gamma (IFN-α and IFN-γ), interferon alfa-2b and pegylated interferon (including pegylated interferon alfa-2a and pegylated interferon alfa-2b); granulocyte macrophage colony stimulating factor (GM-CSF, molgramostim or sargramostim); dendritic cell vaccines and other allogeneic or autologous therapeutic cancer vaccines, including intralesional vaccines containing an oncolytic herpes virus encoding GM-CSF (OncoVex®) or a plasmid encoding human leukocyte antigen-B7 and beta-2 microglobulin agent designed to express allogeneic MHC class I antigens (Allovectin-7®); and antibodies against specific tumor antigens.

33. The pharmaceutical composition of Claim 30 wherein said intralesional chemoablative pharmaceutical composition comprises an IL chemoablative agent comprising primarily a halogenated xanthene in an appropriate pharmaceutical composition, including a 0.1% (w/v) or higher concentration aqueous solution of the halogenated xanthene or mixtures thereof, or a physiologically acceptable salt of the halogenated xanthene.

34. The pharmaceutical composition of Claim 33 wherein the halogenated xanthene is rose bengal (4,5,6,7-tetrachloro-2',4',5',7'-tetraiodofluorescein).

35. The pharmaceutical composition of Claim 34 wherein the halogenated xanthene is rose bengal disodium.

36. The pharmaceutical composition of Claim 33 wherein the halogenated xanthene is selected from the group consisting of erythrosin B, phloxine B, 4,5,6,7-tetrabromo-2',4',5',7'-tetraiodofluorescein, 2',4,5,6,7-pentachloro-4',5',7'-triiodofluorescein, 4,4',5,6,7-pentachloro-2',5',7'-triiodofluorescein, 2',4,5,6,7,7'-hexachloro-4',5'-diiodofluorescein, 4,4',5,5',6,7-hexachloro-2',7'-diiodofluorescein, 2',4,5,5',6,7 - hexachloro-4',7'-diiodofluorescein, 4,5,6,7-tetrachloro-2',4',5'-triiodofluorescein, 4,5,6,7-tetrachloro-2',4',7'-triiodofluorescein, 4,5,6,7-tetrabromo-2',4',5'-triiodofluorescein, and 4,5,6,7-tetrabromo-2',4',7'-triiodofluorescein.

37. The pharmaceutical composition of Claim 33 wherein said halogenated xanthene has a concentration of about 0.1% (w/v) up to about 20% (w/v), and that the pharmaceutical composition includes an electrolyte comprising at least one cation selected from the group consisting of sodium, potassium, calcium and magnesium and at least one anion selected from the group consisting of chloride, phosphate and nitrate, wherein the electrolyte is at a concentration of between about 0.1% (w/v) and about 2% (w/v).

38. The pharmaceutical composition of Claim 37 wherein the concentration of said electrolyte in the IL chemoablative pharmaceutical composition is between 0.5 to 1.5% (w/v).

39. The pharmaceutical composition of Claim 33 wherein said chemoablative pharmaceutical composition has an osmolality of the composition of greater than about 100 mOsm/kg.

40. The pharmaceutical composition of Claim 37 wherein said electrolyte is sodium chloride.

41. The pharmaceutical composition of Claim 33 wherein said pharmaceutical composition comprises a hydrophilic vehicle.

42. The pharmaceutical composition of Claim 33 wherein said pharmaceutical composition has a pH in the range of between about 4 to about 10.

43. The pharmaceutical composition of Claim 42 wherein said pharmaceutical composition has a pH in the range of between about 5 to about 7.

44. The pharmaceutical composition of Claim 30 wherein said pharmaceutical composition is for the treatment of cancers selected from melanoma, breast cancer,

primary and metastatic liver cancer, prostate cancer and small cell and non small cell lung cancer.

45. A pharmaceutical composition for treatment of cancer comprising:

a therapeutically effective amount of an intralesional chemoablative pharmaceutical composition; and

a therapeutically effective amount of a systemic targeted anticancer agent.

46. The pharmaceutical composition of Claim 45 wherein said systemic targeted anticancer agent is selected from the group consisting of drugs that target protein kinases and the receptors that activate them, including afatinib (BIBW 2992), bevacizumab, cetuximab, dasatinib, E7080, erlotinib, gefitinib, imatinib, lapatinib, nilotinib, panitumumab, pazopanib, pegaptanib, ranibizumab, sorafenib, sunitinib, trastuzumab and vandetanib; serine/threonine-selective protein kinase inhibitors, including those targeting the B-Raf/MEK/ERK pathway, such as vemurafenib (also known as PLX4032, RG7204 or RO5185426), GSK2118436 and GSK1120212; aromatase inhibitors, including aminoglutethimide, anastrozole, exemestane, fadrozole, formestane, letrozole, testolactone and vorozole; estrogen receptor antagonists, including lasofoxifene, raloxifene, tamoxifen and toremifene; COX-2 inhibitors, including celecoxib, valdecoxib and rofecoxib; angiogenesis blockers, including IFN-α, IL-12, suramin, and thrombospondin (including thrombospondin 1, ABT-510 and ABT-898); and immune cell therapy, including adoptive T-cell transfer and autologous immune cell therapy.

47. The pharmaceutical composition of Claim 45 wherein said intralesional chemoablative pharmaceutical composition comprises an IL chemoablative agent comprising primarily a halogenated xanthene in an appropriate pharmaceutical composition, including a 0.1% (w/v) or higher concentration aqueous solution of the halogenated xanthene or mixtures thereof, or a physiologically acceptable salt of the halogenated xanthene.

48. The pharmaceutical composition of Claim 47 wherein the halogenated xanthene is rose bengal (4,5,6,7-tetrachloro-2',4',5',7'-tetraiodofluorescein).

49. The pharmaceutical composition of Claim 48 wherein the halogenated xanthene is rose bengal disodium.

50. The pharmaceutical composition of Claim 47 wherein the halogenated xanthene is selected from the group consisting of erythrosin B, phloxine B, 4,5,6,7-tetrabromo-2',4',5',7'-tetraiodofluorescein, 2',4,5,6,7-pentachloro-4',5',7'-triiodofluorescein, 4,4',5,6,7-pentachloro-2',5',7'-triiodofluorescein, 2',4,5,6,7,7'-hexachloro-4',5'-diiodofluorescein, 4,4',5,5',6,7-hexachloro-2',7'-diiodofluorescein, 2',4,5,5',6,7 - hexachloro-4',7'-diiodofluorescein, 4,5,6,7-tetrachloro-2',4',5'-triiodofluorescein, 4,5,6,7-tetrachloro-2',4',7'-triiodofluorescein, 4,5,6,7-tetrabromo-2',4',5'-triiodofluorescein, and 4,5,6,7-tetrabromo-2',4',7'-triiodofluorescein.

51. The pharmaceutical composition of Claim 47 wherein said halogenated xanthene has a concentration of about 0.1% (w/v) up to about 20% (w/v), and that the pharmaceutical composition includes an electrolyte comprising at least one cation selected from the group consisting of sodium, potassium, calcium and magnesium and at least one anion selected from the group consisting of chloride, phosphate and nitrate, wherein the electrolyte is at a concentration of between about 0.1% (w/v) and about 2% (w/v).

52. The pharmaceutical composition of Claim 51 wherein the concentration of said electrolyte in the IL chemoablative pharmaceutical composition is between 0.5 to 1.5% (w/v).

53. The pharmaceutical composition of Claim 47 wherein said chemoablative pharmaceutical composition has an osmolality of the composition of greater than about 100 mOsm/kg.

54. The pharmaceutical composition of Claim 51 wherein said electrolyte is sodium chloride.

55. The pharmaceutical composition of Claim 47 wherein said pharmaceutical composition comprises a hydrophilic vehicle.

56. The pharmaceutical composition of Claim 47 wherein said pharmaceutical composition has a pH in the range of between about 4 to about 10.

57. The pharmaceutical composition of Claim 56 wherein said pharmaceutical composition has a pH in the range of between about 5 to about 7.

58. The pharmaceutical composition of Claim 45 wherein said pharmaceutical composition is for the treatment of cancers selected from melanoma, breast cancer, primary and metastatic liver cancer, prostate cancer and small cell and non small cell lung cancer.

**INTERNATIONAL SEARCH REPORT**

International application No.
PCT/US 12/28412

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USPC - 530/387.3
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B. FIELDS SEARCHED

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Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWest (US Pat, PgPub, EPO, JPO), GoogleScholar (PL, NPL), FreePatentsOnline (US Pat, PgPub, EPO, JPO, WIPO, NPL); search terms: ipilimumab tremelimumab rose Bengal intralesional chemoablative xanthenes

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X<br>---<br>Y | US 2010/0189651 A1 (Stagliano et al.) 29 July 2010 (29.07.2010) para [0011], [0013], [0048], [0253] | 1-3, 16-19, 30-32, 45-46<br>---<br>4-15, 20-29, 33-44, 47-58 |
| Y | US 2008/0118567 A1 (Scott et al.) 22 May 2008 (22.05.2008) para [0017], [0027]- [0032], [0056], [0071], [0075], [0083] | 4-15, 20-29, 33-44, 47-58 |
| A | US 2009/0117199 A1 (Scott et al.) 07 May 2009 (07.05.2009) Abstract | 1-58 |
| A, P | US 2011/0071217 A1 (Singer et al.) 24 March 2011 (24.03.2011) Abstract | 1-58 |

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
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"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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"&" document member of the same patent family

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(75) Inventors/Applicants *(for US only)*: **LANGERMANN, Solomon** [US/US]; 6606 Cross Country Boulevard, Balyimore, MD 21215 (US). **LIU, Linda** [US/US]; 6512 Tipperary Court, Clarksville, MD 21029 (US). **MARSHALL, Shannon** [US/US]; 1503 Frederick Road, Baltimore, MD 21228 (US). **YAO, Sheng** [US/US]; 11748 Bryce Overlook Ct., Columbia, MD 21044 (US).

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*[Continued on next page]*

(54) Title: ANTIBODIES AND OTHER MOLECULES THAT BIND B7-H1 AND PD-1

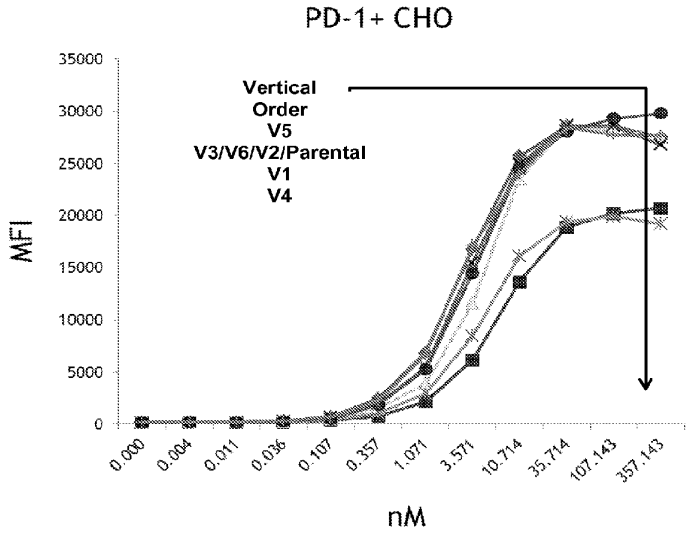



Figure 20

*[Continued on next page]*

WO 2012/145493 A1

TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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**(57) Abstract**: The present invention relates to antibodies and their antigen-binding fragments and to other molecules that are capable of immunospecifically binding to B7-H1 or PD-1. In some embodiments such molecules are additionally capable of modulating the ability of B7-H1 or B7-DC to bind to PD-1 or are capable of affecting the signaling activity of the B7-H1 or PD-1. The invention additionally concerns the uses of such molecules in the diagnosis and treatment of cancer and other diseases.

**Title of the Invention:**

# Antibodies and Other Molecules That Bind B7-H1 and PD-1

**Cross-Reference to Related Applications:**

**[0001]** This application claims priority to United States Patent Application No. 61/477,414 (filed on April 20, 2011; pending), which application is herein incorporated by reference in its entirety.

**Reference to Sequence Listing:**

**[0002]** This application includes one or more Sequence Listings pursuant to 37 C.F.R. 1.821 *et seq.*, which are disclosed in both paper and computer-readable media, and which paper and computer-readable disclosures are herein incorporated by reference in their entirety.

**Background of the Invention:**

**Field of the Invention:**

**[0003]** The present invention relates to antibodies and their antigen-binding fragments and to other molecules that are capable of immunospecifically binding to B7-H1 or PD-1. In some embodiments such molecules are additionally capable of modulating the ability of B7-H1 or B7-DC to bind to PD-1 or are capable of affecting the signaling activity of the B7-H1 or PD-1. The invention additionally concerns the uses of such molecules in the diagnosis and the treatment of cancer and other diseases.

**Description of Related Art:**

**A. Cell Mediated Immune Responses**

**[0004]** The immune system of humans and other mammals is responsible for providing protection against infection and disease. Such protection is provided both by a humoral immune response and by a cell-mediated immune response. The humoral response results in the production of antibodies and other biomolecules that are capable of recognizing and neutralizing foreign targets (antigens). In contrast, the cell-mediated immune response involves the activation of macrophages, natural killer cells (NK), and antigen-specific cytotoxic T-lymphocytes by T cells, and the release of various cytokines in response to the

recognition of an antigen (Dong, C. *et al.* (2003) "*Immune Regulation by Novel Costimulatory Molecules*," Immunolog. Res. 28(1):39-48).

**[0005]** The ability of T cells to optimally mediate an immune response against an antigen requires two distinct signaling interactions (Viglietta, V. *et al.* (2007) "*Modulating Co-Stimulation*," Neurotherapeutics 4:666-675; Korman, A.J. *et al.* (2007) "*Checkpoint Blockade in Cancer Immunotherapy*," Adv. Immunol. 90:297-339). First, antigen that has been arrayed on the surface of antigen-presenting cells (APC) must be presented to an antigen-specific naive $CD4^+$ T cell. Such presentation delivers a signal via the T cell receptor (TCR) that directs the T cell to initiate an immune response that will be specific to the presented antigen. Second, a series of co-stimulatory and inhibitory signals, mediated through interactions between the APC and distinct T cell surface molecules, triggers first the activation and proliferation of the T cells and ultimately their inhibition. Thus, the first signal confers specificity to the immune response whereas the second signal serves to determine the nature, magnitude and duration of the response.

**[0006]** The immune system is tightly controlled by costimulatory and co-inhibitory ligands and receptors. These molecules provide the second signal for T cell activation and provide a balanced network of positive and negative signals to maximize immune responses against infection while limiting immunity to self (Wang, L. *et al.* (March 7, 2011) "*VISTA, A Novel Mouse Ig Superfamily Ligand That Negatively Regulates T Cell Responses*," J. Exp. Med. 10.1084/jem.20100619:1-16; Lepenies, B. *et al.* (2008) "*The Role Of Negative Costimulators During Parasitic Infections*," Endocrine, Metabolic & Immune Disorders - Drug Targets 8:279-288). Of particular importance is binding between the B7.1 (CD80) and B7.2 (CD86) ligands of the Antigen Presenting Cell and the CD28 and CTLA-4 receptors of the $CD4^+$ T-lymphocyte (Sharpe, A.H. *et al.* (2002) "*The B7-CD28 Superfamily*," Nature Rev. Immunol. 2:116-126; Dong, C. et al. (2003) "*Immune Regulation by Novel Costimulatory Molecules*," Immunolog. Res. 28(1):39-48; Lindley, P.S. et al. (2009) "*The Clinical Utility Of Inhibiting CD28-Mediated Costimulation*," Immunol. Rev. 229:307-321). Binding of B7.1 or of B7.2 to CD28 stimulates T cell activation; binding of B7.1 or B7.2 to CTLA-4 inhibits such activation (Dong, C. *et al.* (2003) "*Immune Regulation by Novel Costimulatory Molecules*," Immunolog. Res. 28(1):39-48; Lindley, P.S. *et al.* (2009) "*The Clinical Utility Of Inhibiting CD28-Mediated Costimulation*," Immunol. Rev. 229:307-321; Greenwald, R.J. *et al.* (2005) "*The B7 Family Revisited*," Ann. Rev. Immunol. 23:515-548). CD28 is constitutively

expressed on the surface of T cells (Gross, J., *et al.* (1992) "*Identification And Distribution Of The Costimulatory Receptor CD28 In The Mouse*," J. Immunol. 149:380–388), whereas CTLA4 expression is rapidly up-regulated following T-cell activation (Linsley, P. *et al.* (1996) "*Intracellular Trafficking Of CTLA4 And Focal Localization Towards Sites Of TCR Engagement*," Immunity 4:535–543). Since CTLA4 is the higher affinity receptor (Sharpe, A.H. *et al.* (2002) "*The B7-CD28 Superfamily*," Nature Rev. Immunol. 2:116-126), binding first initiates T cell proliferation (via CD28) and then inhibits it (via nascent expression of CTLA4), thereby dampening the effect when proliferation is no longer needed.

**[0007]** Further investigations into the ligands of the CD28 receptor have led to the identification and characterization of a set of related B7 molecules (the "B7 Superfamily") (Coyle, A.J. *et al.* (2001) "*The Expanding B7 Superfamily: Increasing Complexity In Costimulatory Signals Regulating T Cell Function*," Nature Immunol. 2(3):203-209; Sharpe, A.H. *et al.* (2002) "*The B7-CD28 Superfamily*," Nature Rev. Immunol. 2:116-126; Greenwald, R.J. *et al.* (2005) "*The B7 Family Revisited*," Ann. Rev. Immunol. 23:515-548; Collins, M. *et al.* (2005) "*The B7 Family Of Immune-Regulatory Ligands*," Genome Biol. 6:223.1-223.7; Loke, P. *et al.* (2004) "*Emerging Mechanisms Of Immune Regulation: The Extended B7 Family And Regulatory T Cells*." Arthritis Res. Ther. 6:208-214; Korman, A.J. *et al.* (2007) "*Checkpoint Blockade in Cancer Immunotherapy*," Adv. Immunol. 90:297-339; Flies, D.B. *et al.* (2007) "*The New B7s: Playing a Pivotal Role in Tumor Immunity*," J. Immunother. 30(3):251-260; Agarwal, A. *et al.* (2008) "*The Role Of Positive Costimulatory Molecules In Transplantation And Tolerance*," Curr. Opin. Organ Transplant. 13:366-372; Lenschow, D.J. *et al.* (1996) "*CD28/B7 System of T Cell Costimulation*," Ann. Rev. Immunol. 14:233-258; Wang, S. *et al.* (2004) "*Co-Signaling Molecules Of The B7-CD28 Family In Positive And Negative Regulation Of T Lymphocyte Responses*," Microbes Infect. 6:759-766). There are currently several known members of the family: B7.1 (CD80), B7.2 (CD86), the inducible co-stimulator ligand (ICOS-L), the programmed death-1 ligand (PD-L1; B7-H1), the programmed death-2 ligand (PD-L2; B7-DC), B7-H3, B7-H4 and B7-H6 (Collins, M. *et al.* (2005) "*The B7 Family Of Immune-Regulatory Ligands*," Genome Biol. 6:223.1-223.7; Flajnik, M.F. *et al.* (2012) "*Evolution Of The B7 Family: Co-Evolution Of B7H6 And Nkp30, Identification Of A New B7 Family Member, B7H7, And Of B7's Historical Relationship With The MHC*," Immunogenetics epub doi.org/10.1007/s00251-012-0616-2).

### B. B7-H1 / PD1 Interactions

#### 1. B7-H1

**[0008]** B7-H1 (PD-L1, CD274) is a particularly significant member of the B7 Superfamily as it is pivotally involved in shaping the immune response to tumors (Flies, D.B. *et al.* (2007) "*The New B7s: Playing a Pivotal Role in Tumor Immunity*," J. Immunother. 30(3):251-260; United States Patents Nos. 6,803,192; 7,794,710; United States Patent Application Publication Nos. 2005/0059051; 2009/0055944; 2009/0274666; 2009/0313687; PCT Publication No. WO 01/39722; WO 02/086083). B7-H1 is an approximately 33 kDa type 1 transmembrane protein. It has been speculated to play a major role in suppressing the immune system during particular events such as pregnancy, tissue allografts, autoimmune disease and other disease states such as hepatitis.

**[0009]** B7-H1 is broadly expressed in different human and mouse tissues, such as heart, placenta, muscle, fetal liver, spleen, lymph nodes, and thymus for both species as well as liver, lung, and kidney in mouse only (Martin-Orozco, N. *et al.* (2007) "*Inhibitory Costimulation And Anti-Tumor Immunity*," Semin. Cancer Biol. 17(4):288-298). In humans, B7-H1 protein expression has been found in human endothelial cells (Chen, Y. *et al.* (2005) "*Expression of B7-H1 in Inflammatory Renal Tubular Epithelial Cells*," Nephron. Exp. Nephrol. 102:e81-e92; de Haij, S. *et al.* (2005) "*Renal Tubular Epithelial Cells Modulate T-Cell Responses Via ICOS-L And B7-H1*" Kidney Int. 68:2091-2102; Mazanet, M.M. *et al.* (2002) "*B7-H1 Is Expressed By Human Endothelial Cells And Suppresses T Cell Cytokine Synthesis*," J. Immunol. 169:3581-3588), myocardium (Brown, J.A. *et al.* (2003) "*Blockade Of Programmed Death-1 Ligands On Dendritic Cells Enhances T Cell Activation And Cytokine Production*," J. Immunol. 170:1257-1266), syncyciotrophoblasts (Petroff, M.G. *et al.* (2002) "*B7 Family Molecules: Novel Immunomodulators At The Maternal-Fetal Interface*," Placenta 23:S95-S101), resident macrophages of some tissues, or in macrophages that have been activated with interferon (IFN)-γ or tumor necrosis factor (TNF)-α (Latchman, Y. *et al.* (2001) "*PD-L2 Is A Second Ligand For PD-1 And Inhibits T Cell Activation*," Nat. Immunol 2:261-268), and in tumors (Dong, H. (2003) "*B7-H1 Pathway And Its Role In The Evasion Of Tumor Immunity*," J. Mol. Med. 81:281-287). In the mouse, B7-H1 protein expression is found in heart endothelium, islets cells of the pancreas, small intestines, and placenta (Martin-Orozco, N. *et al.* (2007) "*Inhibitory Costimulation And Anti-Tumor Immunity*," Semin. Cancer Biol. 17(4):288-298).

## 2. PD-1

**[0010]** Programmed Death -1 ("PD-1") is a receptor of B7-H1 and B7-DC. PD-1 is an approximately 31 kD type I membrane protein member of the extended CD28/CTLA4 family of T cell regulators (Ishida, Y. *et al.* (1992) "*Induced Expression Of PD-1, A Novel Member Of The Immunoglobulin Gene Superfamily, Upon Programmed Cell Death*," EMBO J. 11:3887-3895; United States Patent Application Publication No. 2007/0202100; 2008/0311117; 2009/00110667; United States Patents Nos. 6,808,710; 7,101,550; 7,488,802; 7,635,757; 7,722,868; PCT Publication No. WO 01/14557). Compared to CTLA4, PD-1 more broadly negatively regulates immune responses.

**[0011]** PD-1 is expressed on activated T cells, B cells, and monocytes (Agata, Y. *et al.* (1996) "*Expression Of The PD-1 Antigen On The Surface Of Stimulated Mouse T And B Lymphocytes*," Int. Immunol. 8(5):765-772; Yamazaki, T. *et al.* (2002) "*Expression Of Programmed Death 1 Ligands By Murine T Cells And APC*," J. Immunol. 169:5538-5545) and at low levels in natural killer (NK) T cells (Nishimura, H. *et al.* (2000) "*Facilitation Of Beta Selection And Modification Of Positive Selection In The Thymus Of PD-1-Deficient Mice*," J. Exp. Med. 191:891-898; Martin-Orozco, N. *et al.* (2007) "*Inhibitory Costimulation And Anti-Tumor Immunity*," Semin. Cancer Biol. 17(4):288-298).

**[0012]** The extracellular region of PD-1 consists of a single immunoglobulin (Ig)V domain with 23% identity to the equivalent domain in CTLA4 (Martin-Orozco, N. *et al.* (2007) "*Inhibitory Costimulation And Anti-Tumor Immunity*," Semin. Cancer Biol. 17(4):288-298). The extracellular IgV domain is followed by a transmembrane region and an intracellular tail. The intracellular tail contains two phosphorylation sites located in an immunoreceptor tyrosine-based inhibitory motif and an immunoreceptor tyrosine-based switch motif, which suggests that PD-1 negatively regulates TCR signals (Ishida, Y. *et al.* (1992) "*Induced Expression Of PD-1, A Novel Member Of The Immunoglobulin Gene Superfamily, Upon Programmed Cell Death*," EMBO J. 11:3887-3895; Blank, C. *et al.* (Epub 2006 Dec 29) "*Contribution Of The PD-L1/PD-1 Pathway To T-Cell Exhaustion: An Update On Implications For Chronic Infections And Tumor Evasion* Cancer," Immunol. Immunother. 56(5):739-745).

**[0013]** Antibodies capable of immunospecifically binding to murine PD-1 have been reported (see, *e.g.*, Agata, T. *et al.* (1996) "*Expression Of The PD-1 Antigen On The Surface Of Stimulated Mouse T And B Lymphocytes*," Int. Immunol. 8(5):765-772).

### C. The Interactions of B7-H1 and PD-1

**[0014]** Interaction of B7-H1 and PD-1 has been found to provide a crucial negative co-stimulatory signal to T and B cells (Martin-Orozco, N. *et al.* (2007) "*Inhibitory Costimulation And Anti-Tumor Immunity*," Semin. Cancer Biol. 17(4):288-298) and functions as a cell death inducer (Ishida, Y. *et al.* (1992) "*Induced Expression Of PD-1, A Novel Member Of The Immunoglobulin Gene Superfamily, Upon Programmed Cell Death*," EMBO J. 11:3887-3895; Subudhi, S.K. *et al.* (2005) "*The Balance Of Immune Responses: Costimulation Verse Coinhibition*," J. Molec. Med. 83:193-202).

**[0015]** Interaction between low concentrations of the PD-1 receptor and the B7-H1 ligand results in the transmission of an inhibitory signal that strongly inhibits the proliferation of antigen-specific $CD8^+$ T cells; at higher concentrations the interactions with PD-1 do not inhibit T-cell proliferation but markedly reduce the production of multiple cytokines (Sharpe, A.H. *et al.* (2002) "*The B7-CD28 Superfamily*," Nature Rev. Immunol. 2:116-126). T-cell proliferation and cytokine production by both resting and previously activated CD4 and CD8 T cells, and even naive T cells from umbilical-cord blood, have been found to be inhibited by soluble B7-H1-Fc fusion proteins (Freeman, G.J. *et al.* (2000) "*Engagement Of The PD-1 Immunoinhibitory Receptor By A Novel B7 Family Member Leads To Negative Regulation Of Lymphocyte Activation*," J. Exp. Med. 192:1-9; Latchman, Y. *et al.* (2001) "*PD-L2 Is A Second Ligand For PD-1 And Inhibits T Cell Activation*," Nature Immunol. 2:261-268; Carter, L. *et al.* (2002) "*PD-1:PD-L inhibitory pathway affects both CD4(+) and CD8(+) T cells and is overcome by IL-2*," Eur. J. Immunol. 32(3):634-643; Sharpe, A.H. *et al.* (2002) "*The B7-CD28 Superfamily*," Nature Rev. Immunol. 2:116-126).

**[0016]** B7-H1 - PD-1 interactions lead to cell cycle arrest in G0-G1 but do not increase cell death (Latchman, Y. *et al.* (2001) "*PD-L2 Is A Second Ligand For PD-1 And Inhibits T Cell Activation*," Nature Immunol. 2:261-268; Carter, L. *et al.* (2002) "*PD-1:PD-L inhibitory pathway affects both CD4(+) and CD8(+) T cells and is overcome by IL-2*," Eur. J. Immunol. 32(3):634-643). Thus, B7-H1 - PD-1 complexing has the ability to antagonize the B7 - CD28 signal when antigenic stimulation is weak or limiting, and plays a key role in downregulating T-cell responses.

**[0017]** The signal transduction mediated by B7-H1 and PD-1 is complex. Both molecules additionally bind to other proteins. B7-H1 is capable of binding to B7-1 (CD80) (Butte, M.J. *et al.* (2008) "*Interaction of PD-L1 and B7-1*," Molecular Immunol. 45:3567-3572); PD-1 is

capable of binding to B7-DC (PD-L2) (Lázár-Molnár, E. *et al.* (2008) "*Crystal Structure Of The Complex Between Programmed Death-1 (PD-1) And Its Ligand PD-L2*," Proc. Natl. Acad. Sci. (USA) 105(30):10483-10488). B7-1 interacts with CD28 to deliver a co-stimulatory signal for T-cell activation that is important in the early stages of immune response (Elloso, M.M. *et al.* (1999) "Expression and Contribution of B7-1 (CD80) and B7-2 (CD86) in the Early Immune Response to Leishmania major Infection," J. Immunol. 162:6708-6715). B7-DC is a strong stimulator of T cells, enhancing T cell proliferation and IFN-γ production. However, it also exhibits an inhibitory effect on the immune response via its interaction with PD-1 (Ishiwata, K. *et al.* (epub January 10, 2010) "*Costimulator Responses Induced by Nippostrongylus brasiliensis*," J. Immunol. 184:2086-2094). Microbes and tumors appear to have exploited PD-1 and B7-H1 to evade eradication by the immune system. Differences in binding affinities to the various receptors and ligands that interact with PD-1 and B7-H1 have been proposed to provide distinct functional outcomes of blockade of PD-1 and B7-H1 in disease models (Butte, M.J. *et al.* (2008) "*Interaction of PD-L1 and B7*-1," Molecular Immunol. 45:3567-3572). The PD-1 pathway has also been implicated as playing a key role in the impairment of immune function during chronic infection ("T cell exhaustion"), and a blockade of PD-1 function is able to restore many T cell functions (Rodríquez-García, M. *et al.* (November 19, 2010) "*Expression Of PD-L1 And PD-L2 On Human Macrophages Is Up-Regulated By HIV-1 And Differentially Modulated By IL-10*," J. Leukocyte Biol. 89: doi:10.1189/jlb.0610327:1-9).

**[0018]** The role of B7-H1 and PD-1 in inhibiting T cell activation and proliferation has suggested that these biomolecules might serve as therapeutic targets for treatments of inflammation and cancer. The use of anti-PD1 antibodies to treat infections and tumors and up-modulate an adaptive immune response has been proposed (see, United States Patent Application Publication Nos. 2010/0040614; 2010/0028330; 2004/0241745; 2008/0311117; 2009/0217401; United States Patents Nos. 7,521,051; 7,563,869; 7,595,048; PCT Publications Nos. WO 2004/056875; WO 2008/083174). Conversely, agents that modulate the interaction of PD-1 with B7-H1 have been suggested to have utility in up- or down-modulating the immune response (see, United States Patents Nos. 7,029,674; 7,488,802; United States Patent Application Publications Nos. 2007/0122378; 2009/0076250; 2009/0110667; 2009/0263865; 2009/0297518; PCT Publication No. WO 2006/133396). Likewise, the use of anti-B7-H1 antibodies to treat infections and tumors and up-modulate an adaptive immune response has been proposed (United States Patent Application Publication

Nos. 2009/0055944; 2009/0274666; 2009/0317368; United States Patents Nos. 6,803,192; 7,794,710; PCT Publications Nos. WO 01/39722; WO 02/086083).

**[0019]** Nevertheless, despite all such advances a need remains for compositions capable of modulating the interaction between B7-H1 and PD-1. The present invention is directed to such compositions and their use to treat cancer and other diseases and conditions.

**Summary of the Invention:**

**[0020]** The present invention relates to antibodies and their antigen-binding fragments and to other molecules that are capable of immunospecifically binding to B7-H1 or PD-1. In some embodiments such molecules are additionally capable of modulating the ability of B7-H1 to bind to PD-1 or are capable of affecting the signaling activity of the B7-H1 or PD-1. The invention additionally concerns the uses of such molecules in the diagnosis and treatment of cancer and other diseases.

**[0021]** In detail, the invention provides a molecule, comprising an antigen-binding fragment of an antibody that immunospecifically binds to B7-H1 or PD-1, and in particular human B7-H1 or human PD-1, preferably expressed on the surface of a live cell at an endogenous or transfected concentration. The invention particularly concerns the embodiment of such a molecule wherein the antigen-binding fragment binds to B7-H1, and wherein the live cell is a tumor cell, a pathogen-infected cell or an Antigen Presenting Cell as well as the embodiment of such a molecule wherein the antigen-binding fragment binds to PD-1, and wherein the live cell is a T cell.

**[0022]** The present invention relates to antibodies and their antigen-binding fragments and to other molecules that are capable of immunospecifically binding to B7-H1 or PD-1. In some embodiments such molecules are additionally capable of modulating the ability of B7-H1 to bind to PD-1 or are capable of affecting the signaling activity of the B7-H1 or PD-1. The invention additionally concerns the uses of such molecules in the diagnosis and treatment of cancer and other diseases.

**[0023]** In detail, the invention provides a molecule, comprising an antigen-binding fragment of an antibody that immunospecifically binds to B7-H1 or PD-1, and in particular human B7-H1 or human PD-1, preferably expressed on the surface of a live cell at an endogenous or transfected concentration. The invention particularly concerns the embodiment of such a

molecule wherein the antigen-binding fragment binds to B7-H1, and wherein the live cell is a tumor cell, a pathogen-infected cell or an Antigen Presenting Cell as well as the embodiment of such a molecule wherein the antigen-binding fragment binds to PD-1, and wherein the live cell is a T cell.

**[0024]** The invention further concerns the embodiment of such molecules wherein the molecule is a monoclonal antibody, a human antibody, a chimeric antibody or a humanized antibody. The invention includes the embodiments wherein such antibodies are monospecific, bispecific, trispecific or multispecific.

**[0025]** The invention further concerns the embodiment of such molecules or antibodies which binds to B7-H1, and wherein the antigen-binding fragment thereof comprises six CDRs, wherein the CDRs comprise at least one consensus CDR of the CDRs of anti-B7-H1 antibodies 1E12, 1F4, 2G11, 3B6, and 3D10 with all remaining CDRs selected from:

(A) the three light chain and the three heavy chain CDRs of anti-B7-H1 antibody 1E12;
(B) the three light chain and the three heavy chain CDRs of anti-B7-H1 antibody 1F4;
(C) the three light chain and the three heavy chain CDRs of anti-B7-H1 antibody 2G11;
(D) the three light chain and the three heavy chain CDRs of anti-B7-H1 antibody 3B6; or
(E) the three light chain and the three heavy chain CDRs of anti-B7-H1 antibody 3D10.

**[0026]** The invention further concerns the embodiment of such molecules or antibodies which binds to B7-H1, and wherein the antigen-binding fragment thereof comprises six CDRs, wherein the six CDRs are:

(A) the three light chain and the three heavy chain CDRs of anti-B7-H1 antibody 1E12;
(B) the three light chain and the three heavy chain CDRs of anti-B7-H1 antibody 1F4;
(C) the three light chain and the three heavy chain CDRs of anti-B7-H1 antibody 2G11;
(D) the three light chain and the three heavy chain CDRs of anti-B7-H1 antibody 3B6; or
(E) the three light chain and the three heavy chain CDRs of anti-B7-H1 antibody 3D10.

**[0027]** The invention further concerns the embodiment of such antibodies, wherein the antibody binds to B7-H1 and comprises a variable domain of antibody h3D10 Var 1, h3D10 Var 2, h3D10 Var 3, h3D10 Var 4, h3D10 Var 5, h3D10 Var 6, h3D10 Var 7, h3D10 Var 8, h3D10 Var 9, h3D10 Var 10, h3D10 Var 11, h3D10 Var 12, h3D10 Var 13, or h3D10 Var 14.

**[0028]** The invention further concerns the embodiment of the above-described molecules or antibodies, wherein the molecules or antibodies bind to PD-1, and wherein the antigen-binding fragment comprises six CDRs, wherein the CDRs comprise at least one consensus CDR of the CDRs of anti-PD-1 antibodies 1E3, 1E8 and 1H3 with all remaining CDRs selected from:

(A) the three light chain and the three heavy chain CDRs of anti-PD-1 antibody 1E3;

(B) the three light chain and the three heavy chain CDRs of anti-PD-1 antibody 1E8; or

(C) the three light chain and the three heavy chain CDRs of anti-PD-1 antibody 1H3.

**[0029]** The invention further concerns the embodiment of such antibodies, wherein the six CDRs are:

(A) the three light chain and the three heavy chain CDRs of anti-PD-1 antibody 1E3;

(B) the three light chain and the three heavy chain CDRs of anti-PD-1 antibody 1E8; or

(C) the three light chain and the three heavy chain CDRs of anti-PD-1 antibody 1H3.

**[0030]** The invention further concerns the embodiment of such antibodies, wherein the antibody binds to PD-1 and comprises a variable domain of antibody h1H3 Var 1, h1H3 Var 2, h1H3 Var 3, h1H3 Var 4, h1H3 Var 5, h1H3 Var 6, h1H3 Var 7, h1H3 Var 8, h1H3 Var 9, h1H3 Var 10, h1H3 Var 11, h1H3 Var 12, h1H3 Var 13, or h1H3 Var 14.

**[0031]** The invention further concerns the embodiment of the above-described molecules or antibodies, wherein the molecule or antibody is detectably labeled or comprises a conjugated toxin, drug, receptor, enzyme, receptor ligand.

**[0032]** The invention further concerns the embodiment of the above-described molecules or antibodies, wherein the molecule or antibody:

(A) modulates signal transduction mediated by B7-H1 or PD-1;

(B) attenuates the ability of B7-H1 to bind to a B7-H1 receptor or attenuates the ability of PD-1 to bind to a PD-1 ligand;

(C) agonizes B7-H1 or PD-1 mediated signal transduction;

(D) mediates T cell proliferation; or

(E) enhances the production of IFN-γ.

**[0033]** The invention further concerns a pharmaceutical composition comprising a therapeutically effective amount of any of the above-described molecules or antibodies, and a physiologically acceptable carrier or excipient. The invention further concerns the use of

such pharmaceutical composition in the treatment of cancer, autoimmune disease, infectious disease, or a disease affecting T cell number or health. The invention further concerns the use of such pharmaceutical composition, wherein the treatment is prophylactic, and is provided in advance of any symptom of the cancer, the autoimmune disease, the infectious disease, or the disease affecting T cell number or health, or for the treatment of conditions incident to transplantation.

**[0034]** The invention further concerns the use of any of the above-described molecules or antibodies for diagnosing cancer, autoimmune disease (especially graft vs. host disease), infectious disease (especially a chronic viral disease), or a disease affecting T cell number or health in a subject by assaying cells of the subject for their ability to bind to B7-H1 or PD-1.

**[0035]** The invention particularly concerns the embodiment of such molecules, antibodies and compositions, wherein the B7-H1 is human B7-H1 and the PD-1 is human PD-1.

**[0036]** The invention particularly concerns a method for diagnosing a disease (especially cancer) in a subject comprising assaying cells of the subject for their ability to bind to any of the above-described B7-H1 binding molecules, wherein the method provides a cytologic assay for diagnosing the presence of the disease in the subject.

**[0037]** The invention additionally concerns a method for diagnosing a disease (especially a disease affecting T cell number and/or health) in a subject comprising assaying cells of the subject for their ability to bind to a PD-1 binding molecule, wherein the method provides a cytologic assay for diagnosing the presence and/or progression of the disease in the subject, or for assessing a subject's response to treatment.

**Brief Description of the Drawings:**

**[0038]** **Figure 1** shows the binding of tested hybridoma supernatants for antibody that bind to B7-H1. Positive control (PC): 1:1000 diluted sera from the mouse used for hybridoma generation; Negative control (NC): 5% milk / PBS. Data is shown for binding to B7-H1-Fc and detection with anti-mouse IgG.

**[0039]** **Figure 2** shows the results of experiments for determining whether the isolated anti-B7-H1 antibodies are capable of modulating the binding of B7-H1 to PD-1. Positive control: clones MIH-1 and 29E.2A3 (both anti-human CD274 (B7-H1); Two negative controls: conditioned media from an unrelated hybridoma (random Ab) and vector control (VC).

**[0040]** **Figure 3** shows the median fluorescence intensity (MFI) of tested anti-B7-H1 antibodies for binding to CHO-hB7-H1. None of the tested clones were found to cross-react with the parental CHO line, indicating that the expressed antibody was immunospecific for human B7-H1.

**[0041]** **Figure 4** shows the median fluorescence intensity (MFI) result of a human B7-H1-expressing CHO cell binding assay of selected anti-human B7-H1 antibodies.

**[0042]** **Figure 5** compares the median fluorescence intensity (MFI) result of a human B7-H1-expressing CHO cell binding assay of anti-human B7-H1 antibodies 5H1 and 1E12.

**[0043]** **Figure 6** shows the antigen binding and isotype of the isolated anti-human PD-1 antibodies.

**[0044]** **Figures 7A-7B** show the results of experiments indicating that several of the isolated hybridomas expressed neutralizing anti-human PD-1 antibodies.

**[0045]** **Figure 8** shows the median fluorescence intensity (MFI) of tested anti-PD-1 antibodies for binding to CHO-hPD-1. None of the tested clones were found to cross-react with the parental CHO line, indicating that the expressed antibody was specific for human PD-1.

**[0046]** **Figure 9** shows the median fluorescence intensity (MFI) of a human PD-1-expressing CHO cell binding assay of selected anti-human PD-1 antibodies. Positive control: EH12 (a commercially available anti-human PD-1 antibody from BioLegend); mIgG1: murine IgG negative control.

**[0047]** **Figure 10** shows the median fluorescence intensity (MFI) result of a cell-based competition assay at varying concentrations of anti-human PD-1 antibodies.

**[0048]** **Figure 11** shows the median fluorescence intensity (MFI) result of a cell-based competition assay at a concentration of 20 μg/ml anti-human PD-1 antibody.

**[0049]** **Figure 12** shows the results of an experiment in which CHO cells transfected with human full length PD-1 were pre-incubated with a saturating dose of anti-human PD-1 monoclonal antibodies (mAbs) or control Ig before being stained by biotin-labeled hB7-H1-FC or hB7-DC mIg.

**[0050]** **Figure 13, Panels A-B** show the comparative binding of: (A) the commercially available anti-PD-1 antibody, EH12 with (B) a murine monoclonal antibody which possesses chimeric ("ch") murine anti-human PD-1 Fab regions and a human IgG1 Fc region.

**[0051]** **Figure 14, Panels A-B** show the ability of the anti-PD-1 antibodies of the present invention to exert a blocking effect on biotinylated B7-H1-Fc and biotinylated B7-DC-Fc binding to PD-1.

**[0052]** **Figure 15** shows that a binding curve of 1H3 anti-human PD-1chimeric antibody to CHO.hPD-1 cells, as detected by anti-hIg antibody.

**[0053]** **Figures 16A-16B** shows the results of studies of the ability of the 1H3 anti-human PD-1 chimeric antibody to bind to human primary T cells CD8+ (**Figure 16A**) and CD4+ (**Figure 16B**) relative to the negative control antibody (palivizumab; SYNAGIS®, Medimmune, Inc.).

**[0054]** **Figure 17** shows the ability of the antibodies of the present invention to enhance antigen specific T cell response as measured by CFSE dilution upon tetanus toxin (TT) recall.

**[0055]** **Figures 18A-18D** demonstrate the ability of the humanized 1H3 Variants (h1H3 Var 1 – h1H3 Var 14 to bind to CHO.hPD1 cells .

**[0056]** **Figures 19A-19B** demonstrate the ability of humanized anti-PD-1 antibodies to block interactions between hPD-1-Fc and HEK293 cells expressing B7-H1 (**Figure 19A**) or B7-DC (**Figure 19B**).

**[0057]** **Figure 20** shows binding curves for h1H3 Var 1 - h1H3 Var 6.

**Detailed Description of the Invention:**

**[0058]** The present invention relates to antibodies and their antigen-binding fragments and to other molecules that are capable of immunospecifically binding to B7-H1 or PD-1. In some embodiments such molecules are additionally capable of modulating the ability of B7-H1 or B7-DC to bind to PD-1 or are capable of affecting the signaling activity of B7-H1 or PD-1. The invention additionally concerns the uses of such molecules in the treatment of cancer and other diseases.

**[0059]** A molecule is said to be able to "**immunospecifically bind**" a second molecule if such binding exhibits the specificity and affinity of an antibody to its cognate antigen. Antibodies are said to be capable of "**immunospecifically binding**" to a target region or conformation ("epitope") of an antigen (and in particular, the antigens: B7-H1 or PD-1) if such binding involves the antigen recognition site of the immunoglobulin molecule. An antibody that immunospecifically binds to a particular antigen may bind to other antigens with lower affinity if the other antigen has some sequence or conformational similarity that is recognized by the antigen recognition site as determined by, *e.g.*, immunoassays, BIACORE® assays, or other assays known in the art, but would not bind to a totally unrelated antigen. Preferably, however, antibodies (and their antigen binding fragments) will not cross-react with other antigens. Antibodies may also bind to other molecules in a way that is not immunospecific, such as to FcR receptors, by virtue of binding domains in other regions/domains of the molecule that do not involve the antigen recognition site, such as the Fc region.

**[0060]** As used herein the term "**modulate**" relates to a capacity to alter an effect or result. In particular, the invention relates to molecules (especially antibodies or their antigen-binding fragments that immunospecifically bind human B7-H1 or human PD-1) that are capable of modulating the binding between B7-H1 and PD-1 and/or of modulating the signal transduction that occurs as a consequence of B7-H1-PD-1 binding. Such modulation may result in attenuating or in completely blocking the ability of B7-H1 to bind to PD-1. In a further embodiment, such modulation may attenuate or completely neutralize the ability of B7-H1 or PD-1 to mediate signal transduction. In a further embodiment, such modulation may enhance or otherwise agonize signal transduction through B7-H1 or PD-1, either by 1) enhancing the interaction between B7-H1 and PD-1 and facilitating B7-H1 – PD-1 binding or ii) directly binding to B7-H1 and PD-1 and thus mimicking the activity of the endogenous ligand, *etc*. In a still further embodiment, such modulation may alter the nature of the interaction between B7-H1 and PD-1 so as to alter the nature of the elicited signal transduction. For example, the molecules of the present invention can, by binding to B7-H1 or PD-1, alter the ability of such molecules to bind to other ligands and receptor (*e.g.*, affecting the ability of PD-1 to bind to B7-DC or the ability of B7-H1 to bind to B7-1 (CD80)) and thereby alter their overall activity. Preferably, such modulation will provide at least a 10% change in a measurable immune system activity, more preferably, at least a 50%

change in such activity, or at least a 2-fold, 5-fold, 10-fold, or still more preferably, at least a 100-fold change in such activity.

**[0061]** As used herein, the term "**antibody**" is intended to denote an immunoglobulin molecule that possesses a "variable region" antigen recognition site. The term "variable region" is intended to distinguish such domain of the immunoglobulin from domains that are broadly shared by antibodies (such as an antibody Fc domain). The variable region comprises a "hypervariable region" whose residues are responsible for antigen binding. The hypervariable region comprises amino acid residues from a "Complementarity Determining Region" or "**CDR**" (*i.e.*, typically at approximately residues 24-34 (L1), 50-56 (L2) and 89-97 (L3) in the light chain variable domain and at approximately residues 27-35 (H1), 50-65 (H2) and 95-102 (H3) in the heavy chain variable domain; Kabat *et al., Sequences of Proteins of Immunological Interest,* 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)) and/or those residues from a "hypervariable loop" (*i.e.*, residues 26-32 (L1), 50-52 (L2) and 91-96 (L3) in the light chain variable domain and 26-32 (H1), 53-55 (H2) and 96-101 (H3) in the heavy chain variable domain; Chothia, C. *et al.* (1987) "Canonical Structures For The Hypervariable Regions Of Immunoglobulins," *J. Mol. Biol.* 196:901-917). "Framework Region" or "FR" residues are those variable domain residues other than the hypervariable region residues as herein defined. The term antibody includes monoclonal antibodies, multi-specific antibodies, human antibodies, humanized antibodies, synthetic antibodies, chimeric antibodies, camelized antibodies (*See e.g.*, Muyldermans *et al.*, 2001, *Trends Biochem. Sci.* 26:230; Nuttall *et al.*, 2000, *Cur. Pharm. Biotech.* 1:253; Reichmann and Muyldermans, 1999, *J. Immunol. Meth.* 231:25; International Publication Nos. WO 94/04678 and WO 94/25591; U.S. Patent No. 6,005,079), single-chain Fvs (scFv) (see, *e.g.*, *see* Pluckthun in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenburg and Moore eds. Springer-Verlag, New York, pp. 269-315 (1994)), single chain antibodies, disulfide-linked Fvs (sdFv), intrabodies, and anti-idiotypic (anti-Id) antibodies (including, *e.g.*, anti-Id and anti-anti-Id antibodies to antibodies of the invention). In particular, such antibodies include immunoglobulin molecules of any type (*e.g.*, IgG, IgE, IgM, IgD, IgA and IgY), class (*e.g.*, $IgG_1$, $IgG_2$, $IgG_3$, $IgG_4$, $IgA_1$ and $IgA_2$) or subclass.

**[0062]** As used herein, the term "**antigen binding fragment**" of an antibody refers to one or more portions of an antibody that contain the antibody's Complementarity Determining Regions ("CDRs") and optionally the framework residues that comprise the antibody's

"variable region" antigen recognition site, and exhibit an ability to immunospecifically bind antigen. Such fragments include Fab', $F(ab')_2$, Fv, single chain (ScFv),and mutants thereof, naturally occurring variants, and fusion proteins comprising the antibody's "variable region" antigen recognition site and a heterologous protein (*e.g.*, a toxin, an antigen recognition site for a different antigen, an enzyme, a receptor or receptor ligand, *etc.*). As used herein, the term "**fragment**" refers to a peptide or polypeptide comprising an amino acid sequence of at least 5 contiguous amino acid residues, at least 10 contiguous amino acid residues, at least 15 contiguous amino acid residues, at least 20 contiguous amino acid residues, at least 25 contiguous amino acid residues, at least 40 contiguous amino acid residues, at least 50 contiguous amino acid residues, at least 60 contiguous amino residues, at least 70 contiguous amino acid residues, at least 80 contiguous amino acid residues, at least 90 contiguous amino acid residues, at least 100 contiguous amino acid residues, at least 125 contiguous amino acid residues, at least 150 contiguous amino acid residues, at least 175 contiguous amino acid residues, at least 200 contiguous amino acid residues, or at least 250 contiguous amino acid residues.

**[0063]** Human, chimeric or humanized antibodies are particularly preferred for *in vivo* use in humans, however, murine antibodies or antibodies of other species may be advantageously employed for many uses (for example, *in vitro* or *in situ* detection assays, acute *in vivo* use, *etc.*). Completely human antibodies are particularly desirable for therapeutic treatment of human subjects.

**[0064]** Human antibodies can be made by a variety of methods known in the art including phage display methods described above using antibody libraries derived from human immunoglobulin sequences (*see* U.S. Patent Nos. 4,444,887 and 4,716,111; and International Publication Nos. WO 98/46645, WO 98/50433, WO 98/24893, WO 98/16654, WO 96/34096, WO 96/33735, and WO 91/10741). Human antibodies can be produced using transgenic mice which are incapable of expressing functional endogenous immunoglobulins, but which can express human immunoglobulin genes. For example, the human heavy and light chain immunoglobulin gene complexes may be introduced randomly or by homologous recombination into mouse embryonic stem cells. Alternatively, the human variable region, constant region, and diversity region may be introduced into mouse embryonic stem cells in addition to the human heavy and light chain genes. The mouse heavy and light chain immunoglobulin genes may be rendered non-functional separately or simultaneously with the

introduction of human immunoglobulin loci by homologous recombination. In particular, homozygous deletion of the $J_H$ region prevents endogenous antibody production. The modified embryonic stem cells are expanded and microinjected into blastocysts to produce chimeric mice. The chimeric mice are then bred to produce homozygous offspring which express human antibodies. The transgenic mice are immunized using conventional methodologies with a selected antigen, *e.g.*, all or a portion of a polypeptide of the invention. Monoclonal antibodies directed against the antigen can be obtained from the immunized, transgenic mice using conventional hybridoma technology (see, *e.g.*, U.S. Patent No. 5,916,771). The human immunoglobulin transgenes harbored by the transgenic mice rearrange during B cell differentiation, and subsequently undergo class switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA, IgM and IgE antibodies. For an overview of this technology for producing human antibodies, *see* Lonberg and Huszar (1995, *Int. Rev. Immunol.* 13:65-93, which is incorporated herein by reference in its entirety). For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, *see, e.g.*, International Publication Nos. WO 98/24893, WO 96/34096, and WO 96/33735; and U.S. Patent Nos. 5,413,923, 5,625,126, 5,633,425, 5,569,825, 5,661,016, 5,545,806, 5,814,318, and 5,939,598, which are incorporated by reference herein in their entirety. In addition, companies such as Abgenix, Inc. (Freemont, CA) and Medarex (Princeton, NJ) can be engaged to provide human antibodies directed against a selected antigen using technology similar to that described above.

**[0065]** A "**chimeric antibody**" is a molecule in which different portions of the antibody are derived from different immunoglobulin molecules such as antibodies having a variable region derived from a non-human antibody and a human immunoglobulin constant region. Methods for producing chimeric antibodies are known in the art. *See e.g.*, Morrison, 1985, *Science* 229:1202; Oi *et al.*, 1986, *BioTechniques* 4:214; Gillies *et al.*, 1989, *J. Immunol. Methods* 125:191-202; and U.S. Patent Nos. 6,311,415, 5,807,715, 4,816,567, and 4,816,397. Chimeric antibodies comprising one or more CDRs from a non-human species and framework regions from a human immunoglobulin molecule can be produced using a variety of techniques known in the art including, for example, CDR-grafting (EP 239,400; International Publication No. WO 91/09967; and U.S. Patent Nos. 5,225,539, 5,530,101, and 5,585,089), veneering or resurfacing (EP 592,106; EP 519,596; Padlan, 1991, *Molecular Immunology* 28(4/5):489-498; Studnicka *et al.*, 1994, *Protein Engineering* 7:805; and

Roguska *et al.*, 1994, *Proc. Natl. Acad. Sci. USA* 91:969), and chain shuffling (U.S. Patent No. 5,565,332).

**[0066]** The invention particularly concerns "**humanized antibodies**" (see, *e.g.*, European Patent Nos. EP 239,400, EP 592,106, and EP 519,596; International Publication Nos. WO 91/09967 and WO 93/17105; U.S. Patent Nos. 5,225,539, 5,530,101, 5,565,332, 5,585,089, 5,766,886, and 6,407,213; and Padlan, 1991, *Molecular Immunology* 28(4/5):489-498; Studnicka *et al.*, 1994, *Protein Engineering* 7(6):805-814; Roguska *et al.*, 1994, *PNAS* 91:969-973; Tan *et al.*, 2002, *J. Immunol.* 169:1119-25; Caldas *et al.*, 2000, *Protein Eng.* 13:353-60; Morea *et al.*, 2000, *Methods* 20:267-79; Baca *et al.*, 1997, *J. Biol. Chem.* 272:10678-84; Roguska *et al.*, 1996, *Protein Eng.* 9:895-904; Couto *et al.*, 1995, *Cancer Res.* 55 (23 Supp):5973s-5977s; Couto *et al.*, 1995, *Cancer Res.* 55:1717-22; Sandhu, 1994, *Gene* 150:409-10; Pedersen *et al.*, 1994, *J. Mol. Biol.* 235:959-73; Jones *et al.*, 1986, *Nature* 321:522-525; Reichmann *et al.,* 1988, *Nature* 332:323-329; and Presta, 1992, *Curr. Op. Struct. Biol.* 2:593-596). As used herein, the term "humanized antibody" refers to an immunoglobulin comprising a human framework region and one or more CDR's from a non-human (usually a mouse or rat) immunoglobulin. The non-human immunoglobulin providing the CDR's is called the "donor" and the human immunoglobulin providing the framework is called the "acceptor." Constant regions need not be present, but if they are, they must be substantially identical to human immunoglobulin constant regions, *i.e.*, at least about 85-90%, preferably about 95% or more identical. Hence, all parts of a humanized immunoglobulin, except possibly the CDR's, are substantially identical to corresponding parts of natural human immunoglobulin sequences. A humanized antibody is an antibody comprising a humanized light chain and a humanized heavy chain immunoglobulin. For example, a humanized antibody would not encompass a typical chimeric antibody, because, *e.g.*, the entire variable region of a chimeric antibody is non-human. One says that the donor antibody has been "humanized," by the process of "humanization," because the resultant humanized antibody is expected to bind to the same antigen as the donor antibody that provides the CDR's. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which hypervariable region residues of the recipient are replaced by hypervariable region residues from a non-human species (donor antibody) such as mouse, rat, rabbit or a non-human primate having the desired specificity, affinity, and capacity. In some instances, Framework Region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise

residues which are not found in the recipient antibody or in the donor antibody. These modifications are made to further refine antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable regions correspond to those of a non-human immunoglobulin and all or substantially all of the FRs are those of a human immunoglobulin sequence. The humanized antibody optionally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin that immunospecifically binds to a FcγRIIB polypeptide, that has been altered by the introduction of amino acid residue substitutions, deletions or additions (*i.e.,* mutations).

**[0067]** The antibodies used in the methods of the present invention may be monospecific. Also of interest are bispecific antibodies, trispecific antibodies or antibodies of greater multispecificity that exhibit specificity to different targets in addition to B7-H1 or PD-1. In a preferred embodiment, such multispecific antibodies would exhibit specificity to different immune cell target(s). For example, such antibodies may bind to both B7-H1 and B7-DC and thus modulate both PD-1 dependent responses. Conversely, such antibodies may bind to PD-1 and B7-1 and interfere with both B7-H1 dependent responses. In another embodiment, the multispecific antibody binds to molecules (receptors or ligands) involved in alternative immunomodulatory pathways, such as CTLA4, TIM3, TIM4, OX40, CD40, GITR, 4-1-BB, B7-H4, LIGHT or LAG3, in order to enhance the immunomodulatory effects. Furthermore, the multispecific antibody may bind to effector molecules such as cytokines (*e.g.*, IL-7, IL-15, IL-12, IL-4 TGF-beta, IL-10, IL-17, IFNg, Flt3, BLys) and chemokines (*e.g.*, CCL21), which may be particularly relevant for modulating both acute and chronic immune responses.

**[0068]** Additionally, multispecific antibodies may bind to an antigen that is important for targeting the antibody to a particular cell type or tissue. For example such antibodies that bind both PD-1 and CD27 (or B7-H1 and CD27) can help co-localize activated memory B-cells ($mB_{Act}$) and antigen presenting cells (APCs) so that the PD-1 arrayed by such APCs can interact with ligands on the surface of the $mB_{Act}$ B-cells to promote the survival of the $mB_{Act}$ B-cells. Since a loss of $mB_{Act}$ B-cells is a precipitating event in the progression of HIV-infection to AIDS (Titanji, K. *et al.* (2010) "*Acute Depletion Of Activated Memory B Cells Involves The PD-1 Pathway In Rapidly Progressing SIV-Infected Macaques*," J. Clin. Invest. 120(11):3878-3890), antibodies that bind both PD-1 and CD27 have utility in the treatment of

HIV infection and in preventing or delaying the onset of AIDS. As discussed above, the PD-1 pathway has been implicated as playing a key role in the impairment of immune function during chronic HIV infection ("T cell exhaustion") (Khaitan, A. *et al.* (2011) "*Revisiting Immune Exhaustion During HIV Infection*," Curr. HIV/AIDS Rep. 8:4-11; Rodríquez-García, M. *et al.* (November 19, 2010) "*Expression Of PD-L1 And PD-L2 On Human Macrophages Is Up-Regulated By HIV-1 And Differentially Modulated By IL-10*," J. Leukocyte Biol. 89: doi:10.1189/jlb.0610327:1-9; Grabmeier-Pfistershammer, K. *et al.* (2011) "*Identification of PD-1 as a Unique Marker for Failing Immune Reconstitution in HIV-1–Infected Patients on Treatment*," J Acquir. Immune Defic. Syndr. 56(2):118-124). Macrophages have been shown to contribute significantly to the initial steps of HIV infection (Carter, C. A. *et al.* (2008) "*Cell Biology Of HIV-1 Infection Of Macrophages*," Ann. Rev. Microbiol. 62:425-443; Noursadeghi, M. *et al.* (2006) "*HIV-1 Infection Of Mononuclear Phagocytic Cells: The Case For Bacterial Innate Immune Deficiency In AIDS*," Lancet Infect. Dis. 6:794-804). Accordingly, antibodies (particularly if conjugated to a toxin) that bind to PD-1 and to a macrophage-specific marker (such as CD14, CD68, CD163, TLR2 *etc.*) have utility in preventing HIV infection. Additionally, antibodies that bind to multiple markers of T cell exhaustion (*e.g.*, PD-1 and any or all of: CTLA4, TIM3, TIM4 or LAG-3) have utility in the treatment or diagnosis of immune responsiveness. Other target antigens of interest include cancer cell markers.

**[0069]** Additionally, it has been found that PD-$1^+$ $CD8^+$ cells have anti-HIV activity (Killian, M.S. *et al.* (2011) "*Natural Suppression of Human Immunodeficiency Virus Type 1 Replication Is Mediated by Memory $CD8^+$ T Cells*," J. Virol. 85(4):1696-1705). Thus antibodies that bind to both PD-1 and CD8 have utility in, for example, an *ex vivo* means for isolating and producing an enriched population of such cells for ultimate use in the treatment of HIV infection and AIDS in patients.

**[0070]** Other markers that may be used in such anti-PD-1 or anti-B7-H1 bispecific, trispecific or multispecific antibodies include CD4, CD8, CD25 and CTLA-4 (see, De Keersmaecker, B. *et al.* (2011) ("*Fighting with the Enemy's Weapons? The Role of Costimulatory Molecules in HIV*," Curr. Molec. Med. 566-5240/11: 1-25; and Sarikonda, G. (2011) "*Immunosuppressive Mechanisms During Viral Infectious Diseases*;" Methods in Molec. Biol. 677:431-447, both herein incorporated by reference).

**[0071]** Similarly, although CD4 T cells are required to slow the growth and spread of *M. tuberculosis*, PD-1–mediated inhibition is also required to prevent $CD4^+$ T cells from promoting severe disease (Barber, D.L. *et al.* (2011) "*CD4 T Cells Promote Rather than Control Tuberculosis in the Absence of PD-1–Mediated Inhibition*," J. Immunol. 186:1598-1607; Sakai, S. *et al.* (2010) "*PD-1 – PD-L1 pathway impairs $T_h1$ immune response in the late stage of infection with Mycobacterium bovis bacillus Calmette–Guérin*," Intl. Immunol. 22(12):915-925; Lázár-Molnár, E. *et al.* (2010) "*Programmed Death-1 (PD-1)–Deficient Mice Are Extraordinarily Sensitive To Tuberculosis*," Proc. Natl. Acad. Sci. (USA) 107(30):13402-13407). Thus, antibodies that bind to CD4 and PD-1 have utility in the treatment of tuberculosis and in preventing or delaying the onset of tuberculosis.

**[0072]** DNA sequences coding for preferred human acceptor framework sequences include but are not limited to FR segments from the human germline VH segment VH1-18 and JH6 and the human germline VL segment VK-A26 and JK4. In a specific embodiment, one or more of the CDRs are inserted within framework regions using routine recombinant DNA techniques. The framework regions may be naturally occurring or consensus framework regions, and preferably human framework regions (*see, e.g.*, Chothia *et al.*, 1998, *J. Mol. Biol.* 278: 457-479 for a listing of human framework regions).

**[0073]** A humanized or chimeric antibody of the invention may comprise substantially all of at least one, and typically two, variable domains in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin (*i.e.*, donor antibody) and all or substantially all of the framework regions are those of a human immunoglobulin consensus sequence. Preferably, an antibody of the invention also comprises at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. The constant domains of the antibodies of the invention may be selected with respect to the proposed function of the antibody, in particular the effector function which may be required. In some embodiments, the constant domains of the antibodies of the invention are (or comprise) human IgA, IgD, IgE, IgG or IgM domains. In a specific embodiment, human IgG constant domains, especially of the IgG1 and IgG3 isotypes are used, when the humanized antibodies of the invention is intended for therapeutic uses and antibody effector functions such as antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) activity are needed. For example, PD-1 is highly expressed on T cells as well as rare peripheral T cell lymphomas such as

Angioimmunoblastic T-cell lymphoma (AITL). Anti-PD-1 antibodies with ADCC or CDC activity are particularly relevant as therapeutic agents for treating such cancers. In alternative embodiments, IgG2 and IgG4 isotypes are used when the antibody of the invention is intended for therapeutic purposes and antibody effector function is not required. For example, if you want to increase the activity of T cells by targeting PD-1 on the surface of T cells, then effector functions that would kill the T cell may be undesirable. The invention encompasses Fc constant domains comprising one or more amino acid modifications which alter antibody effector functions such as those disclosed in U.S. Patent Application Publication Nos. 2005/0037000 and 2005/0064514.

**[0074]** In some embodiments, the antibody of the invention contains both the light chain as well as at least the variable domain of a heavy chain. In other embodiments, the antibody of the invention may further comprise one or more of the CH1, hinge, CH2, CH3, and CH4 regions of the heavy chain. The antibody can be selected from any class of immunoglobulins, including IgM, IgG, IgD, IgA and IgE, and any isotype, including $IgG_1$, $IgG_2$, $IgG_3$ and $IgG_4$. In some embodiments, the constant domain is a complement fixing constant domain where it is desired that the antibody exhibit cytotoxic activity, and the class is typically $IgG_1$. In other embodiments, where such cytotoxic activity is not desirable, the constant domain may be of the $IgG_2$ class. The antibody of the invention may comprise sequences from more than one class or isotype, and selecting particular constant domains to optimize desired effector functions is within the ordinary skill in the art.

**[0075]** The framework and CDR regions of a humanized antibody need not correspond precisely to the parental sequences, *e.g.*, the donor CDR or the consensus framework may be mutagenized by substitution, insertion or deletion of at least one residue so that the CDR or framework residue at that site does not correspond to either the consensus or the donor antibody. Such mutations, however, are preferably not extensive. Usually, at least 75% of the humanized antibody residues will correspond to those of the parental framework region (FR) and CDR sequences, more often 90%, and most preferably greater than 95%. Humanized antibodies can be produced using variety of techniques known in the art, including, but not limited to, CDR-grafting (European Patent No. EP 239,400; International Publication No. WO 91/09967; and U.S. Patent Nos. 5,225,539, 5,530,101, and 5,585,089), veneering or resurfacing (European Patent Nos. EP 592,106 and EP 519,596; Padlan, 1991, *Molecular Immunology* 28(4/5):489-498; Studnicka *et al.*, 1994, *Protein Engineering*

7(6):805-814; and Roguska *et al.*, 1994, *Proc. Natl. Acad. Sci.* 91:969-973), chain shuffling (U.S. Patent No. 5,565,332), and techniques disclosed in, *e.g.*, U.S. Patent Nos. 6,407,213, 5,766,886, 5,585,089, International Publication No. WO 9317105, Tan *et al.*, 2002, *J. Immunol.* 169:1119-25, Caldas *et al.*, 2000, *Protein Eng.* 13:353-60, Morea *et al.*, 2000, *Methods* 20:267-79, Baca *et al.*, 1997, *J. Biol. Chem.* 272:10678-84, Roguska *et al.*, 1996, *Protein Eng.* 9:895-904, Couto *et al.*, 1995, *Cancer Res.* 55 (23 Supp):5973s-5977s, Couto *et al.*, 1995, *Cancer Res.* 55:1717-22, Sandhu, 1994, *Gene* 150:409-10, Pedersen *et al.*, 1994, J. *Mol. Biol.* 235:959-73, Jones *et al.*, 1986, *Nature* 321:522-525, Riechmann *et al.*, 1988, *Nature* 332:323, and Presta, 1992, *Curr. Op. Struct. Biol.* 2:593-596. Often, framework residues in the framework regions will be substituted with the corresponding residue from the CDR donor antibody to alter, preferably improve, antigen binding. These framework substitutions are identified by methods well known in the art, *e.g.*, by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions (*see, e.g.*, Queen *et al.*, U.S. Patent No. 5,585,089; U.S. Publication Nos. 2004/0049014 and 2003/0229208; U.S. Patent Nos. 6,350,861; 6,180,370; 5,693,762; 5,693,761; 5,585,089; and 5,530,101 and Riechmann *et al.*, 1988, *Nature* 332:323).

**[0076]** The antibodies of the present invention may be produced by any method known in the art useful for the production of polypeptides, *e.g.*, *in vitro* synthesis, recombinant DNA production, and the like. Preferably, the humanized antibodies are produced by recombinant DNA technology. The antibodies of the invention may be produced using recombinant immunoglobulin expression technology. The recombinant production of immunoglobulin molecules, including humanized antibodies are described in U.S. Patent No. 4,816,397 (Boss *et al.*), U.S. Patent Nos. 6,331,415 and 4,816,567 (both to Cabilly *et al.*), U.K. patent GB 2,188,638 (Winter *et al.*), and U.K. patent GB 2,209,757. Techniques for the recombinant expression of immunoglobulins, including humanized immunoglobulins, can also be found, in Goeddel *et al.*, Gene Expression Technology Methods in Enzymology Vol. 185 Academic Press (1991), and Borreback, Antibody Engineering, W. H. Freeman (1992). Additional information concerning the generation, design and expression of recombinant antibodies can be found in Mayforth, Designing Antibodies, Academic Press, San Diego (1993).

**[0077]** An exemplary process for the production of the recombinant chimeric antibodies of the invention may comprise the following: a) constructing, by conventional molecular

biology methods, an expression vector that encodes and expresses an antibody heavy chain in which the CDRs and variable region of the murine anti-B7-H1 (or anti-PD-1) monoclonal antibody are fused to an Fc region derived from a human immunoglobulin, thereby producing a vector for the expression of a chimeric antibody heavy chain; b) constructing, by conventional molecular biology methods, an expression vector that encodes and expresses an antibody light chain of the murine anti-B7-H1 (or anti-PD-1) monoclonal antibody, thereby producing a vector for the expression of chimeric antibody light chain; c) transferring the expression vectors to a host cell by conventional molecular biology methods to produce a transfected host cell for the expression of chimeric antibodies; and d) culturing the transfected cell by conventional cell culture techniques so as to produce chimeric antibodies.

**[0078]** An exemplary process for the production of the recombinant humanized antibodies of the invention may comprise the following: a) constructing, by conventional molecular biology methods, an expression vector that encodes and expresses an antibody heavy chain in which the CDRs and a minimal portion of the variable region framework that are required to retain donor antibody binding specificity are derived from a non-human immunoglobulin, such as the murine anti-B7-H1 (or anti-PD-1) monoclonal antibody, and the remainder of the antibody is derived from a human immunoglobulin, thereby producing a vector for the expression of a humanized antibody heavy chain; b) constructing, by conventional molecular biology methods, an expression vector that encodes and expresses an antibody light chain in which the CDRs and a minimal portion of the variable region framework that are required to retain donor antibody binding specificity are derived from a non-human immunoglobulin, such as the murine anti-B7-H1 (or anti-PD-1) monoclonal antibody, and the remainder of the antibody is derived from a human immunoglobulin, thereby producing a vector for the expression of humanized antibody light chain; c) transferring the expression vectors to a host cell by conventional molecular biology methods to produce a transfected host cell for the expression of humanized antibodies; and d) culturing the transfected cell by conventional cell culture techniques so as to produce humanized antibodies.

**[0079]** With respect to either exemplary method, host cells may be co-transfected with such expression vectors, which may contain different selectable markers but, with the exception of the heavy and light chain coding sequences, are preferably identical. This procedure provides for equal expression of heavy and light chain polypeptides. Alternatively, a single vector may be used which encodes both heavy and light chain polypeptides. The coding sequences

for the heavy and light chains may comprise cDNA or genomic DNA or both. The host cell used to express the recombinant antibody of the invention may be either a bacterial cell such as *Escherichia coli*, or more preferably a eukaryotic cell (*e.g.*, a Chinese hamster ovary (CHO) cell or a HEK-293 cell). The choice of expression vector is dependent upon the choice of host cell, and may be selected so as to have the desired expression and regulatory characteristics in the selected host cell. Other cell lines that may be used include, but are not limited to, CHO-K1, NSO, and PER.C6 (Crucell, Leiden, Netherlands). Furthermore, codon usage may by optimized when host cell is selected to account for species specific codon usage bias and enhance protein expression. For example, for CHO cell expression the DNA encoding the antibodies may incorporate codons used preferentially by Cricetulus griseus (from where Chinese Hamster ovaries cells are derived. Methods of codon optimization may be employed to facilitate improved expression by a desired host cell (see, *e.g.*, Wohlgemuth, I. *et al.* (2011) "*Evolutionary Optimization Of Speed And Accuracy Of Decoding On The Ribosome*," Philos. Trans. R. Soc. Lond. B Biol. Sci. 366(1580):2979-2986; Jestin, J.L. *et al.* (2009) "*Optimization Models And The Structure Of The Genetic Code*," J. Mol. Evol. 69(5):452-457; Bollenbach, T. *et al.* (2007) "*Evolution And Multilevel Optimization Of The Genetic Code*," Genome Res. 17(4):401-404; Kurland, C.G. *et al.* (1984) "*Optimization Of Translation Accuracy*," Prog. Nucleic Acid Res. Mol. Biol. 31:191-219; Grosjean, H. *et al.* (1982) "*Preferential Codon Usage In Prokaryotic Genes: The Optimal Codon-Anticodon Interaction Energy And The Selective Codon Usage In Efficiently Expressed Genes*," Gene 18(3):199-209).

**[0080]** Any of the above-described antibodies can be used to generate anti-idiotype antibodies using techniques well known to those skilled in the art (see, *e.g.*, Greenspan, N.S. *et al.* (1989) "*Idiotypes: Structure And Immunogenicity*," FASEB J. 7:437-444; and Nisinoff, A. (1991) "*Idiotypes: Concepts And Applications*," J. Immunol. 147(8):2429-2438).

**[0081]** The binding properties of any of the above antibodies can, if desired, be further improved by screening for variants that exhibit such desired characteristics. For example, such antibodies can be generated using various phage display methods known in the art. In phage display methods, functional antibody domains are displayed on the surface of phage particles which carry the polynucleotide sequences encoding them. In a particular embodiment, such phage can be utilized to display antigen binding domains, such as Fab and Fv or disulfide-bond stabilized Fv, expressed from a repertoire or combinatorial antibody

library (*e.g.*, human or murine). Phage expressing an antigen binding domain that binds the antigen of interest can be selected or identified with antigen, *e.g.*, using labeled antigen or antigen bound or captured to a solid surface or bead. Phage used in these methods are typically filamentous phage, including fd and M13. The antigen binding domains are expressed as a recombinantly fused protein to either the phage gene III or gene VIII protein. Examples of phage display methods that can be used to make the immunoglobulins, or fragments thereof, of the present invention include those disclosed in Brinkman, U. *et al.* (1995) "*Phage Display Of Disulfide-Stabilized Fv Fragments*," *J. Immunol. Methods*, 182:41-50, 1995; Ames, R.S. *et al.* (1995) "*Conversion Of Murine Fabs Isolated From A Combinatorial Phage Display Library To Full Length Immunoglobulins*," *J. Immunol. Methods*, 184:177-186; Kettleborough, C.A. *et al.* (1994) "*Isolation Of Tumor Cell-Specific Single-Chain Fv From Immunized Mice Using Phage-Antibody Libraries And The Re-Construction Of Whole Antibodies From These Antibody Fragments*," Eur. J. Immunol., 24:952-958, 1994; Persic, L. *et al.* (1997) "*An Integrated Vector System For The Eukaryotic Expression Of Antibodies Or Their Fragments After Selection From Phage Display Libraries*," *Gene*, 187:9-18; Burton, D.R. *et al.* (1994) "*Human Antibodies From Combinatorial Libraries*," Adv. Immunol. 57:191-280; PCT Publications WO 92/001047; WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; WO 95/20401; and U.S. Patents Nos. 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,637; 5,780,225; 5,658,727; 5,733,743 and 5,969,108

**[0082]** As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies, including humanized antibodies, or any other desired fragments, and expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria, *e.g.*, as described in detail below. For example, techniques to recombinantly produce Fab, Fab' and $F(ab')_2$ fragments can also be employed using methods known in the art such as those disclosed in PCT Publication WO 92/22324; Mullinax, R.L. *et al.* (1992) "*Expression Of A Heterodimeric Fab Antibody Protein In One Cloning Step*," *BioTechniques*, 12(6):864-869; and Sawai *et al.* (1995) "*Direct Production Of The Fab Fragment Derived From The Sperm Immobilizing Antibody Using Polymerase Chain Reaction And cDNA Expression Vectors*," Am. J. Reprod. Immunol. 34:26-34; and Better, M. *et al.* (1988) "*Escherichia coli Secretion Of An Active Chimeric Antibody Fragment*," Science 240:1041-1043). Examples of techniques which can

be used to produce single-chain Fvs and antibodies include those described in U.S. Patent Nos. 4,946,778 and 5,258,498; Huston, J.S. *et al.* (1991) "*Protein Engineering Of Single-Chain Fv Analogs And Fusion Proteins*," Methods in Enzymology 203:46-88; Shu, L. *et al.*, "*Secretion Of A Single-Gene-Encoded Immunoglobulin From Myeloma Cells*," Proc. Natl. Acad. Sci. (USA) 90:7995-7999; and Skerra. A. *et al.* (1988) "*Assembly Of A Functional Immunoglobulin Fv Fragment In Escherichia coli*," Science 240:1038-1040.

**[0083]** Phage display technology can be used to increase the affinity of an antibody of the invention for B7-H1 and/or PD-1. This technique would be useful in obtaining high affinity antibodies that could be used in the combinatorial methods of the invention. This technology, referred to as affinity maturation, employs mutagenesis or CDR walking and re-selection using such receptors or ligands (or their extracellular domains) or an antigenic fragment thereof to identify antibodies that bind with higher affinity to the antigen when compared with the initial or parental antibody (*See, e.g.,* Glaser, S.M. *et al.* (1992) "*Antibody Engineering By Codon-Based Mutagenesis In A Filamentous Phage Vector System*," J. Immunol. 149:3903-3913). Mutagenizing entire codons rather than single nucleotides results in a semi-randomized repertoire of amino acid mutations. Libraries can be constructed consisting of a pool of variant clones each of which differs by a single amino acid alteration in a single CDR and which contain variants representing each possible amino acid substitution for each CDR residue. Mutants with increased binding affinity for the antigen can be screened by contacting the immobilized mutants with labeled antigen. Any screening method known in the art can be used to identify mutant antibodies with increased avidity to the antigen (*e.g.*, ELISA) (see, *e.g.*, Wu, H. *et al.* (1998) "*Stepwise In Vitro Affinity Maturation Of Vitaxin, An Alphav Beta3-Specific Humanized Mab*," Proc. Natl. Acad. Sci. (USA) 95(11):6037-6042; Yelton, D.E. *et al.* (1995) "*Affinity Maturation Of The BR96 Anti-Carcinoma Antibody By Codon-Based Mutagenesis*," J. Immunol. 155:1994-2004). CDR walking which randomizes the light chain may be used possible (see, Schier *et al.* (1996) "*Isolation Of Picomolar Affinity Anti-C-Erbb-2 Single-Chain Fv By Molecular Evolution Of The Complementarity Determining Regions In The Center Of The Antibody Binding Site*," J. Mol. Biol. 263:551-567).

**[0084]** The invention thus contemplates the use of random mutagenesis in concert with methods of phage display to identify improved CDRs and/or variable regions. Phage display technology can alternatively be used to increase (or decrease) CDR affinity by directed

mutagenesis (*e.g.*, affinity maturation or "CDR-walking"). This technique uses the target antigen or an antigenic fragment thereof to identify antibodies having CDRs that bind with higher (or lower) affinity to the antigen when compared with the initial or parental antibody (see, *e.g.*, Glaser, S.M. *et al.* (1992) "*Antibody Engineering By Codon-Based Mutagenesis In A Filamentous Phage Vector System*," J. Immunol. 149:3903-3913). Mutagenizing entire codons rather than single nucleotides results in a semi-randomized repertoire of amino acid mutations. Libraries can be constructed consisting of a pool of variant clones each of which differs by a single amino acid alteration in a single CDR and which contain variants representing each possible amino acid substitution for each CDR residue. Mutants with increased (or decreased) binding affinity for the antigen can be screened by contacting the immobilized mutants with labeled antigen. Any screening method known in the art can be used to identify mutant antibodies with increased (or decreased) avidity to the antigen (e.g., ELISA) (see Wu, H. *et al.* (1998) "*Stepwise In Vitro Affinity Maturation Of Vitaxin, An Alphav Beta3-Specific Humanized Mab*," Proc. Natl. Acad. Sci. (USA) 95(11):6037-6042; Yelton, D.E. *et al.* (1995) "*Affinity Maturation Of The BR96 Anti-Carcinoma Antibody By Codon-Based Mutagenesis*," J. Immunol. 155:1994-2004). CDR walking which randomizes the light chain may be used possible (see, Schier *et al.* (1996) "*Isolation Of Picomolar Affinity Anti-C-Erbb-2 Single-Chain Fv By Molecular Evolution Of The Complementarity Determining Regions In The Center Of The Antibody Binding Site*," J. Mol. Biol. 263:551-567).

**[0085]** Methods for accomplishing such affinity maturation are described for example in: Krause, J.C. *et al.* (2011) "*An Insertion Mutation That Distorts Antibody Binding Site Architecture Enhances Function Of A Human Antibody*," MBio. 2(1) pii: e00345-10. doi: 10.1128/mBio.00345-10; Kuan, C.T. *et al.* (2010) "*Affinity-Matured Anti-Glycoprotein NMB Recombinant Immunotoxins Targeting Malignant Gliomas And Melanomas*," Int. J. Cancer 10.1002/ijc.25645; Hackel, B.J. *et al.* (2010) "*Stability And CDR Composition Biases Enrich Binder Functionality Landscapes*," J. Mol. Biol. 401(1):84-96; Montgomery, D.L. *et al.* (2009) "*Affinity Maturation And Characterization Of A Human Monoclonal Antibody Against HIV-1 gp41*," MAbs 1(5):462-474; Gustchina, E. *et al.* (2009) "*Affinity Maturation By Targeted Diversification Of The CDR-H2 Loop Of A Monoclonal Fab Derived From A Synthetic Naïve Human Antibody Library And Directed Against The Internal Trimeric Coiled-Coil Of Gp41 Yields A Set Of Fabs With Improved HIV-1 Neutralization Potency And Breadth*," Virology 393(1):112-119; Finlay, W.J. *et al.* (2009) "*Affinity Maturation Of A*

*Humanized Rat Antibody For Anti-RAGE Therapy: Comprehensive Mutagenesis Reveals A High Level Of Mutational Plasticity Both Inside And Outside The Complementarity-Determining Regions*," J. Mol. Biol. 388(3):541-558; Bostrom, J. *et al.* (2009) "*Improving Antibody Binding Affinity And Specificity For Therapeutic Development*," Methods Mol. Biol. 525:353-376; Steidl, S. *et al.* (2008) "*In Vitro Affinity Maturation Of Human GM-CSF Antibodies By Targeted CDR-Diversification*," Mol. Immunol. 46(1):135-144; and Barderas, R. *et al.* (2008) "Affinity maturation of antibodies assisted by in silico modeling," Proc. Natl. Acad. Sci. (USA) 105(26):9029-9034.

**[0086]** The invention particularly contemplates the production and use of "**derivatives**" of any of the above-described antibodies and their antigen-binding fragments. The term "derivative" refers to an antibody or antigen-binding fragment thereof that immunospecifically binds to an antigen but which comprises, one, two, three, four, five or more amino acid substitutions, additions, deletions or modifications relative to a "parental" (or wild-type) molecule. Such amino acid substitutions or additions may introduce naturally occurring (*i.e.*, DNA-encoded) or non-naturally occurring amino acid residues. Such amino acids may be glycosylated (*e.g.*, have altered mannose, 2-N-acetylglucosamine, galactose, fucose, glucose, sialic acid, 5-N-acetylneuraminic acid, 5-glycolneuraminic acid, *etc.* content), acetylated, pegylated, phosphorylated, amidated, derivatized by known protecting/blocking groups, proteolytic cleavage, linked to a cellular ligand or other protein, *etc.* In some embodiments, the altered carbohydrate modifications modulate one or more of the following: solubilization of the antibody, facilitation of subcellular transport and secretion of the antibody, promotion of antibody assembly, conformational integrity, and antibody-mediated effector function. In a specific embodiment the altered carbohydrate modifications enhance antibody mediated effector function relative to the antibody lacking the carbohydrate modification. Carbohydrate modifications that lead to altered antibody mediated effector function are well known in the art (for example, *see* Shields, R.L. *et al.* (2002) "*Lack Of Fucose On Human IgG N-Linked Oligosaccharide Improves Binding To Human Fcgamma RIII And Antibody-Dependent Cellular Toxicity.*," J. Biol. Chem. 277(30): 26733-26740; Davies J. *et al.* (2001) "*Expression Of GnTIII In A Recombinant Anti-CD20 CHO Production Cell Line: Expression Of Antibodies With Altered Glycoforms Leads To An Increase In ADCC Through Higher Affinity For FC Gamma RIII*," Biotechnology & Bioengineering 74(4): 288-294). Methods of altering carbohydrate contents are known to those skilled in the art, *see*, *e.g.*, Wallick, S.C. *et al.* (1988) "*Glycosylation Of A VH Residue Of A Monoclonal*

*Antibody Against Alpha (1----6) Dextran Increases Its Affinity For Antigen*," J. Exp. Med. 168(3): 1099-1109; Tao, M.H. *et al.* (1989) "*Studies Of Aglycosylated Chimeric Mouse-Human IgG. Role Of Carbohydrate In The Structure And Effector Functions Mediated By The Human IgG Constant Region*," J. Immunol. 143(8): 2595-2601; Routledge, E.G. *et al.* (1995) "*The Effect Of Aglycosylation On The Immunogenicity Of A Humanized Therapeutic CD3 Monoclonal Antibody*," Transplantation 60(8):847-53; Elliott, S. *et al.* (2003) "*Enhancement Of Therapeutic Protein In Vivo Activities Through Glycoengineering*," Nature Biotechnol. 21:414-21; Shields, R.L. *et al.* (2002) "*Lack Of Fucose On Human IgG N-Linked Oligosaccharide Improves Binding To Human Fcgamma RIII And Antibody-Dependent Cellular Toxicity*.," J. Biol. Chem. 277(30): 26733-26740).

**[0087]** In some embodiments, a humanized antibody is a derivative. Such a humanized antibody comprises amino acid residue substitutions, deletions or additions in one or more non-human CDRs. The humanized antibody derivative may have substantially the same binding, better binding, or worse binding when compared to a non-derivative humanized antibody. In specific embodiments, one, two, three, four, or five amino acid residues of the CDR have been substituted, deleted or added (*i.e.,* mutated).

**[0088]** A derivative antibody or antibody fragment may be modified by chemical modifications using techniques known to those of skill in the art, including, but not limited to, specific chemical cleavage, acetylation, formulation, metabolic synthesis of tunicamycin, *etc.* In one embodiment, an antibody derivative will possess a similar or identical function as the parental antibody. In another embodiment, an antibody derivative will exhibit an altered activity relative to the parental antibody. For example, a derivative antibody (or fragment thereof) can bind to its epitope more tightly or be more resistant to proteolysis than the parental antibody.

**[0089]** Substitutions, additions or deletions in the derivatized antibodies may be in the Fc region of the antibody and may thereby serve to modify the binding affinity of the antibody to one or more FcγR. Methods for modifying antibodies with modified binding to one or more FcγR are known in the art, *see, e.g.*, PCT Publication Nos. WO 04/029207, WO 04/029092, WO 04/028564, WO 99/58572, WO 99/51642, WO 98/23289, WO 89/07142, WO 88/07089, and U.S. Patent Nos. 5,843,597 and 5,642,821. In some embodiments, the invention encompasses antibodies that have altered affinity for an activating FcγR, *e.g.*, FcγRIIIA. Preferably such modifications also have an altered Fc-mediated effector function.

Modifications that affect Fc-mediated effector function are well known in the art (*see* U.S. Patent No. 6,194,551, and WO 00/42072). In one particular embodiment, the modification of the Fc region results in an antibody with an altered antibody-mediated effector function, an altered binding to other Fc receptors (*e.g.*, Fc activation receptors), an altered antibody-dependent cell-mediated cytotoxicity (ADCC) activity, an altered C1q binding activity, an altered complement-dependent cytotoxicity activity (CDC), a phagocytic activity, or any combination thereof.

**[0090]** Derivatized antibodies may be used to alter the half-lives (*e.g.*, serum half-lives) of parental antibodies in a mammal, preferably a human. Preferably such alteration will result in a half-life of greater than 15 days, preferably greater than 20 days, greater than 25 days, greater than 30 days, greater than 35 days, greater than 40 days, greater than 45 days, greater than 2 months, greater than 3 months, greater than 4 months, or greater than 5 months. The increased half-lives of the humanized antibodies of the present invention or fragments thereof in a mammal, preferably a human, results in a higher serum titer of said antibodies or antibody fragments in the mammal, and thus, reduces the frequency of the administration of said antibodies or antibody fragments and/or reduces the concentration of said antibodies or antibody fragments to be administered. Antibodies or fragments thereof having increased *in vivo* half-lives can be generated by techniques known to those of skill in the art. For example, antibodies or fragments thereof with increased *in vivo* half-lives can be generated by modifying (*e.g.*, substituting, deleting or adding) amino acid residues identified as involved in the interaction between the Fc domain and the FcRn receptor. The humanized antibodies of the invention may be engineered to increase biological half-lives (*see, e.g.* U.S. Patent No. 6,277,375). For example, humanized antibodies of the invention may be engineered in the Fc-hinge domain to have increased *in vivo* or serum half-lives.

**[0091]** Antibodies or fragments thereof with increased *in vivo* half-lives can be generated by attaching to said antibodies or antibody fragments polymer molecules such as high molecular weight polyethyleneglycol (PEG). PEG can be attached to said antibodies or antibody fragments with or without a multifunctional linker either through site-specific conjugation of the PEG to the N– or C- terminus of said antibodies or antibody fragments or via epsilon-amino groups present on lysine residues. Linear or branched polymer derivatization that results in minimal loss of biological activity will be used. The degree of conjugation will be closely monitored by SDS-PAGE and mass spectrometry to ensure proper

conjugation of PEG molecules to the antibodies. Unreacted PEG can be separated from antibody-PEG conjugates by, *e.g.*, size exclusion or ion-exchange chromatography.

**[0092]** The antibodies of the invention may also be modified by the methods and coupling agents described by Davis *et al.* (*See* U.S. Patent No. 4,179,337) in order to provide compositions that can be injected into the mammalian circulatory system with substantially no immunogenic response.

**[0093]** The invention encompasses modification of framework residues of the humanized antibodies of the invention. Framework residues in the framework regions may be substituted with the corresponding residue from the CDR donor antibody to alter, preferably improve, antigen binding. These framework substitutions are identified by methods well known in the art, *e.g.*, by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. (*See*, *e.g.*, U.S. Patent No. 5,585,089; and Riechmann, L. *et al.* (1988) "*Reshaping Human Antibodies For Therapy*," Nature 332:323-327).

**[0094]** The present invention also encompasses anti-human B7-H1 and anti-human PD-1 antibodies (and more preferably, humanized antibodies) and antigen-binding fragments thereof that are recombinantly fused or chemically conjugated (including both covalently and non-covalently conjugations) to a heterologous molecule (*i.e.*, an unrelated molecule). The fusion does not necessarily need to be direct, but may occur through linker sequences.

**[0095]** The Fc portion of the fusion the fusion protein may be varied by isotype or subclass, may be a chimeric or hybrid, and/or may be modified, for example to improve effector functions, control of half-life, tissue accessibility, augment biophysical characteristics such as stability, and improve efficiency of production (and less costly). Many modifications useful in construction of disclosed fusion proteins and methods for making them are known in the art, see for example Mueller, J.P. *et al.* (1997) "*Humanized Porcine VCAM-Specific Monoclonal Antibodies With Chimeric Igg2/G4 Constant Regions Block Human Leukocyte Binding To Porcine Endothelial Cells*," Mol. Immun. 34(6):441-452, Swann, P.G. (2008) "*Considerations For The Development Of Therapeutic Monoclonal Antibodies*," Curr. Opin. Immun. 20:493-499 (2008), and Presta, L.G. (2008) "*Molecular Engineering And Design Of Therapeutic Antibodies*," Curr. Opin. Immun. 20:460-470. In some embodiments the Fc

region is the native IgG1, IgG2, or IgG4 Fc region. In some embodiments the Fc region is a hybrid, for example a chimeric consisting of IgG2/IgG4 Fc constant regions. Modications to the Fc region include, but are not limited to, IgG4 modified to prevent binding to Fc gamma receptors and complement, IgG1 modified to improve binding to one or more Fc gamma receptors, IgG1 modified to minimize effector function (amino acid changes), IgG1 with altered/no glycan (typically by changing expression host), and IgG1 with altered pH-dependent binding to FcRn. The Fc region may include the entire hinge region, or less than the entire hinge region.

**[0096]** The therapeutic outcome in patients treated with rituximab (a chimeric mouse/human IgG1 monoclonal antibody against CD20) for non-Hodgkin's lymphoma or Waldenstrom's macroglobulinemia correlated with the individual's expression of allelic variants of Fcγ receptors with distinct intrinsic affinities for the Fc domain of human IgG1. In particular, patients with high affinity alleles of the low affinity activating Fc receptor CD16A (FcγRIIIA) showed higher response rates and, in the cases of non-Hodgkin's lymphoma, improved progression-free survival. In another embodiment, the Fc domain may contain one or more amino acid insertions, deletions or substitutions that reduce binding to the low affinity inhibitory Fc receptor CD32B (FcγRIIB) and retain wild-type levels of binding to or enhance binding to the low affinity activating Fc receptor CD16A (FcγRIIIA).

**[0097]** Another embodiment includes IgG2-4 hybrids and IgG4 mutants that have reduce binding to FcR which increase their half-life. Representative IG2-4 hybrids and IgG4 mutants are described in Angal, S. *et al.* (1993) "*A Single Amino Acid Substitution Abolishes The Heterogeneity Of Chimeric Mouse/Human (IgG4) Antibody*," Molec. Immunol. 30(1):105-108; Mueller, J.P. *et al.* (1997) "*Humanized Porcine VCAM-Specific Monoclonal Antibodies With Chimeric IgG2/G4 Constant Regions Block Human Leukocyte Binding To Porcine Endothelial Cells*," Mol. Immun. 34(6):441-452; and U.S. Patent No. 6,982,323. In some embodiments the IgG1 and/or IgG2 domain is deleted for example, Angal et al. describe IgG1 and IgG2 having serine 241 replaced with a proline

**[0098]** In a preferred embodiment, the Fc domain contains amino acid insertions, deletions or substitutions that enhance binding to CD16A. A large number of substitutions in the Fc domain of human IgG1 that increase binding to CD16A and reduce binding to CD32B are known in the art and are described in Stavenhagen, J.B. *et al.* (2007) "*Fc Optimization Of Therapeutic Antibodies Enhances Their Ability To Kill Tumor Cells In Vitro And Controls*

*Tumor Expansion In Vivo Via Low-Affinity Activating Fcgamma Receptors*," Cancer Res. 57(18):8882-8890. Exemplary variants of human IgG1 Fc domains with reduced binding to CD32B and/or increased binding to CD16A contain F243L, R929P, Y300L, V305I or P296L substitutions. These amino acid substitutions may be present in a human IgG1 Fc domain in any combination. In one embodiment, the human IgG1 Fc domain variant contains a F243L, R929P and Y300L substitution. In another embodiment, the human IgG1 Fc domain variant contains a F243L, R929P, Y300L, V305I and P296L substitution. In another embodiment, the human IgG1 Fc domain variant contains an N297Q substitution, as this mutation abolishes FcR binding.

**[0099]** In one embodiment such heterologous molecules are polypeptides having at least 10, at least 20, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90 or at least 100 amino acids. Such heterologous molecules may alternatively be enzymes, hormones, cell surface receptors, drug moieties, such as: **toxins** (such as abrin, ricin A, pseudomonas exotoxin (*i.e.*, PE-40), diphtheria toxin, ricin, gelonin, or pokeweed antiviral protein), **proteins** (such as tumor necrosis factor, interferon (*e.g.*, α-interferon, β-interferon), nerve growth factor, platelet derived growth factor, tissue plasminogen activator, or an apoptotic agent (*e.g.*, tumor necrosis factor-α, tumor necrosis factor-β)), **biological response modifiers** (such as, for example, a lymphokine (*e.g.*, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6")), granulocyte macrophage colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or macrophage colony stimulating factor, ("M-CSF")), or growth factors (*e.g.*, growth hormone ("GH"))), **cytotoxins** (*e.g.*, a cytostatic or cytocidal agent, such as paclitaxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, monomethyl auristatin F (MMAF), monomethyl auristatin E (MMAE; *e.g.*, vedotin) and puromycin and analogs or homologs thereof), **antimetabolites** (*e.g.*, methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), **alkylating agents** (*e.g.*, mechlorethamine, thioepa chlorambucil, melphalan, BiCNU® (carmustinc; BSNU) and lomustinc (CCNU), cyclothosphamidc, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cisdichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (*e.g.*, daunorubicin (formerly daunomycin) and doxorubicin), **antibiotics** (*e.g.*,

dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), or **anti-mitotic** agents (*e.g.*, vincristine and vinblastine).

**[00100]** Techniques for conjugating such therapeutic moieties to antibodies are well known; *see*, *e.g.*, Arnon *et al.*, "*Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy*", in MONOCLONAL ANTIBODIES AND CANCER THERAPY, Reisfeld *et al.* (eds.), 1985, pp. 243-56, Alan R. Liss, Inc.); Hellstrom *et al.*, "*Antibodies For Drug Delivery*", in CONTROLLED DRUG DELIVERY *(2nd Ed.)*, Robinson *et al.* (eds.), 1987, pp. 623-53, Marcel Dekker, Inc. ); Thorpe, "A*ntibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review*", in MONOCLONAL ANTIBODIES '84: BIOLOGICAL AND CLINICAL APPLICATIONS, Pinchera *et al.* (eds.), 1985, pp. 475-506); "*Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy*", in MONOCLONAL ANTIBODIES FOR CANCER DETECTION AND THERAPY, Baldwin *et al.* (eds.), 1985, pp. 303-16, Academic Press; Thorpe *et al.* (1982) "*The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates*," Immunol. Rev. 62:119-158; Carter, P.J. *et al.* (2008) "*Antibody-Drug Conjugates for Cancer Therapy*," Cancer J. 14(3):154-169; Alley, S.C. *et al.* (2010) "*Antibody-Drug Conjugates: Targeted Drug Delivery For Cancer*," Curr. Opin. Chem. Biol. 14(4):529-537; Carter, P. *et al.* (2005) "*Designer Antibody-Based Therapeutics For Oncology*," Amer. Assoc. Cancer Res. Educ. Book. 2005(1):147-154; Carter, P.J. *et al.* (2008) "*Antibody-Drug Conjugates For Cancer Therapy*," Cancer J. 14(3):154-169; Chari, R.V.J. (2008) "*Targeted Cancer Therapy: Conferring Specificity To Cytotoxic Drugs*," Acc. Chem Res. 41(1):98-107; Doronina, S.O. *et al.* (2003) "*Development Of Potent Monoclonal Antibody Auristatin Conjugates For Cancer Therapy*," Nat. Biotechnol. 21(7):778-784; Ducry, L. *et al.* (2010) "*Antibody-Drug Conjugates: Linking Cytotoxic Payloads To Monoclonal Antibodies*," Bioconjug Chem. 21(1):5-13; Senter, P.D. (2009) "*Potent Antibody Drug Conjugates For Cancer Therapy*," Curr. Opin. Chem. Biol. 13(3):235-244; and Teicher, B.A. (2009) "*Antibody-Drug Conjugate Targets*," Curr Cancer Drug Targets. 9(8):982-1004.

**[00101]** Any of the molecules of the present invention can be fused to marker sequences, such as a peptide, to facilitate purification. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, the hemagglutinin "HA" tag, which corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson, I.A. *et al.* (1984) "*The Structure Of An Antigenic Determinant In A Protein*," Cell, 37:767-778) and the "flag" tag

(Knappik, A. *et al.* (1994) "*An Improved Affinity Tag Based On The FLAG Peptide For The Detection And Purification Of Recombinant Antibody Fragments*," Biotechniques 17(4):754-761).

**[00102]** The present invention also encompasses antibodies or their antigen-binding fragments that are conjugated to a diagnostic or therapeutic agent or any other molecule for which serum half-life is desired to be increased. The antibodies can be used diagnostically (*in vivo*, *in situ* or *in vitro*) to, for example, monitor the development or progression of a disease, disorder or infection as part of a clinical testing procedure to, *e.g.*, determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, radioactive materials, positron emitting metals, and nonradioactive paramagnetic metal ions. The detectable substance may be coupled or conjugated either directly to the antibody or indirectly, through an intermediate (such as, for example, a linker known in the art) using techniques known in the art. *See*, for example, U.S. Patent No. 4,741,900 for metal ions which can be conjugated to antibodies for use as diagnostics according to the present invention. Such diagnosis and detection can be accomplished by coupling the antibody to detectable substances including, but not limited to, various enzymes, enzymes including, but not limited to, horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; prosthetic group complexes such as, but not limited to, streptavidin/biotin and avidin/biotin; fluorescent materials such as, but not limited to, umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; luminescent material such as, but not limited to, luminol; bioluminescent materials such as, but not limited to, luciferase, luciferin, and aequorin; radioactive material such as, but not limited to, bismuth ($^{213}$Bi), carbon ($^{14}$C), chromium ($^{51}$Cr), cobalt ($^{57}$Co), fluorine ($^{18}$F), gadolinium ($^{153}$Gd, $^{159}$Gd), gallium ($^{68}$Ga, $^{67}$Ga), germanium ($^{68}$Ge), holmium ($^{166}$Ho), indium ($^{115}$In, $^{113}$In, $^{112}$In, $^{111}$In), iodine ($^{131}$I, $^{125}$I, $^{123}$I, $^{121}$I), lanthanium ($^{140}$La), lutetium ($^{177}$Lu), manganese ($^{54}$Mn), molybdenum ($^{99}$Mo), palladium ($^{103}$Pd), phosphorous ($^{32}$P), praseodymium ($^{142}$Pr), promethium ($^{149}$Pm), rhenium ($^{186}$Re, $^{188}$Re), rhodium ($^{105}$Rh), ruthemium ($^{97}$Ru), samarium ($^{153}$Sm), scandium ($^{47}$Sc), selenium ($^{75}$Se), strontium ($^{85}$Sr), sulfur ($^{35}$S), technetium ($^{99}$Tc), thallium ($^{201}$Ti), tin ($^{113}$Sn, $^{117}$Sn), tritium ($^{3}$H), xenon ($^{133}$Xe), ytterbium ($^{169}$Yb, $^{175}$Yb), yttrium ($^{90}$Y), zinc ($^{65}$Zn);

positron emitting metals using various positron emission tomographies, and nonradioactive paramagnetic metal ions.

**[00103]** The molecules of the present invention can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980. Such heteroconjugate antibodies may additionally bind to haptens (such as fluorescein, *etc.*), or to cellular markers (*e.g.*, 4-1-BB, B7-H4, CD4, CD8, CD14, CD25, CD27, CD40, CD68, CD163, CTLA4, GITR, LAG-3, OX40, TIM3, TIM4, TLR2, LIGHT, ICOS, B7-H3, B7-H7, B7-H7CR, CD70, CD47, *etc.*) or to cytokines (*e.g.*, IL-7, IL-15, IL-12, IL-4 TGF-beta, IL-10, IL-17, IFNγ, Flt3, BLys) or chemokines (*e.g.*, CCL21), *etc.*

**[00104]** The molecules of the present invention may be attached to solid supports, which are particularly useful for immunoassays or purification of the target antigen or of other molecules that are capable of binding to target antigen that has been immobilized to the support *via* binding to an antibody or antigen-binding fragment of the present invention. Such solid supports include, but are not limited to, glass, cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene.

**[00105]** The present invention additionally includes nucleic acid molecules (DNA or RNA) that encode any such antibodies, fusion proteins or fragments, as well as vector molecules (such as plasmids) that are capable of transmitting or of replication such nucleic acid molecules. The nucleic acids can be single-stranded, double-stranded, may contain both single-stranded and double-stranded portions.

### A. Preferred Modulator Compositions of the Present Invention

**[00106]** The invention particularly concerns antibodies that immunospecifically bind to B7-H1 or to PD-1 and/or modulate the ability of B7-H1 to bind to PD-1 in a subject. As used herein, a "**subject**" is preferably a mammal such as a non-primate (*e.g.*, cows, pigs, horses, cats, dogs, rats etc.) and a primate (*e.g.*, monkey and human), most preferably a human. The invention thus particularly relates to humanized antibodies, and antigen-binding fragments thereof, that immunospecifically bind to human B7-H1 or to human PD-1 and modulate the ability of B7-H1 to bind to PD-1 in a human or in human tissue (*in situ* or *ex vivo*).

**[00107]** Most preferably, such antibodies and antigen-binding fragments will possess sufficient avidity to modulate the ability of B7-H1 (especially when expressed at an

endogenous concentration and) arrayed on the surface of a subject's APC to bind to PD-1 (especially when expressed at an endogenous concentration and) arrayed on the surface of a T cell of such subject and *vice versa*. The term "endogenous concentration" refers to the level at which an endogenous molecule is natively expressed (*i.e.*, in the absence of expression vectors or recombinant promoters) in a normal, cancer or infected cell.

**[00108]** In one embodiment, such modulation will comprise inhibiting or otherwise interfering with the binding of such (preferably endogenously expressed and) arrayed B7-H1 and such (preferably endogenously expressed and) arrayed PD-1. In an alternative embodiment, such modulation will comprise an enhancement or otherwise facilitate the binding of endogenously expressed and arrayed B7-H1 and endogenously expressed and arrayed PD-1. In yet another embodiment, such modulation includes direct agonism whereby binding of the anti-B7-H1 or anti-PD-1 triggers signal transduction through the corresponding receptor.

### (1) Preferred Anti-Human B7-H1 Antibodies and Their CDRs

**[00109]** In accordance with the present invention, such molecules can be produced by screening hybridoma lines for those that produce antibody that are immunospecific for human B7-H1, and then optionally screening amongst such lines for those exhibiting modulating activity (*e.g.*, neutralizing activity, agonizing activity, altered signal transducing activity, *etc.*). The invention particularly provides anti-human B7-H1 clones: 1E12, 1F4, 2G11, 3B6, and 3D10.

**[00110]** The antibodies expressed by the anti-human B7-H1 clones were sequenced to reveal their variable domains. CDR sequences are shown in bold and underlined:

**Anti-Human B7-H1 Clone 1E12**

**Light Chain Variable Region:**

DIVMTQSHKL MSTSVGDRVS ITC**KASQDVG TAVA**WYQQKP GQSPKLLIY**W ASTRHT**GVPD RFTGSGSGTD FTLTISNVQS EDLADYFC**QQ DSSYPLT**FGA GTKVELK **(SEQ ID NO:1)**

**Heavy Chain Variable Region:**

EVKLQESGPS LVKPSQTLSL TCSVT**GYSIT SDYWN**WIRKF PGNKLEYVG**Y ISYTGSTYYN PSLKS**RISIT RDTSKNQYYL QLNSVTSEDT ATYYCAR**YGG WLSPF**DYWGQ GTTLTVSS **(SEQ ID NO:2)**

**Anti-Human B7-H1 Clone 1F4**

**Light Chain Variable Region:**

DIVTTQSHKL MSTSVGDRVS ITC**KASQDVG TAVA**WYQQKP GQSPKLLIY**W ASTRHT**GVPD RFTGSGSGTD FTLTISNVQS EDLADYFC**QQ DSSYPLT**FGA GTKVELK **(SEQ ID NO:3)**

**Heavy Chain Variable Region:**

EVQLQESGPG LVAPSQSLSI TCTVS**GFSLT TYSIN**WIRQP PGKGLEWLG**V MWAGGGTNSN SVLKS**RLIIS KDNSKSQVFL KMNSLQTDDT ARYYCAR**YYG NSPYYAI**DYW GQGTSVTVSS **(SEQ ID NO:4)**

**Anti-Human B7-H1 Clone 2G11**

**Light Chain Variable Region:**

DIVMTQSPSS LAVSVGEKVS MGC**KSSQSLL YSSNQKNSLA** WYQQKPGQSP KLLID**WASTR ES**GVPDRFTG SGSGTDFTLT ISSVKAEDLA VYYC**QQYYGY PLT**FGAGTKL ELK **(SEQ ID NO:5)**

**Heavy Chain Variable Region:**

EVKLQESGPS LVKPSQTLSL TCSVT**GYSII SDYWN**WIRKF PGNKLEYLG**Y ISYTGSTYYN PSLKS**RISIT RDTSKNQYYL QLNSVTTEDT ATYYCA**RRGG WLLPF**DYWGQ GTTLTVSS **(SEQ ID NO:6)**

**Anti-Human B7-H1 Clone 3B6**

**Light Chain Variable Region:**

DIVMTQSPAI MSASPGEKVT MTC**SASSSIR YMH**WYQQKPG TSPKRWIS**DT SKLTS**GVPAR FSGSGSGTSY ALTISSMEAE DAATYYC**HQR SSYPWT**FGGG TKLEIK **(SEQ ID NO:7)**

**Heavy Chain Variable Region:**

EVKLQESGPS LVKPGASVKL SCKAS**GYTFT SYDIN**WVKQR PGQGLEWIG**W IFPRDNNTKY NENFKG**KATL TVDTSSTTAY MELHSLTSED SAVYFCTK**EN WVGDF**DYWGQ GTTLTLSS **(SEQ ID NO:8)**

**Anti-Human B7-H1 Clone 3D10:**

**Light Chain Variable Region:**

QIVLSQSPAI LSASPGEKVT MTC**RASSSVS YIY**WFQQKPG SSPKPWIY**AT FNLAS**GVPAR FSGSGSGTSY SLTISRVETE DAATYYC**QQW SNNPLT**FGAG TKLELK **(SEQ ID NO:9)**

**Heavy Chain Variable Region:**

EVQLQQSGPD LVTPGASVRI SCQAS**GYTFP DYYMN**WVKQS HGKSLEWIG**D IDPNYGGTTY NQKFKG**KAIL TVDRSSSTAY MELRSLTSED SAVYYCAR**GA L**TDWGQGTSL TVSS **(SEQ ID NO:10)**

### (2) Preferred Anti-Human PD-1 Antibodies and Their CDRs

**[00111]** Alternatively such antibodies can be produced by screening hybridoma lines for those that produce antibody that are immunospecific for human PD-1, and then screening amongst such lines for those exhibiting modulating activity (*e.g.*, neutralizing activity, agonizing activity, altered signal transducing activity, *etc.*). The invention particularly provides anti-human PD-1 clones: 1E3, 1E8, and 1H3.

**[00112]** The antibodies expressed by the anti-human PD-1 clones were sequenced to reveal their variable domains. CDR sequences are shown in bold and underlined:

**Anti-Human PD-1 Clone 1E3:**

**Light Chain Variable Region:**

DIQMTQFPSS LCASQGGKVT VTC**KASQDIN NYMA**WYQHKP GKGPRLLIH**Y TSTLLS**GIPS RFSGSGSGRD YSFSISNLEP EDIATYYC**LQ YDNLWT**FGGG TKLEIK **(SEQ ID NO:11)**

**Heavy Chain Variable Region:**

EVQLQQSGPV LVKPGASVKM SCKAS**GYTFT DYYMN**WVKQS HGKSLEWIG**N INPYNGGTTY NQKFKG**KATL TVDKSSRTAY MEINSLTSED SAVYYCAR**GR IYDGSLDY**WG QGTALTVSS **(SEQ ID NO:12)**

**Anti-Human PD-1 Clone 1E8:**

**Light Chain Variable Region:**

DIVMTQSQKF MSTSVGDRVS VTC**KASQSVD TNVA**WYQQKP GQSPKALIF**S ASYRYS**GVPD RFTGSGSGTD FTLTINSVQS EDLAEYFC**QQ YNSYPYT**FGS GTKLEIK **(SEQ ID NO:13)**

**Heavy Chain Variable Region:**

QVQLQQSGAE LAKPGASVRL SCKAS**GYTFT NYWMH**WVKQR PGQGLEWIG**H INPSSGFTTY NQNFKD**KATL TADKSSNTAY MQLSSLTYED SAVYFCAR**ED YDVDY**WGQGT TLTVSS **(SEQ ID NO:14)**

**Anti-Human PD-1 Clone 1H3:**

**Light Chain Variable Region:**

QIVLTQSPAL MSASPGEKVT MTC**SASSSVS YMY**WYQQKPR SSPKPWIY**LT SNLAS**GVPAR FSGSGSGTSY SLTISSMEAE DAATYYC**QQW SSNPFT**FGSG TKLEIK **(SEQ ID NO:15)**

**Heavy Chain Variable Region:**

EVQLVESGGG LVKPGGSLKL SCAAS**GFTFS DYGMH**WVRQA PEKGLEWVA**Y ISSGSYTIYY TDTVKG**RFTI SRDNAKNTLF LQMTSLRSED TAMYYCAR**RG YGSFYEYYFD Y**WGQGTTLTV SS **(SEQ ID NO:16)**

### (3) Consensus CDRs of the Anti-Human B7-H1 and Anti-Human PD-1 Antibodies of the Present Invention

**[00113]** Analyses of the CDRs of the identified antibodies were conducted in order to identify consensus CDR sequences and likely variant CDR sequences that would provide similar binding attributes. Such variant CDRs were computed using Blosum62.iij analysis according to **Table 1**. **Table 1** presents the Blosum62.iij substitution scores. The higher the score the more conservative the substitution and thus the more likely the substitution will not affect function.

**Table 1**

| | A | R | N | D | C | Q | E | G | H | I | L | K | M | F | P | S | T | W | Y | V |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | +4 | -1 | -2 | -2 | 0 | -1 | -1 | 0 | -2 | -1 | -1 | -1 | -1 | -2 | -1 | +1 | 0 | -3 | -2 | 0 |
| **R** | -1 | +5 | 0 | -2 | -3 | +1 | 0 | -2 | 0 | -3 | -2 | +2 | -1 | -3 | -2 | -1 | -1 | -3 | -2 | -3 |
| **N** | -2 | 0 | +6 | +1 | -3 | 0 | 0 | 0 | +1 | -3 | -3 | 0 | -2 | -3 | -2 | +1 | 0 | -4 | -2 | -3 |
| **D** | -2 | -2 | +1 | +6 | -3 | 0 | +2 | -1 | -1 | -3 | -4 | -1 | -3 | -3 | -1 | 0 | -1 | -4 | -3 | -3 |
| **C** | 0 | -3 | -3 | -3 | +9 | -3 | -4 | -3 | -3 | -1 | -1 | -3 | -1 | -2 | -3 | -1 | -1 | -2 | -2 | -1 |
| **Q** | -1 | +1 | 0 | 0 | -3 | +5 | +2 | -2 | 0 | -3 | -2 | +1 | 0 | -3 | -1 | 0 | -1 | -2 | -1 | -2 |
| **E** | -1 | 0 | 0 | +2 | -4 | +2 | +5 | -2 | 0 | -3 | -3 | +1 | -2 | -3 | -1 | 0 | -1 | -3 | -2 | -2 |
| **G** | 0 | -2 | 0 | -1 | -3 | -2 | -2 | +6 | -2 | -4 | -4 | -2 | -3 | -3 | -2 | 0 | -2 | -2 | -3 | -3 |
| **H** | -2 | 0 | +1 | -1 | -3 | 0 | 0 | -2 | +8 | -3 | -3 | -1 | -2 | -1 | -2 | -1 | -2 | -2 | +2 | -3 |
| **I** | -1 | -3 | -3 | -3 | -1 | -3 | -3 | -4 | -3 | +4 | +2 | -3 | +1 | 0 | -3 | -2 | -1 | -3 | -1 | +3 |
| **L** | -1 | -2 | -3 | -4 | -1 | -2 | -3 | -4 | -3 | +2 | +4 | -2 | +2 | 0 | -3 | -2 | -1 | -2 | -1 | +1 |
| **K** | -1 | +2 | 0 | -1 | -3 | +1 | +1 | -2 | -1 | -3 | -2 | +5 | -1 | -3 | -1 | 0 | -1 | -3 | -2 | -2 |
| **M** | -1 | -1 | -2 | -3 | -1 | 0 | -2 | -3 | -2 | +1 | +2 | -1 | +5 | 0 | -2 | -1 | -1 | -1 | -1 | +1 |
| **F** | -2 | -3 | -3 | -3 | -2 | -3 | -3 | -3 | -1 | 0 | 0 | -3 | 0 | +6 | -4 | -2 | -2 | +1 | +3 | -1 |
| **P** | -1 | -2 | -2 | -1 | -3 | -1 | -1 | -2 | -2 | -3 | -3 | -1 | -2 | -4 | +7 | -1 | -1 | -4 | -3 | -2 |
| **S** | +1 | -1 | +1 | 0 | -1 | 0 | 0 | 0 | -1 | -2 | -2 | 0 | -1 | -2 | -1 | +4 | +1 | -3 | -2 | -2 |
| **T** | 0 | -1 | 0 | -1 | -1 | -1 | -1 | -2 | -2 | -1 | -1 | -1 | -1 | -2 | -1 | +1 | +5 | -2 | -2 | 0 |
| **W** | -3 | -3 | -4 | -4 | -2 | -2 | -3 | -2 | -2 | -3 | -2 | -3 | -1 | +1 | -4 | -3 | -2 | +11 | +2 | -3 |
| **Y** | -2 | -2 | -2 | -3 | -2 | -1 | -2 | -3 | +2 | -1 | -1 | -2 | -1 | +3 | -3 | -2 | -2 | +2 | +7 | -1 |
| **V** | 0 | -3 | -3 | -3 | -1 | -2 | -2 | -3 | -3 | +3 | +1 | -2 | +1 | -1 | -2 | -2 | 0 | -3 | -1 | +4 |

**[00114]** The present invention permits the formation of novel antibodies and antigen-binding fragments having 1, 2, 3, 4, 5 or 6 variant CDRs. Because the methods of the present invention have identified a substantial number of distinct CDRs, the invention permits a recognition of CDR residues that are likely to be required in any variant of a particular identified CDR. Such residues are shown in boldface in **Table 2**, **Table 3, Table 4** and **Table 5.** For those residues that are found to vary among the compared CDRs, the substitution scores of **Table 1** provide a means for determining the identities of permitted substitutions. For example, if a particular residue of a particular CDR is found to vary as R or S, then since R and S have a substitution score of -1, any substitution for R or S having a substitution score of -1 or greater are as likely as the observed variants (R or S) (or are more likely than R or S) to create a variant CDR having binding attributes that are sufficiently

similar to those of the particular CDR to permit the variant CDR to be employed in lieu thereof so as to form a functional anti-B7-H1 or anti-PD-1 antibody or antigen-binding fragment. For each position, the selection of a residue having a higher substitution score is preferred over the selection of a residue having a lower substitution score.

**[00115] Table 2** presents an analysis of the light chain CDRs of the anti-B7-H1 antibodies and provides the consensus sequence of the observed and preferred variant light chain anti-B7-H1 CDRs of the present invention.

**Table 2: Anti-Human B7-H1 Light Chain CDRs**

**Light Chain CDR1**

| Antibody | Sequence | | | | | | | | | | | | | SEQ ID NO |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 3D10 | R | A | S | S | | | S | V | S | Y | I | Y | | **17** |
| 3B6 | S | A | S | S | | | S | I | R | Y | M | H | | **18** |
| 1E12 | K | A | S | Q | | D | V | G | T | A | V | A | | **19** |
| 2G11 | K | S | S | Q | SLLYSS | N | Q | K | N | S | L | A | | **20** |
| 1F4 | K | A | S | Q | | D | V | G | T | A | V | A | | **21** |
| **Light Chain CDR1 Consensus Sequence:** | X 1 | X 2 | **S** | X 3 | X 4 | X 5 | X 6 | X 7 | X 8 | X 9 | X 10 | X 11 | | **22** |

$X_1$ are substitutions of R/K/S or substitutions having an equal or greater substitution score (*i.e.*, ≥ +2): R, S or K

$X_2$ are substitutions of A/S or substitutions having an equal or greater substitution score (*i.e.*, ≥ +1): A or S

$X_3$ are substitutions of Q/S or substitutions having an equal or greater substitution score (*i.e.*, ≥ 0): R, N, D, Q, E, H, K, M, or S

$X_4$ is SLLYSS (**SEQ ID NO: 23**) or is absent

$X_5$ is absent or substitutions of D/N or substitutions having an equal or greater substitution score (*i.e.*, ≥ +1): N or D

$X_6$ are substitutions of S/V/Q or substitutions having an equal or greater substitution score (*i.e.*, ≥ 0): A, Q, E, L, K, M, P, S, T, Y, or V

$X_7$ are substitutions of V/I/G/K or substitutions having an equal or greater substitution score (*i.e.*, ≥ -4): Any Amino Acid

$X_8$ are substitutions of S/R/T/N or substitutions having an equal or greater substitution score (*i.e.*, ≥ 0): R, N, S, or T

$X_9$ are substitutions of Y/A/S or substitutions having an equal or greater substitution score (*i.e.*, ≥ -2): A, R, N, C, Q, E, H, I, L, K, M, F, S, T, Y, or V

$X_{10}$ are substitutions of I/M/L/V or substitutions having an equal or greater substitution score (*i.e.*, ≥ +1): I, L, M, or V

$X_{11}$ are substitutions of Y/A/H or substitutions having an equal or greater substitution score (*i.e.*, ≥ -2): A, R, N, Q, E, H, I, K, M, F, S, T, or Y

**Light Chain CDR2**

| Antibody | Sequence | | | | | | | | | | | | | | | SEQ ID NO |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 3D10 | A | T | F | N | L | A | S | | | | | | | | | **24** |
| 3B6 | D | T | S | K | L | T | S | | | | | | | | | **25** |
| 1E12 | W | A | S | T | R | H | T | | | | | | | | | **26** |
| 2G11 | W | A | S | T | R | E | S | | | | | | | | | **27** |
| 1F4 | W | A | S | T | R | H | T | | | | | | | | | **28** |
| **Light Chain CDR2 Consensus Sequence:** | X 1 | X 2 | X 3 | X 4 | X 5 | X 6 | X 7 | | | | | | | | | **29** |

$X_1$ are substitutions of A/D/W or substitutions having an equal or greater substitution score (*i.e.*, ≥ -4): Any Amino Acid

**Table 2: Anti-Human B7-H1 Light Chain CDRs**

$X_2$ are substitutions of T/A or substitutions having an equal or greater substitution score (*i.e.*, ≥ 0): A, C, G, S, T, or V
$X_3$ are substitutions of F/S or substitutions having an equal or greater substitution score (*i.e.*, ≥ -2): A, C, H, I, L, M, F, S, T, Y, or V
$X_4$ are substitutions of N/K/T or substitutions having an equal or greater substitution score (*i.e.*, ≥ -1): R, N, D, Q, E, K, S, or T
$X_5$ are substitutions of L/R or substitutions having an equal or greater substitution score (*i.e.*, ≥ -2): A, R, Q, L, K, M, S, T, or Y
$X_6$ are substitutions of A/T/H/E or substitutions having an equal or greater substitution score (*i.e.*, ≥ -2): A, R, N, D, Q, E, G, H, K, M, F, P, S, T, or Y

$X_7$ are substitutions of S/T or substitutions having an equal or greater substitution score (*i.e.*, ≥ +1): A, N, S, or T

**Light Chain CDR3**

| Antibody | Sequence | | | | | | | | | | | | | | SEQ ID NO |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 3D10 | Q | Q | W | S | N | N | P | L | T | | | | | | **30** |
| 3B6 | H | Q | R | S | S | Y | P | W | T | | | | | | **31** |
| 1E12 | Q | Q | D | S | S | Y | P | L | T | | | | | | **32** |
| 2G11 | Q | Q | Y | Y | G | Y | P | L | T | | | | | | **33** |
| 1F4 | Q | Q | D | S | S | Y | P | L | T | | | | | | **34** |
| **Light Chain CDR3 Consensus Sequence:** | X 1 | **Q** | X 2 | X 3 | X 4 | X 5 | **P** | X 6 | **T** | | | | | | **35** |

$X_1$ are substitutions of Q/H or substitutions having an equal or greater substitution score (*i.e.*, ≥ 0): R, N, Q, E, or H
$X_2$ are substitutions of W/R/D/Y or substitutions having an equal or greater substitution score (*i.e.*, ≥ -4): Any Amino Acid
$X_3$ are substitutions of S/Y or substitutions having an equal or greater substitution score (*i.e.*, ≥ -2): A, R, N, C, Q, E, H, I, L, K, M, F, S, T, Y, or V
$X_4$ are substitutions of N/S/G or substitutions having an equal or greater substitution score (*i.e.*, ≥ 0): N, G, or S
$X_5$ are substitutions of N/Y or substitutions having an equal or greater substitution score (*i.e.*, ≥ 0): A, R, N, D, C, Q, E, G, H, I, L, K, M, F, P, S, T, W, Y, or V
$X_6$ are substitutions of K/S or substitutions having an equal or greater substitution score (*i.e.*, ≥ -2): A, R, N, D, Q, E, G, H, K, M, P, S, T, or Y

**[00116]** **Table 3** presents an analysis of the heavy chain CDRs of the anti-B7-H1 antibodies and provides the consensus sequence of the observed and preferred variant anti-B7-H1 heavy chain CDRs of the present invention.

**Table 3: Anti-Human B7-H1 Heavy Chain CDRs**

**Heavy Chain CDR1**

| Antibody | Sequence | | | | | | | | | | | | | | SEQ ID NO |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 3D10 | G | Y | T | F | P | D | Y | Y | M | N | | | | | **36** |
| 3B6 | G | Y | T | F | T | S | Y | D | I | N | | | | | **37** |
| 1E12 | G | Y | S | I | T | S | D | Y | W | N | | | | | **38** |
| 2G11 | G | Y | S | I | I | S | D | Y | W | N | | | | | **39** |
| 1F4 | G | F | S | L | T | T | Y | S | I | N | | | | | **40** |
| **Heavy Chain CDR1 Consensus Sequence:** | **G** | X 1 | X 2 | X 3 | X 4 | X 5 | X 6 | X 7 | X 8 | **N** | | | | | **41** |

**Table 3: Anti-Human B7-H1 Heavy Chain CDRs**

$X_1$ are substitutions of Y/F or substitutions having an equal or greater substitution score (*i.e.*, ≥ +3): Y or F

$X_2$ are substitutions of T/S or substitutions having an equal or greater substitution score (*i.e.*, ≥ +1): T or S

$X_3$ are substitutions of F/I/L or substitutions having an equal or greater substitution score (*i.e.*, ≥ 0): I, L, M, or F

$X_4$ are substitutions of P/T/I or substitutions having an equal or greater substitution score (*i.e.*, ≥ -3): A, R, N, D, C, Q, E, H, I, L, K, M, P, S, T, W, Y, or V

$X_5$ are substitutions of D/S/T or substitutions having an equal or greater substitution score (*i.e.*, ≥ -1): N, D, Q, E, K, P, S, or T

$X_6$ are substitutions of Y/D or substitutions having an equal or greater substitution score (*i.e.*, ≥ -3): A, R, N, D, C, Q, E, G, H, I, L, K, M, F, P, S, T, Y, or V

$X_7$ are substitutions of Y/D/S or substitutions having an equal or greater substitution score (*i.e.*, ≥ 0): A, R, N, D, C, Q, E, G, H, I, L, K, M, F, P, S, T, Y, or V

$X_8$ are substitutions of M/I/W or substitutions having an equal or greater substitution score (*i.e.*, ≥ -3): A, R, C, Q, E, H, I, L, K, M, F, S, T, W, Y, or V

**Heavy Chain CDR2**

| **Antibody** | **Sequence** | | | | | | | | | | | | | | | | | **SEQ ID NO** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 3D10 | D | I | D | P | N | Y | G | G | T | T | Y | N | Q | K | F | K | G | **42** |
| 3B6 | W | I | F | P | R | D | N | N | T | K | Y | N | E | N | F | K | G | **43** |
| 1E12 | Y | I | S | | Y | T | G | S | T | Y | Y | N | P | S | L | K | S | **44** |
| 2G11 | Y | I | S | | Y | T | G | S | T | Y | Y | N | P | S | L | K | S | **45** |
| 1F4 | V | M | W | | A | G | G | G | T | N | S | N | S | V | L | K | S | **46** |
| **Heavy Chain CDR2 Consensus Sequence:** | X 1 | X 2 | X 3 | X 4 | X 5 | X 6 | X 7 | X 8 | **T** | X 9 | X 10 | **N** | X 11 | X 12 | X 13 | **K** | X 14 | **47** |

$X_1$ are substitutions of D/W/Y/V or substitutions having an equal or greater substitution score (*i.e.*, ≥ -4): Any Amino acid

$X_2$ are substitutions of I/M or substitutions having an equal or greater substitution score (*i.e.*, ≥ +1): I, L, M, F, or V

$X_3$ are substitutions of D/F/S/W or substitutions having an equal or greater substitution score (*i.e.*, ≥ -4): Any Amino Acid

$X_4$ is P or absent

$X_5$ are substitutions of N/R/Y/A or substitutions having an equal or greater substitution score (*i.e.*, ≥ -2): A, R, N, Q, E, H, K, M, S, T, Y, or V

$X_6$ are substitutions of Y/D/T/G or substitutions having an equal or greater substitution score (*i.e.*, ≥ -3): A, R, N, D, C, Q, E, G, H, K, M, F, P, S, T, Y, or V

$X_7$ are substitutions of N/G or substitutions having an equal or greater substitution score (*i.e.*, ≥ 0): N, G, or S

$X_8$ are substitutions of N/G/S or substitutions having an equal or greater substitution score (*i.e.*, ≥ 0): N, G, or S

$X_9$ are substitutions of K/T/Y/N or substitutions having an equal or greater substitution score (*i.e.*, ≥ -2): A, R, N, Q, E, H, K, M, S, T, or Y

$X_{10}$ are substitutions of Y/S or substitutions having an equal or greater substitution score (*i.e.*, ≥ -2): A, R, N, C, Q, E, H, I, L, K, M, F, S, T, W, Y, or V

$X_{11}$ are substitutions of Q/E/P/S or substitutions having an equal or greater substitution score (*i.e.*, ≥ -1): A, D, Q, E, K, P, S, or T

$X_{12}$ are substitutions of K/N/S/V or substitutions having an equal or greater substitution score (*i.e.*, ≥ -3): A, R, N, D, C, Q, E, G, H, I, L, K, M, F, P, S, T, Y, or V

$X_{13}$ are substitutions of F/L or substitutions having an equal or greater substitution score (*i.e.*, ≥ 0): I, L, M, or F

$X_{14}$ are substitutions of G/S or substitutions having an equal or greater substitution score (*i.e.*, ≥ 0): A, G, or S

**Table 3: Anti-Human B7-H1 Heavy Chain CDRs**

| Heavy Chain CDR3 | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Antibody** | **Sequence** | | | | | | | | | | | | | | | **SEQ ID NO** |
| 3D10 | | | G | A | L | | | | | | | | | | | **48** |
| 3B6 | E | N | W | V | G | D | F | | | | | | | | | **49** |
| 1E12 | Y | G | G | W | L | S | P | F | | | | | | | | **50** |
| 2G11 | R | G | G | W | L | L | P | F | | | | | | | | **51** |
| 1F4 | Y | Y | G | N | S | P | Y | Y | AI | | | | | | | **52** |
| **Heavy Chain CDR3 Consensus Sequence:** | X 1 | X 2 | X 3 | X 4 | X 5 | X 6 | X 7 | X 8 | X 9 | | | | | | | **53** |

$X_1$ are substitutions of E/Y/R or substitutions having an equal or greater substitution score (*i.e.*, ≥ -2): A, R, N, Q, E, H, K, M, S, T, or Y
$X_2$ are substitutions of N/G/Y or substitutions having an equal or greater substitution score (*i.e.*, ≥ -3): A, R, N, D, C, Q, E, G, H, K, M, F, P, S, T, Y, or V
$X_3$ are substitutions of G/W or substitutions having an equal or greater substitution score (*i.e.*, ≥ -2): Q, G, H, T, or W
$X_4$ are substitutions of A/V/W/N or substitutions having an equal or greater substitution score (*i.e.*, ≥ -4): Any Amino Acid
$X_5$ are substitutions of L/G/S or substitutions having an equal or greater substitution score (*i.e.*, ≥ -4): Any Amino Acid
$X_6$ are substitutions of D/S/L/P or substitutions having an equal or greater substitution score (*i.e.*, ≥ -4): Any Amino Acid
$X_7$ are substitutions of F/P/Y or substitutions having an equal or greater substitution score (*i.e.*, ≥ -4): Any Amino Acid
$X_8$ are substitutions of F/Y or substitutions having an equal or greater substitution score (*i.e.*, ≥ +3): F or Y
$X_9$ is AI or absent

**[00117]** Thus, in addition to antibodies and antigen-binding fragments thereof that possess the CDRs of the anti-B7-H1 antibodies: 1E12, 1F4, 2G11, 3B6, and 3D10, the invention additionally provides antibodies and antigen-binding fragments thereof that possess CDRs having the above-described light and/or heavy chain consensus sequences.

**[00118]** **Table 4** presents an analysis of the light chain CDRs of the anti-PD-1 antibodies and provides the consensus sequence of the observed and preferred variant light chain anti-PD-1 CDRs of the present invention.

**Table 4: Anti-Human PD-1 Light Chain CDRs**

| Light Chain CDR1 | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Antibody** | **Sequence** | | | | | | | | | | | | | | | **SEQ ID NO** |
| 1H3 | S | A | S | S | | S | V | S | Y | M | Y | | | | | **54** |
| 1E3 | K | A | S | Q | D | I | N | N | Y | M | A | | | | | **55** |
| 1E8 | K | A | S | Q | S | V | D | T | N | V | A | | | | | **56** |
| **Light Chain CDR1 Consensus Sequence:** | X 1 | **A** | **S** | X 2 | X 3 | X 4 | X 5 | X 6 | X 7 | X 8 | X 9 | | | | | **57** |

$X_1$ are substitutions of K/S or substitutions having an equal or greater substitution score (*i.e.*, ≥ 0): N, Q, E, K, or S
$X_2$ are substitutions of S/Q or substitutions having an equal or greater substitution score (*i.e.*, ≥ 0): N, D, Q, E, K, or S
$X_3$ is absent or are substitutions of D/S or substitutions having an equal or greater substitution score (*i.e.*, ≥ 0): N, D, Q, E, or S

**Table 4: Anti-Human PD-1 Light Chain CDRs**

$X_4$ are substitutions of S/I/V or substitutions having an equal or greater substitution score (*i.e.*, ≥ -2): A, C, I, L, M, F, S, T, Y, or V
$X_5$ are substitutions of V/N/D or substitutions having an equal or greater substitution score (*i.e.*, ≥ -3): A, R, N, D, C, Q, E, G, H, I, K, M, F, P, S, T, Y, or V
$X_6$ are substitutions of S/N/T or substitutions having an equal or greater substitution score (*i.e.*, ≥ 0): N, S, or T
$X_7$ are substitutions of Y/N or substitutions having an equal or greater substitution score (*i.e.*, ≥ -2): A, R, N, D, Q, E, G, H, K, M, P, S, T, or Y
$X_8$ are substitutions of M/V or substitutions having an equal or greater substitution score (*i.e.*, ≥ +1): I, L, M, or V
$X_9$ are substitutions of Y/A or substitutions having an equal or greater substitution score (*i.e.*, ≥ -2): A, R, N, C, Q, E, H, I, L, K, M, F, S, T, Y, or V

**Light Chain CDR2**

| **Antibody** | **Sequence** | | | | | | | | | | | | | | **SEQ ID NO** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1H3 | L | T | S | N | L | A | S | | | | | | | | **58** |
| 1E3 | Y | T | S | T | L | L | S | | | | | | | | **59** |
| 1E8 | S | A | S | Y | R | Y | S | | | | | | | | **60** |
| **Light Chain CDR2 Consensus Sequence:** | X 1 | X 2 | **S** | X 3 | X 4 | X 5 | **S** | | | | | | | | **61** |

$X_1$ are substitutions of L/Y/S or substitutions having an equal or greater substitution score (*i.e.*, ≥ -2): A, R, C, Q, I, L, K, M, F, S, T, Y, or V
$X_2$ are substitutions of T/A or substitutions having an equal or greater substitution score (*i.e.*, ≥ 0): A, S, T, or V
$X_3$ are substitutions of N/T/Y or substitutions having an equal or greater substitution score (*i.e.*, ≥ 2): A, R, N, Q, E, H, K, M, S, T, or Y
$X_4$ are substitutions of L/R or substitutions having an equal or greater substitution score (*i.e.*, ≥ -2): A, R, N, Q, L, K, M, S, T, or Y
$X_5$ are substitutions of A/L/Y or substitutions having an equal or greater substitution score (*i.e.*, ≥ -2): A, R, C, Q, I, L, K, M, F, S, T, Y, or V

**Light Chain CDR3**

| **Antibody** | **Sequence** | | | | | | | | | | | | | | **SEQ ID NO** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1H3 | Q | Q | W | S | S | N | P | F | T | | | | | | **62** |
| 1E3 | L | Q | Y | D | N | L | | W | T | | | | | | **63** |
| 1E8 | Q | Q | Y | N | S | Y | P | Y | T | | | | | | **64** |
| **Light Chain CDR3 Consensus Sequence:** | X 1 | **Q** | X 2 | X 3 | X 4 | X 5 | X 6 | X 7 | **T** | | | | | | **65** |

$X_1$ are substitutions of Q/L or substitutions having an equal or greater substitution score (*i.e.*, ≥ -2): A, R, N, Q, L, K, M, S, T, W, Y, or V
$X_2$ are substitutions of W/Y or substitutions having an equal or greater substitution score (*i.e.*, ≥ +2): W or Y
$X_3$ are substitutions of S/D/N or substitutions having an equal or greater substitution score (*i.e.*, ≥ 0): N, D, Q, E, or S
$X_4$ are substitutions of S/N or substitutions having an equal or greater substitution score (*i.e.*, ≥ +1): N, S, or T
$X_5$ are substitutions of N/L/Y or substitutions having an equal or greater substitution score (*i.e.*, ≥ -3): A, R, N, C, Q, E, H, I, L, K, M, F, P, S, T, Y, or V
$X_6$ is absent or P
$X_7$ are substitutions of F/W/Y or substitutions having an equal or greater substitution score (*i.e.*, ≥ +1): F, W, or Y

**[00119]** **Table 5** presents an analysis of the heavy chain CDRs of the anti-PD-1 antibodies and provides the consensus sequence of the observed and preferred variant anti-PD-1 heavy chain CDRs of the present invention.

**Table 5: Anti-Human PD-1 Heavy Chain CDRs**

**Heavy Chain CDR1**

| Antibody | Sequence | | | | | | | | | | | | | | | SEQ ID NO |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1H3 | G | F | T | F | S | D | Y | G | M | H | | | | | | **66** |
| 1E3 | G | Y | T | F | T | D | Y | Y | M | N | | | | | | **67** |
| 1E8 | G | Y | T | F | T | N | Y | W | M | H | | | | | | **68** |
| **Heavy Chain CDR1 Consensus Sequence:** | **G** | X 1 | **T** | **F** | X 2 | X 3 | **Y** | X 4 | **M** | X 5 | | | | | | **69** |

$X_1$ are substitutions of F/Y or substitutions having an equal or greater substitution score (*i.e.*, ≥ +3): F or Y

$X_2$ are substitutions of S/T or substitutions having an equal or greater substitution score (*i.e.*, ≥ +1): S or T

$X_3$ are substitutions of D/N or substitutions having an equal or greater substitution score (*i.e.*, ≥ +1): N or D

$X_4$ are substitutions of G/Y/W or substitutions having an equal or greater substitution score (*i.e.*, ≥ -3): A, R, C, Q, E, G, H, K, M, F, S, T, W, Y, or V

$X_5$ are substitutions of H/N or substitutions having an equal or greater substitution score (*i.e.*, ≥ +1): N or H

**Heavy Chain CDR2**

| Antibody | Sequence | | | | | | | | | | | | | | | | | SEQ ID NO |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1H3 | Y | I | S | S | G | S | Y | T | I | Y | Y | T | D | T | V | K | G | **70** |
| 1E3 | N | I | N | P | Y | N | G | G | T | T | Y | N | Q | K | F | K | G | **71** |
| 1E8 | H | I | N | P | S | S | G | F | T | T | Y | N | Q | N | F | K | D | **72** |
| **Heavy Chain CDR2 Consensus Sequence:** | x 1 | **I** | X 2 | X 3 | X 4 | X 5 | X 6 | X 7 | X 8 | X 9 | **Y** | X 10 | X 11 | X 12 | X 13 | **K** | X 14 | **73** |

$X_1$ are substitutions of Y/N/H or substitutions having an equal or greater substitution score (*i.e.*, ≥ -2): A, R, N, Q, E, H, K, M, S, T, or Y

$X_2$ are substitutions of S/N or substitutions having an equal or greater substitution score (*i.e.*, ≥ +1):N or S

$X_3$ are substitutions of S/P or substitutions having an equal or greater substitution score (*i.e.*, ≥ -1): A, D, Q, E, K, P, S, or T

$X_4$ are substitutions of G/Y/S or substitutions having an equal or greater substitution score (*i.e.*, ≥ -3): A, R, N, D, C, Q, E, G, H, K, M, F, P, S, T, W, Y, or V

$X_5$ are substitutions of S/N or substitutions having an equal or greater substitution score (*i.e.*, ≥ 0): N or S

$X_6$ are substitutions of Y/G or substitutions having an equal or greater substitution score (*i.e.*, ≥ -3): A, R, N, D, C, Q, E, G, H, K, M, F, P, S, T, W, Y, or V

$X_7$ are substitutions of T/G/F or substitutions having an equal or greater substitution score (*i.e.*, ≥ -3): A, R, N, D, C, Q, E, G, H, K, M, F, S, T, W, Y, or V

$X_8$ are substitutions of I/T or substitutions having an equal or greater substitution score (*i.e.*, ≥ -1): A, C, I, L, M, T, or V

$X_9$ are substitutions of Y/T or substitutions having an equal or greater substitution score (*i.e.*, ≥ -2): A, R, N, C, Q, E, H, I, L, K, M, F, S, T, W, Y, or V

$X_{10}$ are substitutions of T/N or substitutions having an equal or greater substitution score (*i.e.*, ≥ 0): N, S, or T

$X_{11}$ are substitutions of D/Q or substitutions having an equal or greater substitution score (*i.e.*, ≥ 0): N, D, Q, E, or S

$X_{12}$ are substitutions of T/K/N or substitutions having an equal or greater substitution score (*i.e.*, ≥ -1): R, N, D, Q, E, K, S, or T

$X_{13}$ are substitutions of V/F or substitutions having an equal or greater substitution score (*i.e.*, ≥ -1): I, L, M, W, Y, or V

$X_{14}$ are substitutions of G/D or substitutions having an equal or greater substitution score (*i.e.*, ≥ -1): N, D, G, or S

**Table 5: Anti-Human PD-1 Heavy Chain CDRs**

| Heavy Chain CDR3 | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Antibody** | **Sequence** | | | | | | | | | | | | | | | **SEQ ID NO** |
| 1H3 | R | G | Y | G | S | F | Y | E | YYF | | D | Y | | | | **74** |
| 1E3 | G | R | I | Y | D | G | S | L | | | D | Y | | | | **75** |
| 1E8 | E | D | Y | D | V | | | | | | D | Y | | | | **76** |
| **Heavy Chain CDR3 Consensus Sequence:** | X 1 | X 2 | X 3 | X 4 | X 5 | X 6 | X 7 | X 8 | X 9 | | **D** | **Y** | | | | **77** |

$X_1$ are substitutions of R/G/E or substitutions having an equal or greater substitution score (*i.e.*, ≥ -2): A, R, N, D, Q, E, G, H, K, P, S, or T
$X_2$ are substitutions of G/R/D or substitutions having an equal or greater substitution score (*i.e.*, ≥ -2): A, R, N, D, Q, E, G, H, K, P, S, or T
$X_3$ are substitutions of Y/I or substitutions having an equal or greater substitution score (*i.e.*, ≥ -1): I, L, M, F, Y, or V
$X_4$ are substitutions of Y/G/D or substitutions having an equal or greater substitution score (*i.e.*, ≥ -3): A, R, N, D, C, Q, E, G, H, K, M, F, P, S, T, W, Y, or V
$X_5$ are substitutions of S/D/V or substitutions having an equal or greater substitution score (*i.e.*, ≥ -3): A, R, N, D, C, Q, E, G, H, I, K, M, F, P, S, T, Y, or V
$X_6$ is absent or are substitutions of F/G or substitutions having an equal or greater substitution score (*i.e.*, ≥ -3): A, R, N, D, C, Q, E, G, H, K, M, F, S, T, W, Y, or V
$X_7$ is absent or are substitutions of Y/S or substitutions having an equal or greater substitution score (*i.e.*, ≥ -2): A, R, N, C, Q, E, H, I, L, K, M, F, S, T, Y, or V
$X_8$ is absent or are substitutions of E/L or substitutions having an equal or greater substitution score (*i.e.*, ≥ -3): A, R, N, Q, E, H, I, L, K, M, F, P, S, T, W, Y, or V
$X_9$ is absent or is YYF

**[00120]** Thus, in addition to antibodies and antigen-binding fragments thereof that possess the CDRs of the anti-PD-1 antibodies: 1E3, 1E8, and 1H3, the invention additionally provides antibodies and antigen-binding fragments thereof that possess CDRs having the above-described light and/or heavy chain consensus sequences.

**[00121]** The present invention encompasses antibodies or fragments thereof comprising an amino acid sequence of a variable heavy chain and/or variable light chain that is at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to the amino acid sequence of the variable heavy chain and/or light chain of the mouse monoclonal antibody produced by any of the above clones, and which exhibit immunospecific binding to B7-H1 or PD-1. The present invention further encompasses antibodies or fragments thereof that comprise a CDR that is at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to the amino acid sequence of a CDR of the above-listed clones and which exhibit immunospecific binding to B7-H1 or PD-1. The determination of percent identity of two amino acid sequences can be determined by BLAST protein comparison.

**[00122]** In a specific embodiment, an antibody or an antigen-binding fragment thereof of the present invention will comprise one, two, three, four, five, or more preferably, all six of the CDRs of the above-described preferred antibodies and will exhibit the ability to bind to human B7-H1 or PD-1.

### B. Therapeutic and Prophylactic Uses of the Preferred Compositions of the Present Invention

**[00123]** The invention particularly relates to the therapeutic and/or prophylactic use of molecules (especially antibodies or their antigen-binding fragments) that immunospecifically bind human B7-H1 or human PD-1, and/or that are capable of modulating the binding between B7-H1 and PD-1 as such molecules (B7-H1 or PD-1) are endogenously expressed and arrayed in a subject (*e.g.*, a human patient) and/or are capable of modulating signaling *via* PD-1 or B7-H1.

**[00124]** As used herein, the terms "**treat**," "**treating**," "**treatment**" and "**therapeutic use**" refer to the elimination, reduction or amelioration of one or more symptoms of a disease or disorder exacerbated by the interactions of B7-H1 and PD-1. As used herein, a "**therapeutically effective amount**" refers to that amount of a therapeutic agent sufficient to mediate a clinically relevant elimination, reduction or amelioration of such symptoms. An effect is clinically relevant if its magnitude is sufficient to impact the health or prognosis of a recipient subject. A therapeutically effective amount may refer to the amount of therapeutic agent sufficient to delay or minimize the onset of disease, *e.g.*, delay or minimize the spread of cancer. A therapeutically effective amount may also refer to the amount of the therapeutic agent that provides a therapeutic benefit in the treatment or management of a disease. Further, a therapeutically effective amount with respect to a therapeutic agent of the invention means that amount of therapeutic agent alone, or in combination with other therapies, that provides a therapeutic benefit in the treatment or management of a disease, *e.g.*, sufficient to enhance the therapeutic efficacy of a therapeutic antibody sufficient to treat or manage a disease.

**[00125]** As used herein, the term "**prophylactic agent**" refers to an agent that can be used in the prevention of a disorder or disease prior to the detection of any symptoms of such disorder or disease. A "**prophylactically effective**" amount is the amount of prophylactic agent sufficient to mediate such protection. A prophylactically effective amount may also refer to the amount of the prophylactic agent that provides a prophylactic benefit in the

prevention of disease. Further, a prophylactically effective amount with respect to a prophylactic agent of the invention means that amount of prophylactic agent alone, or in combination with other agents, that provides a prophylactic benefit in the prevention of disease.

**[00126]** The dosage amounts and frequencies of administration provided herein are encompassed by the terms therapeutically effective and prophylactically effective. The dosage and frequency further will typically vary according to factors specific for each patient depending on the specific therapeutic or prophylactic agents administered, the severity and type of cancer, the route of administration, as well as age, body weight, response, and the past medical history of the patient. Suitable regimens can be selected by one skilled in the art by considering such factors and by following, for example, dosages reported in the literature and recommended in the *Physician's Desk Reference* (56$^{th}$ ed., 2002).

### 1. Uses of Up-Modulators of the Immune System

**[00127]** In a preferred embodiment, such antibodies and fragments bind to these antigens at one or more sites to B7-H1 or PD-1 proximal to and disruptive of the B7-H1 – PD-1 binding site. As discussed above, interactions between PD-1 and B7-H1 inhibit the proliferation of T cells and reduce the production of multiple cytokines (see, Sharpe, A.H. et al. (2002) "*The B7-CD28 Superfamily*," Nature Rev. Immunol. 2:116-126). Thus, in a preferred embodiment, the administration of the molecules of the present invention to a subject **up-modulates** the immune system of the subject by antagonizing B7-H1 – PD-1 binding. In another embodiment, the avidity and/or affinity of the anti-PD-1 antibody may be such that it only binds to (and blocks B7-H1 binding to) T cells that express very high levels of PD-1, which are the exhausted or dysfunctional T cells, and thus allow specific targeting of this cell population. Thus, the invention relates to the use of the antibodies of the present invention to mediate increased production of IFN-γ. Thus, it relates to the use of such antibodies in the treatment of diseases and conditions that are treatable with IFN-γ, such as ovarian and other forms of cancer, chronic granulomatous disease, osteopetrosis, Friedreich's ataxia, *etc.* Additionally, the invention particularly relates to the use of the antibodies of the present invention to mediate increased T cell proliferation. Thus, it relates to the use of such antibodies in the treatment of diseases and conditions that are treatable by increasing T cell proliferation, such as: AIDS; severe combined immunodeficiency (SCID); Omenn syndrome; Cartilage-hair hypoplasia; T cell loss or ablation incident to organ or tissue transplantation or

chemotherapy; hypogammaglobulinemia; X-linked agammaglobulinemia; Transient hypogammaglobulinemia; dysgammaglobulinemia; IgA deficiency ·IgG deficiency;·IgM deficiency;·hyper IgM syndrome;·Wiskott-Aldrich syndrome;·hyper-IgE syndrome; common variable immunodeficiency; ICF syndrome; thymic hypoplasia (*e.g.*; Di George's syndrome; Nezelof syndrome; Ataxia telangiectasia); purine nucleoside phosphorylase deficiency; adenosine deaminase deficiency;·ZAP70 deficiency;·Bare lymphocyte syndrome; leukopenia; lymphocytopenia (*e.g.*; idiopathic CD4+ lymphocytopenia); or a complement deficiency. The invention particularly relates to the use of the antibodies of the present invention to mediate *both* increased production of IFN-γ and increased T cell proliferation.

**[00128]** Up-modulation of the immune system is particularly desirable in the treatment of cancers and chronic infections, and thus the present invention has utility in the treatment of such disorders. Both PD-1 and B7-H1 are over-expressed upon HIV infection (Xu, Huanbin *et al.* (2010) "*Increased B7-H1 Expression on Dendritic Cells Correlates with Programmed Death 1 Expression on T Cells in Simian Immunodeficiency Virus-Infected Macaques and May Contribute to T Cell Dysfunction and Disease Progression*," J. Immunol. 185:7340-7348; Grabmeier-Pfistershammer, K. *et al.* (2011) "*Identification of PD-1 as a Unique Marker for Failing Immune Reconstitution in HIV-1–Infected Patients on Treatment*," J Acquir. Immune Defic. Syndr. 56(2):118-124). Thus, expression of B7-H1 on such cells may be used to diagnose HIV in humans. Thus, the anti-PD-1 and anti-B7-H1 antibodies of the present invention have particular utility as therapeutics for HIV infection and AIDS treatment. As used herein, the term "cancer" refers to a neoplasm or tumor resulting from abnormal uncontrolled growth of cells. As used herein, cancer explicitly includes, leukemias and lymphomas. The term "cancer" refers to a disease involving cells that have the potential to metastasize to distal sites and exhibit phenotypic traits that differ from those of non-cancer cells, for example, formation of colonies in a three-dimensional substrate such as soft agar or the formation of tubular networks or web-like matrices in a three-dimensional basement membrane or extracellular matrix preparation. Non-cancer cells do not form colonies in soft agar and form distinct sphere-like structures in three-dimensional basement membrane or extracellular matrix preparations.

**[00129]** Cancers and related disorders that can be treated or prevented by methods and compositions of the present invention include, but are not limited to, the following: leukemias including, but not limited to, acute leukemia, acute lymphocytic leukemia, acute myelocytic

leukemias such as myeloblastic, promyelocytic, myelomonocytic, monocytic, erythroleukemia leukemias and myelodysplastic syndrome, chronic leukemias such as but not limited to, chronic myelocytic (granulocytic) leukemia, chronic lymphocytic leukemia, hairy cell leukemia; polycythemia vera; lymphomas such as, but not limited to, Hodgkin's disease or non-Hodgkin's disease lymphomas (*e.g.*, diffuse anaplastic lymphoma kinase (ALK) negative, large B-cell lymphoma (DLBCL); diffuse anaplastic lymphoma kinase (ALK) positive, large B-cell lymphoma (DLBCL); anaplastic lymphoma kinase (ALK) positive, ALK+ anaplastic large-cell lymphoma (ALCL), acute myeloid lymphoma (AML)); multiple myelomas such as, but not limited to, smoldering multiple myeloma, nonsecretory myeloma, osteosclerotic myeloma, plasma cell leukemia, solitary plasmacytoma and extramedullary plasmacytoma; Waldenström's macroglobulinemia; monoclonal gammopathy of undetermined significance; benign monoclonal gammopathy; heavy chain disease; bone and connective tissue sarcomas such as, but not limited to, bone sarcoma, osteosarcoma, chondrosarcoma, Ewing's sarcoma, malignant giant cell tumor, fibrosarcoma of bone, chordoma, periosteal sarcoma, soft-tissue sarcomas, angiosarcoma (hemangiosarcoma), fibrosarcoma, Kaposi's sarcoma, leiomyosarcoma, liposarcoma, lymphangiosarcoma, neurilemmoma, rhabdomyosarcoma, synovial sarcoma; brain tumors including but not limited to, glioma, astrocytoma, brain stem glioma, ependymoma, oligodendroglioma, nonglial tumor, acoustic neurinoma, craniopharyngioma, medulloblastoma, meningioma, pineocytoma, pineoblastoma, primary brain lymphoma; breast cancer including, but not limited to, adenocarcinoma, lobular (small cell) carcinoma, intraductal carcinoma, medullary breast cancer, mucinous breast cancer, tubular breast cancer, papillary breast cancer, Paget's disease, and inflammatory breast cancer; adrenal cancer, including but not limited to, pheochromocytom and adrenocortical carcinoma; thyroid cancer such as but not limited to papillary or follicular thyroid cancer, medullary thyroid cancer and anaplastic thyroid cancer; pancreatic cancer, including but not limited to, insulinoma, gastrinoma, glucagonoma, vipoma, somatostatin-secreting tumor, and carcinoid or islet cell tumor; pituitary cancers including but not limited to, Cushing's disease, prolactin-secreting tumor, acromegaly, and diabetes insipius; eye cancers including, but not limited to, ocular melanoma such as iris melanoma, choroidal melanoma, and cilliary body melanoma, and retinoblastoma; vaginal cancers, including, but not limited to, squamous cell carcinoma, adenocarcinoma, and melanoma; vulvar cancer, including but not limited to, squamous cell carcinoma, melanoma, adenocarcinoma, basal cell carcinoma, sarcoma, and Paget's disease; cervical cancers including, but not limited to, squamous cell carcinoma, and adenocarcinoma; uterine cancers

including, but not limited to, endometrial carcinoma and uterine sarcoma; ovarian cancers including, but not limited to, ovarian epithelial carcinoma, borderline tumor, germ cell tumor, and stromal tumor; esophageal cancers including, but not limited to, squamous cancer, adenocarcinoma, adenoid cyctic carcinoma, mucoepidermoid carcinoma, adenosquamous carcinoma, sarcoma, melanoma, plasmacytoma, verrucous carcinoma, and oat cell (small cell) carcinoma; stomach cancers including, but not limited to, adenocarcinoma, fungating (polypoid), ulcerating, superficial spreading, diffusely spreading, malignant lymphoma, liposarcoma, fibrosarcoma, and carcinosarcoma; colon cancers; rectal cancers; liver cancers including, but not limited to, hepatocellular carcinoma and hepatoblastoma, gallbladder cancers including, but not limited to, adenocarcinoma; cholangiocarcinomas including, but not limited to, papillary, nodular, and diffuse; lung cancers including but not limited to, non-small cell lung cancer, squamous cell carcinoma (epidermoid carcinoma), adenocarcinoma, large-cell carcinoma and small-cell lung cancer; testicular cancers including, but not limited to, germinal tumor, seminoma, anaplastic, classic (typical), spermatocytic, nonseminoma, embryonal carcinoma, teratoma carcinoma, choriocarcinoma (yolk-sac tumor), prostate cancers including, but not limited to, adenocarcinoma, leiomyosarcoma, and rhabdomyosarcoma; penal cancers; oral cancers including, but not limited to, squamous cell carcinoma; basal cancers; salivary gland cancers including, but not limited to, adenocarcinoma, mucoepidermoid carcinoma, and adenoidcystic carcinoma; pharynx cancers including, but not limited to, squamous cell cancer, and verrucous; skin cancers including, but not limited to, basal cell carcinoma, squamous cell carcinoma and melanoma, superficial spreading melanoma, nodular melanoma, lentigo malignant melanoma, acral lentiginous melanoma; kidney cancers including, but not limited to, renal cell cancer, adenocarcinoma, hypernephroma, fibrosarcoma, transitional cell cancer (renal pelvis and/ or uterer); Wilms' tumor; bladder cancers including, but not limited to, transitional cell carcinoma, squamous cell cancer, adenocarcinoma, carcinosarcoma. In addition, cancers include myxosarcoma, osteogenic sarcoma, endotheliosarcoma, lymphangioendotheliosarcoma, mesothelioma, synovioma, hemangioblastoma, epithelial carcinoma, cystadenocarcinoma, bronchogenic carcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma and papillary adenocarcinomas (for a review of such disorders, *see* Fishman *et al.*, 1985, *Medicine*, 2d Ed., J.B. Lippincott Co., Philadelphia and Murphy *et al.*, 1997, *Informed Decisions: The Complete Book of Cancer Diagnosis, Treatment, and Recovery*, Viking Penguin, Penguin Books U.S.A., Inc., United States of America).

**[00130]** Accordingly, the methods and compositions of the invention are also useful in the treatment or prevention of a variety of cancers or other abnormal proliferative diseases, including (but not limited to) the following: carcinoma, including that of the bladder, breast, colon, kidney, liver, lung, ovary, pancreas, stomach, cervix, thyroid and skin; including squamous cell carcinoma; hematopoietic tumors of lymphoid lineage, including leukemia, acute lymphocytic leukemia, acute lymphoblastic leukemia, B-cell lymphoma, T-cell lymphoma, Berketts lymphoma; hematopoietic tumors of myeloid lineage, including acute and chronic myelogenous leukemias and promyelocytic leukemia; tumors of mesenchymal origin, including fibrosarcoma and rhabdomyoscarcoma; other tumors, including melanoma, seminoma, tetratocarcinoma, neuroblastoma and glioma; tumors of the central and peripheral nervous system, including astrocytoma, neuroblastoma, glioma, and schwannomas; tumors of mesenchymal origin, including fibrosafcoma, rhabdomyoscarama, and osteosarcoma; and other tumors, including melanoma, xenoderma pegmentosum, keratoactanthoma, seminoma, thyroid follicular cancer and teratocarcinoma. It is also contemplated that cancers caused by aberrations in apoptosis would also be treated by the methods and compositions of the invention. Such cancers may include, but are not be limited to, follicular lymphomas, carcinomas with p53 mutations, hormone dependent tumors of the breast, prostate and ovary, and precancerous lesions such as familial adenomatous polyposis, and myelodysplastic syndromes. In specific embodiments, malignancy or dysproliferative changes (such as metaplasias and dysplasias), or hyperproliferative disorders, are treated or prevented by the methods and compositions of the invention in the ovary, bladder, breast, colon, lung, skin, pancreas, or uterus. In other specific embodiments, sarcoma, melanoma, or leukemia is treated or prevented by the methods and compositions of the invention.

**[00131]** Cancer cells acquire a characteristic set of functional capabilities during their development, albeit through various mechanisms. Such capabilities include evading apoptosis, self-sufficiency in growth signals, insensitivity to anti-growth signals, tissue invasion/metastasis, limitless explicative potential, and sustained angiogenesis. The term "cancer cell" is meant to encompass both pre-malignant and malignant cancer cells. In some embodiments, cancer refers to a benign tumor, which has remained localized. In other embodiments, cancer refers to a malignant tumor, which has invaded and destroyed neighboring body structures and spread to distant sites. In yet other embodiments, the cancer is associated with a specific cancer antigen (e.g., pan-carcinoma antigen (KS 1/4), ovarian

carcinoma antigen (CA125), prostate specific antigen (PSA), carcinoembryonic antigen (CEA), CD19, CD20, HER2/neu, etc.).

**[00132]** The antibodies and antibody fragments of the present invention are particularly useful for the treatment of cancers that are associated with cells (*e.g.*, exhausted T cells, B cells, monocytes, *etc.*) that express abnormally high levels of PD-1 (Youngblood, B. (2011) "*Chronic Virus Infection Enforces Demethylation Of The Locus That Encodes PD-1 In Antigen-Specific CD8(+) T Cells*," Immunity 35(3):400-412; Spahn, J. *et al.* (2011) "*Ineffective CD8(+) T-Cell Immunity To Adeno-Associated Virus Can Result In Prolonged Liver Injury And Fibrogenesis*," Amer. J. Pathol. 179(5):2370-2381; Wang, C. *et al.* (2011) "*Phenotype, Effector Function, And Tissue Localization Of PD-1-Expressing Human Follicular Helper T Cell Subsets*," BMC Immunol. 12:53, 1-15; Eichbaum, Q. (2011) "*PD-1 Signaling In HIV And Chronic Viral Infection Potential For Therapeutic Intervention?*" Curr. Med. Chem. 18(26):3971-3980; Hallett, W.H. *et al.* (2011) "*Immunosuppressive Effects Of Multiple Myeloma Are Overcome By PD-L1 Blockade*," Biol Blood Marrow Transplant. 17(8):1133-1145; Ni, L. *et al.* (2010) "*PD-1 Modulates Regulatory T Cells And Suppresses T-Cell Responses In HCV-Associated Lymphoma*," Immunol. Cell. Biol. 89(4):535-539; Inozume, T. *et al.* (2010) "*Selection Of CD8+PD-1+ Lymphocytes In Fresh Human Melanomas Enriches For Tumor-Reactive T Cells*," J. Immunother. 33(9):956-964; and Jin, H.T. *et al.* (2010) "*Cooperation Of Tim-3 And PD-1 In CD8 T-Cell Exhaustion During Chronic Viral Infection*," Proc. Natl. Acad. Sci. (USA) 107(33):14733-14738).

**[00133]** Similar to its application to tumors as discussed above, the antibodies and antigen-binding fragments of the present invention can be used alone, or as an adjuvant, in combination with vaccines or with antimibrobial agents, to stimulate the immune response against toxins or self-antigens or against pathogens (*e.g.*, viruses, such as HIV, HTLV, hepatitis virus, influenza virus, respiratory syncytial virus, vaccinia virus, rabies virus; bacteria, such as those of *Mycobacteria*, *Staphylococci*, *Streptococci*, *Pneumonococci*, *Meningococci*, *Conococci*, *Klebsiella, Proteus, Serratia, Pseudomonas, Legionella, Corynebacteria*, *Salmonella, Vibrio*, *Clostridia*, *Bacilli*, *Pasteurella*, *Leptospirosis*, *Bordatella*, and particularly such pathogens associated with cholera, tetanus, botulism, anthrax, plague, and Lyme disease; or fungal or parasitic pathogens, such as *Candida (albicans, krusei, glabrata, tropicalis,* etc.), *Cryptococcus*, *Aspergillus (jumigatus, niger,* etc.), Genus Mucorales *(mucor, absidia, rhizophus), Sporothrix (schenkii), Blastomyces*

*(dermatitidis), Paracoccidioides (brasiliensis), Coccidioides (immitis)* and *Histoplasma (capsulatum), Entamoeba, histolytica, Balantidium coli, Naegleria fowleri, Acanthamoeba* sp., *Giardia lambia, Cryptosporidium* sp., *Pneumocystis carinii, Plasmodium vivax, Babesia microti, Trypanosoma brucei, Trypanosoma cruzi, Toxoplasma gondi, etc.)., Sporothrix, Blastomyces, Paracoccidioides, Coccidioides, Histoplasma, Entamoeba, Histolytica, Balantidium, Naegleria, Acanthamoeba, Giardia, Cryptosporidium, Pneumocystis, Plasmodium, Babesia,* or *Trypanosoma, etc.* Thus, the antibodies and antigen-binding fragments of the present invention have utility in the treatment of infectious disease.

### 2. Uses of Down-Modulators of the Immune System

**[00134]** In an alternative embodiment, the anti-B7-H1 or anti-PD-1 antibodies of the present invention are employed to produce anti-idiotypic peptides or antibodies (Wallmann, J. *et al.* (2010) "*Anti-Ids in Allergy: Timeliness of a Classic Concept*," World Allergy Organiz. J. 3(6):195–201; Nardi, M. *et al.* (2000) "*Antiidiotype Antibody Against Platelet Anti-Gpiiia Contributes To The Regulation Of Thrombocytopenia In HIV-1-ITP Patients*," J. Exp. Med. 191(12):2093-2100) or mimetics (Zang, Y.C. *et al.* (2003) "*Human Anti-Idiotypic T Cells Induced By TCR Peptides Corresponding To A Common CDR3 Sequence Motif In Myelin Basic Protein-Reactive T Cells*," Int. Immunol. 15(9):1073-1080; Loiarro, M. *et al.* (Epub 2010 Apr 8) "*Targeting TLR/IL-1R Signalling In Human Diseases*," Mediators Inflamm. 2010:674363) of B7-H1 or PD-1. Such molecules serve as surrogates for B7-H1 or PD-1, and thus their administration to a subject **down-modulates** the immune system of such subject by mimicking or facilitating B7-H1 – PD-1 binding. Such molecules have utility in the treatment of graft vs. host disease. Similarly, agonist antibodies that i) enhance binding between such antibodies and such receptor/ligand or ii) trigger signal transduction when bound directly to B7-H1 or PD-1, have utility as agonists of B7-H1-PD-1 signaling and thus have utility in the treatment of inflammation and autoimmune disease, by directly or indirectly agonizing receptor activity.

**[00135]** Bi-specific antibodies, exhibiting immunospecific binding to both PD-1 and B7-H1 are capable of binding to both APC and T-cells, and thus facilitate the co-localization of APCs and T-cells. Such co-localization facilitates the ability of such cells to bind together via B7-H1 and PD-1 molecules that are not complexed with antibody, or by co-inhibitory molecules. Such binding provides down modulation of the immune system of the recipient.

**[00136]** Down-modulation of the immune system is desirable in the treatment of inflammatory and auto-immune diseases, and graft vs. host disease (GvHD). Examples of autoimmune disorders that may be treated by administering the antibodies of the present invention include, but are not limited to, alopecia areata, ankylosing spondylitis, antiphospholipid syndrome, autoimmune Addison's disease, autoimmune diseases of the adrenal gland, autoimmune hemolytic anemia, autoimmune hepatitis, autoimmune oophoritis and orchitis, autoimmune thrombocytopenia, Behcet's disease, bullous pemphigoid, cardiomyopathy, celiac sprue-dermatitis, chronic fatigue immune dysfunction syndrome (CFIDS), chronic inflammatory demyelinating polyneuropathy, Churg-Strauss syndrome, cicatrical pemphigoid, CREST syndrome, cold agglutinin disease, Crohn's disease, discoid lupus, essential mixed cryoglobulinemia, fibromyalgia-fibromyositis, glomerulonephritis, Graves' disease, Guillain-Barre, Hashimoto's thyroiditis, idiopathic pulmonary fibrosis, idiopathic thrombocytopenia purpura (ITP), IgA neuropathy, juvenile arthritis, lichen planus, lupus erthematosus, Ménière's disease, mixed connective tissue disease, multiple sclerosis, Neuromyelitis optica (NMO), type 1 or immune-mediated diabetes mellitus, myasthenia gravis, pemphigus vulgaris, pernicious anemia, polyarteritis nodosa, polychrondritis, polyglandular syndromes, polymyalgia rheumatica, polymyositis and dermatomyositis, primary agammaglobulinemia, primary biliary cirrhosis, psoriasis, psoriatic arthritis, Raynauld's phenomenon, Reiter's syndrome, Rheumatoid arthritis, sarcoidosis, scleroderma, Sjögren's syndrome, stiff-man syndrome, systemic lupus erythematosus, lupus erythematosus, takayasu arteritis, temporal arteristis/ giant cell arteritis, transverse myelitis, ulcerative colitis, uveitis, vasculitides such as dermatitis herpetiformis vasculitis, vitiligo, and Wegener's granulomatosis.

**[00137]** Examples of inflammatory disorders which can be prevented, treated or managed in accordance with the methods of the invention include, but are not limited to, asthma, encephilitis, inflammatory bowel disease, chronic obstructive pulmonary disease (COPD), allergic disorders, septic shock, pulmonary fibrosis, undifferentiated spondyloarthropathy, undifferentiated arthropathy, arthritis, inflammatory osteolysis, and chronic inflammation resulting from chronic viral or bacterial infections.

**[00138]** Thus, the antibodies and antigen-binding fragments of the present invention have utility in the treatment of inflammatory and auto-immune diseases.

### C. Methods of Administration

**[00139]** Various delivery systems are known and can be used to administer the therapeutic or prophylactic compositions of the present invention, *e.g.*, encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the antibody or fusion protein, receptor-mediated endocytosis (*see*, *e.g.*, Wu and Wu, 1987, *J. Biol. Chem.* 262:4429-4432), construction of a nucleic acid as part of a retroviral or other vector, *etc.*

**[00140]** Methods of administering a humanized antibody of the invention include, but are not limited to, injection, as by parenteral administration (*e.g.*, intradermal, intramuscular, intraperitoneal, intravenous and subcutaneous), epidural, and mucosal (*e.g.*, intranasal and oral routes). In a specific embodiment, the antibodies of the invention are administered intramuscularly, intravenously, or subcutaneously. The compositions may be administered by any convenient route, for example, by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.*, oral mucosa, rectal and intestinal mucosa, *etc.*) and may be administered together with other biologically active agents. Administration can be systemic or local. In addition, pulmonary administration can also be employed, *e.g.*, by use of an inhaler or nebulizer, and formulation with an aerosolizing agent. *See*, *e.g.*, U.S. Patent Nos. 6,019,968; 5,985, 20; 5,985,309; 5,934,272; 5,874,064; 5,855,913; 5,290,540; and 4,880,078; and PCT Publication Nos. WO 92/19244; WO 97/32572; WO 97/44013; WO 98/31346; and WO 99/66903. In a specific embodiment, it may be desirable to administer the pharmaceutical compositions of the invention locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion, by injection, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. Preferably, when administering an antibody of the invention, care must be taken to use materials to which the antibody or the fusion protein does not absorb.

**[00141]** In some embodiments, the humanized or chimeric antibodies of the invention are formulated in liposomes for targeted delivery of the antibodies of the invention. Liposomes are vesicles comprised of concentrically ordered phopsholipid bilayers which encapsulate an aqueous phase. Liposomes typically comprise various types of lipids, phospholipids, and/or surfactants. The components of liposomes are arranged in a bilayer configuration, similar to the lipid arrangement of biological membranes. Liposomes are particularly preferred delivery vehicles due, in part, to their biocompatibility, low immunogenicity, and low

toxicity. Methods for preparation of liposomes are known in the art and are encompassed within the invention, *see*, *e.g.*, Epstein *et al.*, 1985, *Proc. Natl. Acad. Sci. USA*, 82: 3688; Hwang *et al.*, 1980 *Proc. Natl. Acad. Sci. USA*, 77: 4030-4; U.S. Patent Nos. 4,485,045 and 4,544,545.

**[00142]** The invention also encompasses methods of preparing liposomes with a prolonged serum half-life, *i.e.*, enhanced circulation time, such as those disclosed in U.S. Patent No. 5,013,556. Preferred liposomes used in the methods of the invention are not rapidly cleared from circulation, *i.e.*, are not taken up into the mononuclear phagocyte system (MPS). The invention encompasses sterically stabilized liposomes which are prepared using common methods known to one skilled in the art. Although not intending to be bound by a particular mechanism of action, sterically stabilized liposomes contain lipid components with bulky and highly flexible hydrophilic moieties, which reduces the unwanted reaction of liposomes with serum proteins, reduces oposonization with serum components and reduces recognition by MPS. Sterically stabilized liposomes are preferably prepared using polyethylene glycol. For preparation of liposomes and sterically stabilized liposome, *see, e.g.,* Bendas *et al.*, 2001 *BioDrugs*, 15(4): 215-224; Allen *et al.*, 1987 *FEBS Lett.* 223: 42-6; Klibanov *et al.*, 1990 *FEBS Lett.*, 268: 235-7; Blum *et al.*, 1990, *Biochim. Biophys. Acta.*, 1029: 91-7; Torchilin *et al.*, 1996, *J. Liposome Res.* 6: 99-116; Litzinger *et al.*, 1994, *Biochim. Biophys. Acta*, 1190: 99-107; Maruyama *et al.*, 1991, *Chem. Pharm. Bull.*, 39: 1620-2; Klibanov *et al.*, 1991, *Biochim Biophys Acta*, 1062; 142-8; Allen *et al.*, 1994, *Adv. Drug Deliv. Rev*, 13: 285-309. The invention also encompasses liposomes that are adapted for specific organ targeting, *see*, *e.g.*, U.S. Patent No. 4,544,545, or specific cell targeting, see, e.g., U.S. Patent Application Publication No. 2005/0074403. Particularly useful liposomes for use in the compositions and methods of the invention can be generated by reverse phase evaporation method with a lipid composition comprising phosphatidylcholine, cholesterol, and PEG derivatized phosphatidylethanolamine (PEG-PE). Liposomes are extruded through filters of defined pore size to yield liposomes with the desired diameter. In some embodiments, a fragment of an antibody of the invention, *e.g.*, F(ab'), may be conjugated to the liposomes using previously described methods, *see*, *e.g.*, Martin *et al.*, 1982, *J. Biol. Chem.* 257: 286-288.

**[00143]** The humanized or chimeric antibodies of the invention may also be formulated as immunoliposomes. Immunoliposomes refer to a liposomal composition, wherein an antibody of the invention or a fragment thereof is linked, covalently or non-covalently to the liposomal

surface. The chemistry of linking an antibody to the liposomal surface is known in the art and encompassed within the invention, *see*, *e.g.*, U.S. Patent No. 6,787,153; Allen *et al.*, 1995, Stealth Liposomes, Boca Rotan: CRC Press, 233-44; Hansen *et al.*, 1995, *Biochim. Biophys. Acta*, 1239: 133-144. In most preferred embodiments, immunoliposomes for use in the methods and compositions of the invention are further sterically stabilized. Preferably, the humanized antibodies of the invention are linked covalently or non-covalently to a hydrophobic anchor, which is stably rooted in the lipid bilayer of the liposome. Examples of hydrophobic anchors include, but are not limited to, phospholipids, *e.g.*, phosoatidylethanolamine (PE), phospahtidylinositol (PI). To achieve a covalent linkage between an antibody and a hydrophobic anchor, any of the known biochemical strategies in the art may be used, *see*, *e.g.*, J. Thomas August, *ed.*, 1997, Gene Therapy: Advances in Pharmacology, Volume 40, Academic Press, San Diego, CA, p. 399-435. For example, a functional group on an antibody molecule may react with an active group on a liposome associated hydrophobic anchor, *e.g.*, an amino group of a lysine side chain on an antibody may be coupled to liposome associated N-glutaryl-phosphatidylethanolamine activated with water-soluble carbodiimide; or a thiol group of a reduced antibody can be coupled to liposomes via thiol reactive anchors, such as pyridylthiopropionylphosphatidylethanolamine. *See*, *e.g.*, Dietrich *et al.*, 1996, *Biochemistry*, 35: 1100-1105; Loughrey *et al.*, 1987, *Biochim. Biophys. Acta*, 901: 157-160; Martin *et al.*, 1982, *J. Biol. Chem*. 257: 286-288; Martin *et al.*, 1981, *Biochemistry*, 20: 4429-38. Although not intending to be bound by a particular mechanism of action, immunoliposomal formulations comprising an antibody of the invention are particularly effective as therapeutic agents, since they deliver the antibody to the cytoplasm of the target cell, *i.e.*, the cell comprising the receptor to which the antibody binds. The immunoliposomes preferably have an increased half-life in blood, specifically target cells, and can be internalized into the cytoplasm of the target cells thereby avoiding loss of the therapeutic agent or degradation by the endolysosomal pathway.

**[00144]** The immunoliposomal compositions of the invention comprise one or more vesicle forming lipids, an antibody of the invention or a fragment or derivative thereof, and, optionally, a hydrophilic polymer. A vesicle forming lipid is preferably a lipid with two hydrocarbon chains, such as acyl chains and a polar head group. Examples of vesicle forming lipids include phospholipids, *e.g.*, phosphatidylcholine, phosphatidylethanolamine, phosphatidic acid, phosphatidylinositol, sphingomyelin, and glycolipids, *e.g.*, cerebrosides, gangliosides. Additional lipids useful in the formulations of the invention are known to one

skilled in the art and encompassed within the invention. In some embodiments, the immunoliposomal compositions further comprise a hydrophilic polymer, *e.g.*, polyethylene glycol, and ganglioside GM1, which increases the serum half-life of the liposome. Methods of conjugating hydrophilic polymers to liposomes are well known in the art and encompassed within the invention. For a review of immunoliposomes and methods of preparing them, *see, e.g.*, U.S. Patent Application Publication No. 2003/0044407; PCT International Publication No. WO 97/38731, Vingerhoeads *et al.*, 1994, *Immunomethods*, 4: 259-72; Maruyama, 2000, *Biol. Pharm. Bull.* 23(7): 791-799; Abra *et al.*, 2002, *Journal of Liposome Research*, 12(1&2): 1-3; Park, 2002, *Bioscience Reports*, 22(2): 267-281; Bendas *et al.*, 2001 *BioDrugs*, 14(4): 215-224, J. Thomas August, *ed.*, 1997, Gene Therapy: Advances in Pharmacology, Volume 40, Academic Press, San Diego, CA, p. 399-435.

**[00145]** The invention also provides that the humanized or chimeric antibodies of the invention are packaged in a hermetically sealed container, such as an ampoule or sachette, indicating the quantity of antibody. In one embodiment, the antibodies of the invention are supplied as a dry sterilized lyophilized powder or water free concentrate in a hermetically sealed container and can be reconstituted, *e.g.*, with water or saline to the appropriate concentration for administration to a subject. Preferably, the antibodies of the invention are supplied as a dry sterile lyophilized powder in a hermetically sealed container at a unit dosage of at least 5 mg, more preferably at least 10 mg, at least 15 mg, at least 25 mg, at least 35 mg, at least 45 mg, at least 50 mg, or at least 75 mg. The lyophilized antibodies of the invention should be stored at between 2 and 8°C in their original container and the antibodies should be administered within 12 hours, preferably within 6 hours, within 5 hours, within 3 hours, or within 1 hour after being reconstituted. In an alternative embodiment, antibodies of the invention are supplied in liquid form in a hermetically sealed container indicating the quantity and concentration of the antibody, fusion protein, or conjugated molecule. Preferably, the liquid form of the antibodies are supplied in a hermetically sealed container at least 1 mg/ml, more preferably at least 2.5 mg/ml, at least 5 mg/ml, at least 8 mg/ml, at least 10 mg/ml, at least 15 mg/ml, at least 25 mg/ml, at least 50 mg/ml, at least 100 mg/ml, at least 150 mg/ml, at least 200 mg/ml of the antibodies.

**[00146]** The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the condition, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be

extrapolated from dose-response curves derived from *in vitro* or animal model test systems. For antibodies encompassed by the invention, the dosage administered to a patient is typically 0.01 mg/kg to 100 mg/kg of the patient's body weight. Preferably, the dosage administered to a patient is between 0.01 mg/kg and 20 mg/kg, 0.01 mg/kg and 10 mg/kg, 0.01 mg/kg and 5 mg/kg, 0.01 and 2 mg/kg, 0.01 and 1 mg/kg, 0.01 mg/kg and 0.75 mg/kg, 0.01 mg/kg and 0.5 mg/kg, 0.01 mg/kg to 0.25 mg/kg, 0.01 to 0.15 mg/kg, 0.01 to 0.10 mg/kg, 0.01 to 0.05 mg/kg, or 0.01 to 0.025 mg/kg of the patient's body weight. In particular, the invention contemplates that the dosage administered to a patient is 0.2 mg/kg, 0.3 mg/kg, 1 mg/kg, 3 mg/kg, 6 mg/kg or 10 mg/kg. A dose as low as 0.01 mg/kg is predicted to would show appreciable pharmacodynamic effects. Dose levels of 0.10 – 1 mg/kg are predicted to be most appropriate. Higher doses (*e.g.*, 1-30 mg/kg) would also be expected to be active. Generally, human antibodies have a longer half-life within the human body than antibodies from other species due to the immune response to the foreign polypeptides. Thus, lower dosages of human antibodies and less frequent administration is often possible. Further, the dosage and frequency of administration of antibodies of the invention or fragments thereof may be reduced by enhancing uptake and tissue penetration of the antibodies by modifications such as, for example, lipidation.

**[00147]** In yet another embodiment, the compositions can be delivered in a controlled release or sustained release system. Any technique known to one of skill in the art can be used to produce sustained release formulations comprising one or more antibodies of the invention. *See*, *e.g.*, U.S. Patent No. 4,526,938; PCT publication WO 91/05548; PCT publication WO 96/20698; Ning *et al.*, 1996, "Intratumoral Radioimmunotheraphy of a Human Colon Cancer Xenograft Using a Sustained-Release Gel," *Radiotherapy & Oncology* 39:179-189, Song *et al.*, 1995, "Antibody Mediated Lung Targeting of Long-Circulating Emulsions," *PDA Journal of Pharmaceutical Science & Technology* 50:372-397; Cleek *et al.*, 1997, "Biodegradable Polymeric Carriers for a bFGF Antibody for Cardiovascular Application," *Pro. Int'l. Symp. Control. Rel. Bioact. Mater.* 24:853-854; and Lam *et al.*, 1997, "Microencapsulation of Recombinant Humanized Monoclonal Antibody for Local Delivery," *Proc. Int'l. Symp. Control Rel. Bioact. Mater.* 24:759-760. In one embodiment, a pump may be used in a controlled release system (*See* Langer, *supra*; Sefton, 1987, *CRC Crit. Ref. Biomed. Eng.* 14:20; Buchwald *et al.*, 1980, Surgery 88:507; and Saudek *et al.*, 1989, *N. Engl. J. Med.* 321:574). In another embodiment, polymeric materials can be used to achieve controlled release of antibodies (*see e.g.*, Medical Applications of Controlled Release, Langer

and Wise (eds.), CRC Pres., Boca Raton, Florida (1974); Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, 1983, J., *Macromol. Sci. Rev. Macromol. Chem.* 23:61; *See* also Levy *et al.*, 1985, *Science* 228:190; During *et al.*, 1989, Ann. Neurol. 25:351; Howard *et al.*, 1989, *J. Neurosurg.* 7 1:105); U.S. Patent No. 5,679,377; U.S. Patent No. 5,916,597; U.S. Patent No. 5,912,015; U.S. Patent No. 5,989,463; U.S. Patent No. 5,128,326; PCT Publication No. WO 99/15154; and PCT Publication No. WO 99/20253). Examples of polymers used in sustained release formulations include, but are not limited to, poly(2-hydroxy ethyl methacrylate), poly(methyl methacrylate), poly(acrylic acid), poly(ethylene-co-vinyl acetate), poly(methacrylic acid), polyglycolides (PLG), polyanhydrides, poly(N-vinyl pyrrolidone), poly(vinyl alcohol), polyacrylamide, poly(ethylene glycol), polylactides (PLA), poly(lactide-co-glycolides) (PLGA), and polyorthoesters. In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target (*e.g.*, the lungs), thus requiring only a fraction of the systemic dose (*see*, *e.g.*, Goodson, in Medical Applications of Controlled Release, *supra*, vol. 2, pp. 115-138 (1984)). In another embodiment, polymeric compositions useful as controlled release implants are used according to Dunn *et al.* (*See* U.S. 5,945,155). This particular method is based upon the therapeutic effect of the in situ controlled release of the bioactive material from the polymer system. The implantation can generally occur anywhere within the body of the patient in need of therapeutic treatment. In another embodiment, a non-polymeric sustained delivery system is used, whereby a non-polymeric implant in the body of the subject is used as a drug delivery system. Upon implantation in the body, the organic solvent of the implant will dissipate, disperse, or leach from the composition into surrounding tissue fluid, and the non-polymeric material will gradually coagulate or precipitate to form a solid, microporous matrix (*See* U.S. 5,888,533). Controlled release systems are discussed in the review by Langer (1990, *Science* 249:1527-1533). Any technique known to one of skill in the art can be used to produce sustained release formulations comprising one or more therapeutic agents of the invention. *See*, *e.g.*, U.S. Patent No. 4,526,938; International Publication Nos. WO 91/05548 and WO 96/20698; Ning *et al.*, 1996, *Radiotherapy & Oncology* 39:179-189; Song *et al.*, 1995, *PDA Journal of Pharmaceutical Science &* Technology 50:372-397; Cleek *et al.*, 1997, *Pro. Int'l. Symp. Control. Rel. Bioact. Mater.* 24:853-854; and Lam *et al.*, 1997, *Proc. Int'l. Symp. Control Rel. Bioact. Mater.* 24:759-760.

**[00148]** In a specific embodiment wherein the therapeutic or prophylactic composition of the invention is a nucleic acid encoding an antibody of the invention or an antigen-binding fragment thereof, the nucleic acid can be administered *in vivo* to promote expression of its encoded antibody, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, *e.g.*, by use of a retroviral vector (*See* U.S. Patent No. 4,980,286), or by direct injection, or by use of microparticle bombardment (*e.g.*, a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox-like peptide which is known to enter the nucleus (*See e.g.*, Joliot *et al.*, 1991, *Proc. Natl. Acad. Sci. USA* 88:1864-1868), *etc.* Alternatively, a nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression by homologous recombination.

**[00149]** Treatment of a subject with a therapeutically or prophylactically effective amount of antibodies of the invention can include a single treatment or, preferably, can include a series of treatments.

### D. Pharmaceutical Compositions

**[00150]** The compositions of the invention include bulk drug compositions useful in the manufacture of pharmaceutical compositions (*i.e.*, compositions that are suitable for administration to a subject or patient) which can be used in the preparation of unit dosage forms. Such compositions comprise a prophylactically or therapeutically effective amount of a prophylactic and/or therapeutic agent disclosed herein or a combination of those agents and a pharmaceutically acceptable carrier. Preferably, compositions of the invention comprise a prophylactically or therapeutically effective amount of humanized antibodies of the invention and a pharmaceutically acceptable carrier.

**[00151]** In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant (*e.g.*, Freund's adjuvant (complete and incomplete), excipient, surfactant, cryoprotectant or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions

and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol, polysorbate-80 and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like.

**[00152]** Generally, the ingredients of compositions of the invention are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

**[00153]** The compositions of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include, but are not limited to, those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, *etc.*

### E. Kits

**[00154]** The invention provides a pharmaceutical pack or kit comprising one or more containers filled with humanized antibodies of the invention. Additionally, one or more other prophylactic or therapeutic agents useful for the treatment of a disease can also be included in the pharmaceutical pack or kit. The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

**[00155]** The present invention provides kits that can be used in the above methods. In one embodiment, a kit comprises one or more humanized antibodies of the invention. In another embodiment, a kit further comprises one or more other prophylactic or therapeutic agents useful for the treatment of cancer, in one or more containers. In another embodiment, a kit further comprises one or more cytotoxic antibodies that bind one or more cancer antigens associated with cancer. In certain embodiments, the other prophylactic or therapeutic agent is a chemotherapeutic. In other embodiments, the prophylactic or therapeutic agent is a biological or hormonal therapeutic.

### F. Diagnostic Methods

**[00156]** The antibodies of the invention and their antigen-binding fragments can be used for diagnostic purposes, such as to detect, diagnose, or monitor diseases, disorders or infections associated with B7-H1 or PD-1 expression. The invention provides for the detection or diagnosis of a disease, disorder or infection, particularly an autoimmune disease comprising: (a) assaying the expression of B7-H1 or of PD-1 in cells or in a tissue sample of a subject using one or more antibodies (or fragments thereof) that immunospecifically bind to such antigens; and (b) comparing the level of the antigen with a control level, *e.g.*, the level in a normal tissue sample, or before treatment, whereby an increase or decrease in the assayed level of antigen compared to the control level of the antigen is indicative of the disease, disorder or infection, or of the subject's response to treatment. Thus, the invention also provides for **monitoring the progression of a disease**, disorder or infection, comprising: (a) assaying the expression of B7-H1 or of PD-1 in cells or in a tissue sample of a subject at one point in time using one or more antibodies (or fragments thereof) that immunospecifically bind to such antigens; and (b) comparing the level of expression of B7-H1 or of PD-1 in cells or in a tissue sample of a subject at another point in time, or over a time course, whereby an increase or decrease in the assayed level of antigen is indicative of the progression of disease, disorder or infection. The invention additionally provides for **monitoring the response to treatment**, comprising: (a) assaying the expression of B7-H1 or of PD-1 in cells or in a tissue sample of a subject prior to treatment with one or more antibodies (or fragments thereof) that immunospecifically bind to such antigens; and (b) assaying the expression of B7-H1 or of PD-1 in cells or in a tissue sample of a subject prior at one or more time points after treatment, and comparing the level of the antigen over time, whereby an increase or decrease in the assayed level of antigen compared to the pre-treatment level of the antigen is indicative of a response to treatment. Such antibodies and fragments are preferably employed in

immunoassays, such as the enzyme linked immunosorbent assay (ELISA), the radioimmunoassay (RIA) and fluorescence-activated cell sorting (FACS).

**[00157]** One aspect of the invention relates to the use of such antibodies and fragments, and particularly such antibodies and fragments that bind to human B7-H1, as reagents for IHC analysis in cells of an *in vitro* or *in situ* tissue sample or *in vivo*. For example, since B7-H1 is expressed by cancer cells but not by normal tissue (Dong, H. (2003) "*B7-H1 Pathway And Its Role In The Evasion Of Tumor Immunity*," J. Mol. Med. 81:281-287), detection of its presence on a cell by such cell's binding to such antibodies or fragments is indicative and diagnostic of a cancer cell. Thus, the present invention provides a cytologic assay for diagnosing the presence of cancer in a subject.

**[00158]** The presence of B7-H1 on tumor cells has been found to enhance apoptosis of T cells that respond to the tumor (United States Patent No. 7,794,710). Thus, by determining the extent or degree to which tumor cells of a cancer patient exhibit B7-H1 on their surfaces, the present invention provides a means for determining the clinical significance of the cancer, and the extent to which it will be refractory to an immune response.

**[00159]** Similarly, B7-H1 is expressed on lymphoid and mucosal dendritic cells (both myeloid and plasmacytoid dendritic cells), and its expression significantly increases after SIV infection (Xu, Huanbin *et al.* (2010) "*Increased B7-H1 Expression on Dendritic Cells Correlates with Programmed Death 1 Expression on T Cells in Simian Immunodeficiency Virus-Infected Macaques and May Contribute to T Cell Dysfunction and Disease Progression*," J. Immunol. 185:7340-7348). Thus, expression of B7-H1 on such cells may be used to diagnose HIV in humans. Additionally, it has been found that PD-1 expression on CD8+ cells is increased in the context of HIV infection (Killian, M.S. *et al.* (2011) "*Natural Suppression of Human Immunodeficiency Virus Type 1 Replication Is Mediated by Memory CD8$^+$ T Cells*," J. Virol. 85(4):1696-1705). Thus antibodies that bind to both PD-1 and CD8 have particular utility in the diagnosis of HIV infection and AIDS progression.

**[00160]** A further aspect of the invention relates to the use of such antibodies and fragments, and particularly such antibodies and fragments that bind to human PD-1. PD-1 has particular utility as a marker of chronic immune activation and T-cell exhaustion. Its expression is enhanced on T cells of viremic HIV-infected patient and correlates with the viral load in these patients (Khaitan, A. *et al.* (2011) "*Revisiting Immune Exhaustion During HIV*

*Infection*," Curr. HIV/AIDS Rep. 8:4-11; Grabmeier-Pfistershammer, K. *et al.* (2011) "*Identification of PD-1 as a Unique Marker for Failing Immune Reconstitution in HIV-1–Infected Patients on Treatment*," J Acquir. Immune Defic. Syndr. 56(2):118-124). Thus, PD-1 has particular utility as a marker of HIV progression. Most preferably PD-1 expression will be assessed using flow cytometry. Through the use of such antibodies and fragments, T cells (which express PD-1) can be analyzed for multiple parameters (*e.g.*, cell counting, cell size, phenotype and cellular health, *etc.*) even though present in heterogeneous preparations. Thus, in concert with antibodies to PD-1 and their antigen-binding fragments, such methods can be used to diagnose the extent or severity of AIDS, leukemias, and other disease affecting T cell number and health. Methods of flow cytometry that may be adapted to the purposes of the present invention are disclosed in and in Peters, J.M. *et al.* (2011) "*Multiparameter Flow Cytometry In The Diagnosis And Management Of Acute Leukemia*," Arch. Pathol. Lab. Med. 135(1):44-54; Meyerson, H.J. (2010) "A *Practical Approach To The Flow Cytometric Detection And Diagnosis Of T-Cell Lymphoproliferative Disorders*," Lab. Hematol. 16(3):32-52; Vandewoestyne, M. *et al.* (Epub 2010 Aug 3) *Laser Capture Microdissection In Forensic Research: A Review*," Int. J. Legal. Med. 124(6):513-521; Ornatsky, O. *et al.* (Epub 2010 Jul 21) "*Highly Multiparametric Analysis By Mass Cytometry*," J. Immunol. Meth. 361(1-2):1-20; Mach, W.J. *et al.* (Epub 2010 Jul 13) "*Flow Cytometry And Laser Scanning Cytometry, A Comparison Of Techniques*," J. Clin. Monit. Comput. 24(4):251-259; and Chattopadhyay, P.K. *et al.* (2010) "*Good Cell, Bad Cell: Flow Cytometry Reveals T-Cell Subsets Important In HIV Disease*," Cytometry A. 77(7):614-622; and in United States Patents Nos. 7,876,436; 7,847,923; 7,842,244; 7,746,466; 7,590,500; 7,527,978; 7,507,548; 7,491,502; 7,486,387; 7,479,630; 7,465,543; 7,354,773; 6,794,152 and 6,784,981

**[00161]** Thus, the antibodies and fragments of the present invention have utility in the detection and diagnosis of a disease, disorder, or infection in a human. In one embodiment, such diagnosis comprises: a) administering to a subject (for example, parenterally, subcutaneously, or intraperitoneally) an effective amount of a labeled antibody or antigen-binding fragment that immunospecifically binds to B7-H1 or PD-1; b) waiting for a time interval following the administration for permitting the labeled molecule to preferentially concentrate at sites in the subject where B7-H1 or PD-1 is expressed (and for unbound labeled molecule to be cleared to background level); c) determining background level; and d) detecting the labeled antibody in the subject, such that detection of labeled antibody above the background level indicates that the subject has the disease, disorder, or infection. In

accordance with this embodiment, the antibody is labeled with an imaging moiety which is detectable using an imaging system known to one of skill in the art. Background level can be determined by various methods including, comparing the amount of labeled molecule detected to a standard value previously determined for a particular system.

**[00162]** It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. *In vivo* tumor imaging is described in S.W. Burchiel *et al.*, "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments." (Chapter 13 in Tumor Imaging: The Radiochemical Detection of Cancer, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982).

**[00163]** Depending on several variables, including the type of label used and the mode of administration, the time interval following the administration for permitting the labeled molecule to preferentially concentrate at sites in the subject and for unbound labeled molecule to be cleared to background level is 6 to 48 hours or 6 to 24 hours or 6 to 12 hours. In another embodiment the time interval following administration is 5 to 20 days or 5 to 10 days.

**[00164]** In one embodiment, monitoring of a disease, disorder or infection is carried out by repeating the method for diagnosing the disease, disorder or infection, for example, one month after initial diagnosis, six months after initial diagnosis, one year after initial diagnosis, etc.

**[00165]** Presence of the labeled molecule can be detected in the subject using methods known in the art for *in vivo* scanning. These methods depend upon the type of label used. Skilled artisans will be able to determine the appropriate method for detecting a particular label. Methods and devices that may be used in the diagnostic methods of the invention include, but are not limited to, computed tomography (CT), whole body scan such as position emission tomography (PET), magnetic resonance imaging (MRI), and sonography.

**[00166]** In a specific embodiment, the molecule is labeled with a radioisotope and is detected in the patient using a radiation responsive surgical instrument (Thurston *et al.*, U.S. Patent No. 5,441,050). In another embodiment, the molecule is labeled with a fluorescent compound and is detected in the patient using a fluorescence responsive scanning instrument. In another embodiment, the molecule is labeled with a positron emitting metal and is detected

in the patient using positron emission-tomography. In yet another embodiment, the molecule is labeled with a paramagnetic label and is detected in a patient using magnetic resonance imaging (MRI).

**[00167]** Having now generally described the invention, the same will be more readily understood through reference to the following examples, which are provided by way of illustration and are not intended to be limiting of the present invention unless specified.

## Example 1
## Isolation and Characterization of Anti-Human B7-H1 Antibodies

**[00168]** In order to isolate high affinity neutralizing anti-human B7-H1 antibodies, mice were first immunized and then boosted with human B7-H1-Fc. Splenocytes from anti-B7-H1 positive animals were fused with myeloma cells following standard protocol. The resulting murine hybridomas were screened for those that express B7-H1-immunoreactive monoclonal antibodies. Antibodies were additionally evaluated to determine whether they were IgG or IgM antibodies. Accordingly, B7-H1-Fc or negative control was immobilized to a solid support. Hybridoma supernatants were then placed in contact with the support, and the presence of anti-B7-H1 antibody was determined using labeled anti-mouse IgG or anti-mouse IgM. **Figure 1** shows the results of tested hybridoma supernatants and indicates the isolation of multiple hybridoma lines that express antibody immunoreactive with human B7-H1.

**[00169]** The identified hybridomas were screened to determine whether their expressed antibodies were neutralizing antibodies and as such capable of blocking the binding between B7-H1 and PD-1. PD-1-Fc was immobilized to a solid support, which was then incubated in the presence of diluted conditioned media containing biotinylated B7-H1-Fc. The ability of the B7-H1 and PD-1 to bind to one another was detected by assaying for the binding of streptavidin-horse radish peroxidase (SA-HRP) to the solid support. Anti-B7-H1 antibody capable of modulating the binding of B7-H1 to PD-1 thus mediate a decrease in SA-HAS binding in this assay. The results of the experiment are shown in **Figure 2**, and indicate that several of the isolated hybridomas expressed human B7-H1 neutralizing antibodies. Antibody MIH-1 (anti-human CD274 (B7-H1) (Chen, Y. *et al.* (Epub 2005 Nov 11) "*Expression Of B7-H1 In Inflammatory Renal Tubular Epithelial Cells*," Nephron. Exp. Nephrol. 102(3-4):e81-e92) was used as a positive control. 29E.2AE is an anti-PD-1 antibody but was shown to be non-neutralizing in this assay. Conditioned media from an unrelated hybridoma (rand Ab) and a vector control (VC) were used as negative controls.

**[00170]** In order to determine whether the expressed neutralizing antibodies were capable of binding to B7-H1 that was arrayed on the surface of a cell, a cell binding assay was conducted. Supernatant from each hybridoma clone was diluted 1:4 and incubated in the presence of parental CHO cells and a clonal CHO line that overexpressed full length human B7-H1. After permitting binding to occur, the cells were washed and the presence of residual cell-bound anti-B7-H1 antibody was detected using fluorescently labeled anti-mouse IgG antibody. The median fluorescence intensity (MFI) for binding to CHO-hB7-H1 is shown in **Figure 3**. None of the tested clones were found to cross-react with the parental CHO line, indicating that the expressed antibody was specific for human B7-H1.

**[00171]** As a further assessment, different concentrations of three clones were compared to anti-B7-H1 antibody MIH-1. Antibody was purified using protein G and assessed for ability to bind to B7-H1 when present at endogenous levels and as arrayed on the surface of an APC. CHO cells that expressed human B7-H1 were incubated with varying concentrations (10, 1 or 0.1 μg/ml) of anti-B7-H1 antibody followed by incubation with APC-conjugated donkey anti-mouse antibody. Binding was reported by measuring median fluorescence intensity. The results show that the tested antibodies exhibited greater avidity toward B7-H1 than the control antibody (MIH1) (**Figure 4**) and that antibody 1E12 exhibited greater avidity toward B7-H1 than a control anti-B7-H1 antibody (5H1) (Dong, H. *et al.* (2002) "*Tumor-Associated B7-H1 Promotes T-Cell Apoptosis: A Potential Mechanism Of Immune Evasion*," Nature Med. 8(8):793-800) (**Figure 5**).

**[00172]** In summary, the data show that multiple anti-human B7-H1-expressing hybridomas were obtained. All clones are IgG antibodies that recognize B7-H1-Fc. Clones 1B3, 1D11, 1E2, 1E4, 1E10, 2A6, 2E12, 2F2, 2F5, 2F11, 3A4, and 3B1 are weak in the screening binding assay. Low signal could be due to low expression levels and/or weak affinity.

**[00173]** Significantly, several clones (*e.g.*, clones: 1D5, 1E12, 1F4, 2A7, 2G11, 3B6, 3D10) showed strong neutralizing activity. All are neutralizing at all concentrations tested and appear to bind to antigen well. MFI for binding to CHO-B7-H1 cells is as follows: 1D5 = 50,821; 1E12 = 56,152; 1F4 = 62,015; 2A7 = 49,008; 2G11 = 55,947; 3B6 = 59,638; 3D10 = 53,114.

**Example 2**
**Isolation and Characterization of Anti-Human PD-1 Antibodies**

**[00174]** In order to isolate high affinity neutralizing anti-human PD-1 antibodies, mice were first immunized and then boosted with human PD-1-Fc. Splenocytes from anti-PD-1 positive animals were fused with myeloma cells following standard protocol. The resulting murine hybridomas were screened for those that express high affinity human PD-1-immunoreactive monoclonal antibodies. Antibodies were additionally evaluated to determine whether they were IgG or IgM antibodies. Accordingly, PD-1-Fc or negative control (B7-H4-Fc) was immobilized to a solid support. Hybridoma supernatants were then placed in contact with the support, and the presence of anti-PD-1 antibody was determined using labeled anti-mouse IgG or anti-mouse IgM. **Figure 6** shows the antigen binding and isotype of the isolated anti-human PD-1 antibodies and indicates the isolation of multiple hybridoma lines that express antibody immunoreactive with human PD-1.

**[00175]** The identified hybridomas were screened to determine whether their expressed antibodies were neutralizing antibodies and as such capable of blocking the binding between B7-DC and PD-1. B7-DC-Fc, a fusion protein that binds PD-1, was immobilized to a solid support, which was then incubated in the presence of diluted conditioned media containing biotinylated PD-1-Fc. The ability of the B7-DC and PD-1 to bind to one another was detected by assaying for the binding of streptavidin-horse radish peroxidase (SA-HRP) to the solid support. Anti-PD-1 antibody capable of blocking the binding of B7-DC to PD-1 thus mediates a decrease in SA-HRP binding in this assay. The results of the experiment are shown in **Figures 7A** and **7B**, and indicate that several of the isolated hybridomas expressed human PD-1 neutralizing antibodies (**Figure 7B** shows the same graph as **Figure 7A**, but at a different scale).

**[00176]** A cell binding assay was conducted in order to determine whether the expressed neutralizing antibodies were capable of binding to PD-1 arrayed on a cell surface. Supernatant from each hybridoma clone was diluted 1:4 and incubated in the presence of parental CHO cells and a clonal CHO line that overexpressed full length human PD-1. After permitting binding to occur, the cells were washed and the presence of residual cell-bound anti-PD-1 antibody was detected using fluorescently labeled anti-mouse IgG antibody. The median fluorescence intensity (MFI) for binding to CHO-hPD-1 is shown in **Figure 8**. The results demonstrate that antibodies 1E3, 1E8, and 1H3 were particularly capable of binding to

human PD-1 expressed on a cell surface. None of the tested clones were found to cross-react with the parental CHO line, indicating that the expressed antibody was specific for human PD-1. J116 is commercially available anti-human PD-1 control antibody (eBioscience, Inc.). Isotyping revealed that 1E3, 1E8, 1H3 are IgG1 / kappa.

**[00177]** Two clones (1E3 and 1H3) were selected for further study. As a further assessment, different concentrations of two clones were compared to anti-PD-1 antibodies M3 and EH-12. Antibody was purified using protein G and assessed for ability to bind to PD-1 expressed on the surfaces of CHO cells. CHO-hPD1 cells were stained with unlabeled anti-PD-1 antibody (10, 1, and 0.1 μg/mL) followed by APC-conjugated donkey anti-mouse antibody and the median fluorescence intensity was reported. The results of the assay are reported in **Figure 9**. The negative control is murine IgG (mIgG1); the positive controls are M3 (a neutralizing monoclonal antibody against human PD-1 (Wu, K. *et al.* (2009) "*Kupffer Cell Suppression of CD8+ T Cells in Human Hepatocellular Carcinoma Is Mediated by B7-H1/Programmed Death-1 Interactions*," Cancer Res 69(20):8067-8075) and anti-PD-1 antibody EH12 (Dorfman, D.M. *et al.* (2006) "*Programmed Death-1 (PD-1) Is A Marker Of Germinal Center-Associated T Cells And Angioimmunoblastic T-Cell Lymphoma*," Am. J. Surg. Pathol. 30(7):802-810).

**[00178]** A cell-based competition assay was performed by incubating 50,000 CHO-hPD1 cells for 30 minutes with anti-PD-1 antibody (present at concentrations ranging from 0-0.1 mg/ml). 10 μg/ml of APC-labeled B7-DC-Fc was then added and the incubation continued for an additional 30 minutes, after which the fluorescence of bound B7-DC-Fc was measured. The median fluorescence intensity is shown in **Figure 10**. The competition was repeated at a fixed antibody concentration of 20 μg/mL (**Figure 11**).

**[00179]** In summary, the results show that clones 1E3, 1E8, and 1H3 exhibit neutralizing activity and recognize antigen well. Clone 1E6 is able to cross-link PD-1 without neutralizing it, thereby giving enhanced binding. However this clone does not appear to bind PD-1 on the cell surface.

**Example 3**
**Production of Humanized Antibodies: General Methodology**

**[00180]** As indicated above, for certain purposes, including for example, use in the *in vivo* treatment of human disease, it is preferred to employ a humanized derivative of the above-described anti-human B7-H1 and/or anti-human PD-1 antibodies.

**[00181]** To form such derivatives, the framework sequences of the 3D10 or 1H3 antibodies (the "Parental" sequences) were first aligned with framework sequences of a set of "Acceptor" human antibodies in order to identify differences in the framework sequences. Humanization was accomplished by substituting non-matching framework residues between the Parental and the Acceptor. Substitutions at potentially important positions such as those in the Vernier zone, the VH/VL inter-chain interface or CDR canonical class determining positions were analyzed for prospective back mutations (see, Foote, J. *et al.* (1992) "*Antibody Framework Residues Affecting The Conformation Of The Hypervariable Loops*," J. Molec. Biol. 224:487-499). A total 14 humanized variant sequences were identified.

**[00182]** The Conserved Domain Database (COD) (Marchler-Bauer, *et al.* (2011) "*COD: A Conserved Domain Database For The Functional Annotation Of Proteins*," Nucleic Acids Res. 39:D225-D229) was used to determine the domain content of each amino-acid chain and the approximate boundaries of each domain. Variable domain boundaries were exactly determined along with the boundaries of the complementarity-determining regions (CDRs) according to several commonly used definitions (Kabat, E.A. *et al.* (1991) "*Sequences of Proteins of Immunological Interest*," Fifth Edition. NIH Publication No. 91-3242; Chothia, C. *et al.* (1987) "*Canonical Structures For The Hypervariable Regions Of Immunoglobulins*," J. Mol. Biol. 196:901-917); Honegger, A. *et al.* (2001) "*Yet Another Numbering Scheme For Immunoglobulin Variable Domains: An Automatic Modeling And Analysis Tool*," J. Molec. Biol. 309(3):657-670; Chothia's CDR definition (Chothia, C. *et al.* (1987) "*Canonical Structures For The Hypervariable Regions Of Immunoglobulins*," J. Mol. Biol. 196:901-917) will be used below with respect to such humanized sequences.

**[00183]** Multiple alignments of the Parental sequence to the mouse and human germline sequences were generated using MAFFT (Katoh, K. *et al.* (2002) "*MAFFT: A Novel Method For Rapid Multiple Sequence Alignment Based On Fast Fourier Transform*," Nucleic Acids Res. 30: 3059-3066) and entries in each alignment were ordered according to the sequence

identity to the Parental sequence. Reference sets were reduced to a unique set of sequences by clustering at 100% sequence identity and excluding redundant entries.

**[00184]** The optimal Acceptor framework selection was based on the overall Parental antibodies sequence identity to the Acceptor across the framework of both chains; however the positions that compose the VH/VL inter-chain interface are of particular interest. Additionally, the CDR-loops lengths and CDR positions responsible for the discrete set of canonical structures that has been defined for 5 of the CDRs (Chothia, C. *et al.* (1987) "*Canonical Structures For The Hypervariable Regions Of Immunoglobulins*," J. Mol. Biol. 196:901-917; Martin, A.C. *et al.* (1996) "*Structural Families In Loops Of Homologous Proteins: Automatic Classification, Modelling And Application To Antibodies*," J. Molec. Biol 263:800-815; Al-Laziniki, B. *et al.* (1997) "*Standard Conformations For The Canonical Structures Of Immunoglobulins*," J. Molec. Biol. 273:927-948) were compared to the germlines, in order to determine which germline frameworks had both the same interface residues and were known to support similar CDR-loop conformations. **Table 6** and **Table 7** show the conserved positions within the VH/VL interface and the positions which determine the CDR canonical class (respectively), with numbering according to Chothia's definition.

**Table 6**
**Conserved Positions Within The VH/VL Interface of Antibody 1H3**

| Domain | Positions |
|---|---|
| VL | 34, 36, 38, 43, 44, 46, 87, 88, 89, 91, 96, 98 |
| VH | 35, 37, 39, 45, 47, 91 , 93, 95 100-100K*, 101, 103 |
| * The numbering of the position one N-terminal to position 101 differs by CDR H3 length | |

**Table 7**
**Positions Determining CDR Canonical Class of Antibody 1H3**

| Domain | Positions |
|---|---|
| L1 | 2, 25, 29, 30, 30D*, 33, 71 |
| L2 | 34 |
| L3 | 90, 94, 95, 97 |
| H1 | 24, 26, 29, 34, 94 |
| H2 | 54, 55, 71 |
| * If CDR L1 is long enough to contain the position | |

**[00185]** Based on the parent antibody's sequence alignment to the human germlines the closest matching entries were identified. The choice of the preferred human germline was based on the ordered criteria: (1) Sequence identity across the framework; (2) Identical or compatible inter-chain interface residues; (3) Support loops with the Parental CDRs

canonical conformations; (4) The combination of heavy and light germlines are found in expressed antibodies; and (5) Presence of N-glycosylation sites that have to be removed.

**[00186]** A structural model of antibody 1H3's Fv-region was generated. Candidate structural template fragments for the framework (FR) and complementarity-determining regions (CDRs) as well as the full Fv were scored, ranked and selected from an antibody database based on their sequence identity to the target, as well as qualitative crystallographic measures of the template structure such as the resolution, in Angstroms (Å).

**[00187]** In order to structurally align the CDRs to the FR templates, 5 residues on either side of the CDR were included in the CDR template. An alignment of the fragments was generated based on overlapping segments and a structural sequence alignment generated. The template fragments along with the alignment were processed by MODELLER (Sali, A. *et al.* (1993) "*Comparative Protein Modelling By Satisfaction Of Spatial Restraints*," J. Molec. Biol. 234:779-815). This protocol creates conformational restraints derived from the set of aligned structural templates. An ensemble of structures which satisfied the constraints was created by conjugate gradient and simulated annealing optimization procedures. Model structures were selected from this ensemble on the basis of an energy score, derived from the score of the proteins structure and the satisfaction of the conformational constraints. The models were inspected and the side chains of the positions which differed between the target and template were optimized using a side chain optimization algorithm and energy minimized. A suite of visualization and computational tools were used to assess the CDRs conformational variability, local packing and surface analysis to select one or more preferred models.

**[00188]** A structural model of the Parental antibody was constructed and inspected for imperfections such as poor atomic packing, strain in bond lengths, bond angles or dihedral angles. These imperfections may indicate potential issues with the structural stability of the antibody. The modeling protocol seeks to minimize such imperfections. The initial structural model of the Humanized Fv contains all safe substitutions (*i.e.*, substitutions that should not affect binding affinity or stability) and cautious substitutions (*i.e.*, the position substitution is made but the position may be important for binding affinity). Substitutions at positions that are considered to be associated with a risk a decreased binding affinity or reduced stability are not altered. The template search and selection was performed separately to the Parental template search in order to create a good stand-alone model rather than a

closely matching variant model of the Parental. As the assessment of potential substitutions was performed the model was updated to reflect the preferred substitutions and the effect of back mutations.

## Example 4
## Production of Humanized Anti-Human B7-H1 Antibodies

**[00189]** To illustrate the production of such humanized derivatives, humanized derivatives of anti-human B7-H1 antibody 3D10 was produced in accordance with the above-described procedure.

**[00190]** Sequence alignments comparing 3D10 light chain variable domains to the human germ lines were generated using: **IGKV3** light chain germlines (IGKV3-11*01, IGKV3-11*02, IGKV3-NL5*01, IGKV3D-11*01, IGKV3-NL4*01, IGKV3D-7*01, IGKV3D-20*01, IGKV3-20*01, IGKV3-20*02, and IGKV3-15*01), **IGKV1** light chain germlines (IGKV1-9*01, IGKV1-39*01, IGKV1D-13*01, IGKV1-16*01, IGKV1-8*01, IGKV1-13*02, IGKV1-NL1*01, IGKV1D-43*01, IGKV1-27*01, and IGKV1-12*01) and **IGKJ** light chain germlines (IGKJ4*01, IGKJ2*02, IGKJ2*01, IGKJ2*04, IGKJ2*03, IGKJ5*01, IGKJ1*01, and IGKJ3*01).

**[00191]** Sequence alignments comparing 3D10 heavy chain variable domains to the human germ lines were generated using: **IGHV1** heavy chain germlines (IGHV1-2*02, IGHV1-2*04, IGHV1-f*01, IGHV1-48*01, IGHV1-2*03, IGHV1-2*01, IGHV1-46*02, IGHV1-2*05, IGHV1-3*01, and IGHV1-8*01), **IGHV3** heavy chain germlines (IGHV3-49*04, IGHV3-49*01, IGHV3-49*02, IGHV3-49*03, IGHV3-64*01, IGHV3-64*02, IGHV3-72*01, IGHV3-66*01, and IGHV3-23*01), and **IGHJ** heavy chain germlines (IGHJ3*02, IGHJ6*01, IGHJ3*01, IGHJ6*03, IGHJ5*02, IGHJ5*01, IGHJ4*01, IGHJ1*01, IGHJ6*04, and IGHJ2*01).

### (A) Humanization of the Light Chain

**[00192]** Based on the above criteria, the light chain of antibody 3D10 was found to be most similar to the light chain of germline IGKV3-11*01 (**SEQ ID NO:78**):

EIVLTQSPAT LSLSPGERAT LSCRASQSVS SYLAWYQQKP GQAPRLLIYD
ASNRATGIPA RFSGSGSGTD FTLTISSLEP EDFAVYYCQQ RSNWP

and IGKV1-9*01 (**SEQ ID NO:79**):

DIQLTQSPSF LSASVGDRVT ITCRASQGIS SYLAWYQQKP GKAPKLLIYA
ASTLQSGVPS RFSGSGSGTE FTLTISSLQP EDFATYYCQQ LNSYP

with IGKV3-11*01 being preferred, as shown in **Table 8** (CDR residues of antibody 3D10 are shown in italics, identical aligned residues are shown underlined):

**Table 8**
**Alignment of Light Chain Variable Regions**

| Light Chain | SEQ ID NO. | Sequence |
|---|---|---|
| | | 1 10 20 30 40 |
| 3D10 | 9 | QIVLSQSPAI LSASPGEKVT MTC***RASSSVS*** ***YIY***WFQQKPG |
| IGKV3-11*01 | 78 | EIVLTQSPAT LSLSPGERAT LSCRASQSVSS YLAWYQQKPG |
| IGKV1-9*01 | 79 | DIQLTQSPSF LSASVGDRVT ITCRASQGISS YLAWYQQKPG |
| | | 41 50 60 70 80 |
| 3D10 | 9 | SSPKPWIY***AT*** ***FNLAS***GVPAR FSGSGSGTSY SLTISRVETE |
| IGKV3-11*01 | 78 | QAPRLLIYDA SNRATGIPAR FSGSGSGTDF TLTISSLEPE |
| IGKV1-9*01 | 79 | KAPKLLIYAA STLQSGVPSR FSGSGSGTEF TLTISSLQPE |
| | | 81 90 100 106 |
| 3D10 | 9 | DAATYYC***QQW*** ***SNNPLT***FGAG TKLELK |
| IGKV3-11*01 | 78 | DFAVYYCQQR SNWP---- -------- |
| IGKV1-9*01 | 79 | **D**FATYYCQQL NSYP---- -------- |

**[00193]** The J-segment genes were compared to the Parental sequence over FR4, and J-segment IGKJ4*01 (**SEQ ID NO:80**: LTFGGGTKVEIK) was selected for the light chain.

**[00194]** As shown above, the light chain of antibody 3D10 has a short ten residue CDR L1 similar to canonical class I loops. Human germlines do not have such a short CDR Ll. Each of the selected germlines, IGKV3-11*01 and IGKV1-9*01, have shorter L1 loops containing the correct framework residues to support this type of loop. A good overall sequence similarity is observed between both Acceptor frameworks and the Parental sequence, however important differences are observed at the interface positions Y33 and P45. Residue Y33 is in CDR LI and whilst the two selected Acceptor families do not contain tyrosine at this position, other germline sequences do. The difference between the Acceptor frameworks and the Parental sequence at position P45 is a consequence of the Parental germline possessing a FR2 dissimilar to any human germline. As a result changes in this region were proposed regardless of the Acceptor framework chosen, so IGKV3-11*01 and IGKV1-9*01 were advanced as the Acceptor frameworks.

**[00195]** Three humanized chains were created for each of the two preferred Acceptor frameworks, IGKV3-11*01 and IGKV1-9*01. The three LC1 chains are derived from IGKV3-11*01; the three LC2 chains are derived from IGKV1-9*01. The first humanized chain for each Acceptor framework contained all humanizing substitutions deemed possible and is the most human of the three chains. The second humanized chain for each Acceptor framework contained several back mutations at positions that alter the charge, potentially interfere with the core packing or could affect the conformation of the CDRs. The third chain, for each of the Acceptor frameworks, contained the most back mutations, including substitutions that alter the charge and could potentially alter the binding affinity. The sequences of these six humanized chains are shown below.

LC1_1 **(SEQ ID NO:81)** EIVLTQSPAT LSLSPGERAT LSCRASSSVS YIYWFQQKPG QAPRLLIYAA FNRATGIPAR FSGSGSGTDY TLTISSLEPE DFAVYYCQQW SNNPLTFGQG TKVEIK

LC1_2 **(SEQ ID NO:82)** EIVLTQSPAT LSLSPGERAT LSCRASSSVS YIYWFQQKPG QSPRPLIYAA FNRATGIPAR FSGSGSGTDY TLTISSLEPE DFAVYYCQQW SNNPLTFGQG TKVEIK

LC1_3 **(SEQ ID NO:83)** QIVLTQSPAT LSLSPGERAT LSCRASSSVS YIYWFQQKPG QSPRPLIYAT FNLASGIPAR FSGSGSGTSY TLTISRLEPE DFAVYYCQQW SNNPLTFGQG TKVEIK

LC2_1 **(SEQ ID NO:84)** DIQLTQSPSS LSASVGDRVT ITCRASSGVS YIYWFQQKPG KAPKLLIYAA FNLASGVPSR FSGSGSGTEY TLTISSLQPE DFATYYCQQW SNNPLTFGQG TKVEIK

LC2_2 **(SEQ ID NO:85)** DIQLTQSPSS LSASVGDRVT ITCRASSGVS YIYWFQQKPG KAPKPLIYAA FNLASGVPSR FSGSGSGTEY TLTISSLQPE DFATYYCQQW SNNPLTFGQG TKVEIK

LC2_3 **(SEQ ID NO:86)** QIQLTQSPSI LSASVGDRVT ITCRASSSVS YIYWFQQKPG KAPKPLIYAT FNLASGVPSR FSGSGSGTSY TLTISSLQPE DFATYYCQQW SNNPLTFGQG TKVEIK

**[00196]** The sequences for these six humanized chains are shown in **Table 9** with differences relative to the Parental 3D10 light chain shown in boldface and underline.

**Table 9**
**Humanized Light Chains of Antibody 3D10**

| Light Chain | SEQ ID NO | Sequence |
|---|---|---|
| LC1_1 | 81 | **E**IVL**T**QSPA**T** LS**L**SPGE**RA**T **LS**CRASSSVS YIYWFQQKPG **QA**P**RLL**IYA**A** FN**R**A**T**G**I**PAR FSGSGSGT**D**Y **T**LTIS**SL**E**P**E D**F**A**V**YYCQQW SNNPLTFG**Q**G TK**V**E**I**K |
| LC1_2 | 82 | **E**IVL**T**QSPA**T** LS**L**SPGE**RA**T **LS**CRASSSVS YIYWFQQKPG **Q**SP**R**P**L**IYA**A** FN**R**A**T**G**I**PAR FSGSGSGT**D**Y **T**LTIS**SL**E**P**E D**F**A**V**YYCQQW SNNPLTFG**Q**G TK**V**E**I**K |
| LC1_3 | 83 | QIVL**T**QSPA**T** LS**L**SPGE**RA**T **LS**CRASSSVS YIYWFQQKPG **Q**SP**R**P**L**IYAT FNLASG**I**PAR FSGSGSGTSY **T**LTISR**L**E**P**E D**F**A**V**YYCQQW SNNPLTFG**Q**G TK**V**E**I**K |
| LC2_1 | 84 | **D**I**Q**L**T**QSP**SS** LSAS**V**G**DR**VT **I**TCRASSGVS YIYWFQQKPG **KA**P**KLL**IYA**A** FNLASGVP**S**R FSGSGSGT**E**Y **T**LTIS**SLQP**E D**F**ATYYCQQW SNNPLTFG**Q**G TK**V**E**I**K |
| LC2_2 | 85 | **D**I**Q**L**T**QSP**SS** LSAS**V**G**DR**VT **I**TCRASSGVS YIYWFQQKPG **KA**P**KPL**IYA**A** FNLASGVP**S**R FSGSGSGT**E**Y **T**LTIS**SLQP**E D**F**ATYYCQQW SNNPLTFG**Q**G TK**V**E**I**K |
| LC2_3 | 86 | **D**I**Q**L**T**QSP**S**I LSAS**V**G**DR**VT **I**TCRASSSVS YIYWFQQKPG **KA**P**KPL**IYAT FNLASGVP**S**R FSGSGSGTSY **T**LTIS**SLQP**E D**F**ATYYCQQW SNNPLTFG**Q**G TK**V**E**I**K |

**(B) Humanization of the Heavy Chain**

**[00197]** In light of the above-discussed criteria, two candidate germline Acceptor frameworks: IGHV1-2*02 (IGHV1 Germline) and IGHV3-49*04 (IGHV3 Germline) were selected for the humanization of the heavy chain of antibody 3D10.

**[00198]** Acceptor Framework IGHV 1-2*02 was selected due to its sequence similarity to the Parental sequence and the fact that it contained very similar residues involved in the core packing of the domain. Acceptor Framework IGHV3-49*04 was selected after consideration and rejection of other germline sequences similar to IGHV 1-2*02. As Acceptor Framework IGHV3-49*04 is slightly more dissimilar to the Parental sequence than IGHV 1-2*02, a greater number of substitutions were required, however this germline Acceptor framework can support the Parental CDRs. The sequences of Acceptor Framework IGHV 1-2*02 and Acceptor Framework IGHV3-49*04 are shown below:

**[00199]** Acceptor Framework IGHV 1-2*02 (**SEQ ID NO:87**):

QVQLVQSGAE VKKPGASVKV SCKASGYTFT GYYMHWVRQA PGQGLEWMGW
INPNSGGTNY AQKFQGRVTM TRDTSISTAY MELSRLRSDD TAVYYCAR

**[00200]** Acceptor Framework IGHV3-49*04 (**SEQ ID NO:88**):

EVQLVESGGG LWQPGRSLRL SCTASGFTFG DYAMSWVRQA PGKGLEWVGF
IRSKAYGGTT EYAASVKGRF TISRDDSKSI AYLQMNSLKT EDTAVYYCTR

**[00201]** **Table 10** shows the alignment of these sequences with the heavy chain of antibody 3D10 (CDR residues of antibody 3D10 are shown in italics, identical aligned residues are shown underlined):

**Table 10**
**Alignment of Heavy Chain Variable Regions**

| Heavy Chain | SEQ ID NO. | Sequence |
|---|---|---|
| | | 1 10 20 30 40 |
| 3D10 | 10 | EVQLQQSGPD LVTPGASVRI SCQAS***GYTFP DYYMN***WVKQS |
| IGHV 1-2*02 | 87 | QVQLVQSGAE VKKPGASVKV SCKASGYTFT GYYMHWVRQA |
| IGHV3-49*04 | 88 | EVQLVESGGG LWQPGRSLRL SCTASGFTFG DYAMSWVRQA |
| | | 41 50 60 70 80 |
| 3D10 | 10 | HGKSLEWIG***D IDP--NYGGTTY NQKFKG***KAIL TVDRSSSTAY |
| IGHV 1-2*02 | 87 | PGQGLEWMGW INP--NSGGTNY AQKFQGRVTM TRDTSISTAY |
| IGHV3-49*04 | 88 | PGKGLEWVGF IRSKAYGGTTEY AASVKGRFTI SRDDSKSIAY |
| | | 81 90 100 110 114 |
| 3D10 | 10 | MELRSLTSED SAVYYCAR***GA L***TDWGQGTSL TVSS |
| IGHV 1-2*02 | 87 | MELSRLRSDD TAVYYCAR |
| IGHV3-49*04 | 88 | LQMNSLKTED TAVYYCTR |

**[00202]** The J-segment genes were compared to the Parental sequence over FR4, and J-segment IGHJ3*02 (**SEQ ID NO:89**: DAFDIWGQGTMVTVSS) was selected for the heavy chain.

**[00203]** Three humanized chains were created for each of the two preferred Acceptor frameworks, IGHV1-2*02 and IGHV3-49*04. The three HC1 chains are derived from IGHV1-2*02; the three HC2 chains are derived from IGHV3-49*04). The first humanized chain for each Acceptor framework contained all humanizing substitutions deemed possible and is the most human of the three chains. The second humanized chain for each Acceptor framework contained several back mutations at positions that alter the charge, potentially interfere with the core packing or could affect the conformation of the CDRs. The third chain, for each of the Acceptor frameworks, contained the most back mutations, including

substitutions that alter the charge and could potentially alter the binding affinity. The sequences for these six humanized chains are shown below:

HC1_1 **(SEQ ID NO:90)** QVQLVQSGAE VKKPGASVKV SCKASGYTFP DYYMNWVRQA PGQGLEWMGD IDPNYGGTNY AQKFQGRVTM TRDTSISTAY MELSRLRSDD TAVYYCARGA LTDWGQGTMV TVSS

HC1_2 **(SEQ ID NO:91)** QVQLVQSGAE VKKPGASVKV SCKASGYTFP DYYMNWVRQA PGQSLEWMGD IDPNYGGTNY NQKFQGRVTM TRDTSISTAY MELSRLRSDD TAVYYCARGA LTDWGQGTMV TVSS

HC1_3 **(SEQ ID NO:92)** EVQLVQSGAE VKKPGASVKV SCKASGYTFP DYYMNWVRQA PGQSLEWMGD IDPNYGGTNY NQKFQGRVTM TVDRSSSTAY MELSRLRSDD TAVYYCARGA LTDWGQGTMV TVSS

HC2_1 **(SEQ ID NO:93)** EVQLVESGGG LVQPGRSLRL SCTASGYTFP DYYMNWVRQA PGKGLEWVGD IDPNYGGTTY AASVKGRFTI SVDRSKSIAY LQMSSLKTED TAVYYCTRGA LTDWGQGTMV TVSS

HC2_2 **(SEQ ID NO:94)** EVQLVESGGG LVQPGRSLRL SCTASGYTFP DYYMNWVRQA PGKGLEWVGD IDPNYGGTTY NASVKGRFTI SVDRSKSIAY LQMSSLKTED TAVYYCARGA LTDWGQGTMV TVSS

HC2_3 **(SEQ ID NO:95)** EVQLVESGGG LVQPGRSLRL SCTASGYTFP DYYMNWVRQA PGKGLEWVGD IDPNYGGTTY NQSVKGRFTI SVDRSKSIAY LQMSSLKTED TAVYYCARGA LTDWGQGTMV TVSS

**[00204]** The sequences for these six humanized chains are shown in **Table 11** with differences relative to the Parental 3D10 heavy chain shown in boldface and underline.

**Table 11**
**Humanized Heavy Chains of Antibody 3D10**

| Heavy Chain | SEQ ID NO | Sequence |
|---|---|---|
| HC1_1 | 90 | **Q**VQL**V**QSG**AE** **VKK**PGASV**KV** SC**K**ASGYTFP DYYMNWV**R**Q**A** **P**G**QG**LEW**M**GD IDPNYGGT**N**Y **A**QKF**Q**G**RVTM** T**R**D**T**S**I**STAY MEL**SR**L**R**S**D**D **T**AVYYCARGA LTDWGQGT**MV** TVSS |
| HC1_2 | 91 | **Q**VQL**V**QSG**AE** **VKK**PGASV**KV** SC**K**ASGYTFP DYYMNWV**R**Q**A** **P**G**Q**SLEW**M**GD IDPNYGGT**N**Y NQKF**Q**G**RVTM** T**R**D**T**S**I**STAY MEL**SR**L**R**S**D**D **T**AVYYCARGA LTDWGQGT**MV** TVSS |
| HC1_3 | 92 | EVQL**V**QSG**AE** **VKK**PGASV**KV** SC**K**ASGYTFP DYYMNWV**R**Q**A** **P**G**Q**SLEW**M**GD IDPNYGGT**N**Y NQKF**Q**G**RVTM** TVDRSSSTAY MEL**SR**L**R**S**D**D **T**AVYYCARGA LTDWGQGT**MV** TVSS |
| HC2_1 | 93 | EVQL**VE**SG**GG** LV**Q**PG**R**S**LRL** SC**T**ASGYTFP DYYMNWV**R**Q**A** **P**GK**G**LEW**V**GD IDPNYGGTTY **AASV**KG**RFTI** **S**VDRS**K**S**I**AY **LQMS**SL**KT**ED **T**AVYYCTRGA LTDWGQGT**MV** TVSS |

| | | |
|---|---|---|
| HC2_2 | 94 | EVQLVESGGG LVQPGRSLRL SCTASGYTFP DYYMNWVRQA PGKGLEWVGD IDPNYGGTTY NASVKGRFTI SVDRSKSIAY LQMSSLKTED TAVYYCARGA LTDWGQGTMV TVSS |
| HC2_3 | 95 | EVQLVESGGG LVQPGRSLRL SCTASGYTFP DYYMNWVRQA PGKGLEWVGD IDPNYGGTTY NQSVKGRFTI SVDRSKSIAY LQMSSLKTED TAVYYCARGA LTDWGQGTMV TVSS |

### (C) Humanized Derivatives of Antibody 3D10

**[00205]** A search confirmed that antibodies with a combination of germlines, similar to the pairing of IGKV3-11*01 with IGHV1-2*02, existed. The pairing is labeled Acceptor 1. Additionally, antibodies with a combination of germ lines, similar to the pairing of IGKV 1-9*01 with IGHV3-49*04 were found. The pairing is labeled Acceptor 2.

**[00206]** The above-described light and heavy humanized chains were combined to create 14 variant humanized antibodies, whose sequences are described in **Table 12**.

**Table 12**
**Humanized 3D10 Antibodies**

| Antibody | Light Chain | SEQ ID NO. | Heavy Chain | SEQ ID NO. |
|---|---|---|---|---|
| h3D10 Var 1 | LC1_1 | 81 | HC1_1 | 90 |
| h3D10 Var 2 | LC1_2 | 82 | HC1_2 | 91 |
| h3D10 Var 3 | LC1_3 | 83 | HC1_2 | 91 |
| h3D10 Var 4 | LC1_2 | 82 | HC1_3 | 92 |
| h3D10 Var 5 | LC1_3 | 83 | HC1_3 | 92 |
| h3D10 Var 6 | LC2_1 | 84 | HC2_1 | 93 |
| h3D10 Var 7 | LC2_2 | 85 | HC2_2 | 94 |
| h3D10 Var 8 | LC2_3 | 86 | HC2_2 | 94 |
| h3D10 Var 9 | LC2_2 | 85 | HC2_3 | 95 |
| h3D10 Var 10 | LC2_3 | 86 | HC2_3 | 95 |
| h3D10 Var 11 | LC1_1 | 81 | HC2_1 | 93 |
| h3D10 Var 12 | LC2_1 | 84 | HC1_1 | 90 |
| h3D10 Var 13 | LC1_3 | 83 | HC2_3 | 95 |
| h3D10 Var 14 | LC2_3 | 86 | HC1_3 | 92 |

## Example 5
## Production of Humanized Anti-Human PD-1 Antibodies

**[00207]** To illustrate the production of such humanized derivatives, humanized derivatives of anti-human PD-1 antibody 1H3 were produced in accordance with the above-described procedure (a chimeric antibody incorporating the variable region of 1H3 and a human IgG1 Fc region was used as the Parental antibody).

**[00208]** Sequence alignments comparing 1H3 light chain variable domains to the human germ lines were generated using: **IGKV3** light chain germlines (IGKV3-11*01, IGKV3-11*02, IGKV3D-11*01, IGKV3D-20*01, IGKV3-NL4*01, IGKV3D-7*01, IGKV3-20*01, IGKV3-NL5*01, IGKV3-15*01, IGKV3-NL1*01, IGKV3-20*01, IGKV3-NL2*01, IGKV3-NL3*01), **IGKV1** light chain germlines (IGKV1-9*01, IGKV1D-43*01, IGKV1-39*01, IGKV1D-13*02, IGKV1-8*01, IGKV1D-13*01, IGKV1-12*01, IGKV1D-16*01, IGKV1-5*01, and IGKV1-NL1*01) and **IGKJ** light chain germlines (IGKJ2*02, IGKJ2*01, IGKJ2*04, IGKJ2*03, IGKJ5*01, IGKJ4*01, IGKJ3*01, and IGKJ1*01).

**[00209]** Sequence alignments comparing 1H3 heavy chain variable domains to the human germ lines were generated using: **IGHV3** heavy chain germlines (IGHV3-48*01, IGHV3-48*02, IGHV3-48*03, IGHV3-11*01, IGHV3-21*01, IGHV3-11*03, IGHV3-30*03, IGHV3-9*01, IGHV3-7*01, and IGHV3-30*10), **IGHV1** heavy chain germlines (IGHV1-3*01, IGHV1-69*08, IGHV1-69*11, IGHV1-46*01, IGHV1-69*05, IGHV1-69*06, IGHV1-69*01, IGHV1-46*02, IGHV1-69*02, and IGHV1-69*10), and **IGHJ** heavy chain germlines (IGHJ6*01, IGHJ6*03, IGHJ4*01, IGHJ6*04, IGHJ5*02, IGHJ3*02, IGHJ5*01, IGHJ3*01, IGHJ2*01, and IGHJ1*01).

**[00210]** Based on overall sequence identity, matching interface positions and similarly classed CDR canonical positions, two germline families were identified for each of the light and heavy chain as possible Acceptor frameworks (IGKV3 and IGKV1 for the light chain and 1GHV3 and IGHV1 for the heavy chain). Antibody 1H3 was found to be most similar to the light chain germline 1GKV3-11*01 and heavy IGHV3-48*01. Based on overall sequence identity, matching interface positions and similarly classed CDR canonical positions, two germline families were identified for each of the light and heavy chain as possible Acceptor frameworks (IGKV3 and IGKV1 for the light chain and IGHV3, IGHV1 for the heavy chain.

### (A) Humanization of the Light Chain

**[00211]** Antibody 1H3 has a short 10 residue CDR L1 and falls into canonical class I. No human germ line has such a short CDR L1, but the closest germlines in each selected family (IGKV3-II*01 and IGKV1-9*01 have short L1 loops and contain the correct framework residues to support a class I LI loop. The overall sequence similarity was good for both Acceptor frameworks; however there are differences at two interface positions (Y33 and P45). Y33 lies in CDR Ll and while the two Acceptor families do not contain tyrosine at this position other potential acceptor families do. The difference at P45 was tied in with a

number of other differences in FR2 of the Parental light chain. In short, the Parental germline belongs to a mouse germline family with a FR2 dissimilar to anything in the human germlines.

**[00212]** Germ lines 1GKV3-11 *01 and IGKV 1-9*01 were selected as light chain Acceptor frameworks. The sequences of the light chains of 1GKV3-11 *01 and IGKV 1-9*01 are shown above as **SEQ ID NO:78** and **SEQ ID NO:79**, respectively. The alignments of these sequences with the light chain of antibody 1H3 are shown in **Table 13** (CDR residues of antibody 1H3 are shown in italics, identical aligned residues are shown underlined):

**Table 13**
**Alignment of Light Chain Variable Regions**

| Light Chain | SEQ ID NO. | Sequence |
|---|---|---|
| | | 1 10 20 30 40 |
| 1H3 | **15** | QIVLTQSPAL MSASPGEKVT MTC***SASSSVS YMY***WYQQKPR |
| IGKV3-11*01 | **78** | EIVLTQSPAT LSLSPGERAT LSCRASQSVSS YLAWYQQKPG |
| IGKV1-9*01 | **79** | DIQLTQSPSF LSASVGDRVT ITCRASQGISS YLAWYQQKPG |
| | | 41 50 60 70 80 |
| 1H3 | **15** | SSPKPWIY***LT SNLAS***GVPAR FSGSGSGTSY SLTISSMEAE |
| IGKV3-11*01 | **78** | QAPRLLIYDA SNRATGIPAR FSGSGSGTDF TLTISSLEPE |
| IGKV1-9*01 | **79** | KAPKLLIYAA STLQSGVPSR FSGSGSGTEF TLTISSLQPE |
| | | 81 90 100 106 |
| 1H3 | **15** | DAATYYC***QQW SSNPFT***FGSG TKLEIK |
| IGKV3-11*01 | **78** | DFAVYYCQQR SNWP------ ------ |
| IGKV1-9*01 | **79** | DFATYYCQQL NSYP------ ------ |

**[00213]** The J-segment genes were compared to the Parental sequence over FR4, and J-segment IGKJ2*02 (**SEQ ID NO:96**: CTFGQGTKLEIK) was selected for the light chain.

**[00214]** Two humanized chains were created for each of the two preferred Acceptor frameworks, IGKV3-11*01 and IGKV1-9*01. The two LC1 chains are derived from IGKV3-11*01; the two LC2 chains are derived from IGKV1-9*01. The first humanized chain for each Acceptor framework contained all humanizing substitutions deemed possible and is the most human of the three chains. The second humanized chain for each Acceptor framework contained several back mutations at positions that alter the charge, potentially

interfere with the core packing or could affect the conformation of the CDRs. The sequences of these four humanized chains are shown below:

LC1_1 **(SEQ ID NO:97)** EIVLTQSPAT LSLSPGERAT LSCRASSSVS YMYWYQQKPG QAPRLLIYLA SNRATGIPAR FSGSGSGTDY TLTISSLEPE DFAVYYCQQW SSNPFTFGQG TKLEIK

LC1_2 **(SEQ ID NO:98)** QIVLTQSPAT LSLSPGERAT LSCSASSSVS YMYWYQQKPG QAPRLLIYLT SNRATGIPAR FSGSGSGTDY TLTISSLEPE DFAVYYCQQW SSNPFTFGQG TKLEIK

LC2_1 **(SEQ ID NO:99)** DIQLTQSPSS LSASVGDRVT ITCRASSSVS YMYWYQQKPG KAPKLLIYLA SNLASGVPSR FSGSGSGTEY TLTISSLEPE DFATYYCQQW SSNPFTFGQG TKLEIK

LC2_2 **(SEQ ID NO:100)** QIQLTQSPSS LSASVGDRVT ITCSASSSVS YMYWYQQKPG KAPKLLIYLT SNLASGVPSR FSGSGSGTEY TLTISSLEPE DFATYYCQQW SSNPFTFGQG TKLEIK

**[00215]** The sequences for these four humanized chains are shown in **Table 14** with differences relative to the Parental 1H3 Var1 light chain shown in boldface and underline.

**Table 14**
**Humanized Light Chains of Antibody 1H3**

| Light Chain | SEQ ID NO | Sequence |
|---|---|---|
| LC1_1 | **97** | **E**IVLTQSPA**T** **L**S**L**SPGE**RA**T **LS**C**R**ASSSVS YMYWYQQKP**G** **QA**P**RLL**IYL**A** SN**R**A**T**G**I**PAR FSGSGSGT**D**Y **T**LTISS**L**E**P**E D**F**A**V**YYCQQW SSNPFTFG**Q**G TKLEIK |
| LC1_2 | **98** | QIVLTQSPA**T** **L**S**L**SPGE**RA**T **LS**CSASSSVS YMYWYQQKP**G** **QA**P**RLL**IYLT SN**R**A**T**G**I**PAR FSGSGSGT**D**Y **T**LTISS**L**E**P**E D**F**A**V**YYCQQW SSNPFTFG**Q**G TKLEIK |
| LC2_1 | **99** | **D**I**Q**LTQSP**SS** **L**SAS**V**G**DR**VT **I**TC**R**ASSSVS YMYWYQQKP**G** **KA**PK**LL**IYL**A** SNLASGVP**S**R FSGSGSGT**E**Y **T**LTISS**L**E**P**E D**F**ATYYCQQW SSNPFTFG**Q**G TKLEIK |
| LC2_2 | **100** | QI**Q**LTQSP**SS** **L**SAS**V**G**DR**VT **I**TCSASSSVS YMYWYQQKP**G** **KA**PK**LL**IYLT SNLASGVP**S**R FSGSGSGT**E**Y **T**LTISS**L**E**P**E D**F**ATYYCQQW SSNPFTFG**Q**G TKLEIK |

**(B) Humanization of the Heavy Chain**

**[00216]** In light of the above-discussed criteria, two candidate germline Acceptor frameworks: IGHV3-48*01 (IGHV3 Germline) and IGHV1-3*01 (IGHV1 Germline) were selected for the humanization of the heavy chain of antibody 1H3 Var1.

**[00217]** Acceptor Framework 1GHV3-48*01 was selected as the primary heavy Acceptor framework due to its overall sequence similarity. The interface residues match except for H35, a position at where some variation is allowed and it contains the correct residues for determining canonical class for CDR H1. CDR H2 does not fall into any sequence-based canonical class due to a tyrosine at position 56. The choice of the second heavy chain Acceptor framework was made after removing any germ line closely related to IGHV3-48*01. The closest germ line then became IGHV1-3*01. The number of differences to take into account becomes larger as the packing of the lower core is different, however the germline should support the CDRs and function as a suitable Acceptor framework. It was noted that the Parental sequence contains a cysteine at the conserved position 60, where all human germlines have a tyrosine. The sequences of Acceptor Framework 1GHV3-48*01 and Acceptor Framework IGHV1-3*01 are shown below:

**[00218]** Acceptor Framework 1GHV3-48*01 (**SEQ ID NO:101**):

EVQLVESGGG LVQPGGSLRL SCAASGFTFS SYSMNWVRQA PGKGLEWVSY
ISSSSSTIYY ADSVKGRFTI SRDNAKNSLY LQMNSLRAED TAVYYCAR

**[00219]** Acceptor Framework IGHV1-3*01 (**SEQ ID NO:102**):

QVQLVQSGAE VKKPGASVKV SCKASGYTFT SYAMHWVRQA PGQRLEWMGW
INAGNGNTKY SQKFQGRVTI TRDTSASTAY MELSSLRSED TAVYYCAR

**[00220]** **Table 15** shows the alignment of these sequences with the heavy chain of antibody 1H3 (CDR residues of antibody 1H3 are shown in italics, identical aligned residues are shown underlined):

**Table 15**
**Alignment of Heavy Chain Variable Regions**

| Heavy Chain | SEQ ID NO. | Sequence |
|---|---|---|
| | | 1 10 20 30 40 |
| 1H3 | **16** | EVQLVESGGG LVKPGGSLKL SCAAS***GFTFS DYGMH***WVRQA |
| IGHV3-48*01 | **101** | EVQLVESGGG LVQPGGSLRL SCAASGFTFS SYSMNWVRQA |
| IGHV1-3*01 | **102** | QVQLVQSGAE VKKPGASVKV SCKASGYTFT SYAMHWVRQA |
| | | 41 50 60 70 80 |

| | | |
|---|---|---|
| 1H3 | **16** | PEKGLEWVA***Y ISSGSYTIYY TDTVKG***RFTI SRDNAKNTLF |
| IGHV3-48*01 | **101** | PGKGLEWVSY ISSSSSTIYY ADSVKGRFTI SRDNAKNSLY |
| IGHV1-3*01 | **102** | PGQRLEWMGW INAGNGNTKY SQKFQGRVTI TRDTSASTAY |
| | | 81 90 100 110 120 |
| 1H3 | **16** | LQMTSLRSED TAMYYCAR***RG YGSFYEYYFD Y***WGQGTTLTV SS |
| IGHV3-48*01 | **101** | LQMNSLRAED TAVYYCAR |
| IGHV1-3*01 | **102** | MELSSLRSED TAVYYCAR |

**[00221]** The J-segment genes were compared to the Parental sequence over FR4, and J-segment IGHJ6*01 (**SEQ ID NO:103**: WGQGTTVTV) was selected for the heavy chain.

**[00222]** Three humanized chains were created for each of the two preferred Acceptor frameworks, IGHV3-48*01 and IGHV1-3*01. The three HC1 chains are derived from IGHV3-48*01; the three HC2 chains are derived from IGHV1-3*01). The first humanized chain for each Acceptor framework contained all humanizing substitutions deemed possible and is the most human of the three chains. The second humanized chain for each Acceptor framework contained several back mutations at positions that alter the charge, potentially interfere with the core packing or could affect the conformation of the CDRs. The third chain, for each of the Acceptor frameworks, contained the most back mutations, including substitutions that alter the charge and could potentially alter the binding affinity. The sequences of these six humanized chains are shown below:

HC1_1 **(SEQ ID NO:104)**
EVQLVESGGG LVQPGGSLRL SCAASGFTFS DYGMHWVRQA
PGKGLEWVSY ISSGSSTIYY ADSVKGRFTI SRDNAKNTLY
LQMSSLRAED TAVYYCARRG YGSFYEYYFD YWGQGTTVTV
SS

HC1_2 **(SEQ ID NO:105)**
EVQLVESGGG LVQPGGSLRL SCAASGFTFS DYGMHWVRQA
PGKGLEWVAY ISSGSYTIYY ADSVKGRFTI SRDNAKNTLY
LQMSSLRAED TAVYYCARRG YGSFYEYYFD YWGQGTTVTV
SS

HC1_3 **(SEQ ID NO:106)**
EVQLVESGGG LVQPGGSLRL SCAASGFTFS DYGMHWVRQA
PGKGLEWVAY ISSGSYTIYS ADSVKGRFTI SRDNAKNTLY
LQMSSLRAED TAVYYCARRG YGSFYEYYFD YWGQGTTVTV
SS

HC2_1 **(SEQ ID NO:107)**
QVQLVQSGAE VKKPGASVKV SCKASGFTFS DYGMHWVRQA
PGQRLEWMGY ISSGSSTIYY SQKFQGRVTI TRDNSASTLY
MELSSLRSED TAVYYCARRG YGSFYEYYFD YWGQGTTLTV
SS

HC2_2 **(SEQ ID NO:108)** EVQLVQSGAE VKKPGASVKV SCAASGFTFS DYGMHWVRQA PGQRLEWMGY ISSGSYTIYY SQKFQGRVTI TRDNSASTLY MELSSLRSED TAVYYCARRG YGSFYEYYFD YWGQGTTLTV SS

HC2_3 **(SEQ ID NO:109)** EVQLVQSGAE VKKPGASVKV SCAASGFTFS DYGMHWVRQA PGQRLEWVAY ISSGSYTIYY SQKFQGRVTI TRDNSASTLY MELSSLRSED TAVYYCARRG YGSFYEYYFD YWGQGTTLTV SS

**[00223]** The sequences for these six humanized chains are shown in **Table 16** with differences relative to the Parental 1H3 heavy chain shown in boldface and underline.

**Table 16**
**Humanized Heavy Chains of Antibody 3H1**

| Heavy Chain | SEQ ID NO | Sequence |
|---|---|---|
| HC1_1 | **104** | EVQLVESGGG LV**Q**PGGSL**R**L SCAASGFTFS DYGMHWVRQA P**G**KGLEWV**S**Y ISSGS**S**TIY**Y** **A**D**S**VKGRFTI SRDNAKNTL**Y** LQM**S**SLR**A**ED TA**V**YYCARRG YGSFYEYYFD YWGQGTT**V**TV SS |
| HC1_2 | **105** | EVQLVESGGG LV**Q**PGGSL**R**L SCAASGFTFS DYGMHWVRQA P**G**KGLEWVAY ISSGSYTIY**Y** **A**D**S**VKGRFTI SRDNAKNTL**Y** LQM**S**SLR**A**ED TA**V**YYCARRG YGSFYEYYFD YWGQGTT**V**TV SS |
| HC1_3 | **106** | EVQLVESGGG LV**Q**PGGSL**R**L SCAASGFTFS DYGMHWVRQA P**G**KGLEWVAY ISSGSYTIY**S** **A**D**S**VKGRFTI SRDNAKNTL**Y** LQM**S**SLR**A**ED TA**V**YYCARRG YGSFYEYYFD YWGQGTT**V**TV SS |
| HC2_1 | **107** | **Q**VQLV**Q**SG**AE** **VKK**PG**A**S**V**KV SC**K**ASGFTFS DYGMHWVRQA P**GQR**LEW**MG**Y ISSGS**S**TIY**Y** **SQKFQ**GR**V**TI **T**RDN**SAS**TL**Y** **MELS**SLRSED TA**V**YYCARRG YGSFYEYYFD YWGQGTTLTV SS |
| HC2_2 | **108** | EVQLV**Q**SG**AE** **VKK**PG**A**S**V**KV SCAASGFTFS DYGMHWVRQA P**GQR**LEW**MG**Y ISSGSYTIY**Y** **SQKFQ**GR**V**TI **T**RDN**SAS**TL**Y** **MELS**SLRSED TA**V**YYCARRG YGSFYEYYFD YWGQGTTLTV SS |
| HC2_3 | **109** | EVQLV**Q**SG**AE** **VKK**PG**A**S**V**KV SCAASGFTFS DYGMHWVRQA P**GQR**LEW**V**AY ISSGSYTIY**Y** **SQKFQ**GR**V**TI **T**RDN**SAS**TL**Y** **MELS**SLRSED TA**V**YYCARRG YGSFYEYYFD YWGQGTTLTV SS |

### (C) Humanized Derivatives of Antibody 1H3

**[00224]** A search confirmed that antibodies with a combination of germlines close to the pairing of IGKV3-11*01 with heavy IGHV3-48*01 exist. The pairing is labeled Acceptor 1. Subsequently, antibodies with a similar pairing to IGKVI-9*01 with IGHVI-3*01 were found. The pairing is labeled Acceptor 2.

**[00225]** The above-described light and heavy humanized chains were combined to create 14 variant humanized antibodies, whose sequences are described in **Table 17**.

| Table 17<br>Humanized 1H3 Antibodies | | | | |
|---|---|---|---|---|
| **Antibody** | **Light Chain** | **SEQ ID NO.** | **Heavy Chain** | **SEQ ID NO.** |
| h1H3 Var 1 | LC1_1 | 97 | HC1_1 | 104 |
| h1H3 Var 2 | LC1_1 | 97 | HC1_2 | 105 |
| h1H3 Var 3 | LC1_1 | 97 | HC1_3 | 106 |
| h1H3 Var 4 | LC1_2 | 98 | HC1_1 | 104 |
| h1H3 Var 5 | LC1_2 | 98 | HC1_2 | 105 |
| h1H3 Var 6 | LC1_2 | 98 | HC1_3 | 106 |
| h1H3 Var 7 | LC2_1 | 99 | HC2_1 | 107 |
| h1H3 Var 8 | LC2_1 | 99 | HC2_2 | 108 |
| h1H3 Var 9 | LC2_1 | 100 | HC2_3 | 109 |
| h1H3 Var 10 | LC2_2 | 100 | HC2_1 | 107 |
| h1H3 Var 11 | LC2_2 | 99 | HC2_2 | 108 |
| h1H3 Var 12 | LC2_2 | 99 | HC2_2 | 108 |
| h1H3 Var 13 | LC1_1 | 96 | HC2_1 | 107 |
| h1H3 Var 14 | LC2_1 | 98 | HC1_1 | 104 |

## Example 6
## Characterization of 1H3 Anti-Human PD-1 Antibody

**[00226]** In order to evaluate the properties of the anti-PD-1 antibodies of the present invention, a construct was produced which possesses chimeric ("ch") murine anti-human PD-1 Fab regions of antibody 1H3 and a human IgG1 Fc region (1H3 Construct). The construct was tested for its ability to bind human PD-1.

**[00227]** **Figure 12** shows the results of an experiment in which CHO cells transfected with human full length PD-1 were pre-incubated with a saturated dose of PD-1 mAbs before being stained by biotin-labeled anti-hB7-H1-Fc or anti-hB7-DC mFc. **Figure 12**, shows that a murine monoclonal antibody construct which possesses chimeric ("ch") murine anti-human PD-1 Fab regions of antibody 1H3 and a human IgG1 Fc region blocks binding of B7-H1-Fc and B7-DC-Fc to cells expressing human PD-1, as a result of antibody binding to the cells. M1, m3 and 1E8 are all positive control anti-PD-1 antibodies. On non-reducing gels, the Construct migrated as a single band of approximately 200 MW; this band was replaced with band at approximately 52 MW under reducing conditions.

**[00228]** The 1H3 Construct exhibited an Affinity $K_D$ of 2.19 nM, an "on rate" $K_a$ of 0.734 x $10^{-5}$ /Ms, and an "off rate" $K_d$ of 1.61 x $10^{-4}$ /s. The $EC_{50}$ of the construct was found to be 75 ng. **Figure 13** compares the binding obtained with this construct to that of the commercially available anti-PD-1 antibody, EH12. The construct was found to be capable of completely

blocking the ability of hPD-1 (expressed by CHO cells) to bind to B7-H1-Fc or B7-DC-Fc as shown **in Figure 14**. **Figure 15** shows that the chimeric 1H3 Construct was able to bind to hPD-1-Fc and block the binding of such hPD-1-Fc to hB7-H1 expressed by CHO cells.

**[00229]** The ability of the 1H3 Construct to bind to human primary T cells was assessed relative to a control antibody (palivizumab; SYNAGIS®, Medimmune, Inc.); 1H3 demonstrated enhanced binding to both CD8 and CD4 cells (**Figures 16A-16B**).

**[00230]** To illustrate the functional characteristics of the antibodies of the present invention, the ability of Construct 1H3, and of chimeric antibody constructs having the FAB regions of antibody 1H3 ("1H3 Construct"), 1E3 ("1E3 Construct"), and antibody 3D10 ("3D10 construct") to enhance T cell activity was assessed. Immature dendritic cells (DC) were exposed to TNFα and PGE2 for two days (cells were incubated in the presence of 50 μg/ml tetanus toxoid (TT) overnight at the second day of maturation). The resultant cells were found to have become mature DC as determined by their acquired ability to express B7-H1 and B7-DC. The matured DC cells were then incubated for two weeks in the presence of carboxyfluorescein succinimidyl ester (CFSE)-labeled autologous T cells and 100 ng/ml TT and the above-described antibody constructs. As shown in **Figure 17**, the antibodies of the present invention were capable of block B7-H1-PD-1 interactions as measured by expansion of antigen-specific memory T cells. At Day 7, an analysis of the cytokines present in the cell supernatants was conducted (**Table 18**).

**Table 18**

| Cytokine (pg/ml) | Control Ig | 1H3 Construct | 1E3 Construct | 3H10 Construct |
|---|---|---|---|---|
| G-CSF | 8.04 | 18.41 | 17.39 | 20.40 |
| GM-CSF | 35.12 | 379.05 | 162.30 | 445.78 |
| IFN-γ | 61.78 | 5967.64 | 1247.81 | 5337.78 |
| IL-2 | 12.72 | 12.25 | 9.45 | 13.96 |
| IL-4 | 3.16 | 9.93 | 6.98 | 9.59 |
| IL-5 | 3.51 | 31.34 | 9.40 | 110.40 |
| IL-6 | 125.40 | 418.24 | 134.56 | 124.31 |
| IL-7 | 7.88 | 21.81 | 13.67 | 11.93 |
| IL-8 | 5027.28 | 7978.03 | 5010.73 | 4292.56 |
| IL-10 | 6.99 | 29.45 | 15.30 | 18.33 |
| IL-12p70 | 23.18 | 106.55 | 74.84 | 110.13 |
| IL-13 | 18.90 | 378.05 | 79.33 | 738.62 |
| IL-17 | 368.98 | 503.01 | 407.81 | 421.37 |
| MCP-1 | 2174.36 | 9954.89 | 4792.30 | 5895.81 |
| MIP-1β* | 1453.85 | 5750.63 | 9197.32 | 8511.53 |

| Table 18 | | | | |
|---|---|---|---|---|
| **Cytokine (pg/ml)** | **Control Ig** | **1H3 Construct** | **1E3 Construct** | **3H10 Construct** |
| TNF-α | 51.75 | 486.39 | 353.89 | 949.85 |
| * day 6 | | | | |

**[00231]** As shown in **Table 18**, both of the anti-hPD-1 antibody constructs promoted both Th1 and Th2 responses. Very low amount of IL-1β, IL-2, IL-4, IL-7, IL-10 and G-CSF were found in the supernatants. All mAbs have very low endotoxin, less than 0.01 EU/mg; additionally, the DC and T cells were kept in serum-free media. Intracellular IFN-γ staining of Day 7 cells revealed that whereas only 0.15% of control cells were IFN-$\gamma^+$, 1.9% of cells that had been incubated with the 1H3 Construct, 0.91% of cells that had been incubated with the 1E3 Construct, and 3.2% of cells that had been incubated with the 3D10 Construct were IFN-$\gamma^+$.

**[00232]** Thus, in sum, the 1H3 Construct was found to mediate approximately a 7-fold increase in T cell proliferation and approximately a 12 fold increase in IFN-γ production per cell. The cumulative effect of such action is an approximately 100 fold increase in IFN-γ secretion.

**[00233]** As further functional characterization, monocyte-derived DC were matured by incubation with TNFα and PGE2. Cells were then pulsed for 2 hours in the presence of a pool of mixed class I and class II restricted CEF peptides (*i.e.*, peptides of **C**ytomegalovirus, **E**pstein-Barr virus and in**f**luenza virus). , and incubated with CFSE-labeled autologous T cell (LD column, 95% purify) for two weeks. The treated cells were then incubated for two weeks in the presence of CFSE-labeled autologous T cells (LD column, 95% purify) and the above-described antibody constructs. On Day 7, the percentage of CFSE-diluted T cells was found to be 40% for cells incubated with the control antibody, 37% for cells incubated with the 1H3 Construct, 50% for cells incubated with the 3D10 Construct and 57% for cells incubated with antibody CA-18C3 (an anti-IL-1α-specific monoclonal antibody). On Day 11, the percentage of CFSE-diluted T cells was again assessed. The percentage of CFSE-diluted T cells was then found to be 17% for cells incubated with the control antibody and 38% for cells incubated with the 1H3 Construct. The percentage of CFSE-diluted T cells was then found to be 27% for cells incubated with antibody CA-18C3.

**[00234]** The supernatants of the treated cells were also analyzed for IL-2 and IFN-γ cytokines on day 7 (**Table 19**).

| Table 19 | | | | |
|---|---|---|---|---|
| **Cytokine (pg/ml)** | **Control Ig** | **1H3 Construct** | **1E3 Construct** | **3H10 Construct** |
| IFN-γ | 26816.44 | 39423.11 | 39658.19 | 31954.72 |
| IL-2 | 432.03 | 868.17 | 1182.30 | 1379.07 |

**[00235]** The supernatants of the treated cells were also analyzed for IFN-γ, TNFα and GM-CSF cytokines on day 11. The 1H3 Construct was found to mediate an enhancement of all three cytokines relative to a control antibody (**Table 20**).

| Table 20 | | | |
|---|---|---|---|
| **Cytokine (pg/ml)** | **Control Ig** | **1H3 Construct** | **3H10 Construct** |
| GM-CSF | 52.75 | 150.66 | 70.71 |
| IFN-γ | 223.15 | 786.11 | 228.50 |
| TNF-α | 21.06 | 46.26 | 51.75 |

**[00236]** As further functional characterization, monocyte-derived DC (obtained from HLA-A2 positive donor PBMCs) were matured by exposure to TNFα and PGE2, and the matured DC were then pulsed with HLA-A2 restricted MART-1 and Flu M1 peptides for 2 hours. Afterwards, the cells were incubated for two weeks in the presence of CFSE-labeled autologous T cell (LD column, 95% purify) and the above-described 1H3Construct or 3D10 Construct. The effect of the MART-1 and M1 peptides on cytokine production is shown in **Table 21**.

| Table 21 | | | |
|---|---|---|---|
| **Cytokine (pg/ml)** | **Control Ig** | **1H3 Construct** | **3H10 Construct** |
| IFN-γ | 0 | 357.64 | 0 |
| IL-17 | 0 | 225.81 | 19.76 |

## Example 7
## Characterization of Humanized Anti-PD-1 Antibodies

**[00237]** The above-described humanized 1H3_var 1 –1H3_var 14 antibodies (see **Table 17**) were evaluated to confirm their ability to bind to human PD-1 and their therapeutic

capabilities. Polypeptides encoding the antibodies were expressed in CHO cells and the titers of functional antibodies were determined by ELISA (**Table 22**).

**Table 22**

| Antibody | Light Chain | Heavy Chain | Antibody Titer (ng/ml) |
|---|---|---|---|
| h1H3 Var 1 | LC1_1 | HC1_1 | 623 |
| h1H3 Var 2 | LC1_1 | HC1_2 | 6520 |
| h1H3 Var 3 | LC1_1 | HC1_3 | 1344 |
| h1H3 Var 4 | LC1_2 | HC1_1 | 1160 |
| h1H3 Var 5 | LC1_2 | HC1_2 | 8840 |
| h1H3 Var 6 | LC1_2 | HC1_3 | 1906 |
| h1H3 Var 7 | LC2_1 | HC2_1 | 35 |
| h1H3 Var 8 | LC2_1 | HC2_2 | 22 |
| h1H3 Var 9 | LC2_1 | HC2_3 | 4 |
| h1H3 Var 10 | LC2_2 | HC2_1 | 232 |
| h1H3 Var 11 | LC2_2 | HC2_2 | 66 |
| h1H3 Var 12 | LC2_2 | HC2_2 | 17 |
| h1H3 Var 13 | LC1_1 | HC2_1 | 219 |
| h1H3 Var 14 | LC2_1 | HC1_1 | 134 |
| 1H3 Parental Control Antibody | -- | -- | 158 |
| Control | -- | -- | 11 |

**[00238]** To ensure that binding was specific for human PD-1, binding was assessed using CHO cells that had been transfected to express human PD-1. The results of such binding experiments are shown in **Figures 18A-18D**. By repeating such binding experiments in the presence of 1, 3, 10, 30, 100, 300, 1000 or 3000 ng of the h1H3 variant, it was found that the ability of such humanized antibodies to bind to PD-1 was in all cases dependent upon antibody concentration.

**[00239]** In order to demonstrate the ability of the humanized anti-PD-1 antibodies of the present invention to block interactions between PD-1 and its natural ligands, PD-1-expressing HEK293 cells were incubated in the presence of B7-H1 (or B7-DC) and selected h1H3 variants. The h1H3 variant antibodies were found to be capable of blocking the binding of the B7-H1 to the HEK293 cells (**Figure 19A** (B7-H1); **Figure 19B** (B7-DC) (Ctl = synagis, WT = chimeric 1H3).

**[00240]** **Table 23** provides the results obtained in a 500 ml scale transient expression and purification procedure. Non-reducing gels showed that the expressed antibodies predominantly migrated as a single band of approximately 160kD; when analyzed in reducing

gels, this band was replaced by bands of approximately 60 kD and 30 kD. The results show that Acceptor 1 h1H3 variants: h1H3 Var1, h1H3 Var3, h1H3 Var4 and h1H3 Var6 exhibited good binding to human PD-1 whereas Acceptor 2 h1H3 variants: h1H3 Var7 - h1H3 Var14 exhibited poorer binding to human PD-1. Accordingly, the heavy and light chains from Acceptor 1 h1H3 variants: h1H3 Var1 - h1H3 Var6 were cloned into the Double Gene Vector (DGV; Lonza Biologics, Berkshire, UK; Bebbington, C.R. *et al.* (1992) "*High-Level Expression Of A Recombinant Antibody From Myeloma Cells Using A Glutamine Synthetase Gene As An Amplifiable Selectable Marker*," Biotechnology (NY) 10(2):169-175) and transfected into CHO cells to permit the production of stable antibody-producing cell lines.

**Table 23**

| h1H3 Variant | Concentration (mg/ml) | Volume (ml) | Total (mg) | EU/μg | $EC_{50}$ (nM) |
|---|---|---|---|---|---|
| h1H3 Var 1 | 1.31 | 1.5 | 2.0 | 0.012 * | 6 |
| h1H3 Var 2 | 0.99 | 1.7 | 1.7 | 0.003 | 3 |
| h1H3 Var 3 | 0.61 | 1.6 | 1.0 | 0.005 | 3 |
| h1H3 Var 4 | 1.67 | 1.6 | 2.7 | 0.003 | 6 |
| h1H3 Var 5 | 1.44 | 1.5 | 2.2 | 0.003 | 3 |
| h1H3 Var 6 | 0.69 | 1.6 | 1.1 | 0.008 | 3 |
| Parental | | | | | 3 |
| * Commercial very low endotoxin level <0.01 EU/μg | | | | | |

**[00241]** Analysis of binding at antibody concentrations of 0.5, 1.5, 5, 15, 50, 150, and 500 ng/ml as well as 1.5, 5, 15, and 50 μg/ml revealed that binding was concentration dependent. The PD-1-specific binding ability of the variants was determined at antibody concentrations ranging from 0 to approximately 350 nM. **Figure 20** shows the resultant curves for h1H3 Var 1 - h1H3 Var 6, and indicate that these antibodies bind PD-1. Antibodies h1H3 Var 1 and h1H3 Var 4 exhibit reduced binding relative to the Parental antibody. In contrast, h1H3 Var 2, h1H3 Var 3, h1H3 Var 5 and h1H3 Var 6 exhibited binding that was comparable to that of the Parental antibody. The EC50 data for these antibodies is shown in **Table 23**.

**[00242]** All publications and patents mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference in its entirety. While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of

the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth.

**What Is Claimed Is:**

Claim 1. A molecule, comprising an antigen-binding fragment of an antibody that immunospecifically binds to B7-H1 or PD-1.

Claim 2. The molecule of claim 1, wherein said immunospecifically bound B7-H1 or PD-1 is expressed on the surface of a live cell at an endogenous or transfected concentration.

Claim 3. The molecule of any of claims 1-2, wherein said B7-H1 is human B7-H1 and said PD-1 is human PD-1.

Claim 4. The molecule of any of claims 1-3, wherein said antigen-binding fragment binds to B7-H1, and wherein said live cell is a tumor cell, a pathogen-infected cell or an Antigen Presenting Cell.

Claim 5. The molecule of any of claims 1-3, wherein said antigen-binding fragment binds to PD-1, and wherein said live cell is a T cell.

Claim 6. The molecule of any of claims 1-5, wherein said molecule is a monoclonal antibody, a human antibody, a chimeric antibody or a humanized antibody.

Claim 7. The antibody of claim 6, wherein said antibody is a bispecific, trispecific or multispecific antibody.

Claim 8. The molecule of any of claims 3 or 4, or the antibody of any of claims 6 or 7, wherein molecule or antibody binds to B7-H1, and wherein said antigen-binding fragment thereof comprises six CDRs, wherein said CDRs comprise at least one consensus CDR of the CDRs of anti-B7-H1 antibodies 1E12, 1F4, 2G11, 3B6, and 3D10 with all remaining CDRs selected from:

(A) the three light chain and the three heavy chain CDRs of anti-B7-H1 antibody 1E12;

(B) the three light chain and the three heavy chain CDRs of anti-B7-H1 antibody 1F4;

(C) the three light chain and the three heavy chain CDRs of anti-B7-H1 antibody 2G11;

(D) the three light chain and the three heavy chain CDRs of anti-B7-H1 antibody 3B6; or

(E) the three light chain and the three heavy chain CDRs of anti-B7-H1 antibody 3D10.

Claim 9. The molecule or antibody of claim 8, wherein said six CDRs are:

(A) the three light chain and the three heavy chain CDRs of anti-B7-H1 antibody 1E12;

(B) the three light chain and the three heavy chain CDRs of anti-B7-H1 antibody 1F4;

(C) the three light chain and the three heavy chain CDRs of anti-B7-H1 antibody 2G11;

(D) the three light chain and the three heavy chain CDRs of anti-B7-H1 antibody 3B6; or

(E) the three light chain and the three heavy chain CDRs of anti-B7-H1 antibody 3D10.

Claim 10. The antibody of any of claims 6-9, wherein said antibody binds to B7-H1 and comprises a variable domain of antibody h3D10 Var 1, h3D10 Var 2, h3D10 Var 3, h3D10 Var 4, h3D10 Var 5, h3D10 Var 6, h3D10 Var 7, h3D10 Var 8, h3D10 Var 9, h3D10 Var 10, h3D10 Var 11, h3D10 Var 12, h3D10 Var 13, or h3D10 Var 14.

Claim 11. The molecule of any of claims 3 or 5, or the antibody of any of claims 6 or 7, wherein molecule or antibody binds to PD-1, and wherein said antigen-binding fragment comprises six CDRs, wherein said CDRs comprise at least one consensus CDR of the CDRs of anti-PD-1 antibodies 1E3, 1E8 and 1H3 with all remaining CDRs selected from:

(A) the three light chain and the three heavy chain CDRs of anti-PD-1 antibody 1E3;

(B) the three light chain and the three heavy chain CDRs of anti-PD-1 antibody 1E8; or

(C) the three light chain and the three heavy chain CDRs of anti-PD-1 antibody 1H3.

Claim 12. The molecule or antibody of claim 11, wherein said six CDRs are:

(A) the three light chain and the three heavy chain CDRs of anti-PD-1 antibody 1E3;

(B) the three light chain and the three heavy chain CDRs of anti-PD-1 antibody 1E8; or

(C) the three light chain and the three heavy chain CDRs of anti-PD-1 antibody 1H3.

Claim 13. The antibody of any of claims 6, 7, 11 or 12, wherein said antibody binds to PD-1 and comprises a variable domain of antibody h1H3 Var 1, h1H3 Var 2, h1H3 Var 3, h1H3 Var 4, h1H3 Var 5, h1H3 Var 6, h1H3 Var 7, h1H3 Var 8, h1H3 Var 9, h1H3 Var 10, h1H3 Var 11, h1H3 Var 12, h1H3 Var 13, or h1H3 Var 14.

Claim 14. The molecule of any of claims 1-5, 8-9, 11-12, or the antibody of any of claims 6-13, wherein said molecule or antibody is detectably labeled or comprises a conjugated toxin, drug, receptor, enzyme, receptor ligand.

Claim 15. The molecule of any of claims 1-5, 8-9, 11-12 or 14, or the antibody of any of claims 6-14, wherein said molecule or antibody:

(A) modulates signal transduction mediated by B7-H1 or PD-1;

(B) attenuates the ability of B7-H1 to bind to a B7-H1 receptor or attenuates the ability of PD-1 to bind to a PD-1 ligand;

(C) agonizes B7-H1 or PD-1 mediated signal transduction;

(D) mediates T cell proliferation; or

(E) enhances the production of IFN-γ.

Claim 16. A pharmaceutical composition comprising a therapeutically effective amount of the molecule of claim 15, and a physiologically acceptable carrier or excipient.

Claim 17. Use of the pharmaceutical composition of claim 16 in the treatment of cancer, autoimmune disease, infectious disease, or a disease affecting T cell number or health.

Claim 18. The use of claim 17, wherein said treatment is prophylactic, and is provided in advance of any symptom of said cancer, said autoimmune disease, said infectious disease, or said disease affecting T cell number or health.

Claim 19. Use of the molecule of any of claims 1-5, 8-9, 11-12 or 14, or the antibody of any of claims 6-14, for diagnosing cancer, autoimmune disease, infectious disease, or a disease affecting T cell number or health in a subject by assaying cells of said subject for their ability to bind to B7-H1 or PD-1.

Claim 20. The use of any of claims 17-19, wherein:

(A) said infectious disease is a chronic viral disease; or

(B) said autoimmune disease is a graft vs host disease.

Figure 1




Figure 2



EX1002 - Page 1110

Figure 3



EX1002 - Page 1111

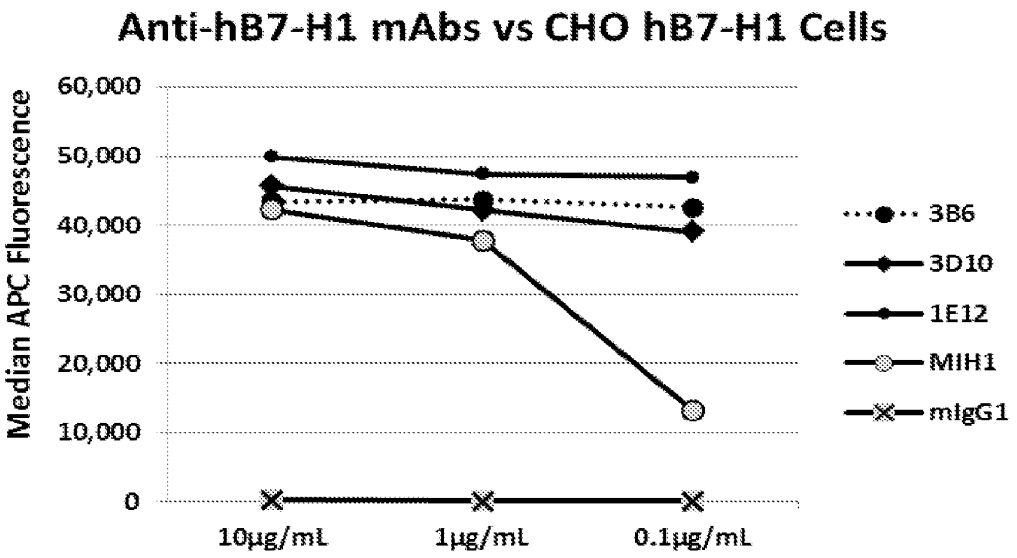



Figure 4

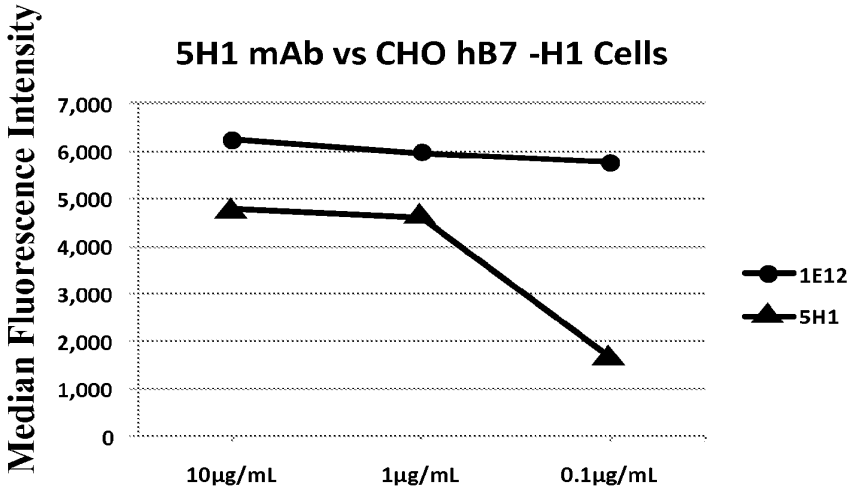



Figure 5

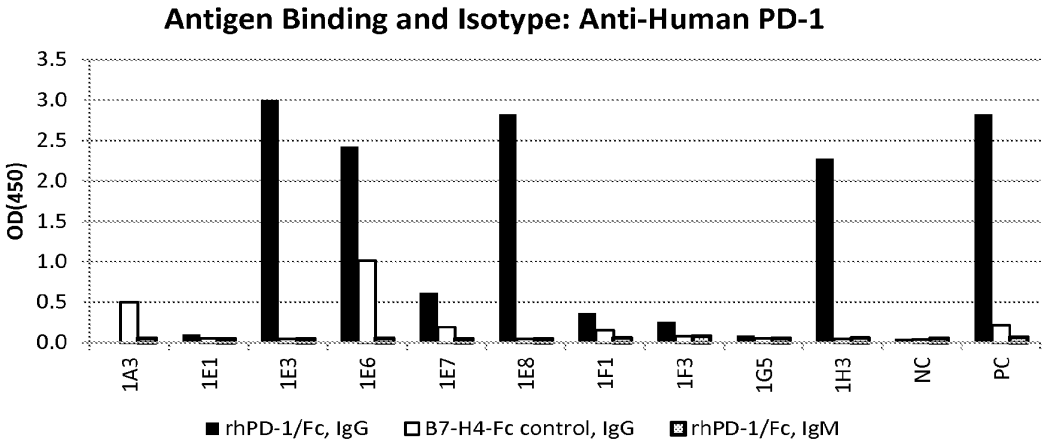

Antigen Binding and Isotype: Anti-Human PD-1
OD(450)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
1A3
1E1
1E3
1E6
1E7
1E8
1F1
1F3
1G5
1H3
NC
PC
rhPD-1/Fc, IgG
B7-H4-Fc control, IgG
rhPD-1/Fc, IgM

**Figure 6**

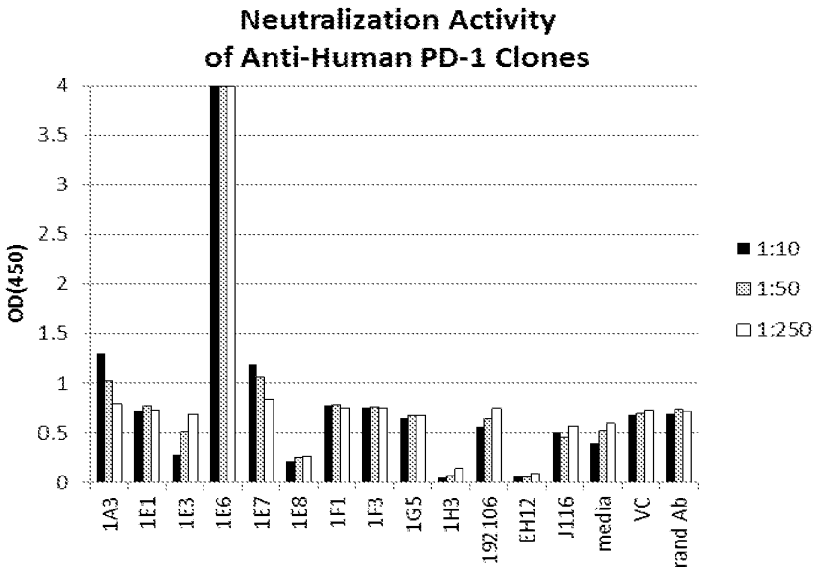



Figure 7A

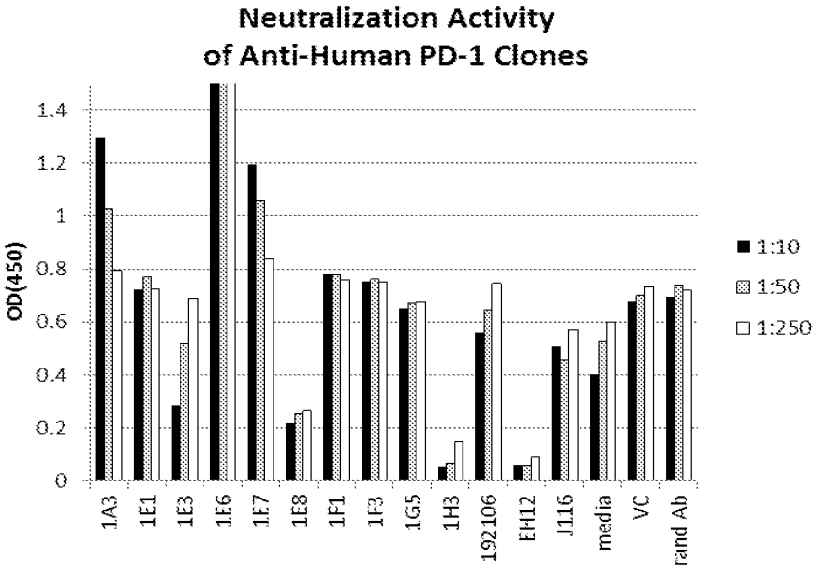



Figure 7B

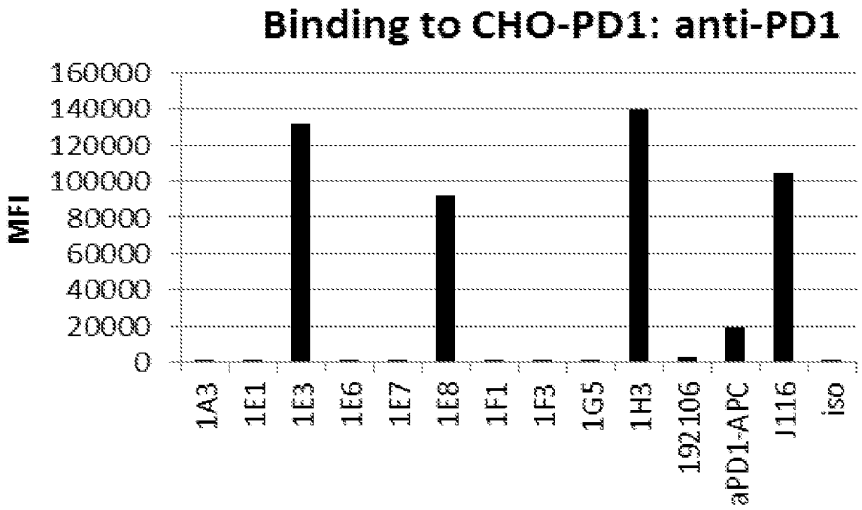

Binding to CHO-PD1: anti-PD1
MFI
160000
140000
120000
100000
80000
60000
40000
20000
0
1A3
1E1
1E3
1E6
1E7
1E8
1F1
1F3
1G5
1H3
192.106
aPD1-APC
J116
iso

**Figure 8**

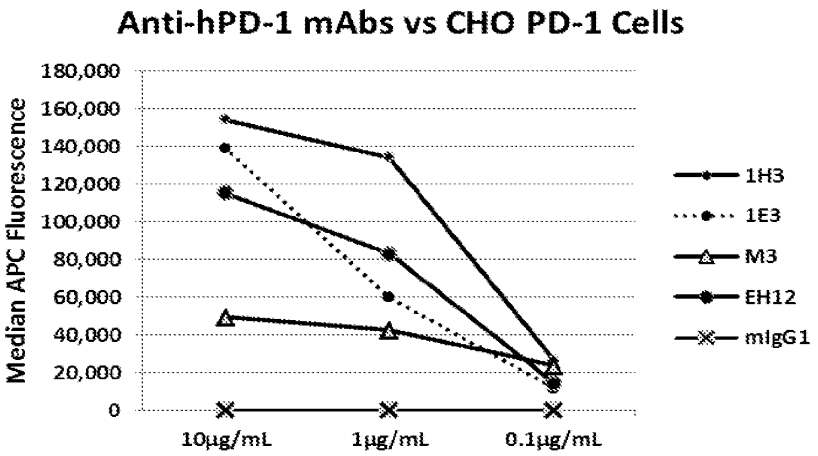
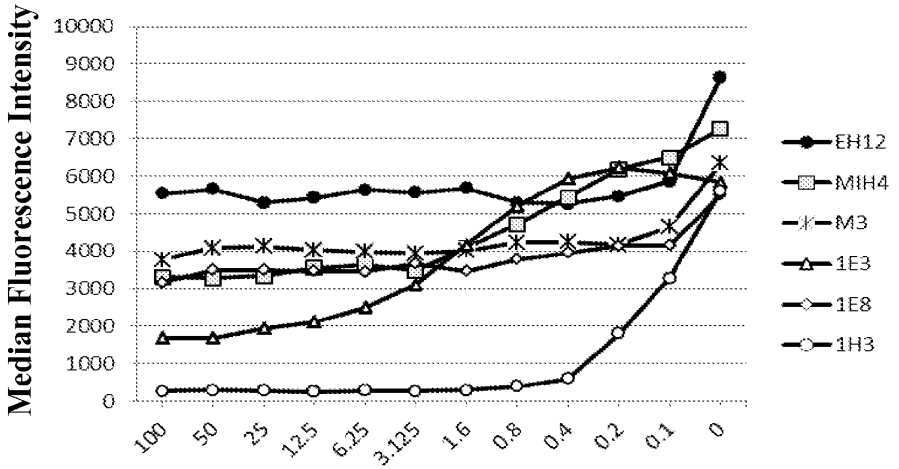



Figure 9




hPD-1 mAb Competition Assay

Figure 10

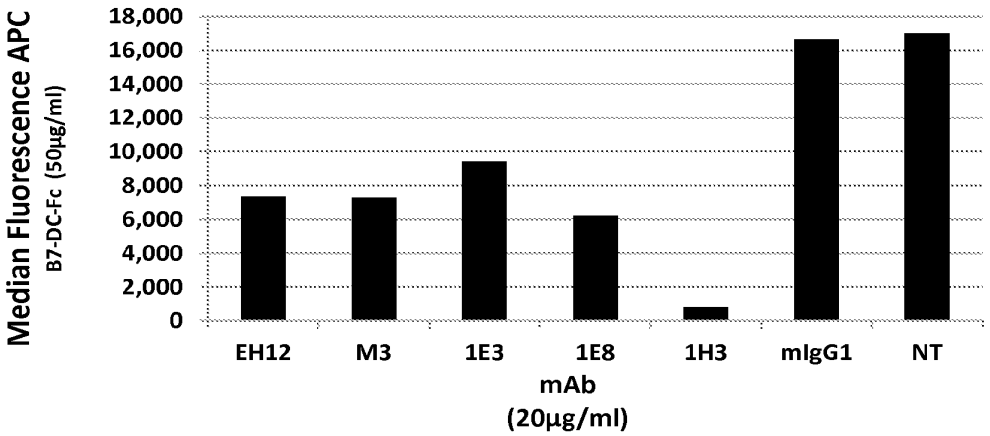



Figure 11

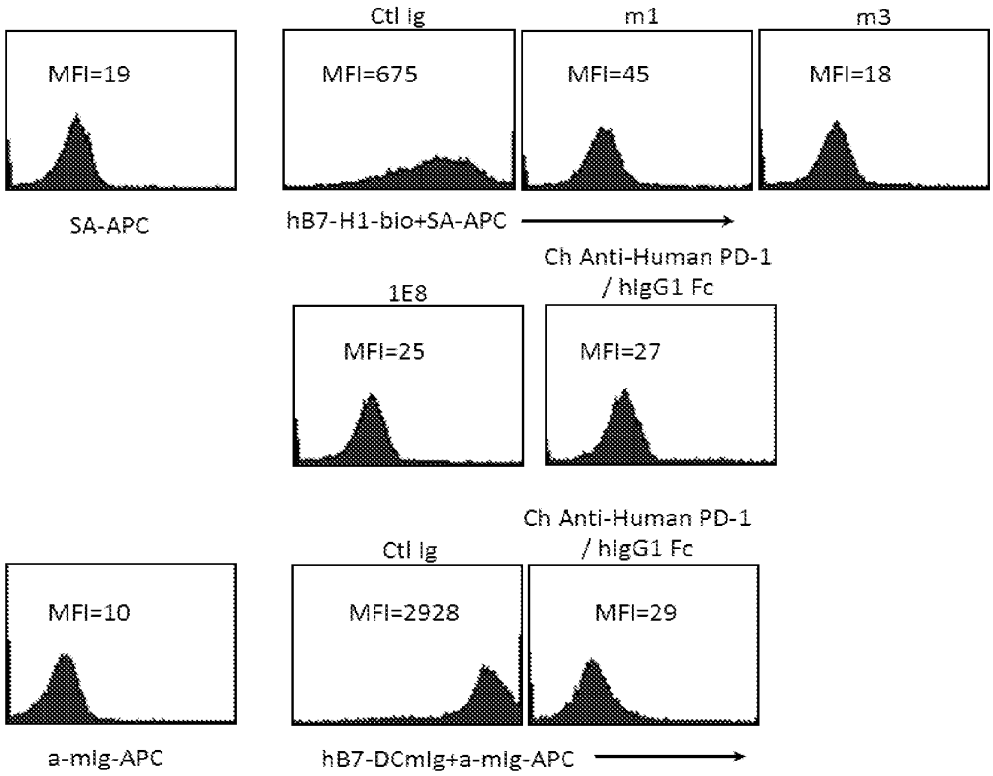
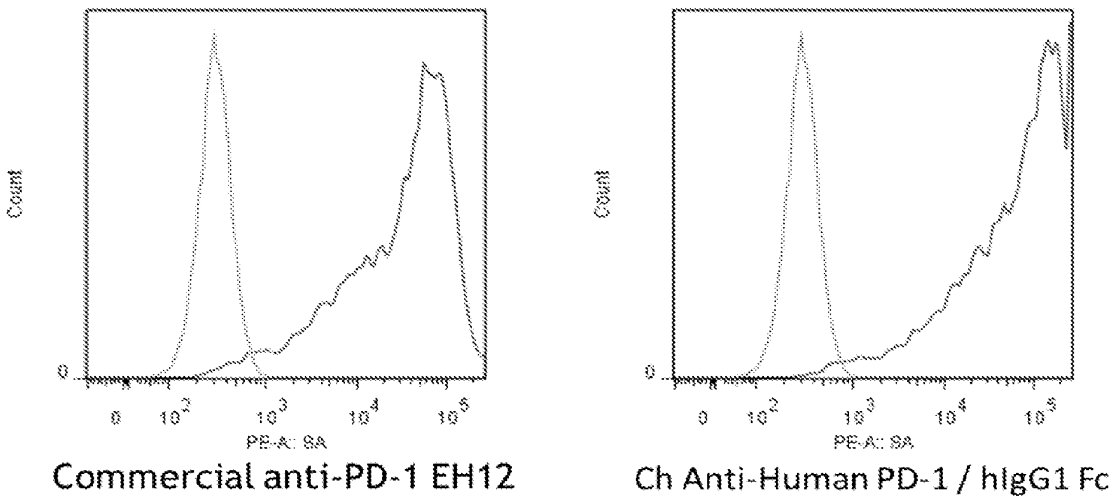
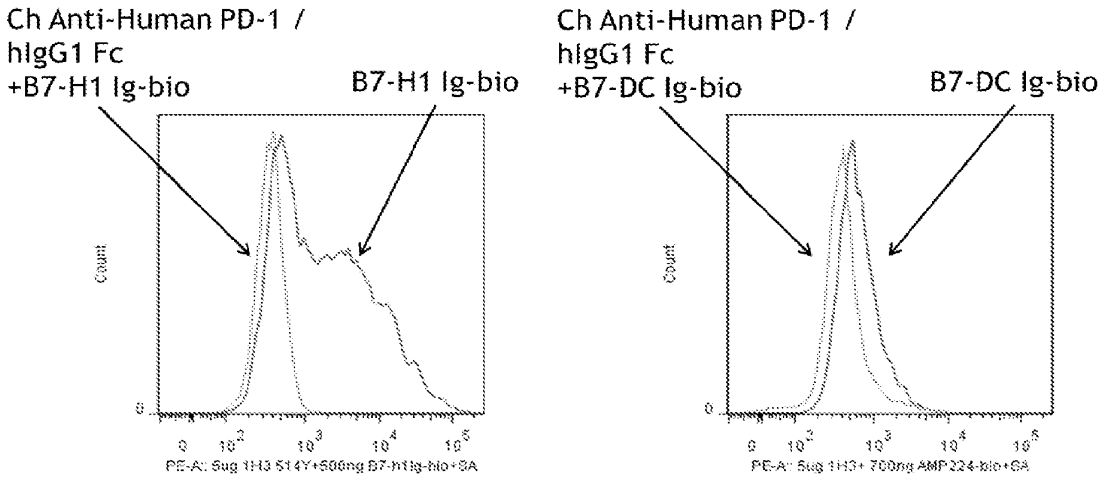



Figure 12




A B

Figure 13




A B

Figure 14

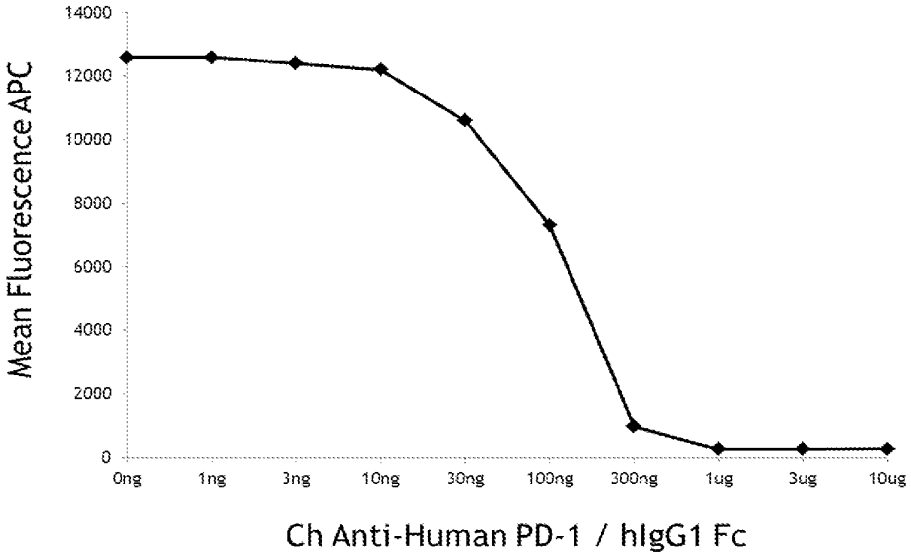

14000
12000
10000
8000
6000
4000
2000
0
Mean Fluorescence APC
0ng
1ng
3ng
10ng
30ng
100ng
300ng
1ug
3ug
10ug
Ch Anti-Human PD-1 / hIgG1 Fc

**Figure 15**

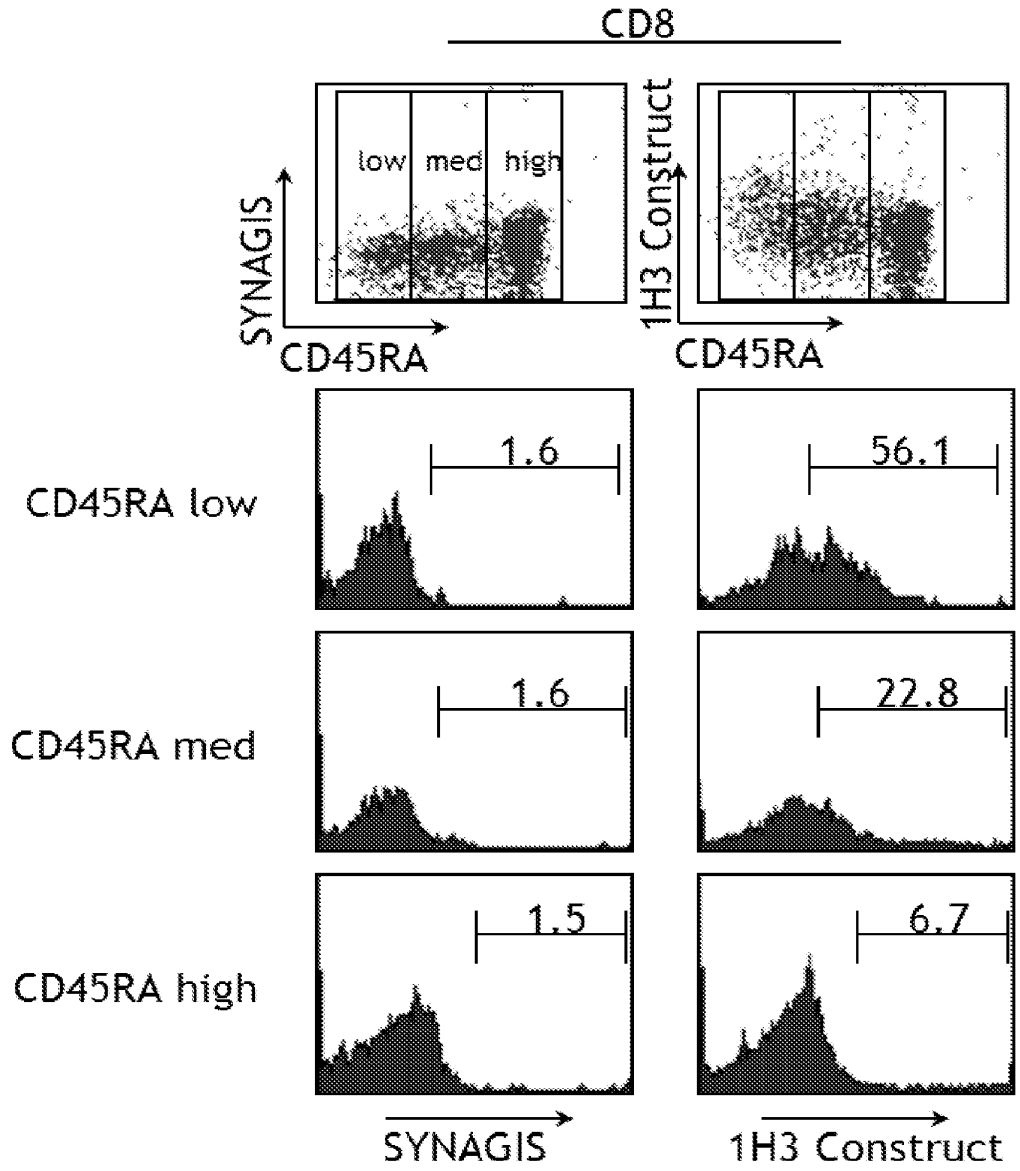

CD8
low
med
high
SYNAGIS
CD45RA
1H3 Construct
CD45RA
CD45RA low
1.6
56.1
CD45RA med
1.6
22.8
CD45RA high
1.5
6.7
SYNAGIS
1H3 Construct

**Figure 16A**

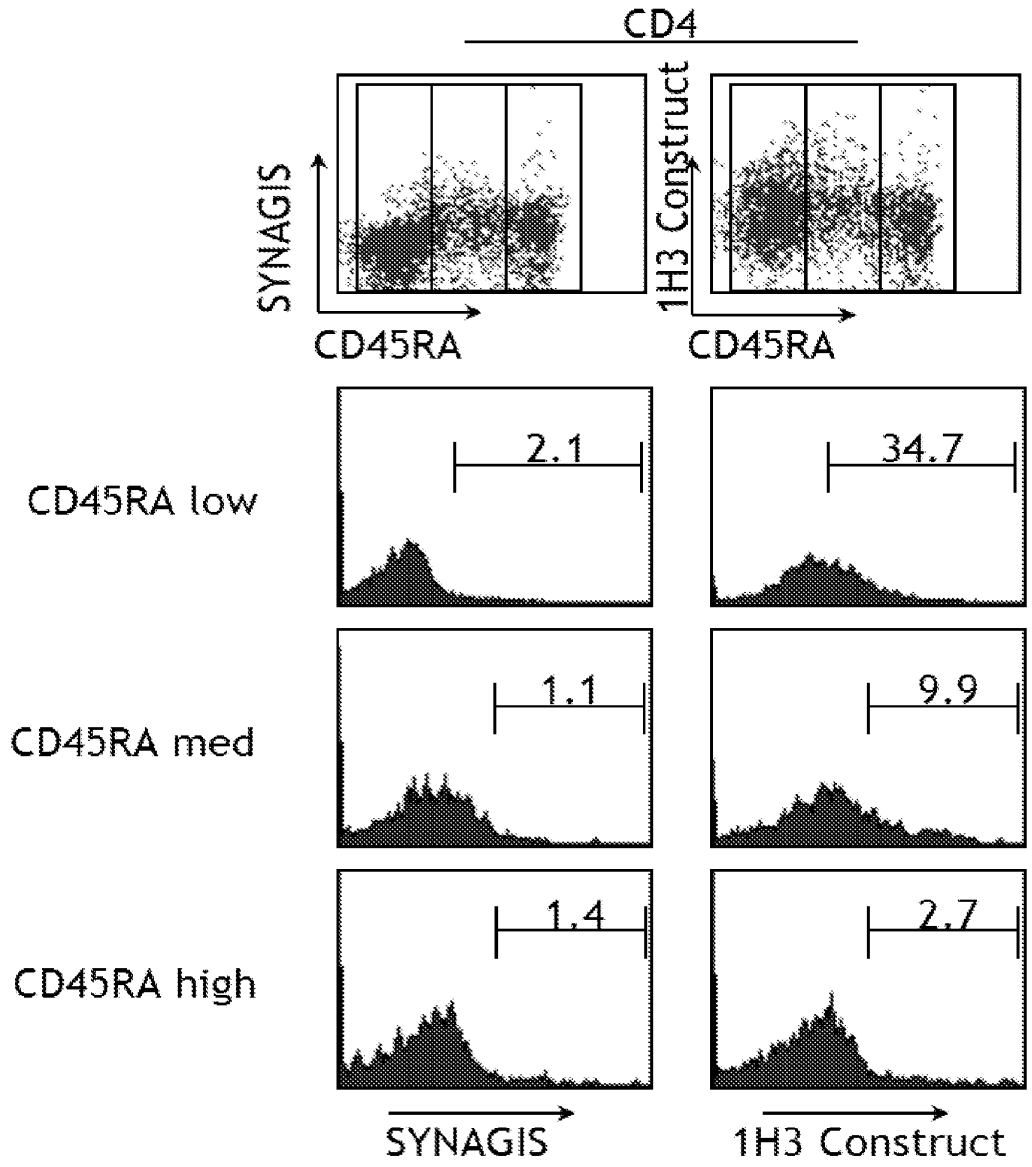

CD4
SYNAGIS
CD45RA
1H3 Construct
CD45RA
CD45RA low
2.1
34.7
CD45RA med
1.1
9.9
CD45RA high
1.4
2.7
SYNAGIS
1H3 Construct

**Figure 16B**

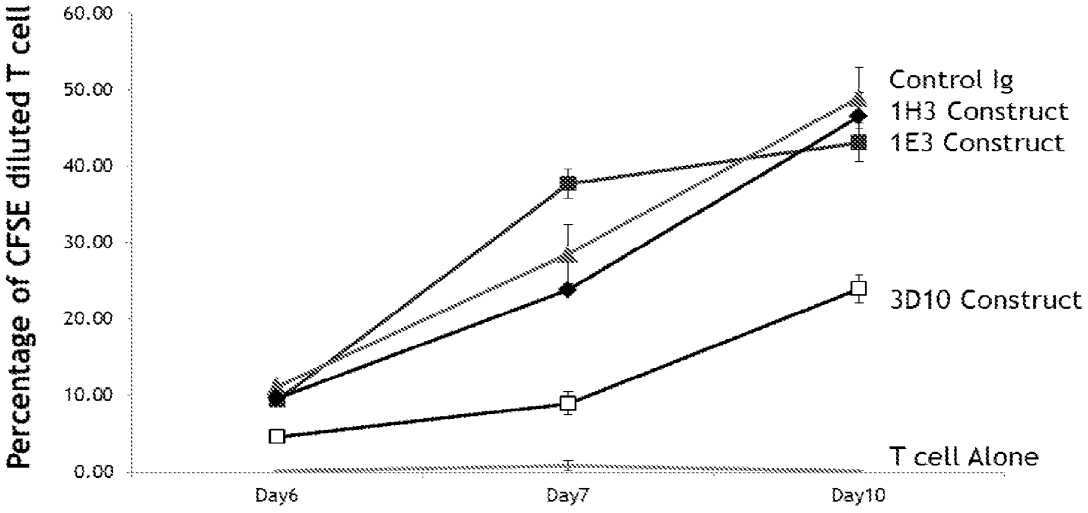

Percentage of CFSE diluted T cell
60.00
50.00
40.00
30.00
20.00
10.00
0.00
Day6
Day7
Day10
Control Ig
1H3 Construct
1E3 Construct
3D10 Construct
T cell Alone

**Figure 17**

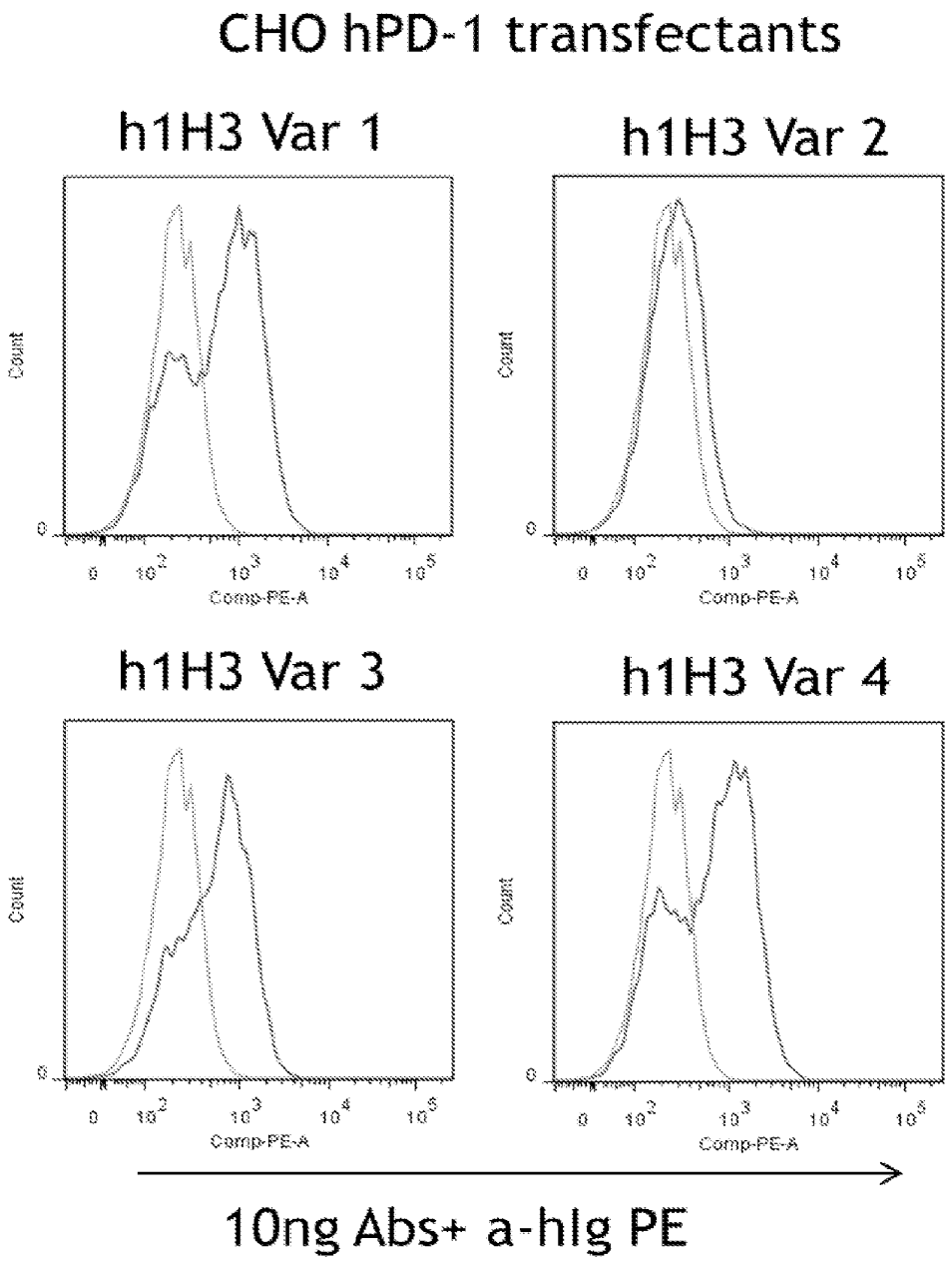

CHO hPD-1 transfectants
h1H3 Var 1
h1H3 Var 2
h1H3 Var 3
h1H3 Var 4
Count
Comp-PE-A
0
$10^2$
$10^3$
$10^4$
$10^5$
10ng Abs+ a-hIg PE

**Figure 18A**

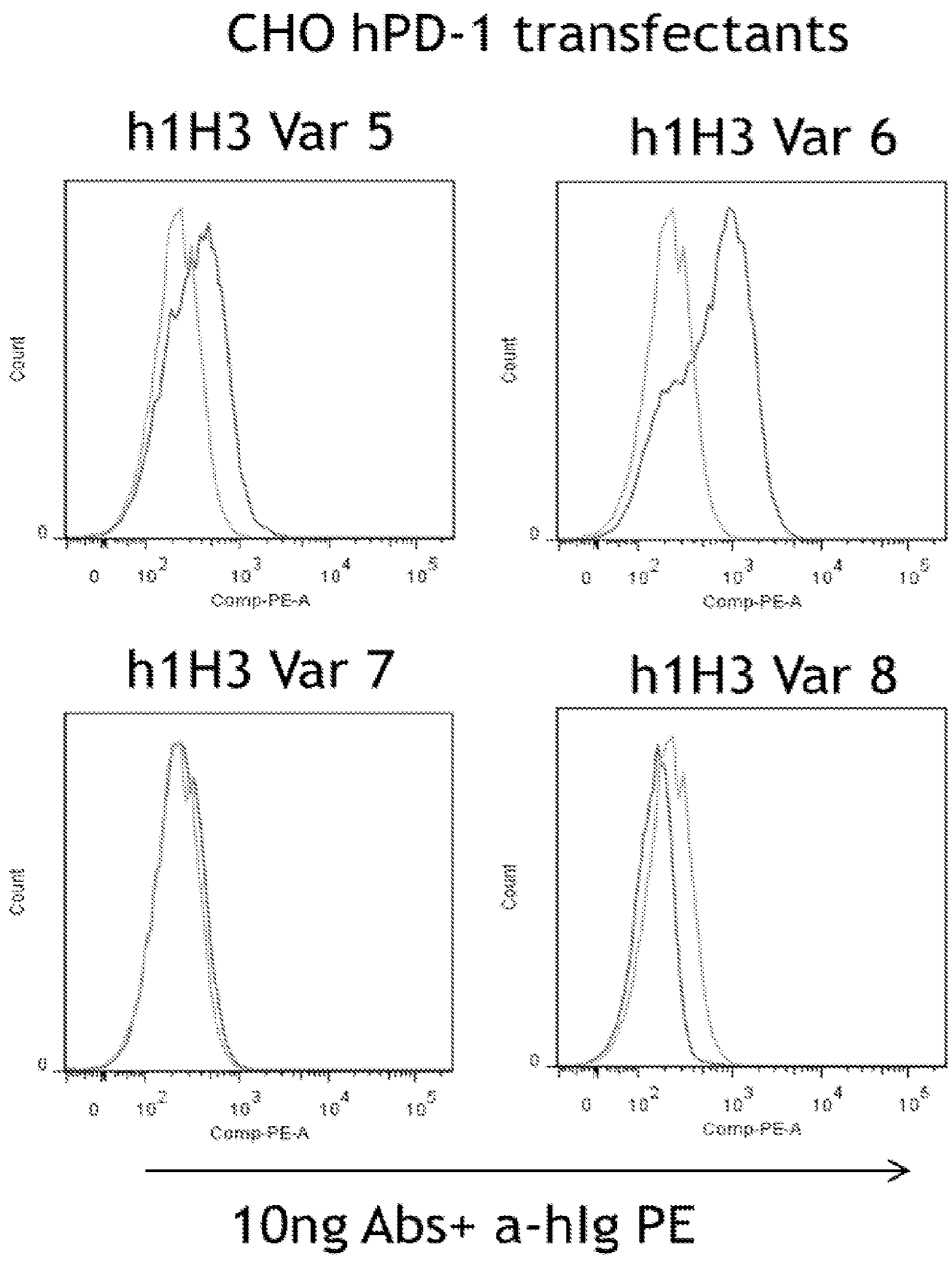

CHO hPD-1 transfectants
h1H3 Var 5
h1H3 Var 6
h1H3 Var 7
h1H3 Var 8
Count
Comp-PE-A
0
$10^2$
$10^3$
$10^4$
$10^5$
10ng Abs+ a-hIg PE

**Figure 18B**

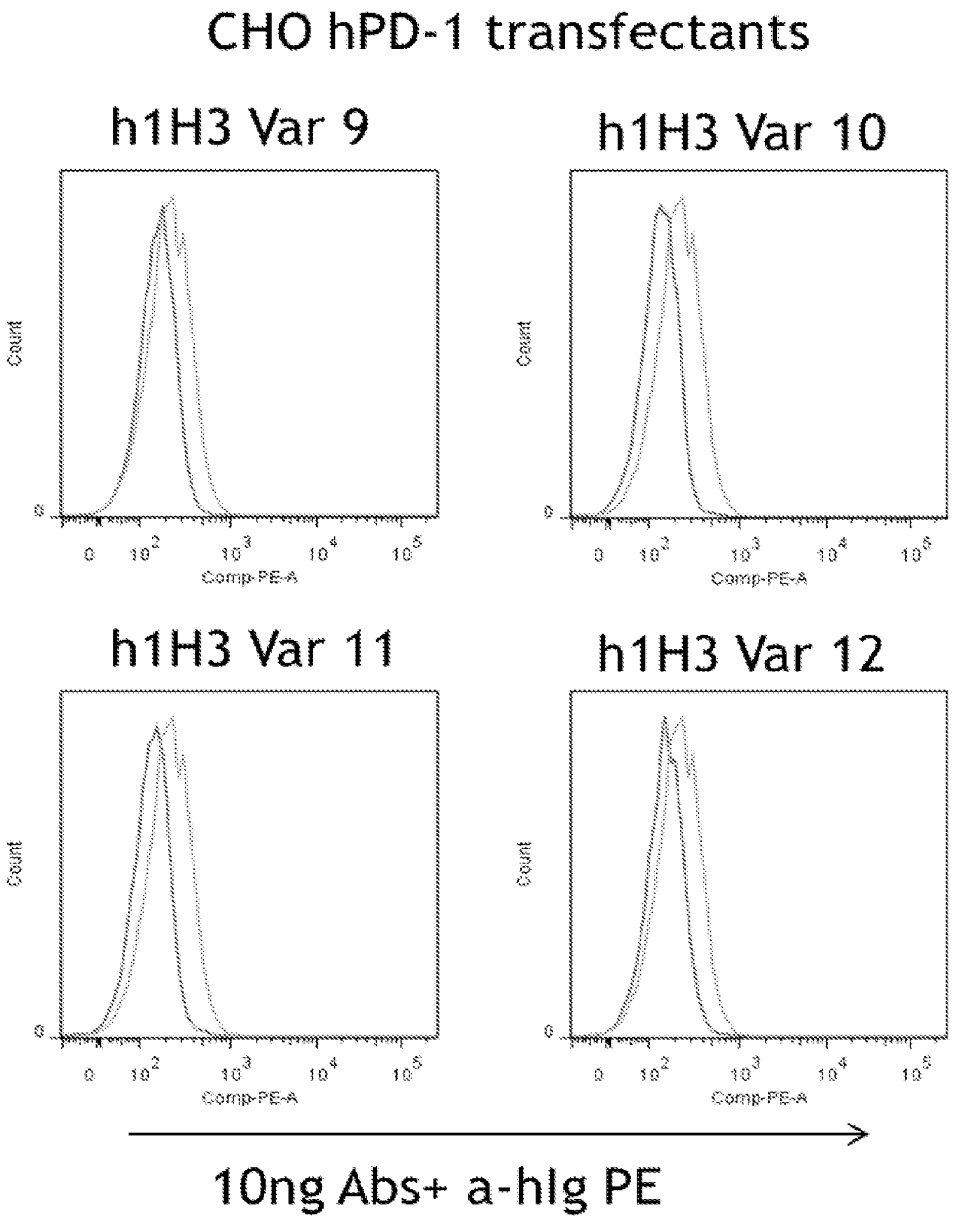

CHO hPD-1 transfectants
h1H3 Var 9
h1H3 Var 10
h1H3 Var 11
h1H3 Var 12
Count
Comp-PE-A
10ng Abs+ a-hIg PE

**Figure 18C**

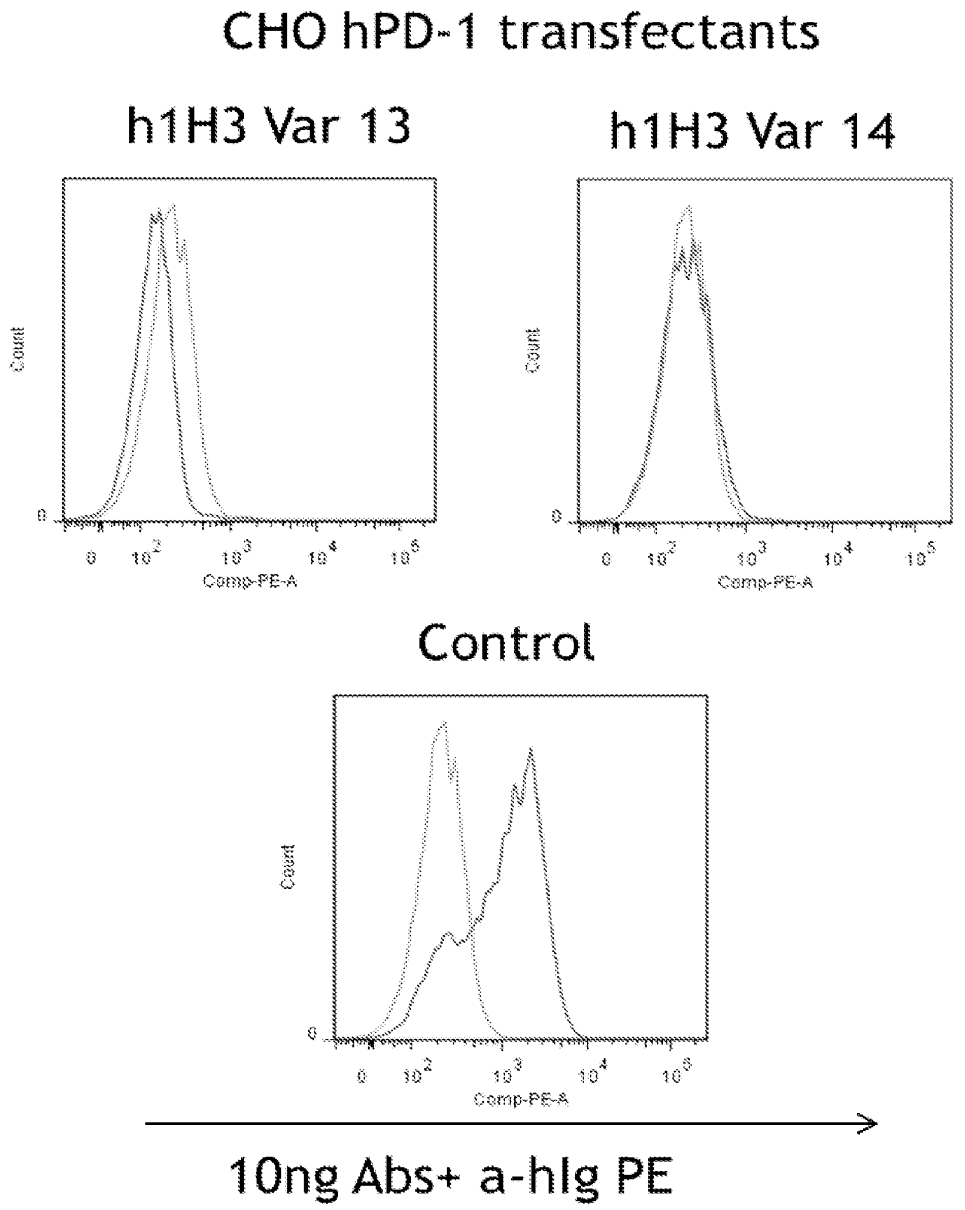

CHO hPD-1 transfectants
h1H3 Var 13
h1H3 Var 14
Count
Comp-PE-A
Control
10ng Abs+ a-hIg PE

**Figure 18D**

PD-1 + CHO

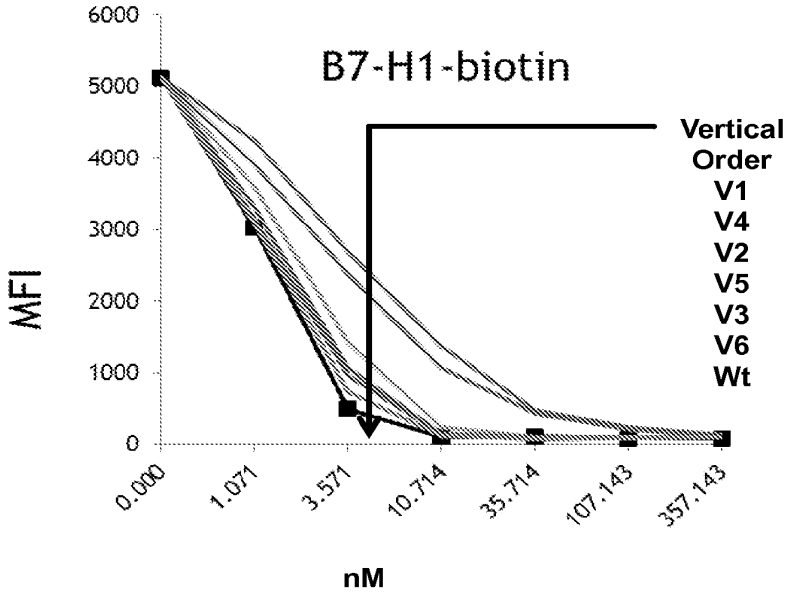



Figure 19A

PD-1 + CHO

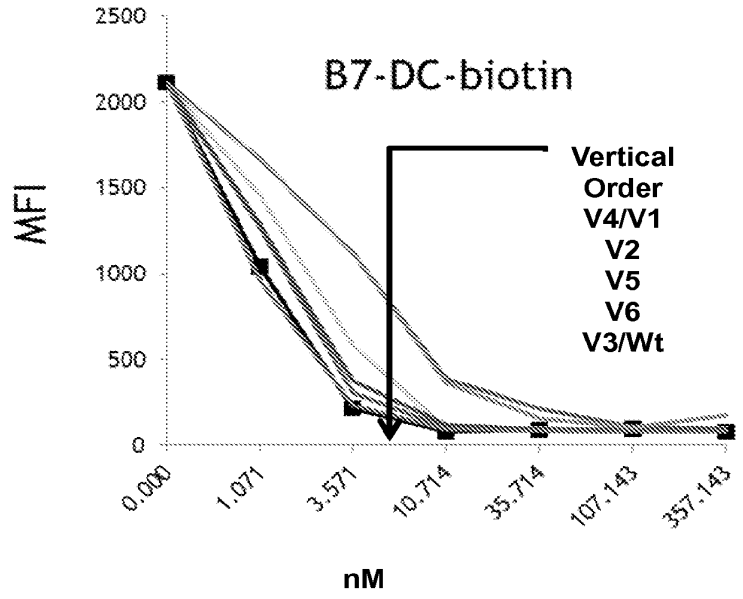



Figure 19B

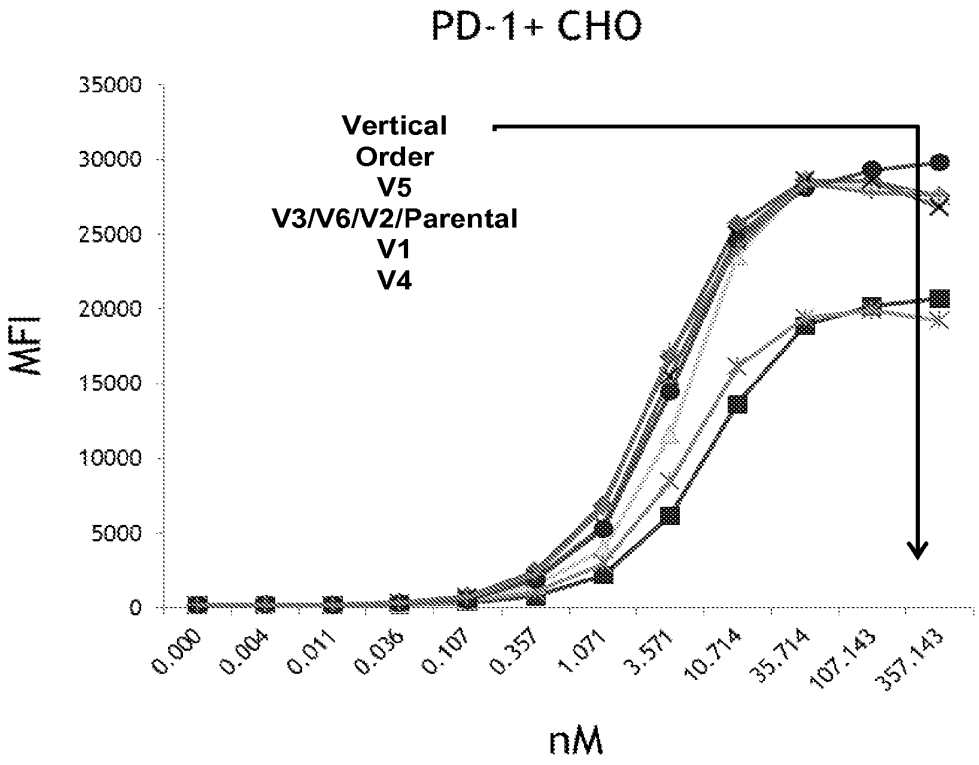

PD-1+ CHO
Vertical
Order
V5
V3/V6/V2/Parental
V1
V4
MFI
35000
30000
25000
20000
15000
10000
5000
0
0.000
0.004
0.011
0.036
0.107
0.357
1.071
3.571
10.714
35.714
107.143
357.143
nM

**Figure 20**

**INTERNATIONAL SEARCH REPORT**

International application No.
PCT/US 12/34223

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 39/395; C07K 16/28; C07K 16/30 (2012.01)
USPC - 424/172.1; 530/388.2
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
USPC - 424/172.1; 530/388.2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 424/130.1, 174.1; 530/388.8 (text search, see terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST(PGPB,USPT,EPAB,JPAB); Google Scholar (text search, see terms below)
Search Terms: B7-H1, PD-L1, CD274, programmed death-1 ligand, antibody, binding fragment, human, cell surface, cell memebrane

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | DONG et al., Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. Nature Medicine, August 2002, vol 8, No 9, pp 793 -800. Especially Figure 2a legend; Figure 2d legend; page 794, col 2, para 1; page 798, section entitled "Monoclonal antibodies and fusion proteins"; page 799, section entitled "Transfection" | 1-3 |
| A | THOMPSON et al., Tumor B7-H1 is associated with poor prognosis in renal cell carcinoma patients with long-term follow-up. Cancer Res, 1 April 2006, Vol 66, No 7, pp 3381-5. | 1-3 |

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 03 September 2012 (03.09.2012) | 21 SEP 2012 |
| Name and mailing address of the ISA/US<br>Mail Stop PCT, Attn: ISA/US, Commissioner for Patents<br>P.O. Box 1450, Alexandria, Virginia 22313-1450<br>Facsimile No. 571-273-3201 | Authorized officer:<br>Lee W. Young<br>PCT Helpdesk: 571-272-4300<br>PCT OSP: 571-272-7774 |

Form PCT/ISA/210 (second sheet) (July 2009)

**INTERNATIONAL SEARCH REPORT**

International application No.
PCT/US 12/34223

**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 4-20
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I-II: claims 1-3, drawn to a molecule, compnsmg an antigen-binding fragment of an antibody that immunospecifically binds to B7-HI and PD-I, respectively.

The inventions listed as Groups I-II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The inventions of Group I do not include the inventive concept of a molecule, compnsmg an antigen-binding fragment of an antibody that immunospecifically binds to PD-I, as required by Group II.

---please see extra sheet---

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-3, restricted to B7-HI

**Remark on Protest**

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (2)) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/34223

Continuation of Box No. III Observations where unity of invention is lacking

The inventions of Group II do not include the inventive concept of a molecule, comprising an antigen-binding fragment of an antibody that immunospecifically binds to B7-HI, as required by Group I.

In addition, a molecule comprising an antigen-binding fragment of an antibody that immunospecifically binds to B7-HI was known in the art at the time of the invention, as evidenced by an article titled "Tumor B7-H1 is associated with poor prognosis in renal cell carcinoma patients with long-term follow-up" by Thompson, et al. (Cancer Res. 2006, 66(7):3381-3385) (hereinafter "Thompson") (Abstract, "We did immunohistochemistry with anti-B7-H1"; pg 3382, col 1, "anti-B7-H1 (clone 5H1)" and "To validate the specificity of 5H1 for B7-H1 in paraffin specimens, we did immunohistochemical blocking studies in a competition assay. .... Thus, the anti-B7-H1 antibody employed in our study shows an appropriate level of specificity for the B7-H1 protein in paraffin-embedded tumor specimens").

Groups I-II therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
31 January 2013 (31.01.2013)

WIPO | PCT

(10) International Publication Number
WO 2013/014668 A1

(51) International Patent Classification:
*C07K 16/28* (2006.01) *A61K 39/395* (2006.01)

(21) International Application Number:
PCT/IL2012/050267

(22) International Filing Date:
23 July 2012 (23.07.2012)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/511,055 24 July 2011 (24.07.2011) US

(71) Applicant *(for all designated States except US)*: **CURETECH LTD.** [IL/IL]; 42 Hayarkon Street, 81227 Yavne (IL).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **ROTEM-YEHUDAR, Rinat** [IL/IL]; 1 Yaakov Meridor Street, 69411 Tel Aviv (IL). **SCHICKLER, Michael** [IL/IL]; 19 Hamagen, 76804 Mazkeret Batya (IL).

(74) Agents: **WEBB & CO.** et al.; P.O. Box 2189, 76121 Rehovot (IL).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

**Published**:

— *with international search report (Art. 21(3))*

— *with sequence listing part of description (Rule 5.2(a))*

WO 2013/014668 A1

(54) **Title**: VARIANTS OF HUMANIZED IMMUNOMODULATORY MONOCLONAL ANTIBODIES

(57) **Abstract**: The present invention relates to humanized monoclonal antibodies, pharmaceutical compositions comprising same, and use thereof for the treatment of a variety of indications, particularly cancer and immunodeficiency disorders. In particular, the present invention provides modified antibodies or fragments thereof having specific amino acid modifications compared to the humanized monoclonal immunomodulatory antibody termed hBAT-1.

# VARIANTS OF HUMANIZED IMMUNOMODULATORY MONOCLONAL ANTIBODIES

## FIELD OF THE INVENTION

The present invention relates to the field of immunotherapy and more specifically concerns humanized monoclonal antibodies useful for therapy of a variety of indications, particularly in the treatment of cancer and immunodeficiency disorders.

## BACKGROUND OF THE INVENTION

The rapid increase of knowledge in recent years about the molecular and cellular bases of immune regulation, particularly at the level of T cell responses, provides a new arsenal of immunotherapeutic approaches including the development of anti-tumor vaccines. Certain monoclonal antibodies were shown to have immunomodulatory activity including the ability to bind determinants on the surface of T cells and to induce proliferation, activation, maturation or differentiation of these cells.

BAT (also referred to as mBAT-1 or BAT-1) is a murine monoclonal antibody generated against a membrane preparation of a Burkitt lymphoma cell line (Daudi) that was shown to exhibit antitumor and immunostimulatory effects towards various types of tumors (Hardy et al., 2001, Int. J. Oncol. 19:897). This monoclonal antibody was initially disclosed in U.S. Patent No. 5,897,862 to Hardy et al. BAT-1 is secreted by the hybridoma cell line having CNCM Accession No. I-1397. The immunomodulatory effect of murine BAT was studied also in vitro. Murine BAT activates CD4+ T cells and induces the secretion of IFN-γ from these cells (Hardy et al., 2000, Int. Immunol. 12:1623 and Quaglino E. et al., 2005, Vaccine 9:23(25):3280-7). In addition, it was found that BAT triggers the proliferation of T cells and increases their cytolytic activity (Hardy, B. et al., 1997, Hum. Antibodies, 8:95).

The polynucleotide and amino-acid sequences of murine BAT are disclosed in WO 00/58363, to Hardy et al., and U.S. Patent No. 7,695,715.

A number of humanized monoclonal antibodies (mAbs) based on murine BAT are disclosed in U.S. Patent No. 7,332,582, the contents of which are incorporated herein by reference. In particular embodiments of US 7,332,582, the humanized monoclonal antibodies

comprise a light chain variable region selected from SEQ ID NO: 1-4 (denoted $BATRK_D$, $BATRK_A$, $BATRK_B$ and $BATRK_C$, respectively) and a heavy chain variable region selected from SEQ ID NO: 5-9 (denoted $BATRH_C$, $BATRH_A$, $BATRH_B$, $BATRH_D$ and $BATRH_E$, respectively). The amino acid sequence of the light and heavy chain antibody variants are depicted in Table 1 herein below. The residues which differ in said variable regions are depicted with a gray background (positions 2, 30, 69, 77, 97 and 98 of the light region and positions 35, 69, 70 and 71 of the heavy chain).

According to U.S. 7,332,582, the humanized monoclonal BAT antibodies appear to induce a greater antitumor effect than those induced by the parent murine BAT antibody. Among various model systems tested, the BAT antitumor activity was studied in SCID (severe combined immunodeficiency disease) mice, beige mice that are deficient in NK cells and nude mice that are deficient in T cells (Hardy, B., 1997, Proc. Natl. Acad. Sci. USA 94:5756). All mice were injected intravenously with murine B16 melanoma cells that subsequently developed tumors in the lungs. BAT antibodies exerted an antitumor effect only in SCID mice that were engrafted with either murine or human lymphocytes. In the athymic nude mice and the beige mice BAT antibodies exerted an antitumor activity, though this activity was less effective as compared to the antitumor activity of BAT antibodies in the wild- type mice.

It should be borne in mind that BAT antibodies are not expected to target the tumor cells themselves but rather the immune-functioning cells of the subject or patient, in order to modulate the immune response in a beneficial way.

Berger et al. (2008) discloses administration of the humanized monoclonal antibody CT-011, which is based on mBAT-1, to patients with advanced hematologic malignancies, and associated pharmacokinetics (Berger et al. Clin. Cancer Res. 2008;14(10) May 15, 2008).

WO 09/101611 relates to methods for inhibiting tumor growth, increasing survival of a subject having a tumor and inducing protection against tumor recurrence in a mammal, comprising administering a humanized monoclonal antibody comprising CDR regions derived from the murine monoclonal antibody designated mBAT-1, in combination with at least one chemotherapeutic agent.

Nowhere in the background art is it taught or suggested that use of a humanized mBAT-1 monoclonal antibody comprising at least one site specific amino acid modification will be

advantageous for the therapy of a variety of indications, particularly in the treatment of cancer and immuno-deficiency related diseases and disorders.

## SUMMARY OF THE INVENTION

The present invention provides mutated humanized monoclonal antibodies or fragments thereof, having site specific amino acid modifications including but not limited to, amino acid substitutions and deletions, compared to the known humanized monoclonal antibodies hBAT-1. The invention further provides pharmaceutical compositions comprising the modified antibodies or fragments thereof, and use thereof for the treatment of cancer and immunodeficiency disorders. In some embodiments, the modified antibodies of the invention retain the immunomodulatory activity of hBAT-1, bind B lymphoblastoid cells and induce proliferation and activation of peripheral blood lymphocytes.

The antibodies of the present invention demonstrate superior features, e.g., enhanced bioactivity and stability and/or reduced immunogenicity, by virtue of a specific modification of at least one amino acid of the hBAT-1 antibody variants, as detailed herein below.

**Table 1- The variable and constant region of the known hBAT-1 light and heavy chain**

| Ig Chain | Region | Amino acid sequence | | SEQ ID NO: |
|---|---|---|---|---|
| Light Chain | Variable | EIVL**T**QSPSSLSASVGDRV**T**ITCSARSSVS YMHW**F**QQKPGKAPKLWIYRTSNLASGVPSR FSGSGSGT**SYC**LTI**NS**LQPEDFATYYCQQR SS**F**PLT**F**GGGTKLEIK | $BATRK_D$ | 1 |
| | Variable | EIVL**T**QSPSSLSASVGDRV**T**ITCSARSSVS YMHW**Y**QQKPGKAPKLWIYRTSNLASGVPSR FSGSGSGT**DFT**LTI**NS**LQPEDFATYYCQQR SS**F**PLT**F**GGGTKLEIK | $BATRK_A$ | 2 |
| | Variable | EIVL**T**QSPSSLSASVGDRV**T**ITCSARSSVS YMHW**F**QQKPGKAPKLWIYRTSNLASGVPSR FSGSGSGT**DYT**LTI**NS**LQPEDFATYYCQQR SS**F**PLT**F**GGGTKLEIK | $BATRK_B$ | 3 |
| | Variable | EIVL**T**QSPSSLSASVGDRV**T**ITCSARSSVS YMHW**F**QQKPGKAPKLWIYRTSNLASGVPSR FSGSGSGT**DYC**LTI**NS**LQPEDFATYYCQQR SS**F**PLT**F**GGGTKLEIK | $BATRK_C$ | 4 |
| | Constant | RTVAAPSVFIFPPSDEQLKSGTASVVCLLN NFYPREAKVQWKVDNALQSG**NS**QESVTEQD | | 10 |

| | | | | |
|---|---|---|---|---|
| | | SK**DS**TYSLSSTLTLSKADYEKHKVYACEVT HQGLSSPVTKSFNRGEC | | |
| | Variable ($BATRK_D$) + Constant | EIVL**T**QSPSSLSASVGDRV**T**ITCSARSSVS YMHWFQQKPGKAPKLWIYRTSNLASGVPSR FSGSGSGTSY**C**LTI**NS**LQPEDFATYYCQQR SS**F**PLT**F**GGGTKLEIKRTVAAPSVFIFPPS DEQLKSGTASVVCLLNNFYPREAKVQWKVD NALQSG**NS**QESVTEQDSK**DS**TYSLSSTLTL SKADYEKHKVYACEVTHQGLSSPVTKSFNR GEC | | 11 |
| Heavy Chain | Variable | QVQLVQSGSELKKPGASVKISCKASGYTFT NYGMNWVRQAPGQGLQWMGWINT**DS**GESTY AEEFKGRFVFSLDTSVNTAYLQITSLTAED TGMYFCVRVGYDALDY**W**GQGTLVTVSS | $BATRH_C$ | 5 |
| | Variable | QVQLVQSGSELKKPGASVKISCKASGYTFS NYGMNWVRQAPGQGLQWMGWINT**DS**GESTY AEEFKGRFVFSLDTSVSTAYLQITSLTAED TGMYFCAKVGYDALDY**W**GQGTLVTVSS | $BATRH_A$ | 6 |
| | Variable | QVQLVQSGSELKKPGASVKISCKASGYTFT NYGMNWVRQAPGQGLQWMGWINT**DS**GESTY AEEFKGRFVFSLDTSVSTAYLQITSLTAED TGMYFCAKVGYDALDY**W**GQGTLVTVSS | $BATRH_B$ | 7 |
| | Variable | QIQLVQSGSELKKPGASVKISCKASGYTFT NYGMNWVRQAPGQGLQWMGWINT**DS**GESTY AEEFKGRFVFSLDTSVNTAYLQITSLTAED TGMYFCVRVGYDALDY**W**GQGTLVTVSS | $BATRH_D$ | 8 |
| | Variable | QIQLVQSGSELKKPGASVKISCKASGYTFT NYGMNWVRQAPGQGLQWMGWINT**DS**GESTY AEEFKGRFAFSLDTSVNTAYLQITSLTAED TGMYFCVRVGYDALDY**W**GQGTLVTVSS | $BATRH_E$ | 9 |
| | Constant | AS**T**KGP**S**VFPLAPSSKSTSGGTAALGCLVK DYFPEPVTVSW**NS**GALTSGVHTFPAVLQSS GLYSLSSVVTVPSSSLGTQTYICNV**NH**KPS NTKVDKRVEPKSC**DK**TH**T**CPPCPAPELLGG PSVFLFPPKPKDTL**M**ISRTPEVTCVVVDVS HE**DP**EVKFNWYV**DG**VEVHNAKTKPREEQY**N** **S**TYRVVSVLTVLHQDWL**NG**KEYKCKVSNKA LPAPIEKTISKAKGQPREPQVYTLPPSREE MTKNQVSLTCLVKGFYPSDIAVEWES**NG**QP ENNYKTTPPVL**DSDG**SFFLYSKLTVDKSRW QQGNVFSCSV**M**HEALH**NH**YTQKSLSLSPG**K** | | 12 |
| | Variable ($BATRH_C$) + Constant | QVQLVQSGSELKKPGASVKISCKASGYTFT NYGMNWVRQAPGQGLQWMGWINT**DS**GESTY AEEFKGRFVFSLDTSVNTAYLQITSLTAED TGMYFCVRVGYDALDY**W**GQGTLVTVSSAS**T** KGP**S**VFPLAPSSKSTSGGTAALGCLVKDYF PEPVTVSW**NS**GALTSGVHTFPAVLQSSGLY SLSSVVTVPSSSLGTQTYICNV**NH**KPSNTK VDKRVEPKSC**DK**TH**T**CPPCPAPELLGGPSV | | 13 |

| | | | | |
|---|---|---|---|---|
| | | FLFPPKPKDTL**M**ISRTPEVTCVVVDVSHE**D**<br>**P**EVKFNWYV**DG**VEVHNAKTKPREEQY**NS**TY<br>RVVSVLTVLHQDWL**NG**KEYKCKVSNKALPA<br>PIEKTISKAKGQPREPQVYTLPPSREEMTK<br>NQVSLTCLVKGFYPSDIAVEWES**NG**QPENN<br>YKTTPPVL**DSDG**SFFLYSKLTVDKSRWQQG<br>NVFSCSV**M**HEALH**NH**YTQKSLSLSPG**K** | | |

Table 1 lists the amino acid sequences of the variable and constant regions of the light and heavy chain of hBAT-1 antibody variants. The at least one amino acid which, in some embodiments, is substituted to produce the antibodies of the invention, is depicted in bold. In particular embodiments, the at least one amino acid is selected from the group consisting of: the amino acids in positions 5, 20, 71, 75, 76, 93 and 97 of SEQ ID NO: 1; the amino acids in positions 54, 55 and 107 of SEQ ID NO: 5, amino acids in positions 157, 158, 169, 170 of SEQ ID NO: 11; and the amino acids in positions 120, 124, 159, 160, 203, 204, 221, 222, 225, 252, 270, 271, 280, 281, 297, 298, 315, 316, 384, 385, 399, 400, 401, 402, 434, 435, 447 and 428 of SEQ ID NO: 13.

According to one aspect, the present invention provides an antibody or antigen binding fragment thereof comprising a light chain variable region selected from the group consisting of SEQ ID NO: 1, 2, 3, and 4, and a heavy chain variable region selected from the group consisting of SEQ ID NO: 5, 6, 7, 8, and 9, wherein the antibody or antigen binding fragment thereof comprises at least one amino acid substitution at a position selected from: Phe 97 and Phe 93 of the light chain variable region to Ala, Leu or Val, or a substitution of Trp 107 of the heavy chain variable region to Ala, Leu, Val or Tyr.

In some embodiments of the invention, the antibody or antigen binding fragment thereof comprises an amino acid substitution of Phe 97 of the light chain variable region and an amino acid substitution of Trp 107 of the heavy chain variable region. In another embodiment, the antibody or antigen binding fragment thereof comprises an amino acid substitution of Phe 93 of the light chain variable region and an amino acid substitution of Trp 107 of the heavy chain variable region.

In another embodiment, the light chain variable region comprises the amino acid sequence selected from the group consisting of: SEQ ID NO: 14, 15, 16, 17, 18, 19, 20 and 21. In one embodiment, the light chain variable region comprises the amino acid sequence as set forth in SEQ ID NO: 14. In another embodiment, the light chain variable region comprises

the amino acid sequence as set forth in SEQ ID NO: 15. In a particular embodiment, the light chain variable region comprises the amino acid sequence as set forth in SEQ ID NO: 29. In yet another particular embodiment, the light chain variable region comprises the amino acid sequence as set forth in SEQ ID NO: 30.

In another embodiment, the heavy chain variable region comprises the amino acid sequence selected from the group consisting of: SEQ ID NO: 24, 25, 26, 27 and 28. In a particular embodiment, the heavy chain variable region comprises the amino acid sequence as set forth in SEQ ID NO: 24. In yet another particular embodiment, the heavy chain variable region comprises the amino acid sequence as set forth in SEQ ID NO: 31.

In yet another embodiment, the antibody or antigen binding fragment thereof further comprises at least one amino acid substitution at a position selected from the group consisting of: Thr 5, Thr 20, Cys 71, Asn 75, Ser 76 of the light chain variable region, and Asp 54 and Ser 55 of the heavy chain variable region.

According to another aspect, the present invention provides an antibody or antigen binding fragment thereof comprising a light chain variable region selected from the group consisting of SEQ ID NO: 1, 2, 3 and 4, and a heavy chain variable region selected from the group consisting of SEQ ID NO: 5, 6, 7, 8 and 9, wherein the antibody or antigen binding fragment thereof comprises at least one amino acid modification at a position selected from the group consisting of: Thr 5, Thr 20, Cys 71, Asn 75, Ser 76, Phe 93 and Phe 97 of the light chain variable region and Asp 54, Ser 55 and Trp 107 of the heavy chain variable region. Each possibility represents a separate embodiment of the present invention.

According to some embodiments of the invention, the amino acid modification is an amino acid substitution. According to one embodiment, the amino acid substitution is a conservative substitution. According to another embodiment, the amino acid substitution is a non-conservative substitution. According to another embodiment, the amino acid modification is an amino acid deletion. According to some embodiments, the modified antibodies of the invention comprise a combination of amino acid modifications, such as, an amino acid substitution of one residue and an amino acid deletion of another residue. According to another embodiment, the modified antibody comprises at least one amino acid substitution and at least one amino acid deletion.

According to various embodiments of the invention, the modified antibody or fragment

thereof comprises a combination of a heavy chain variable region and a light chain variable region, the combination is selected from the group consisting of: SEQ ID NO: 5/SEQ ID NO: 1; SEQ ID NO: 6/SEQ ID NO: 2; SEQ ID NO: 7/SEQ ID NO: 2; SEQ ID NO: 7/SEQ ID NO: 3; SEQ ID NO: 8/SEQ ID NO: 3; and SEQ ID NO: 7/SEQ ID NO: 1. According to particular embodiments of the invention, the antibody or antigen binding fragment thereof comprises a combination of variable regions corresponding to SEQ ID NO: 5/SEQ ID NO: 1. The combination of the heavy and light chain variable region comprises, in one embodiment, at least one amino acid modification at a position selected from the group consisting of: Thr 5, Thr 20, Cys 71, Asn 75, Ser 76, Phe 93 and Phe 97 of the light chain variable region and Asp 54, Ser 55 and Trp 107 of the heavy chain variable region. In another embodiment, the combination of the heavy and light chain variable region comprises at least one amino acid modification at a position selected from: Phe 93 and Phe 97 of the light chain variable region and Trp 107 of the heavy chain variable region. Each possibility represents a separate embodiment of the present invention.

According to another embodiment of the invention, the antibody comprises a light chain constant region as set forth in SEQ ID NO: 10. According to yet another embodiment, the antibody comprises a modified light constant region comprising at least one amino acid modification at a position selected from the group consisting of: Asn 51, Ser 52, Asp 63 and Ser 64 of SEQ ID NO: 10. Each possibility represents a separate embodiment of the present invention.

According to another embodiment of the invention, the antibody comprises a light chain as set forth in SEQ ID NO: 11. According to yet another embodiment, the antibody comprises a modified light chain as set forth in SEQ ID NO: 11 comprising an amino acid modification at a position selected from the group consisting of: Asn 157, Ser 158, Asp 169 and Ser 170 of SEQ ID NO: 11. Each possibility represents a separate embodiment of the present invention.

According to another embodiment of the invention, the antibody comprises a heavy chain constant region as set forth in SEQ ID NO: 12. According to yet another embodiment, the antibody comprises a modified heavy chain constant region comprising at least one amino acid modification at a position selected from the group consisting of: Thr 3, Ser 7, Asn 42, Ser 43, Asn 86, His 87, Asp 104, Lys 105, Thr 108, Met 135, Asp 153, Pro 154, Asp 163, Gly 164, Asn 180, Ser 181, Asn 198, Gly 199, Asn 267, Gly 268, Asp 282, Ser 283, Asp 284, Ser

285, Asn 317, His 318, Lys 330 and Met 311 of SEQ ID NO: 12. Each possibility represents a separate embodiment of the present invention.

According to another embodiment of the invention, the antibody comprises a heavy chain as set forth in SEQ ID NO: 13. According to yet another embodiment, the antibody comprises a heavy chain as set forth in SEQ ID NO: 13 comprising at least one amino acid modification at a position selected from the group consisting of: Thr 120, Ser 124, Asn 159, Ser 160, Asn 203, His 204, Asp 221, Lys 222, Thr 225, Met 252, Asp 270, Pro 271, Asp 280, Gly 281, Asn 297, Ser 298, Asn 315, Gly 316, Asn 384, Gly 385, Asp 399, Ser 400, Asp 401, Ser 402, Asn 434, His 435, Lys 447 and Met 428 of SEQ ID NO: 13. Each possibility represents a separate embodiment of the present invention.

According to another aspect the present invention provides an antibody or antigen binding fragment thereof, the antibody comprises a light chain variable region selected from the group consisting of SEQ ID NO: 1, 2, 3 and 4 and or a heavy chain variable region selected from the group consisting of SEQ ID NO: 5, 6, 7, 8 and 9, or an amino acid sequence at least 85% identical thereto, wherein the antibody comprises at least one amino acid modification in a position selected from the group consisting of:

(i) Thr 5, Thr 20, Cys 71, Asn 75, Ser 76, Phe 93 and Phe 97 of the light chain variable region having the amino acid sequence of SEQ ID NO: 1-4;

(ii) Asp 54, Ser 55 and Trp 107 of the heavy chain variable region having the amino acid sequence of SEQ ID NO: 5-9;

(iii) Asn 51, Ser 52, Asp 63 and Ser 64 of the light chain constant region having the amino acid sequence of SEQ ID NO: 10; and

(iv) Thr 3, Ser 7, Asn 42, Ser 43, Asn 86, His 87, Asp 104, Lys 105, Thr 108, Met 135, Asp 153, Pro 154, Asp 163, Gly 164, Asn 180, Ser 181, Asn 198, Gly 199, Asn 267, Gly 268, Asp 282, Ser 283, Asp 284, Ser 285, Asn 317, His 318, Lys 330 and Met 311 of the heavy chain constant region having the amino acid sequence of SEQ ID NO: 12.

In another embodiment of the invention, the antibody comprises a light chain variable region selected from the group consisting of SEQ ID NO: 1, 2, 3 and 4, or a heavy chain variable region selected from the group consisting of SEQ ID NO: 5, 6, 7, 8 and 9, or an amino acid sequence at least 88%, at least 90%, at least 92%, at least 93%, at least 94%, at

least 95%, at least 96%, at least 97%, at least 98% or 99% identical thereto, wherein each possibility represents a separate embodiment of the present invention.

According to various embodiments of the invention, the antibody or antigen binding fragment thereof comprises a combination of modified amino acids, including but not limited to, at least two amino acid modifications, at least three amino acid modifications, at least four amino acid modifications or at least five amino acid modifications wherein each possibility represent a separate embodiment of the present invention.

According to another embodiment, the antibody or antigen binding fragment thereof comprises at least one amino acid modification at a position selected from the group consisting of: Thr 5, Thr 20, Cys 71, Asn 75, Ser 76, Phe 93 and Phe 97 of SEQ ID NO: 1-4; and at least one amino acid modification at a position selected from the group consisting of: Asp 54, Ser 55 and Trp 107 of SEQ ID NO: 5-9. Each possibility represents a separate embodiment of the present invention.

According to another embodiment, the light chain variable region has one amino acid modification selected from the group consisting of: Thr 5, Thr 20, Cys 71, Asn 75, Ser 76, Phe 93 and Phe 97 of any one of SEQ ID NO: 1, 2, 3, 4 or 11. According to yet another embodiment, the heavy chain variable region has one modified amino acid selected from the group consisting of: Asp 54, Ser 55 and Trp 107 of any one of SEQ ID NO: 5, 6, 7, 8, 9 or 13.

In a particular embodiment, the amino acid modification is at position Cys 71 of any one of SEQ ID NO: 1, 4 or 11. In another embodiment, Cys 71 is substituted with an amino acid selected from the group consisting of: Ser, Val, Gly, Thr, Ile, Leu and Ala. Without wishing to be bound by any theory or mechanism of action, the substitution of said Cystine residue reduces the risk of undesirable disulfide bonds.

Without wishing to be bound by any theory or mechanism of action, modifying one or both amino acid of the following amino acid pairs: Asn-Ser; Asp-Ser; Asn-His; or Asn-Gly, reduces the risk of deamidation thereby increasing the solubility of the antibody of the invention. In a specific embodiment, the amino acid modification is at a position selected from the group consisting of: Asn 75, Ser 76 of any one of SEQ ID NO: 1, 2, 3, 4 or 11. In another embodiment, the amino acid modification is at a position selected from the group consisting of: Asn 157, Ser 158 of SEQ ID NO: 10. In another embodiment, the amino acid modification is at a position selected from the group consisting of: Asn 159, Ser 160, Asn

297, Ser 298 of SEQ ID NO: 11.

In one embodiment, the amino acid pair Asn-Ser is a substituted with Ser-Asn. In another embodiment, the amino acid pair Asn-Ser is substituted with any amino acid pair other than Asp-Ser; Asn-His; or Asn-Gly. In particular embodiments, Asn is substituted with any amino acid selected from Ala, Val, Ser, Leu, Gly, Glu, or Ser. In another particular embodiment, Asn is substituted with any amino acid other than Gln or Asp. In particular embodiments, Ser is substituted with any amino acid selected from Ala, Val, Leu, Gly, Ile, Thr or Asn. In yet another particular embodiment, Ser is substituted with any amino acid selected from Ala, Val, Leu, Gly, Ile or Asn. In another particular embodiment, Ser is substituted with any amino acid other than Gly. Each possibility represents a separate embodiment of the present invention.

In another embodiment, the amino acid modification is at a position selected from the group consisting of: Asp 54, Ser 55 of any one of SEQ ID NO: 5, 6, 7, 8, 9 or 13.

In one embodiment, the amino acid pair Asp-Ser is a substituted with Ser-Asp. In another embodiment, the amino acid pair Asp-Ser is substituted with any amino acid pair other than Asn-Ser; Asn-His; or Asn-Gly. In particular embodiments, Asp is substituted with any amino acid selected from Ala, Val, Ser, Leu, Gly or Ser. In another particular embodiment, Asp is substituted with any amino acid other than Glu or Asn. In particular embodiments, Ser is substituted with any amino acid selected from Ala, Val, Leu, Ile, Gly or Asn. In another particular embodiment, Ser is substituted with any amino acid other than Gly. Each possibility represents a separate embodiment of the present invention.

In another embodiment, the modification is a substitution of at least one residue selected from the residue pairs: Asn 203 and His 204; Asn 315 and Gly 316; Asn 384 and Gly 385; Asn 434 and His 435 of SEQ ID NO: 11. In particular embodiments, the amino acid pair is interchanged (e.g., Asn-His substituted with His-Asn; Asn-Gly substituted with Gly-Asn). In another embodiment, the amino acid pair Asn-his is substituted with any amino acid pair other than Asn-Ser; Asp-Ser; or Asn-Gly. In another embodiment, the amino acid pair Asn-Gly is a substituted with any amino acid pair other than Asn-Ser; Asp-Ser; or Asn-His. Each possibility represents a separate embodiment of the present invention.

In another embodiment, the amino acid modification is at a position selected from Thr 5, Thr 20 of the light chain variable region. In another embodiment, the amino acid modification

is at a position selected from the group consisting of: Thr 3, Ser 7 and Thr 108 of the heavy chain constant region as set forth as SEQ ID NO: 12. In yet another embodiment, the amino acid modification is at a position selected from the group consisting of: Thr 120, Ser 124, Thr 225 of the heavy chain as set forth as SEQ ID NO: 13. In another embodiment, the threonine residue is substituted with any amino acid other than serine. In another embodiment, the serine residue is substituted with any amino acid other than threonine. In a specific embodiment, the threonine and/or serine reside is substituted with an alanine reside. Without wishing to be bound by any theory or mechanism of action, the substitution of said threonine or serine residue reduces the risk of undesirable O-linked glycosylation.

Without wishing to be bound by any theory or mechanism of action, modifying one or both amino acid of the following amino acid pairs: Asp-Ser; Asp-Lys; Asp-Gly; reduces the risk of aspartic acid isomerization thereby preventing or decreasing the risk of degradation. In a specific embodiment, the amino acid modification is at a position selected from the group consisting of: Asp 63-Ser 64 of SEQ ID NO: 10; Asp 169-Ser 170 of SEQ ID NO: 11; Asp 104-Lys 105, Asp 163-Gly 164, Asp 282-Ser 283, Asp 285, Ser 286 of SEQ ID NO: 12; and Asp 221-Lys 222, Asp 280-Gly 281, Asp 339-Ser 400, Asp 401, Ser 402 of SEQ ID NO: 13. In particular embodiments, the amino acid pair is interchanged (e.g., Asp-Ser substituted with Ser-Asp). In another embodiment, the amino acid pair is substituted with any amino acid pair other than Asp-Ser, Asp-Lys or Asp-Gly. In one embodiment, Asp is substituted with Glu. Each possibility represents a separate embodiment of the present invention.

According to various embodiments, the antibody or antigen binding fragment thereof of the invention has an antitumor activity of similar, or greater than, mBAT-1. According to another embodiment, said antibody or antigen binding fragment thereof has an antitumor activity of similar, or greater than, hBAT-1. According to another embodiment, said antibody or antigen binding fragment thereof has enhanced stability as compared to hBAT-1.

According to various embodiments, the fragment of the humanized antibody is selected from the group consisting of: Fv, F (ab'), F (ab') 2, and a single chain antibody.

According to another embodiment, the present invention provides polynucleotide sequences encoding the antibody of the invention or fragments thereof.

According to yet another embodiment there is provided a vector comprising the polynucleotide sequence encoding the antibody of the invention or fragments thereof.

According to a specific embodiment there is provided a vector comprising the polynucleotide sequence encoding the antibody of the invention or fragments thereof selected from the group consisting of: whole humanized antibody, the light chain variable region, the heavy chain variable region, both chains of the variable region.

According to another aspect, there is provided host cells containing a vector comprising the polynucleotide sequence encoding the antibody of the invention or fragments thereof.

According to another aspect, there is provided a pharmaceutical composition comprising as an active ingredient the antibody of the invention or antigen binding fragment thereof, and a pharmaceutically acceptable carrier, diluent or stabilizer.

According to another embodiment, the pharmaceutical composition comprising as an active ingredient the antibody of the invention is used for the treatment of cancer. According to another embodiment, the pharmaceutical composition may be administered either following detection of primary or secondary tumors in a subject, or as preventive therapy of a subject having a high risk of developing cancers. According to some embodiments, the antibody of the invention elicits anti-tumor effects in a variety of tumors.

According to another aspect, the present invention provides a method for treating a disease or a disorder in a subject in need thereof, comprising administering to the subject an effective amount of a pharmaceutical composition comprising the antibody of the invention as an active ingredient or an antigen binding fragment thereof. In some embodiments, the disease or a disorder is cancer. In some embodiments, the disease or a disorder is an immuno-deficiency related disease or disorder.

In one embodiment, the subject has a tumor selected from a solid tumor or a non-solid tumor. In some embodiments, the tumor is a solid tumor. In some embodiments, the tumor is a non-solid tumor. In some embodiments, the non-solid tumor is a hematologic malignancy.

In particular embodiments, the cancer is selected from the group consisting of a colorectal carcinoma, a non-small lung cancer (NSCLC), a small cell lung cancer (SCLC), a breast carcinoma; a melanoma; an ovarian carcinoma, a cervical carcinoma, a pancreatic cancer, a head and neck carcinoma, a gastrointestinal carcinoma, an esophageal tumor, a hepatocellular carcinoma, multiple myeloma, a renal cell carcinoma, a prostate tumor, non-Hodgkin's lymphoma, Hodgkin's disease, mantle cell lymphoma, Kaposi's sarcoma, a squamous cell carcinoma, a basal cell carcinoma, acute myeloid leukemia (AML), chronic

myelocytic leukemia (CML), acute lymphocytic leukemia (ALL), and chronic lymphocytic leukemia (CLL). Each possibility represents a separate embodiment of the present invention.

According to specific embodiments, the cancer is selected from the group consisting of: colorectal carcinoma, melanoma, pancreatic cancer, head and neck carcinoma, esophageal tumor, multiple myeloma, renal cell carcinoma, non-Hodgkin's lymphoma and Hodgkin's disease.

According to various embodiments, the subject is a non-human mammal. According to various preferred embodiments, the subject is a human.

In an additional aspect, the invention provides an antibody or antigen binding fragment thereof of the present invention, for use in treating a tumor. In another embodiment, the invention provides an antibody or antigen binding fragment thereof comprising a light chain variable region selected from the group consisting of SEQ ID NO: 1-4, and a heavy chain variable region selected from the group consisting of SEQ ID NO: 5-9, wherein the antibody or antigen binding fragment thereof comprises at least one amino acid modification at a position selected from the group consisting of: Thr 5, Thr 20, Cys 71, Asn 75, Ser 76, Phe 93 and Phe 97 of the light chain variable region and Asp 54, Ser 55 and Trp 107 of the heavy chain variable region, for use in treating a tumor.

In an additional aspect, the invention provides an antibody or antigen binding fragment thereof of the present invention, for the preparation of a medicament for treating a tumor. In another embodiment, the invention provides an antibody or antigen binding fragment thereof comprising a light chain variable region selected from the group consisting of SEQ ID NO: 1-4, and a heavy chain variable region selected from the group consisting of SEQ ID NO: 5-9, wherein the antibody or antigen binding fragment thereof comprises at least one amino acid modification at a position selected from the group consisting of: Thr 5, Thr 20, Cys 71, Asn 75, Ser 76, Phe 93 and Phe 97 of the light chain variable region and Asp 54, Ser 55 and Trp 107 of the heavy chain variable region, for the preparation of a medicament for treating a tumor.

Other objects, features and advantages of the present invention will become apparent from the following detailed description and appended claims.

## BRIEF DESCRIPTION OF THE DRAWINGS

**Figure 1** is a graph demonstrating $CD4^+$ T lymphocytes viability following incubation with humanized monoclonal antibody BAT-1 (denoted CT-011) and a CT-011 double mutant comprising alanine substitutions at position Phe 97 of the light chain variable region and Trp 107 of the heavy chain variable region.

## DETAILED DESCRIPTION OF THE INVENTION

The present invention provides humanized monoclonal antibodies, pharmaceutical compositions comprising said antibodies, and use thereof for the treatment of a variety of indications, including but not limited to cancer and immunodeficiency disorders. In particular, the present invention provides modified antibodies or fragments thereof having specific amino acid modifications such as, amino acid substitutions, compared to the hBAT-1 humanized monoclonal immunomodulatory antibody variants.

According to another embodiment, there is provided an antibody or antigen binding fragment thereof comprising a light chain variable region at least 85% identical to any one of SEQ ID NO: 1-4, and a heavy chain variable region at least 85% identical to any one of SEQ ID NO: 5-9, the antibody or antigen binding fragment thereof comprises at least one modified amino acid selected from the group consisting of: Thr 5, Thr 20, Cys 71, Asn 75, Ser 76, Phe 93 and Phe 97 in comparison with SEQ ID NO: 1, or at least one modified amino acid selected from the group consisting of: Asp 54, Ser 55 and Trp 107 in comparison with SEQ ID NO: 5. Each possibility represents a separate embodiment of the present invention.

In another embodiment of the invention, the antibody comprises a light chain variable region at least 85%, at least 90%, at least 92%, at least 95% or at least 98% identical to any one of SEQ ID NO: 1-4, and a heavy chain variable region as set forth in any one of SEQ ID NO: 5-9. In another embodiment, the antibody comprises a heavy chain variable region at least 85%, at least 90%, at least 92%, at least 95% or at least 98% identical to any one of SEQ ID NO: 5-9, and a light chain variable region as set forth in any one of SEQ ID NO: 1-4. In another embodiment, the antibody comprises a light chain variable region at least 85%, at least 90%, at least 92%, at least 95% or at least 98% identical to any one of SEQ ID NO: 1-4,

and a heavy chain variable region at least 85%, at least 90%, at least 92%, at least 95% or at least 98% identical to any one of SEQ ID NO: 5-9. Each possibility represents a separate embodiment of the present invention.

The term "at least X percent identical" or "having at least X percent identity" refers to the percent of amino acid residues that are identical in the two compared sequences when the sequences are optimally aligned. Thus, for instance, 85% amino acid sequence identity means that 85% of the amino acids in two or more optimally aligned polypeptide sequences are identical.

According to another embodiment, there is provided an antibody or antigen binding fragment thereof comprising a light chain variable region selected from SEQ ID NO: 1-4, and a heavy chain variable region selected from SEQ ID NO: 5-9, wherein the light chain variable region comprises at least one amino acid modification at a position selected from the group consisting of: Thr 5, Thr 20, Cys 71, Asn 75, Ser 76, Phe 93 and Phe 97; or wherein the heavy chain variable region comprises at least one amino acid modification at a position selected from the group consisting of: Asp 54, Ser 55 and Trp 107. Each possibility represents a separate embodiment of the present invention.

According to one embodiment, the light chain variable region has the amino acid sequence as set forth in SEQ ID NO: 1 ($BATRK_D$). According to another embodiment, the light chain variable region has the amino acid sequence as set forth in SEQ ID NO: 2 ($BATRK_A$). According to another embodiment, the light chain variable region has the amino acid sequence as set forth in SEQ ID NO: 3 ($BATRK_B$). According to another embodiment, the light chain variable region has the amino acid sequence as set forth in SEQ ID NO: 4 ($BATRK_C$).

According to another embodiment, the antibody or a fragment thereof comprises at least one amino acid modification at a position selected from the group consisting of: Thr 5, Thr 20, Cys 71, Asn 75, Ser 76, Phe 93 and Phe 97 of any one of SEQ ID NO: 1 or SEQ ID NO: 4. Each possibility represents a separate embodiment of the present invention.

According to another embodiment, the antibody or a fragment thereof comprises at least one amino acid modification at a position selected from the group consisting of: Thr 5, Thr 20, Asn 75, Ser 76, Phe 93 and Phe 97 of any one of SEQ ID NO: 2 or SEQ ID NO: 3. Each possibility represents a separate embodiment of the present invention.

According to another embodiment, the heavy chain variable region has the amino acid sequence as set forth in SEQ ID NO: 5 ($BATRH_C$). According to another embodiment, the light chain variable region has the amino acid sequence as set forth in SEQ ID NO: 6 ($BATRH_A$). According to another embodiment, the light chain variable region has the amino acid sequence as set forth in SEQ ID NO: 7 ($BATRH_B$). According to another embodiment, the light chain variable region has the amino acid sequence as set forth in SEQ ID NO: 8 ($BATRH_D$). According to another embodiment, the light chain variable region has the amino acid sequence as set forth in SEQ ID NO: 9 ($BATRH_E$).

According to another embodiment, the antibody or antigen binding fragment thereof of the invention comprises an amino acid modification at a position selected from Phe 93 or Phe 97 of the light chain variable region. In another embodiment, the light chain variable region (any one of SEQ ID NO: 1-4) is modified by a substitution of Phe 93 or Phe 97 to any amino acid other than Trp and Tyr. Non limiting examples of amino acid substitutions of Phe and/or Trp include Ala, Leu and Val. In particular embodiments, the light chain variable region comprises the amino acid sequence selected from the group consisting of: SEQ ID NO: 14, 15, 16, 17, 18, 19, 20 and 21. In another particular embodiment, the light chain variable region comprises the amino acid sequence selected from the group consisting of: SEQ ID NO: 14, 16, 18, 20, and 22. In yet another particular embodiment, the light chain variable region comprises the amino acid sequence selected from the group consisting of: SEQ ID NO: 15, 17, 19, 21 and 23. In an additional embodiment, the light chain variable region comprises the amino acid sequence as set forth as SEQ ID NO: 14. In another embodiment, the light chain variable region comprises the amino acid sequence as set forth as SEQ ID NO: 15. In yet another embodiment, the Phe residue is substituted with an Ala residue.

In another embodiment, the light chain variable region comprises an amino acid substitution of Phe 93 and Phe 97 to Ala, Leu or Val. In a particular embodiment the light chain variable region comprises has the amino acid sequence of SEQ ID NO: 22. In yet another embodiment, the light chain variable region comprises an amino acid substitution of Phe 93 and Phe 97 to Ala. In a particular embodiment the light chain variable region comprises has the amino acid sequence of SEQ ID NO: 23.

In another embodiment of the invention, the heavy chain variable region (any one of SEQ ID NO: 5-9) is modified by a substitution of Trp 107 to any amino acid other than Phe. Non limiting examples of amino acid substitutions of Trp include Ala, Leu Tyr and Val. In

particular embodiments, the heavy chain variable region comprises the amino acid sequence selected from the group consisting of: SEQ ID NO: 24, 25, 26, 27 and 28. In another embodiment, the heavy chain variable region comprises the amino acid sequence as set forth as SEQ ID NO: 24. In yet another embodiment, the Trp residue is substituted with an Ala residue.

According to another embodiment, the antibody or antigen binding fragment thereof comprises an amino acid modification at a position selected from Trp 107 of the heavy chain variable region and Phe 97 of the light chain variable region. According to yet another embodiment, the antibody or antigen binding fragment thereof comprises an amino acid modification of Trp 107 of the heavy chain variable region and Phe 97 of the light chain variable region. According to another embodiment, the antibody or antigen binding fragment thereof comprises an amino acid modification at a position selected from Trp 107 of the heavy chain variable region and Phe 93 of the light chain variable region. According to yet another embodiment, the antibody or antigen binding fragment thereof comprises an amino acid modification of Trp 107 of the heavy chain variable region and Phe 93 of the light chain variable region. In a specific embodiment, said modification is a substitution to an alanine residue.

**Table 2- Exemplary variable region of hBAT-1 mutated light and heavy chain comprising F93, F97 or W107 amino acid substitutions.**

| Ig Chain | Based on | Amino acid sequence | Substitution | SEQ ID NO: |
|---|---|---|---|---|
| Light chain variable region | $BATRK_D$ | EIVLTQSPSSLSASVGDRVTITCSARSSVS YMHWFQQKPGKAPKLWIYRTSNLASGVPSR FSGSGSGTSYCLTINSLQPEDFATYYCQQR SS**X**PLTFGGGTKLEIK | F93A, F93L or F93V | 14 |
| | $BATRK_D$ | EIVLTQSPSSLSASVGDRVTITCSARSSVS YMHWFQQKPGKAPKLWIYRTSNLASGVPSR FSGSGSGTSYCLTINSLQPEDFATYYCQQR SSFPLT**X**GGGTKLEIK | F97A, F97L or F97V | 15 |
| | $BATRK_A$ | EIVLTQSPSSLSASVGDRVTITCSARSSVS YMHWYQQKPGKAPKLWIYRTSNLASGVPSR FSGSGSGTDFTLTINSLQPEDFATYYCQQR SS**X**PLTFGGGTKLEIK | F93A, F93L or F93V | 16 |
| | $BATRK_A$ | EIVLTQSPSSLSASVGDRVTITCSARSSVS YMHWYQQKPGKAPKLWIYRTSNLASGVPSR FSGSGSGTDFTLTINSLQPEDFATYYCQQR SSFPLT**X**GGGTKLEIK | F97A, F97L or F97V | 17 |
| | $BATRK_B$ | EIVLTQSPSSLSASVGDRVTITCSARSSVS | F93A, F93L | 18 |

| | | | | |
|---|---|---|---|---|
| | | YMHWFQQKPGKAPKLWIYRTSNLASGVPSR FSGSGSGTDYTLTINSLQPEDFATYYCQQR SS**X**PLTFGGGTKLEIK | or F93V | |
| | $BATRK_B$ | EIVLTQSPSSLSASVGDRVTITCSARSSVS YMHWFQQKPGKAPKLWIYRTSNLASGVPSR FSGSGSGTDYTLTINSLQPEDFATYYCQQR SSFPLT**X**GGGTKLEIK | F97A, F97L or F97V | 19 |
| | $BATRK_C$ | EIVLTQSPSSLSASVGDRVTITCSARSSVS YMHWFQQKPGKAPKLWIYRTSNLASGVPSR FSGSGSGTDYCLTINSLQPEDFATYYCQQR SS**X**PLTFGGGTKLEIK | F93A, F93L or F93V | 20 |
| | $BATRK_C$ | EIVLTQSPSSLSASVGDRVTITCSARSSVS YMHWFQQKPGKAPKLWIYRTSNLASGVPSR FSGSGSGTDYCLTINSLQPEDFATYYCQQR SSFPLT**X**GGGTKLEIK | F97A, F97L or F97V | 21 |
| | $BATRK_D$ | EIVLTQSPSSLSASVGDRVTITCSARSSVS YMHWYQQKPGKAPKLWIYRTSNLASGVPSR FSGSGSGTDFTLTINSLQPEDFATYYCQQR SS**X**PLT**X**GGGTKLEIK | F93 & F97 to A, L or V | 22 |
| | $BATRK_D$ | EIVLTQSPSSLSASVGDRVTITCSARSSVS YMHWYQQKPGKAPKLWIYRTSNLASGVPSR FSGSGSGTDFTLTINSLQPEDFATYYCQQR SS**A**PLT**A**GGGTKLEIK | F93A & F97A | 23 |
| | $BATRK_D$ | EIVLTQSPSSLSASVGDRVTITCSARSSVS YMHWFQQKPGKAPKLWIYRTSNLASGVPSR FSGSGSGTSYCLTINSLQPEDFATYYCQQR SS**A**PLTFGGGTKLEIK | F93A | 29 |
| | $BATRK_D$ | EIVLTQSPSSLSASVGDRVTITCSARSSVS YMHWFQQKPGKAPKLWIYRTSNLASGVPSR FSGSGSGTSYCLTINSLQPEDFATYYCQQR SSFPLT**A**GGGTKLEIK | F97A | 30 |
| Heavy chain variable region | $BATRH_C$ | QVQLVQSGSELKKPGASVKISCKASGYTFT NYGMNWVRQAPGQGLQWMGWINTDSGESTY AEEFKGRFVFSLDTSVNTAYLQITSLTAED TGMYFCVRVGYDALDY**X**GQGTLVTVSS | W107A, W107L, W107Y or W107V | 24 |
| | $BATRH_A$ | QVQLVQSGSELKKPGASVKISCKASGYTFS NYGMNWVRQAPGQGLQWMGWINTDSGESTY AEEFKGRFVFSLDTSVSTAYLQITSLTAED TGMYFCAKVGYDALDY**X**GQGTLVTVSS | W107A, W107L, W107Y or W107V | 25 |
| | $BATRH_B$ | QVQLVQSGSELKKPGASVKISCKASGYTFT NYGMNWVRQAPGQGLQWMGWINTDSGESTY AEEFKGRFVFSLDTSVSTAYLQITSLTAED TGMYFCAKVGYDALDY**X**GQGTLVTVSS | W107A, W107L, W107Y or W107V | 26 |
| | $BATRH_D$ | QIQLVQSGSELKKPGASVKISCKASGYTFT NYGMNWVRQAPGQGLQWMGWINTDSGESTY AEEFKGRFVFSLDTSVNTAYLQITSLTAED TGMYFCVRVGYDALDY**X**GQGTLVTVSS | W107A, W107L, W107Y or W107V | 27 |
| | $BATRH_E$ | QIQLVQSGSELKKPGASVKISCKASGYTFT | W107A, | 28 |

| | | | | |
|---|---|---|---|---|
| | | NYGMNWVRQAPGQGLQWMGWINTDSGESTY<br>AEEFKGRFAFSLDTSVNTAYLQITSLTAED<br>TGMYFCVRVGYDALDY**X**GQGTLVTVSS | W107L,<br>W107Y or<br>W107V | |
| | BATRH$_C$ | QVQLVQSGSELKKPGASVKISCKASGYTFT<br>NYGMNWVRQAPGQGLQWMGWINTDSGESTY<br>AEEFKGRFVFSLDTSVNTAYLQITSLTAED<br>TGMYFCVRVGYDALDY**A**GQGTLVTVSS | W107A | 31 |

According to another embodiment, the present invention provides polynucleotide sequences encoding the antibody of the invention or fragments thereof. Accordingly, the modified antibody is produced by expression of polynucleotides, wherein the polynucleotides may encode the whole humanized antibody or the light chain variable region or the heavy chain variable region or the variable region of both chains of the humanized antibody. Further, the humanized antibody may be expressed in a host cell following co-transfection of distinct vectors each comprising polynucleotides encoding the heavy or the light chain, or by transfection of a single vector comprising both light and heavy chain polynucleotide sequences.

According to yet another embodiment there is provided a vector comprising the polynucleotide sequence encoding the modified antibody of the invention or fragments thereof.

According to yet another embodiment there is provided a vector comprising the polynucleotide sequence encoding the modified antibody of the invention or fragments thereof selected from the group consisting of: whole humanized antibody, the light chain variable region, the heavy chain variable region, both chains of the variable region.

According to yet another embodiment, the vector further comprises at least one sequence encoding a component selected from the group consisting of: resistance genes, promoter, signal peptide, polyA transcription terminator, selection markers, genomic human kappa constant region.

According to yet another preferred embodiment, the components of the vector are selected from the group consisting of: Ampicillin resistance gene, Neomycin resistance gene, HCMV Immediate Early Promoter, the genomic human kappa constant region, a mouse immunoglobulin signal peptide sequence, Kozak sequence, a signal sequence intron, BGH polyA transcription terminator, a Neo/G418 selection marker, a hamster dhfr selection

marker.

According to yet another embodiment, the vector further comprises at least one sequence encoding a component selected from the group consisting of: resistance genes, promoter, signal peptide, polyA transcription terminator, selection markers, the genomic human Ig constant region.

According to yet another preferred embodiment, the components of the vector are selected from the group consisting of: Ampicillin resistance gene, Neomycin resistance gene, HCMV Immediate Early Promoter, the genomic human IgG1 constant region, a mouse immunoglobulin signal peptide sequence, Kozak sequence, a signal sequence intron, BGH polyA transcription terminator, a Neo/G418 selection marker, a hamster dhfr selection marker.

According to another aspect, there is provided host cells containing a vector comprising the polynucleotide sequence encoding the antibody of the invention or fragments thereof for the purposes of storage, propagation, antibody production and therapeutic applications.

According to another embodiment, the host cell may be selected from the group consisting of: CHO, CHO*dhfr*, NSO, NSO/GS, COS, COS7.

Definitions

The term "modified antibodies", "mutated antibodies" and similar grammatical expressions refer to an alteration in an amino acid sequence, for example by substitution or deletion or chemical modification of one or more amino acid residues, as compared to the sequence of the original, known humanized monoclonal antibodies BAT-1 immunoglobulin.

The "humanized monoclonal antibodies hBAT-1" relates to a number of humanized monoclonal antibodies, based on murine BAT (mBAT-1), disclosed in U.S. Patent No. 7,332,582 (the contents of which are incorporated herein by reference). The hBAT-1 mAbs comprise a light chain variable region selected from the group consisting of: SEQ ID NO: 1, 2, 3 and 4 (denoted $BATRK_D$, $BATRK_A$, $BATRK_B$ and $BATRK_C$, respectively) and a heavy chain variable region selected from the group consisting of: SEQ ID NO: 5, 6, 7, 8 and 9 (denoted $BATRH_C$, $BATRH_A$, $BATRH_B$, $BATRH_D$ and $BATRH_E$, respectively). The amino acid sequence of the light and heavy chain antibody variants (SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8 and 9) are depicted in Table 1. The residues which differ in said variable regions are depicted

with a gray background (positions 2, 30, 69, 77, 97 and 98 of the light region and positions 35, 69, 70 and 71 of the heavy chain). The polynucleotide and amino-acid sequences of murine BAT (mBAT-1) are disclosed in U.S. Patent No. 7,695,715, the contents of which are incorporated herein by reference. Particular combinations of a heavy chain variable region and a light chain variable region of the hBAT-1 antibody are selected from the group consisting of: SEQ ID NO: 5/SEQ ID NO: 1; SEQ ID NO: 6/SEQ ID NO: 2; SEQ ID NO: 7/SEQ ID NO: 2; SEQ ID NO: 7/SEQ ID NO: 3; SEQ ID NO: 8/SEQ ID NO: 3; and SEQ ID NO: 7/SEQ ID NO: 1. In specific embodiment of the present invention, the mutated antibodies are based on the above particular combinations of heavy and light chain variable regions and comprise at least one site specific modification such as substitution selected from the disclosed amino acids.

An "amino acid modification" as used herein refers to an alteration in the amino acid, for example by substitution or deletion or chemical modification of said amino acid. Further, the antibodies of the invention may be chemically modified at one or more amino acid residues, either by natural processes, such as processing or other post-translational modifications, or by chemical modification techniques. Chemical modifications include, without limitation, acetylation, acylation, amidation, ADP-ribosylation, glycosylation, GPI anchor formation, covalent attachment of a liquid or lipid derivative, methylation, myristylation, pegylation, prenylation, phosphorylation, ubiqutination, or any similar process.

The term "amino acid" is used in its broadest sense to include naturally occurring amino acids as well as non-naturally occurring amino acids including amino acid analogs. In view of this broad definition, one skilled in the art would know that reference herein to an amino acid includes, for example, naturally occurring proteogenic (L)-amino acids, (D)-amino acids, chemically modified amino acids such as amino acid analogs, naturally occurring non-proteogenic amino acids such as norleucine, and chemically synthesized compounds having properties known in the art to be characteristic of an amino acid. As used herein, the term "proteogenic" indicates that the amino acid can be incorporated into a protein in a cell through a metabolic pathway. The amino acids used in this invention are those which are available commercially or are available by routine synthetic methods. Certain residues may require special methods for incorporation into the peptide, and either sequential, divergent and convergent synthetic approaches to the peptide sequence are useful in this invention. When there is no indication, either the L or D isomers may be used.

Conservative substitution of amino acids as known to those skilled in the art are within the scope of the present invention. Conservative amino acid substitutions includes replacement of one amino acid with another having the same type of functional group or side chain e.g. aliphatic, aromatic, positively charged, negatively charged. One of skill will recognize that individual substitutions, deletions or additions to peptide, polypeptide, or protein sequence which alters, adds or deletes a single amino acid or a small percentage of amino acids in the encoded sequence is a "conservatively modified variant" where the alteration results in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art.

The following six groups each contain amino acids that are conservative substitutions for one another:

1) Alanine (Ala; A), Serine (Ser; S), Threonine (Thr; T);

2) Aspartic acid (Asp; D), Glutamic acid (Glu; E);

3) Asparagine (Asn; N), Glutamine (Gln; Q);

4) Arginine (Arg; R), Histidine (His, H), Lysine (Lys; K);

5) Isoleucine (Ile; I), Leucine (Leu; L), methionine (Met; M), Valine (Val; V); and

6) Phenylalanine (Phe; F), Tyrosine (Tyr; Y), Tryptophan (Trp; W).

According to one embodiment, the amino acid substitution is a conservative substitution.

According to another embodiment, the amino acid substitution is a non-conservative substitution. As used herein, a "non-conservative substitution" is any amino acid substitution other than a conservative substitution described above. Non limiting examples for non-conservative substitutions include substitution of phenylalanine or tryptophan to alanine, leucine or valine.

In a specific embodiment the amino acid modification substitutions of the invention (i.e., to produce the modified antibody of the invention) is other that the substitutions described in US Pat. No. 7,332,582.

The term "antibody" (also referred to as an "immunoglobulin") is used in the broadest sense and specifically encompasses monoclonal antibodies (including full length monoclonal antibodies) and antibody fragments so long as they exhibit the desired biological activity. "Antibody fragments" comprise a portion of a full length antibody, generally the antigen binding or variable region thereof. Examples of antibody fragments include Fab, Fab', F(ab')2, and Fv fragments; diabodies; linear antibodies; single-chain antibody molecules; and multispecific antibodies formed from antibody fragments.

The basic unit of the naturally occurring antibody structure is a heterotetrameric glycoprotein complex of about 150,000 daltons, composed of two identical light (L) chains and two identical heavy (H) chains, linked together by both noncovalent associations and by disulfide bonds. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Five human antibody classes (IgG, IgA, IgM, IgD and IgE) exist, and within these classes, various subclasses, are recognized on the basis of structural differences, such as the number of immunoglobulin units in a single antibody molecule, the disulfide bridge structure of the individual units, and differences in chain length and sequence. The class and subclass of an antibody is its isotype.

The amino terminal regions of the heavy and light chains are more diverse in sequence than the carboxy terminal regions, and hence are termed the variable domains. This part of the antibody structure confers the antigen-binding specificity of the antibody. A heavy variable (VH) domain and a light variable (VL) domain together form a single antigen-binding site, thus, the basic immunoglobulin unit has two antigen-binding sites. Particular amino acid residues are believed to form an interface between the light and heavy chain variable domains (Chothia et al., J. Mol. Biol. 186, 651-63 (1985); Novotny and Haber, (1985) Proc. Natl. Acad. Sci. USA 82 4592-4596).

The carboxy terminal portion of the heavy and light chains form the constant domains i.e. CH1, CH2, CH3, CL. While there is much less diversity in these domains, there are differences from one animal species to another, and further, within the same individual there are several different isotypes of antibody, each having a different function.

The term "framework region" or "FR" refers to the amino acid residues in the variable domain of an antibody which are other than the hypervariable region amino acid residues as herein defined. The term "hypervariable region" as used herein refers to the amino acid

residues in the variable domain of an antibody which are responsible for antigen binding. The hypervariable region comprises amino acid residues from a "complementarity determining region" or "CDR". The CDRs are primarily responsible for binding to an epitope of an antigen. The extent of FRs and CDRs has been precisely defined (see, Kabat et al., ibid).

The term "acceptor human immunoglobulin" refers to the human immunoglobulin providing the framework for a humanized antibody.

As used herein, the term "humanized antibody" refers to an antibody comprising a framework region from a human antibody and one or more CDRs from a non-human (usually a mouse or rat) immunoglobulin. Parts of a humanized immunoglobulin, except possibly the CDRs, are substantially identical to corresponding parts of natural human immunoglobulin sequences. In some cases however, specific amino acid residues, for example in the framework regions, may be modified, so as to optimize performance of the humanized antibody. Importantly, the humanized antibody is expected to bind to the same antigen as the donor antibody that provides the CDRs. For further details, see e.g. U.S. Pat. No. 5,225,539 assigned to Medical Research Council, UK.

The terms "a framework region from an acceptor human immunoglobulin" and "a framework region derived from an acceptor human immunoblobulin", and similar grammatical expressions are used interchangeably herein to refer to a framework region or portion thereof that has the same amino acid sequence of the acceptor human immunoblobulin.

The term "human antibody" refers to an antibody encoded by a gene actually occurring in a human, or an allele, variant or mutant thereof.

The modified humanized monoclonal antibody of the invention is preferably generated by recombinant DNA technology, utilizing CDR grafting.

The term "mammal" means any mammal, including pet animals, such as dogs and cats; farm animals, such as pigs, cattle, sheep, and goats; laboratory animals, such as mice and rats; primates, such as monkeys, apes, and chimpanzees; and preferably, humans.

Methods of the Invention

Cancer immunotherapeutics are aimed by and large at modulating the response of the immune system to induce or enhance killing of tumor cells and control tumor growth. This approach utilizes using various immunomodulators including monoclonal antibodies that selectively bind to specific determinants on T cells thereby either initiating an activation pathway or inducing an inhibitory effect.

According to another aspect, the present invention provides a method for diagnosis or treatment of a disease or a disorder, particularly cancer, comprising administering to a subject in need thereof, an effective amount of a pharmaceutical composition comprising the antibody of the invention or a fragment thereof as an active ingredient.

All types of tumors may be treated by the methods of the present invention. The tumors may be solid or non-solid.

Some examples of solid tumors that can be treated with the combination of the present invention include carcinomas, sarcomas, blastomas or gliomas. Some examples of such tumors include epidermoid tumors, squamous tumors, such as head and neck tumors, colorectal tumors, prostate tumors, breast tumors, lung tumors, including small cell and non-small cell lung tumors, pancreatic tumors, thyroid tumors, ovarian tumors, liver tumors, esophageal tumors and gastric tumors. Other examples include Kaposi's sarcoma, CNS neoplasms, neuroblastomas, capillary hemangioblastomas, meningiomas and cerebral metastases, melanoma, gastrointestinal and renal carcinomas and sarcomas, rhabdomyosarcoma, glioblastoma, preferably glioblastoma multiforme, and leiomyosarcoma. Examples of vascularized skin cancers include squamous cell carcinoma, basal cell carcinoma and skin cancers that can be treated by suppressing the growth of malignant keratinocytes, such as human malignant keratinocytes.

Some examples of non-solid tumors include leukemias, multiple myelomas and lymphomas. Some examples of leukemias include acute myelocytic leukemia (AML), chronic myelocytic leukemia (CML), acute lymphocytic leukemia (ALL), chronic lymphocytic leukemia (CLL), erythrocytic leukemia or monocytic leukemia. Some examples of lymphomas include lymphomas associated with Hodgkin's disease, Non-Hodgkin's disease or mantle cell lymphoma.

Currently preferred types of tumors are selected from the following group: colorectal carcinoma; lung carcinoma including non-small lung cancer (NSCLC) and small cell lung cancer (SCLC); breast carcinoma; melanoma; ovarian carcinoma; cervical carcinoma, pancreatic cancer; head and neck carcinoma; gastrointestinal carcinoma; esophageal tumors; hepatocellular carcinoma; multiple myeloma; renal cell carcinoma; prostate tumors; non-Hodgkin's lymphoma; Hodgkin's disease; mantle cell lymphoma; Kaposi's sarcoma; squamous cell carcinoma; basal cell carcinoma; acute myeloid leukemia (AML); chronic myelocytic leukemia (CML); acute lymphocytic leukemia (ALL); chronic lymphocytic leukemia (CLL).

The term "antitumor effect" as used herein, refers to a beneficial biological effect, which can be manifested by any one or more of: a decrease or stabilization of tumor volume, a decrease or stabilization of the number of tumor cells, a decrease or stabilization of the rate of tumor growth, a decrease or stabilization of the number of metastases, protection from tumor recurrence, an increase in life expectancy or survival of the subject with the tumor, an increase in life expectancy or survival without disease progression of the subject with the tumor or amelioration of various physiological symptoms associated with the cancerous condition. An "antitumor effect" can also be manifested by the ability to prevent the occurrence of tumor in the first place or the recurrence of the tumor. Given its properties, the methods of the invention can be used in the treatment of acute cancer, of dormant, controlled or stabilized cancer, as well as in cancer prophylaxis.

The term "enhanced survival", as used herein, refers to a prolonged length of time during which the subject or patient is alive following treatment with a method of the invention. Enhanced survival denotes the increased probability of staying free of disease progression for an individual suffering from cancer after a particular treatment. It is also used to describe the elevated percentage of individuals in a group whose disease is likely to remain stable (not showing signs of progression) after a specified duration of time, compared to a control group. It is also used to describe the elevated percentage of individuals in a group whose disease is likely to be cured (not showing signs of disease) after a specified duration of time, compared to a control group. This parameter may be measured by any one of the customary clinical endpoints denoted as "progression-free survival", "overall survival" and "disease free survival" used as an indication of the efficacy of a particular treatment.

The term "tumor recurrence" refers to the re-emergence, reappearance, re-growth or proliferation of a tumor of the same type in either the same location or a different location, following a period during which the growth of the original tumor has been reversed, arrested or inhibited.

The term "enhances or increases lymphocyte survival" as used herein refers to the ability of the antibodies of the invention to prolong the viability of lymphocytes in vitro or in vivo, as compared to the viability of an identical cell population not contacted with the antibody of the invention. With regard to the combination therapy of the invention, he term refers to the ability of a particular combination of treatments to prolong the viability of lymphocytes in vitro or in vivo, as compared to the viability of an identical cell population with only one of the treatments.

An antitumor activity of similar to mBAT-1 or hBAT-1 refers to an antitumor effect of about 5%, or not more than 10%, similar to the antitumor effect of mBAT-1 or hBAT-1. An antitumor activity of greater than mBAT-1 or hBAT-1 refers to an antitumor activity of more than 10%, more than 30% or more than 30%, as compared to the antitumor activity of greater than mBAT-1 or hBAT-1.

According to another embodiment, the antibody of the invention in administered together with, prior to, or following, the administration of other agents, which can act in an additive or synergistic manner with it.

According to yet another embodiment, the antibody of the invention in administered together with, prior to, or following, the administration of agents selected from the group consisting of: cytokines, IL-1 (Interleuken-1), IL-2, IL-6, IFN-α (Interferon- α), cell vaccines, antibodies, T-cell stimulatory antibodies, anti-tumor therapeutic antibodies. Each possibility is a separate embodiment of the present invention.

In another embodiment, the disease or a disorder treated by the methods of the invention is an immuno-deficiency related disease or disorder selected from severe combined immunodeficiency disease, acquired immunodeficiency syndrome, and any disorder that involves depletion, attenuation and/or malfunctioning of lymphocytes, specifically T cells, NK cells, NK-T cells, B cells, monocytes, macrophages or any combination thereof.

In another embodiment, the disease or a disorder is an immunodeficiency, immune malfunction or immune incompetence, collectively referred to hereinafter as

immunodeficiency disorders, established after chemotherapy or irradiation. According to certain embodiments, the pharmaceutical composition comprising the antibody of the invention is used in conjunction with autologous, allogeneic or syngeneic stem cell transplantation derived from the bone marrow, umbilical cord blood or peripheral blood and donor leukocyte infusion.

According to yet another embodiment, the immunodeficiency disorder is selected from the group consisting of: severe combined immunodeficiency disease, acquired immunodeficiency syndrome, X-linked agammaglobulinaemia, common variable immunodeficiency, IgA deficiency, IgG subclass deficiency, Wiskott-Aldrich syndrome, DiGeorge anomaly, Ataxia Telangiectasia, adenosine deaminase deficiency and activation-induced cytidine deaminase deficiency.

According to yet another embodiment, the immunodeficiency disorder is related to viral infection, fungal infection or bacterial infection. According to yet another embodiment, the immunodeficiency disorder is associated with intoxication.

According to yet another embodiment, the method of the invention is used for treating anemia, particularly, aplastic anemia and myelodysplastic syndromes (MDS), primarily for avoiding further complication of the anemia due to immunodeficiency disorders.

Combination therapy with chemotherapy

According to certain aspects of the present invention, administration of the immunostimulatory humanized antibody comprising modified amino acids in conjunction with at least one antitumor chemotherapeutic agent acts to enhance the antitumor effect of chemotherapeutic agents, and vice versa. In preferred embodiments, the combinations of the immunostimulatory antibody together with the at least one chemotherapeutic agent improve the clinical outcome in a significant manner versus each of the treatments alone. In a preferred embodiment, there is synergy when tumors are treated with the humanized antibody of the invention in conjunction with at least one chemotherapeutic agent, and, optionally further in conjunction with radiation.

In other words, according to one aspect of the present invention the antitumor effect of the humanized antibody of the invention is augmented more than expected when combined with at least one chemotherapeutic agent.

Antitumor effect induced by the combinations of the invention includes the prevention, inhibition of the progression of a tumor, reduction of tumor growth and protection against tumor recurrence, including cancerous and noncancerous tumors. The progression of a tumor includes the invasiveness, metastasis, recurrence and increase in size of the tumor. The reduction of tumor growth also includes the destruction or elimination of a tumor leading to complete remission.

In another embodiment, the antibody of the invention is effective for improving tolerability to chemotherapeutic agents. As is known in the art, a major setback for patients undergoing cancer chemotherapy is the appearance of severe and detrimental adverse side effects due to the potent toxicity of most chemotherapeutic agents.

The invention further provides a method of enhancing survival in a subject with a tumor, which comprises administration of the humanized antibody of the invention, either on its own, or optionally, combined with the further administration of one or more chemotherapeutic agents.

The invention further provides a method of reducing or preventing recurrence of a tumor, which comprises administration of the humanized antibody of the invention, either on its own, or optionally, combined with the further administration of one or more chemotherapeutic agents. In some embodiments, combination treatment of experimental animals using the humanized antibody of the invention and chemotherapeutic agents induced a "memory" effect, such that tumor recurrence is inhibited upon re-challenge with the original tumor type.

According to another embodiment, the present invention provides a method of treating a tumor, the method comprising (i) administering to a subject in need thereof an effective amount of an antibody of the invention or a fragment thereof; and (ii) administering to the subject an effective amount of at least one chemotherapeutic agent; thereby treating the tumor.

According to another embodiment, the invention further provides a method of improving tolerability to at least one chemotherapeutic agent, the method comprising

administering to a subject in need thereof an effective amount of an antibody of the invention or a fragment thereof, wherein the subject is undergoing chemotherapy with at least one chemotherapeutic agent; thereby improving tolerability to said chemotherapeutic agent.

The term "tolerability to chemotherapeutic agents" refers to the physiological, physicochemical and immunological capacity of a subject to tolerate the adverse side effects associated with treatment with one or more chemotherapeutic agents. Accordingly, the term "improving tolerability to chemotherapeutic agents" refers to increasing the physiological and physicochemical capacity to such adverse side effects, such that the severity of the adverse side effects is decreased and/or the number of side effects is decreased. Accordingly, "improving tolerability to chemotherapeutic agents" may refer to improving the quality of life of cancer patients treated with chemotherapeutic agents.

According to yet another embodiment of the invention there is provided a method of enhancing survival or inhibiting disease progression in a subject having a tumor, wherein the subject is treated with at least one chemotherapeutic agent, the method comprising administering an effective amount of an antibody of the invention or a fragment thereof, thereby enhancing survival of the subject.

According to yet another embodiment, the invention provides a method of reducing or preventing tumor recurrence, the method comprising administering to a subject in need thereof an effective amount of an antibody of the invention or a fragment thereof, thereby reducing or preventing tumor recurrence. According to one embodiment, the method of reducing or preventing tumor recurrence further comprises administering to the subject at least one chemotherapeutic agent. According to particular embodiments, the subject is undergoing or has completed a course of chemotherapy with at least one chemotherapeutic agent.

According to various embodiments, the administering of the antibody of the invention and of the at least one chemotherapeutic agent is carried out substantially simultaneously, concurrently, alternately, sequentially or successively. In some embodiments, the antibody and the at least one chemotherapeutic agent are administered according to overlapping schedules.

According to particular embodiments, administering of the antibody is carried out prior to initial administration of the at least one chemotherapeutic agent.

According to other embodiments, administering of either or both of the antibody and the at least one chemotherapeutic agent is carried out by a route selected from the group consisting of intravenous, oral, intraperitoneal, subcutaneous, isolated limb perfusion, infusion into an organ and combinations thereof.

In particular embodiments, the methods of the invention further comprise assessing at least one parameter selected from the group consisting of: rate of tumor growth, tumor volume, number of metastases, tumor recurrence and combinations thereof.

It should be noted that according to the teaching of the present invention, the modified humanized antibody of the invention may be administered before, during, or after commencing chemotherapy and, optionally, radiation therapy, as well as any combination thereof, i.e. before and during, before and after, during and after, or before, during, and after commencing the chemotherapy and, optionally, the radiation therapy. For example, the antibody of the invention may be administered between 1 and 30 days prior to or after commencing chemotherapy. The antibody may further be administered between courses of chemotherapy.

In the combination therapy methods of the invention, the antibodies may be administered in parallel to the chemotherapy, for example substantially simultaneously or concurrently. Other administration schedules may also be used, for example, overlapping schedules or those which involve alternately, sequentially or successively administering the two types of treatment.

According to various embodiments, the at least one chemotherapeutic agent is selected from the group consisting of: antimetabolites, platinum-based drugs, mitotic inhibitors, anthracycline antibiotics, topoisomerase inhibitors, anti-angiogenic agents and combinations thereof.

According to another particular embodiment, the at least one chemotherapeutic agent is selected so that the modified hBAT-1 of the invention enhances survival of lymphocytes when used in combination with the chemotherapeutic agent. Typically, the enhanced or increased survival may be conveniently assayed in vitro.

Accordingly, in various embodiments, the chemotherapeutic agent may be selected from an antimetabolite, such as the pyrimidine analog 5-fluorouracil, or cytarabin, or a platinum-based drug, such as oxaliplatin or cisplatin. Further, in various embodiments, the

chemotherapeutic agent may be other than an agent selected from a topoisomerase I inhibitor (such as SN-38) and an alkylating agent (such as cyclophosphamide).

According to some embodiments, the at least one chemotherapeutic agent is an antimetabolite, including purine antagonists, pyrimidine antagonists and folate antagonists. According to some embodiments, the antimetabolite is a pyrimidine antagonist. According to some embodiments, the antimetabolite is selected from the group consisting of: 5-fluorouracil, uracil mustard, uracil, capecitabine, 6-mercaptopurine, methotrexate, gemcitabine, cytarabine, fludarabine, and pemetrexed.

According to some embodiments, the at least one chemotherapeutic agent is 5-fluorouracil. According to some embodiments, the at least one chemotherapeutic agent is cytarabine. According to some embodiments, the at least one chemotherapeutic agent is a platinum-based drug selected from the group consisting of: cisplatin, carboplatin and oxaliplatin. According to yet other embodiments, the at least one chemotherapeutic agent is a mitotic inhibitor selected from the group consisting of: paclitaxel, docetaxel, etoposide, vinblastine, vincristine and vinorelbine. According to yet other embodiments, the at least one chemotherapeutic agent is an anthracycline antibiotic selected from the group consisting of: daunorubicin, respinomycin D and idarubicin. According to some embodiments, the at least one chemotherapeutic agent is an anti-angiogenic agent selected from the group consisting of: bevacizumab, dopamine, tetrathiomolybdate, and antiangiogenic variants of VEGF. According to some embodiments, the at least one chemotherapeutic agent is other than a topoisomerase I inhibitor. According to some embodiments, the at least one chemotherapeutic agent is other than an alkylating agent.

Chemotherapy drugs are divided into several groups based on their effect on cancer cells, the cellular activities or processes the drug interferes with, or the specific phases of the cell cycle the drug affects. Accordingly, chemotherapy drugs fall in one of the following categories: alkylating agents, nitrosoureas, antimetabolites, anthracyclines, topoisomerase I and II inhibitors, mitotic inhibitors, *inter alia* platinum based drugs, steroids and anti-angiogenic agents.

Antimetabolites, also termed "nucleoside analogs", replace natural substances as building blocks in DNA molecules, thereby altering the function of enzymes required for cell metabolism and protein synthesis. In the event that they mimic nutrients required for cell

growth, the cells eventually undergo lysis. If a nucleoside is replaced with a non-functional nucleoside analog, the latter is incorporated into DNA and RNA, finally inducing cell cycle arrest and apoptosis by inhibiting the cell's ability to synthesize DNA. Antimetabolites are cell-cycle specific and are most effective during the S-phase of cell division as they primarily act upon cells undergoing synthesis of new DNA for formation of new cells. The toxicities associated with these drugs are seen in cells that are growing and dividing quickly. Examples of antimetabolites include purine antagonists, pyrimidine antagonists, and folate antagonists. These agents damage cells during the S phase and are commonly used to treat leukemias, tumors of the breast, ovary, and the gastrointestinal tract, as well as other cancers. Specific examples of antimetabolites include 5-fluorouracil (also known as 5FU), capecitabine, 6-mercaptopurine, methotrexate, gemcitabine, cytarabine, fludarabine and pemetrexed.

Platinum-based chemotherapeutic drugs crosslink DNA in several different ways, interfering with cell division by mitosis. The damaged DNA elicits DNA repair mechanisms, which in turn activate apoptosis when repair proves impossible. Most notable among the DNA changes are the 1,2-intrastrand cross-links with purine bases. These include 1,2-intrastrand d(GpG) adducts which form nearly 90% of the adducts and the less common 1,2-intrastrand d(ApG) adducts. 1,3-intrastrand d(GpXpG) adducts occur but are readily excised by the nucleotide excision repair (NER). Other adducts include inter-strand crosslinks and nonfunctional adducts that have been postulated to contribute to the activity of platinum-based drugs. Interaction with cellular proteins, particularly HMG domain proteins, has also been advanced as a mechanism of interfering with mitosis, although this is probably not its primary method of action. Platinum-based chemotherapeutic drugs include cisplatin (also known as cisplatinum or *cis*-diamminedichloridoplatinum II (CDDP), carboplatin and oxaliplatin. Cisplatin is frequently designated as an alkylating agent, though it has no alkyl group and cannot carry out alkylating reactions. It is correctly classified as alkylating-like. Platinum-based chemotherapeutic drugs are used to treat various types of cancers, including sarcomas, some carcinomas (e.g. small cell lung cancer, and ovarian cancer), lymphomas and germ cell tumors.

Mitotic inhibitors interfere with cell division. The most known chemotherapeutic agent in this category is paclitaxel (also known as Taxol®, "plant alkaloid", "taxane" and an "antimicrotubule agent"). Together with docetaxel, it forms the drug category of the taxanes. However, other mitotic inhibitors are known, including, but not limited to etoposide,

vinblastine and vincristine. Paclitaxel acts by interfering with normal microtubule growth during cell division by arrests their function; it hyper-stabilizes their structure. This destroys the cell's ability to use its cytoskeleton in a flexible manner. Specifically, paclitaxel binds to the β subunit of tubulin, the "building block" of microtubules, and the binding of paclitaxel locks these building blocks in place. The resulting microtubule/paclitaxel complex does not have the ability to disassemble. This adversely affects cell function because the shortening and lengthening of microtubules (termed dynamic instability) is necessary for their function as a mechanism to transport other cellular components. For example, during mitosis, microtubules position the chromosomes all through their replication and subsequent separation into the two daughter-cell nuclei. Furthermore, paclitaxel induces programmed cell death (apoptosis) in cancer cells by binding to the apoptosis stopping protein Bcl-2 (B-cell leukemia 2) and thus arresting its function.

Another group of DNA-interacting drugs widely used in anti-cancer chemotherapy is the group of anthracycline antibiotics which includes, *inter alia*, daunorubicin, doxorubicin (also known as Adriamycin® and doxorubicin hydrochloride), respinomycin D and idarubicin. These drugs interact with DNA by intercalation and inhibition of macromolecular biosynthesis thereby inhibiting the progression of the enzyme topoisomerase II, which unwinds DNA for transcription. They stabilize the topoisomerase II complex after it has broken the DNA chain for replication, preventing the DNA double helix from being resealed and thereby stopping the process of replication. It is commonly used in the treatment of a wide range of cancers.

Alkylating antineoplastic agents directly attack DNA. They attach an alkyl group to DNA, cross-linking guanine nucleobases in DNA double-helix strands. This makes the strands unable to uncoil and separate. As this is necessary in DNA replication, the cells can no longer divide. These drugs act nonspecifically. Cyclophosphamide is an alkylating agent, however, it is a highly potent immunosuppressive substance.

Topoisomerase I and II inhibitors interfere with the enzymatic activity of topoisomerase I and 2, respectively, eventually leading to inhibition of both DNA replication and transcription. Examples of topoisomerase I inhibitors include topotecan and irinotecan. Irinotecan, is a prodrug converted to a biologically active metabolite 7-ethyl-10-hydroxy-camptothecin (SN-38) by a carboxylesterase-converting enzyme. One thousand-fold more

potent than its parent compound irinotecan, SN-38 inhibits topoisomerase I activity by stabilizing the cleavable complex between topoisomerase I and DNA, resulting in DNA breaks that inhibit DNA replication and trigger apoptotic cell death. Because ongoing DNA synthesis is necessary for irinotecan to exert its cytotoxic effects, it is also classified as an S-phase-specific agent. Examples of topoisomerase II inhibitors include etoposide and teniposide.

Anti-angiogenic agents interfere with the generation of new blood vessels, eventually leading to the "starvation" of tumors. Non-limiting examples of anti-angiogenic agents include the monoclonal antibody bevacizumab, dopamine and tetrathiomolybdate.

Vascular endothelial growth factor (VEGF) is a 32-42 kDa dimeric glycoprotein which mediates vasodilatation, increased vascular permeability and endothelial cell mitogenesis. Differential exon splicing of the VEGF gene results in three main mRNA species which code for three secreted isoforms (subscripts denote numbers of amino acids): VEGF189, VEGF165, and VEGF121. A number of minor splice variants have also been described (VEGF206, VEGF183, VEGF145 and VEGF148). Variants of VEGF polypeptides and their use in cancer therapy is disclosed for example, in WO/2003/012105.

Combination therapy with Radiation

According to various embodiments, the methods further comprise treating the subject with radiation. According to various embodiments, the methods comprise all of administering the antibody of the invention, administering the at least one chemotherapeutic agent and treating the subject with radiation. According to some embodiments, the antibody, the at least one chemotherapeutic agent and radiation treatment are administered substantially simultaneously, concurrently, alternately, successively or according to overlapping schedules.

The source of radiation that may be used in combination with the modified antibody of the invention and the chemotherapeutic agent(s) can be either external or internal to the patient being treated. When the source is external to the patient, the therapy is known as external beam radiation therapy (EBRT). When the source of radiation is internal to the patient, the treatment is called brachytherapy (BT).

Radiation is administered in accordance with well-known standard techniques using standard equipment manufactured for this purpose, such as AECL Theratron and Varian Clinac.

The distance between the source of the external radiation and the point of entry into the patient may be any distance that represents an acceptable balance between killing target cells and minimizing side effects. Typically, the source of the external radiation is between 70 and 100 cm from the point of entry into the patient.

Brachytherapy is generally carried out by placing the source of radiation in the patient. Typically, the source of radiation is placed approximately 0-3 cm from the tissue being treated. Known techniques include interstitial, intercavitary, and surface brachytherapy. The radioactive seeds can be implanted permanently or temporarily. Some typical radioactive atoms that have been used in permanent implants include iodine-125 and radon. Some typical radioactive atoms that have been used in temporary implants include radium, cesium-137, and iridium-192. Some additional radioactive atoms that have been used in brachytherapy include americium-241 and gold-198.

The dose of radiation depends on numerous factors as is well known in the art. Such factors include the organ being treated, the healthy organs in the path of the radiation that might inadvertently be adversely affected, the tolerance of the patient for radiation therapy, and the area of the body in need of treatment. The dose will typically be between 1 and 100 Gy, and more particularly between 2 and 80 Gy. Some doses that have been reported include 35 Gy to the spinal cord, 15 Gy to the kidneys, 20 Gy to the liver, and 65-80 Gy to the prostate. It should be emphasized, however, that the invention is not limited to any particular dose. The dose will be determined by the treating physician in accordance with the particular factors in a given situation, including the factors mentioned above.

The dose of radiation for brachytherapy can be the same as that mentioned above for external beam radiation therapy. In addition to the factors mentioned above for determining the dose of external beam radiation therapy, the nature of the radioactive atom used is also taken into account in determining the dose of brachytherapy.

Humanized antibody of the invention

As used herein, the terms "BAT" and a BAT antibody" are used in a broad sense and specifically cover antibodies identical to or based on the murine monoclonal antibody known as mBAT-1, or an antigen binding fragment thereof. The monoclonal antibody mBAT-1 is secreted by the hybridoma cell line deposited at the Collection Nationale de Cultures de Microorganismes (CNCM), under Accession No. 1-1397, as disclosed in U.S. Patent No.

5,897,862. Further "BAT" and a BAT antibody" may refer to an antibody, which recognizes the same antigenic epitope as mBAT-1, for example a chimeric antibody as described in U.S. Patent Application Publication No. 2003/0026800. A BAT antibody also includes a humanized antibody, various examples of which are disclosed in WO03/099196 and U.S. Patent Application Publication No. 2008/0025980 and interchangeably denoted "CT-011", "hBAT" and "hBAT-1".

In general, the light chain variable region of the humanized monoclonal antibody is characterized by the formula:

$FR_{L1}$-$CDR_{L1}$-$FR_{L2}$-$CDR_{L2}$-$FR_{L3}$-$CDR_{L3}$-$FR_{L4}$

wherein each FR is independently a framework region of a human antibody and each CDR is independently a complementarity determining region of the monoclonal mBAT-1 antibody.

In general, the heavy chain variable region of the humanized monoclonal antibody is characterized by the formula:

$FR_{H1}$-$CDR_{H1}$-$FR_{H2}$-$CDR_{H2}$-$FR_{H3}$-$CDR_{H3}$-$FR_{H4}$

wherein each FR is independently a framework region of a human antibody and each CDR is independently a complementarity determining region of the monoclonal mBAT-1 antibody.

In particular embodiments, the FRs are derived from the light chain variable region of the human TEL9 antibody, or are modified therefrom in certain amino acid residues. Human TEL-9 antibody was identified in diverse libraries of immunoglobulin heavy (VH) and light (V kappa and V lambda) chain variable (V) genes prepared from peripheral blood lymphocytes of unimmunized donors (Marks et al. J MoI Biol. 1991, 222:581-97). This antibody was shown to bind specifically to the turkey egg-white lysozyme (TEL) antigen.

In particular embodiments, the FRs are derived from the heavy chain variable region of the human hsighv1295 antibody, or modified therefrom in certain amino acid residues. Human hsiggvl295 antibody was isolated from stable hybridomas and Epstein-Barr virus-transformed B cell lines from the synovial fluid or peripheral blood of three patients with rheumatoid arthritis and one patient with systemic lupus erythematosus (Fang et al., J Exp Med. 1994, 179:1445-56).

Compositions, administration and dosages

For use in the methods of the invention, the humanized antibody may be formulated in a conventional manner using one or more pharmaceutically acceptable carriers, stabilizers or excipients (vehicles) to form a pharmaceutical composition as is known in the art, in particular with respect to protein active agents. Carrier(s) are "acceptable" in the sense of being compatible with the other ingredients of the composition and not deleterious to the recipient thereof. Suitable carriers typically include physiological saline or ethanol polyols such as glycerol or propylene glycol.

The antibody may be formulated as neutral or salt forms. Pharmaceutically acceptable salts include the acid addition salts (formed with free amino groups) and which are formed with inorganic acids such as hydrochloric or phosphoric acids, or such organic acids such as acetic, oxalic, tartaric and maleic. Salts formed with the free carboxyl groups may also be derived from inorganic bases such as sodium, potassium, ammonium, calcium, or ferric hydroxides, and organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine and procaine.

The compositions may be suitably formulated for intravenous intramuscular, subcutaneous, or intraperitoneal administration and conveniently comprise sterile aqueous solutions of the antibody, which are preferably isotonic with the blood of the recipient. Such formulations are typically prepared by dissolving solid active ingredient in water containing physiologically compatible substances such as sodium chloride, glycine, and the like, and having a buffered pH compatible with physiological conditions to produce an aqueous solution, and rendering said solution sterile. These may be prepared in unit or multi-dose containers, for example, sealed ampoules or vials.

The compositions may incorporate a stabilizer, such as for example polyethylene glycol, proteins, saccharides (for example trehalose), amino acids, inorganic acids and admixtures thereof. Stabilizers are used in aqueous solutions at the appropriate concentration and pH. The pH of the aqueous solution is adjusted to be within the range of 5.0-9.0, preferably within the range of 6-8. In formulating the antibody, anti-adsorption agent may be used. Other suitable excipients may typically include an antioxidant such as ascorbic acid.

The compositions may be formulated as controlled release preparations which may be achieved through the use of polymer to complex or absorb the proteins. Appropriate polymers

for controlled release formulations include for example polyester, polyamino acids, polyvinyl, pyrrolidone, ethylenevinylacetate, and methylcellulose. Another possible method for controlled release is to incorporate the antibody into particles of a polymeric material such as polyesters, polyamino acids, hydrogels, poly(lactic acid) or ethylene vinylacetate copolymers. Alternatively, instead of incorporating these agents into polymeric particles, it is possible to entrap these materials in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly(methylmethacylate) microcapsules, respectively, or in colloidal drug delivery systems, for example, liposomes, albumin microspheres, microemulsions, nanoparticles, and nanocapsules or in macroemulsions.

When oral preparations are desired, the compositions may be combined with carriers, such as lactose, sucrose, starch, talc magnesium stearate, crystalline cellulose, methyl cellulose, carboxymethyl cellulose, glycerin, sodium alginate or gum arabic.

The humanized antibody of the invention is preferably administered parenterally, generally by intravenous infusion. Administration may also be by intraperitoneal, oral, subcutaneous, or intramuscular routes. Antibodies are generally administered in the range of about 0.1 to about 20 mg/kg of patient weight, commonly about 0.5 to about 10 mg/kg, and often about 1 to about 5 mg/kg. In this regard, it is preferred to use antibodies having a circulating half-life of at least 12 hours, preferably at least 4 days, more preferably up to 21 days. Chimeric and humanized antibodies are expected to have circulatory half-lives of up to four and up to 14-21 days, respectively. In some cases it may be advantageous to administer a large loading dose followed by periodic (e.g., weekly) maintenance doses over the treatment period. Antibodies can also be delivered by slow-release delivery systems, pumps, and other known delivery systems for continuous infusion. Dosing regimens may be varied to provide the desired circulating levels of a particular antibody based on its pharmacokinetics. Thus, doses will be calculated so that the desired circulating level of therapeutic agent is maintained.

Typically, the effective dose will be determined by the condition of the subject, as well as the body weight or surface area of the subject to be treated. The size of the dose and the dosing regimen also will be determined by the existence, nature, and extent of any adverse side effects that accompany the administration of modified antibodies of the invention in a particular subject. In determining the effective amount of the therapeutic composition to be

administered, the physician needs to evaluate *inter alia* circulating plasma levels, toxicity, and progression of the disease.

The term "effective amount" with respect to the humanized antibody and the chemotherapeutic agent(s) of the invention should be understood as meaning an amount of each of these active agents required to achieve a therapeutic effect, without causing excessive or uncontrollable adverse side effects. The effective amount required to achieve the therapeutic end result may depend on a number of factors including, for example, the specific type of the tumor and the severity of the patient's condition, and whether the combination is further co-administered with radiation. The effective amount (dose) of the active agents, in the context of the present invention should be sufficient to effect a beneficial therapeutic response in the subject over time, including inhibition of tumor growth, reduction in the rate of tumor growth, prevention of tumor and metastasis growth and enhanced survival.

In various embodiments of the combination methods of the invention, the antibody and the chemotherapeutic agent may be administered according to any of a number of treatment schedules, also referred to "dosing schedules" and "administration regimens", referring to the frequency of administration and order of administration of each active agent. For example, the antibody and the chemotherapeutic agent may be administered substantially simultaneously i.e. at the same time, using for example a combined dosage form or separate dosage forms. This form of administration may also be referred to as "concomitant" administration. Concurrent administration refers to administration of the active agents within the same general time period, for example on the same day(s) but not necessarily at the same time. For example, one active agent may require administration with food, while the other requires administration in the semi-fasting state. Alternate administration includes administration of one agent during a particular time period, for example over the course of a few days or a week, followed by administration of the other agent during a subsequent identical period of time, and then repeating the pattern for one or more cycles. Sequential or successive administration includes administration of one agent during a first time period, using one or more doses, followed by administration of the other agent during a second time period using one or more doses. An overlapping schedule may also be employed, which includes administration of the active agents on different days over the treatment period, not necessarily according to a regular sequence. Variations on these general guidelines may also be employed, according to the agents used and the condition of the subject.

In some particular combinations, it may be advantageous to use a specific sequence of administration e.g. one agent prior to the other.

Having now generally described the invention, the same will be more readily understood through reference to the following examples, which are provided by way of illustration and are not intended to be limiting of the present invention.

## EXAMPLES

### EXAMPLE 1

$CD4^+$ T lymphocytes viability following incubation with a CT-011 double mutant

A CT-011 double mutant antibody comprising amino acid substitutions of Trp 107 to Ala and Phe 97 to Ala was formed (denoted w222+F97). Representative example of 48 hours activated human $CD4^+$ T lymphocytes incubated with 1ug/ml or 0.1ug/ml CT-011 or CT-011 double mutant W222+F97 or isotype control antibody for additional 72 hours. Cell viability was evaluated by Trypan Blue exclusion.

As seen in Figure 1, lymphocyte viability is higher at the cultures including the double mutant antibody. Y-axis of Figure 1 indicates concentration of viable cells.

### EXAMPLE 2

In Vivo Effect of the modified hBAT-1 in a Murine Tumor Model

To examine whether the modified antibodies of the present invention can transmit the biological effects characteristic of mBAT-1 or hBAT-1, the efficacy of the modified antibodies is studied in vivo.

C57BL mice are inoculated with B16 melanoma cells to induce lung metastases. Increasing amounts (1, 10 and 20 μg) of a modified mAb of the invention is injected on day 12 post tumor-inoculation and compared to an optimal dose of 10 μg mBAT-1 and/or hBAT-

1. Lung weight may be measured on Day 24 post tumor inoculation to indicate the establishment of a tumor. The average lung weight per treatment is indicative of the various tested mAbs.

## EXAMPLE 3

Inhibition of Human Melanoma (SK-28) in SCID Mice by the modified hBAT-1

Mouse and human BAT-1 mAb have been shown to inhibit the formation of human-tumor metastases in the presence of human peripheral blood lymphocytes (hPBL). To estimate the efficacy of modified hBAT-1 in inhibition of human cancer, the modified antibody is studied in a model combining both tumors and lymphocytes of human origin. Severe combined immune-deficient mice (SCID) is engrafted with hPBL to restore immune-competence. Mice are challenged with human melanoma cells (SK-28) and treated with increasing concentrations of the humanized antibody, administered in a single i.v. dose on day 11 post tumor inoculation.

## EXAMPLE 4

Immunotherapy of Human Colorectal Cancer Hepatic Metastases by the modified hBAT-1 mAb in Nude Mice

LIM6 and HM7 are two sub-clones of the human CRC cell line LS174T that were selected for their high mucin synthesis and metastatic potential. The tumor cells are injected into the exposed spleen of anesthetized nude mice. After 1 minute, the spleens are removed and the excisions closed. Low doses of mBAT-1, hBAT-1 and modified hBAT-1 antibodies of the invention are administered 12 days later and mice are sacrificed 35 days post tumor inoculation. The livers are weighed, the number of metastatic nodules counted, and liver tissue processed for histology and Immunohistochemistry study.

## EXAMPLE 5

Co-localization of the modified hBAT with CD4 and CD8

Mouse and human BAT-1 have been shown to bind human lymphocytes, recognizing both CD4+ and CD8+ subsets. To establish the binding specificity of the modified humanized mAbs of the invention, human Peripheral Blood Lymphocytes (PBL) are isolated from the blood of normal donors, as described hereinbelow, and analyzed for co-localization of hBAT with known lymphocyte markers.

Peripheral blood mononuclear cells (PBMC) are isolated by ficoll and incubated in tissue culture plates to remove adherent cells. Isolated are were gated on lymphocytes by size and granularity and on live cells by propidium iodine (PI) exclusion. Binding is performed at 4°C for 1 hr, and determined by flow cytometry on gated lymphocytes.

**EXAMPLE 6**

Binding of the modified hBAT-1 to B Lymphocytes

The modified humanized mAb of the invention are raised against the membranes of Daudi cells, a human B lymphoma cell-line. PBL from normal donors are isolated by ficoll, as known in the art, followed by adherence to tissue culture plates. Non-adherent cells are examined for the co-localization of the antibodies with B-cell markers including CD19 and CD20. Binding is performed at 4°C for 1 hr, and determined by flow cytometry on gated lymphocytes.

**EXAMPLE 7**

Stability of the modified hBAT-1

CT-001 mAbs comprising at least one amino acid modification in a position selected from: Thr 5, Thr 20, Cys 71, Asn 75, Ser 76, Phe 93 and Phe 97 of the light chain variable region having the amino acid sequence of SEQ ID NO: 1-4; Asp 54, Ser 55 and Trp 107 of the heavy chain variable region having the amino acid sequence of SEQ ID NO: 5-9; Asn 51,

Ser 52, Asp 63 and Ser 64 of the light chain constant region having the amino acid sequence of SEQ ID NO: 10; or Thr 3, Ser 7, Asn 42, Ser 43, Asn 86, His 87, Asp 104, Lys 105, Thr 108, Met 135, Asp 153, Pro 154, Asp 163, Gly 164, Asn 180, Ser 181, Asn 198, Gly 199, Asn 267, Gly 268, Asp 282, Ser 283, Asp 284, Ser 285, Asn 317, His 318, Lys 330 and Met 311 of the heavy chain constant region having the amino acid sequence of SEQ ID NO: 12, are formed as known in the art.

The stability of the modified mAbs is evaluated using various biochemical and biophysical techniques that assess size, aggregation state, structure, and intermolecular contacts of the antibodies as known in the art. For instance, the purity and aggregation state of the antibodies may be studied by size exclusion chromatography (SEC) and analytical ultracentrifugation (AUC).

The foregoing description of the specific embodiments will so fully reveal the general nature of the invention that others can, by applying current knowledge, readily modify and/or adapt for various applications such specific embodiments without undue experimentation and without departing from the generic concept, and, therefore, such adaptations and modifications should and are intended to be comprehended within the meaning and range of equivalents of the disclosed embodiments. It is to be understood that the phraseology or terminology employed herein is for the purpose of description and not of limitation. The means, materials, and steps for carrying out various disclosed functions may take a variety of alternative forms without departing from the invention.

## CLAIMS

1. An antibody or antigen binding fragment thereof comprising a light chain variable region selected from the group consisting of SEQ ID NO: 1, 2, 3, and 4, and a heavy chain variable region selected from the group consisting of SEQ ID NO: 5, 6, 7, 8, and 9, wherein the antibody or antigen binding fragment thereof comprises at least one amino acid substitution at a position selected from: Phe 97 and Phe 93 of the light chain variable region to Ala, Leu or Val, or a substitution of Trp 107 of the heavy chain variable region to Ala, Leu, Val or Tyr.

2. The antibody or antigen binding fragment thereof of claim 1, comprising an amino acid substitution of Phe 97 of the light chain variable region and an amino acid substitution of Trp 107 of the heavy chain variable region.

3. The antibody or antigen binding fragment thereof of claim 1, comprising an amino acid substitution of Phe 93 of the light chain variable region and an amino acid substitution of Trp 107 of the heavy chain variable region.

4. The antibody or antigen binding fragment thereof of claim 1, wherein the light chain variable region comprises the amino acid sequence selected from the group consisting of: SEQ ID NO: 14, 15, 16, 17, 18, 19, 20 and 21.

5. The antibody or antigen binding fragment thereof of claim 1, wherein the light chain variable region comprises the amino acid sequence as set forth in any one of SEQ ID NO: 14 or 15.

6. The antibody or antigen binding fragment thereof of claim 1, wherein the light chain variable region comprises the amino acid sequence as set forth in SEQ ID NO: 29.

7. The antibody or antigen binding fragment thereof of claim 1, wherein the light chain variable region comprises the amino acid sequence as set forth in SEQ ID NO: 30.

8. The antibody or antigen binding fragment thereof of claim 1, wherein the heavy chain variable region comprises the amino acid sequence selected from the group consisting of: SEQ ID NO: 24, 25, 26, 27 and 28.

9. The antibody or antigen binding fragment thereof of claim 1, wherein the heavy chain variable region comprises the amino acid sequence as set forth in SEQ ID NO: 24.

10. The antibody or antigen binding fragment thereof of claim 1, wherein the heavy chain

variable region comprises the amino acid sequence as set forth in SEQ ID NO: 31.

11. The antibody or antigen binding fragment thereof of claim 1, further comprising at least one amino acid modification at a position selected from the group consisting of: Thr 5, Thr 20, Cys 71, Asn 75 and Ser 76 of the light chain variable region and Asp 54 and Ser 55 of the heavy chain variable region.

12. The antibody or antigen binding fragment thereof of claim 1, comprising a combination of a heavy chain variable region and a light chain variable region, the combination is selected from the group consisting of: SEQ ID NO: 5/SEQ ID NO: 1, SEQ ID NO: 6/SEQ ID NO: 2, SEQ ID NO: 7/SEQ ID NO: 2, SEQ ID NO: 7/SEQ ID NO: 3, SEQ ID NO: 8/SEQ ID NO: 3, and SEQ ID NO: 7/SEQ ID NO: 1.

13. The antibody or antigen binding fragment thereof of claim 1, comprising a variable regions corresponding to SEQ ID NO: 5/SEQ ID NO: 1.

14. The antibody or antigen binding fragment thereof of claim 1 having an antitumor activity of similar, or greater than, mBAT-1.

15. The antibody or antigen binding fragment thereof of claim 1 having an antitumor activity of similar, or greater than, hBAT-1.

16. The antibody or antigen binding fragment thereof of claim 1, wherein the fragment of the humanized antibody is selected from the group consisting of: Fv, F (ab'), F (ab') 2, and a single chain antibody.

17. A polynucleotide sequences encoding the antibody or fragments thereof according to any one of claims 1 -13.

18. A vector comprising the polynucleotide sequence of claim 17.

19. A host cells containing a vector comprising the polynucleotide sequence of claim 18.

20. A pharmaceutical composition comprising as an active ingredient the antibody of any one of claims 1 - 13 or an antigen binding fragment thereof, and a pharmaceutically acceptable carrier, diluent or stabilizer.

21. The pharmaceutical composition of claim 20 for use in treating cancer.

22. A method for treating cancer or an immunodeficiency disorder in a subject in need thereof, comprising administering to the subject an effective amount of a

pharmaceutical composition according to claim 20.

23. The method of claim 22 wherein the subject has a tumor selected from a solid tumor or a non-solid tumor.

24. The method of claim 22 wherein the subject has a non-solid tumor.

25. The method of claim 24 wherein the subject has a hematologic malignancy.

26. The method of claim 23 wherein the cancer is selected from the group consisting of a colorectal carcinoma, a non-small lung cancer (NSCLC), a small cell lung cancer (SCLC), a breast carcinoma; a melanoma; an ovarian carcinoma, a cervical carcinoma, a pancreatic cancer, a head and neck carcinoma, a gastrointestinal carcinoma, an esophageal tumor, a hepatocellular carcinoma, multiple myeloma, a renal cell carcinoma, a prostate tumor, non-Hodgkin's lymphoma, Hodgkin's disease, mantle cell lymphoma, Kaposi's sarcoma, a squamous cell carcinoma, a basal cell carcinoma, acute myeloid leukemia (AML), chronic myelocytic leukemia (CML), acute lymphocytic leukemia (ALL), and chronic lymphocytic leukemia (CLL).

27. The method of claim 26 wherein the cancer is selected from the group consisting of: colorectal carcinoma, melanoma, pancreatic cancer, head and neck carcinoma, esophageal tumor, multiple myeloma, renal cell carcinoma, non-Hodgkin's lymphoma and Hodgkin's disease.

28. Use of an antibody or antigen binding fragment thereof comprising a light chain variable region selected from SEQ ID NO: 1-4, and a heavy chain variable region selected from SEQ ID NO: 5-9, wherein the antibody or antigen binding fragment thereof comprises at least one amino acid substitution at a position selected from: Phe 93 and Phe 97 of the light chain variable region to Ala, Leu or Val or Trp 107 of the heavy chain variable region to Ala, Leu, Tyr or Val, for the preparation of a medicament for treating a tumor.

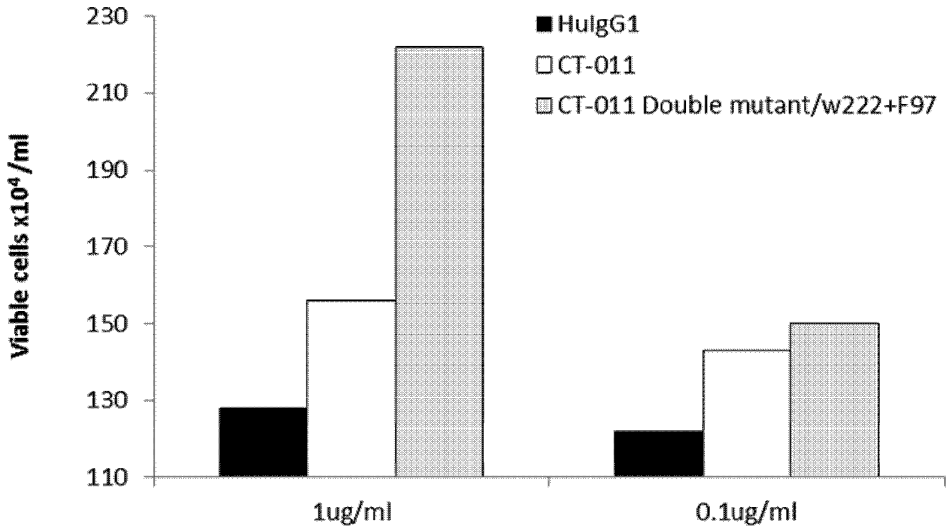

230
210
190
170
150
130
110
Viable cells x10⁴ /ml
HuIgG1
CT-011
CT-011 Double mutant/w222+F97
1ug/ml
0.1ug/ml

Figure 1

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/IL2012/050267

A. CLASSIFICATION OF SUBJECT MATTER
IPC (2012.01) C07K 16/28, A61K 39/395

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC (2012.01) C07K, A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Databases consulted: NCBI, THOMSON INNOVATION, CAPLUS, BIOSIS, EMBASE, MEDLINE, REGISTRY, USGENE, DGENE
Search terms used: Applicant name, sequence search, BAT, BAT-1, CT-011

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
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| A | Berger R, et al., Phase I safety and pharmacokinetic study of CT-011, a humanized antibody interacting with PD-1, in patients with advanced hematologic malignancies. Clin Cancer Res. 2008;14(10):3044-51<br>15 May 2008 (2008/05/15)<br>the whole document | 20-28 |
| A | US 5897862 A MOR RESEARCH APPLIC LTD [IL]<br>27 Apr 1999 (1999/04/27)<br>cited in the application<br>the whole document | 1-28 |
| A | Benson DM Jr, et al., Blood. The PD-1/PD-L1 axis modulates the natural killer cell versus multiple myeloma effect: a therapeutic target for CT-011, a novel monoclonal anti-PD-1 antibody.2010;116(13):2286-94.<br>20 Sep 2010 (2010/09/20)<br>the whole document | 20-28 |

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 17 Oct 2012 | 17 Oct 2012 |

| Name and mailing address of the ISA: | Authorized officer |
|---|---|
| Israel Patent Office<br>The Technology Park, Bldg.5, Malcha, Jerusalem, 96951, Israel<br>Facsimile No. 972-2-5651616 | RON-COHEN Yael<br>yaelrc@justice.gov.il<br>Telephone No. 972-2-5651737 |

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**INTERNATIONAL SEARCH REPORT**
Information on patent family members

International application No.
PCT/IL2012/050267

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**INTERNATIONAL SEARCH REPORT**
Information on patent family members

International application No.
PCT/IL2012/050267

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| | | WO 9520605 A1 | 03 Aug 1995 |

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(74) Agents: **DELAUNEY, Ashton J.** et al.; Bristol-Myers Squibb Company, P.O. Box 4000, Princeton, New Jersey 08543-4000 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

**Declarations under Rule 4.17**:

— *of inventorship (Rule 4.17(iv))*

*[Continued on next page]*

(54) Title: CANCER IMMUNOTHERAPY BY DISRUPTING PD-1/PD-L1 SIGNALING

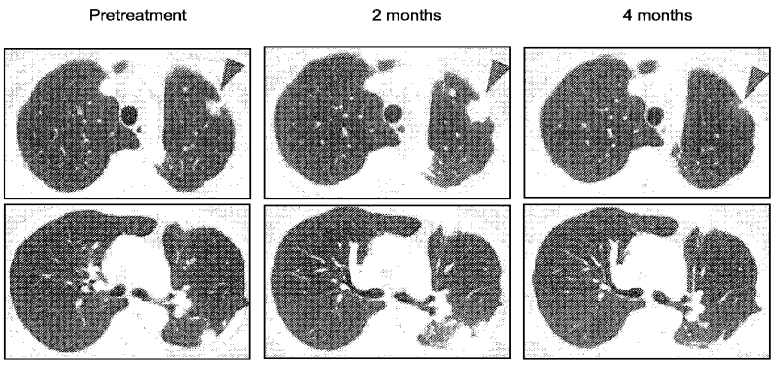



FIG. 7

(57) Abstract: The disclosure provides a method for immunotherapy of a cancer patient, comprises administering to the patient an Ab that inhibits signaling from the PD-1/PD-L1 signaling pathway, or a combination of such Ab and an anti-CTLA-4 Ab. This disclosure also provides a method for immunotherapy of a cancer patient comprising selecting a patient who is a suitable candidate for immunotherapy based on an assessment that the proportion of cells in a test tissue sample from the patient that express PD-L1 on the cell surface exceeds a predetermined threshold level, and administering an anti-PD-1 Ab to the selected subject. The disclosure additionally provides rabbit mAbs that bind specifically to a cell surface-expressed PD-L1 antigen in a FFPE tissue sample, and an automated IHC method for assessing cell surface expression in FFPE tissues using the provided anti-PD-L1 Abs.

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— *with international search report (Art. 21(3))*

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— *with sequence listing part of description (Rule 5.2(a))*

# CANCER IMMUNOTHERAPY BY DISRUPTING PD-1/PD-L1 SIGNALING

This application claims the benefit of U.S. Provisional Patent Application No. 61/647,442, filed May 15, 2012, and U.S. Provisional Patent Application No. 61/790,747, filed March 15, 2013, the contents of which are hereby incorporated herein by reference in their entirety.

Throughout this application, various other publications are referenced in parentheses by author name and date, or by Patent No. or Patent Publication No. Full citations for these publications may be found at the end of the specification immediately preceding the claims. The disclosures of these publications are hereby incorporated in their entireties by reference into this application in order to more fully describe the state of the art as known to those skilled therein as of the date of the invention described and claimed herein. However, the citation of a reference herein should not be construed as an acknowledgement that such reference is prior art to the present invention.

## FIELD OF THE INVENTION

This invention relates to methods for immunotherapy of a cancer patient comprising administering to the patient antibodies that disrupt the PD-1/PD-L1 signaling pathway. A biomarker may be used as part of this treatment for identifying suitable patients for immunotherapy and for predicting the efficacy of anti-PD-1 treatment.

## BACKGROUND OF THE INVENTION

Human cancers harbor numerous genetic and epigenetic alterations, generating neoantigens potentially recognizable by the immune system (Sjoblom *et al.*, 2006). The adaptive immune system, comprised of T and B lymphocytes, has powerful anti-cancer potential, with a broad capacity and exquisite specificity to respond to diverse tumor antigens. Further, the immune system demonstrates considerable plasticity and a memory component. The successful harnessing of all these attributes of the adaptive immune system would make immunotherapy unique among all cancer treatment modalities. However, although an endogenous immune response to cancer is observed in preclinical models and patients, this response is ineffective, and established cancers are viewed as "self" and tolerated by the immune system. Contributing to this state of tolerance, tumors may exploit several distinct mechanisms to actively subvert anti-tumor immunity. These mechanisms include dysfunctional T-cell signaling (Mizoguchi *et al.*, 1992), suppressive regulatory cells (Facciabene *et al.*, 2012), and the co-opting of endogenous "immune

checkpoints," which serve to down-modulate the intensity of adaptive immune responses and protect normal tissues from collateral damage, by tumors to evade immune destruction (Topalian *et al.*, 2011; Mellman *et al.*, 2011).

Until recently, cancer immunotherapy had focused substantial effort on approaches that enhance anti-tumor immune responses by adoptive-transfer of activated effector cells, immunization against relevant antigens, or providing non-specific immune-stimulatory agents such as cytokines. In the past decade, however, intensive efforts to develop specific immune checkpoint pathway inhibitors have begun to provide new immunotherapeutic approaches for treating cancer, including the development of an antibody (Ab), ipilimumab (YERVOY®), that binds to and inhibits Cytotoxic T-Lymphocyte Antigen-4 (CTLA-4) for the treatment of patients with advanced melanoma (Hodi *et al.*, 2010) and, as described herein, the development of Abs that block the inhibitory PD-1 pathway.

Programmed Death-1 (PD-1) is a key immune checkpoint receptor expressed by activated T and B cells and mediates immunosuppression. PD-1 is a member of the CD28 family of receptors, which includes CD28, CTLA-4, ICOS, PD-1, and BTLA. Two cell surface glycoprotein ligands for PD-1 have been identified, Programmed Death Ligand-1 (PD-L1) and Programmed Death Ligand-2 (PD-L2), that are expressed on antigen-presenting cells as well as many human cancers and have been shown to downregulate T cell activation and cytokine secretion upon binding to PD-1 (Freeman *et al.*, 2000; Latchman *et al.*, 2001). Unlike CTLA-4, PD-1 primarily functions in peripheral tissues where activated T-cells may encounter the immunosuppressive PD-L1 (B7-H1) and PD-L2 (B7-DC) ligands expressed by tumor and/or stromal cells (Flies *et al.*, 2011; Topalian *et al.*, 2012a). Inhibition of the PD-1/PD-L1 interaction mediates potent antitumor activity in preclinical models (U.S. Patent Nos. 8,008,449 and 7,943,743), and the use of Ab inhibitors of the PD-1/PD-L1 interaction for treating cancer has entered clinical trials (Brahmer *et al.*, 2010; Topalian *et al.*, 2012b; Brahmer *et al.*, 2012; Flies *et al.*, 2011; Pardoll, 2012; Hamid and Carvajal, 2013).

The promise of the emerging field of personalized medicine is that advances in pharmacogenomics will increasing be used to tailor therapeutics to defined sub-populations, and ultimately, individual patients in order to enhance efficacy and minimize adverse effects. Recent successes include, for example, the development of imatinib mesylate (GLEEVEC®), a protein tyrosine kinase inhibitor that inhibits the bcr-abl

tyrosine kinase, to treat Philadelphia chromosome-positive chronic myelogenous leukemia (CML); crizotinib (XALKORI®) to treat the 5% of patients with late-stage non-small cell lung cancers who express a mutant anaplastic lymphoma kinase (ALK) gene; and vemurafenib (ZELBORAF®), an inhibitor of mutated B-RAF protein (V600E-BRAF) which is expressed in around half of melanoma tumors. However, unlike the clinical development of small molecule agents that target discrete activating mutations found in select cancer populations, a particular challenge in cancer immunotherapy has been the identification of mechanism-based predictive biomarkers to enable patient selection and guide on-treatment management. Advances in validating PD-L1 expression as a biomarker for screening patients for anti-PD-1 immunotherapy are described herein.

## SUMMARY OF THE INVENTION

The present disclosure provides a method for immunotherapy of a subject afflicted with cancer, which method comprises administering to the subject a composition comprising a therapeutically effective amount of an agent that disrupts, reduces or suppresses signaling from an inhibitory immunoregulator. In preferred embodiments, the agent is an Ab. In other preferred embodiments, the inhibitory immunoregulator is a component of the PD-1/PD-L1 signaling pathway. In further preferred embodiments, the Ab disrupts the interaction between PD-1 and PD-L1. In certain embodiments, the Ab is an anti-PD-1 Ab of the invention or an anti-PD-L1 Ab of the invention. In preferred embodiments, the anti-PD-1 Ab of the invention is nivolumab (BMS-936558) and the anti-PD-L1 Ab of the invention is BMS-936559. In certain embodiments, the subject has been pre-treated for the cancer. In other embodiments, the cancer is an advanced, metastatic and/or refractory cancer. In preferred embodiments, the administration of the Ab or antigen-binding portion to the subject thereof induces a durable clinical response in the subject.

This disclosure also provides a method for immunotherapy of a subject afflicted with cancer, which method comprises: (a) selecting a subject that is a suitable candidate for immunotherapy, the selecting comprising (i) optionally providing a test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells, (ii) assessing the proportion of cells in the test tissue sample that express PD-L1 on the cell surface, and (iii) selecting the subject as a suitable candidate based on an assessment that the proportion of cells in the test tissue sample that express PD-L1 on the cell surface exceeds a predetermined threshold level;

and (b) administering a composition comprising a therapeutically effective amount of an agent that disrupts signaling from the PD-1/PD-L1 pathway, for example, an anti-PD-1 or an anti-PD-L1 Ab, to the selected subject.

The disclosure further provides a method for treatment of a subject afflicted with cancer, which method comprises: (a) selecting a subject that is not suitable for immunotherapy with an agent that disrupts signaling from the PD-1/PD-L1 pathway, for example, an anti-PD-1 or an anti-PD-L1 Ab, the selecting comprising (i) optionally providing a test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells; (ii) assessing the proportion of cells in the test tissue sample that express PD-L1 on the cell surface; and (iii) selecting the subject as not suitable for said immunotherapy based on an assessment that the proportion of cells in the test tissue sample that express PD-L1 on the cell surface is less than a predetermined threshold level; and (b) administering a standard-of-care therapeutic other than an agent that disrupts signaling from the PD-1/PD-L1 pathway to the selected subject.

In addition, the disclosure provides a method for selecting a cancer patient for immunotherapy with an agent that disrupts signaling from the PD-1/PD-L1 pathway, for example, an anti-PD-1 or an anti-PD-L1 Ab, which method comprises: (a) optionally providing a test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells; (b) assaying the test tissue sample to determine the proportion of cells therein that express PD-L1 on the cell surface; (c) comparing the proportion of cells that express PD-L1 on the cell surface with a predetermined threshold proportion; and (d) selecting the patient for immunotherapy based on an assessment that PD-L1 is expressed in cells of the test tissue sample.

This disclosure further provides a method for predicting the therapeutic effectiveness of an agent that disrupts signaling from the PD-1/PD-L1 pathway, for example, an anti-PD-1 or an anti-PD-L1 Ab, for treating a cancer patient, which method comprises: (a) optionally providing a test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells; (b) assaying the test tissue sample to determine the proportion of cells therein that express PD-L1 on the cell surface; (c) comparing the proportion of cells that express PD-L1 on the cell surface with a predetermined threshold value; and (d)

predicting the therapeutic effectiveness of the agent, wherein if the proportion of cells that express PD-L1 on the cell surface exceeds the threshold proportion the agent is predicted to be effective in treating the patient, and wherein if the proportion of cells that express PD-L1 on the cell surface is below the threshold proportion the agent is predicted to not be effective in treating the patient.

The present disclosure also provides a method for determining an immunotherapeutic regimen comprising an agent that disrupts signaling from the PD-1/PD-L1 pathway, for example, an anti-PD-1 or an anti-PD-L1 Ab, for treating a cancer patient, which method comprises: (a) optionally providing a test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells; (b) assaying the test tissue sample to determine the proportion of cells therein that express PD-L1 on the cell surface; (c) comparing the proportion of cells that express PD-L1 on the cell surface with a predetermined threshold proportion; and (d) determining an immunotherapeutic regimen comprising the agent based on the determination that the proportion of cells that express PD-L1 on the cell surface exceeds the predetermined threshold proportion.

In certain embodiments of the methods described herein, the test tissue sample is a formalin-fixed and paraffin-embedded (FFPE) sample. In certain other embodiments, assessing the proportion of cells in the test tissue sample that express PD-L1 on the cell surface is achieved by immunohistochemical (IHC) staining of the FFPE sample. In preferred embodiments, the mAb 28-8 or 5H1 is used in an automated IHC assay to bind to PD-L1 on the surface of cells in the test tissue sample. In preferred embodiments of any of the methods disclosed herein, the cancer is melanoma (MEL), renal cell carcinoma (RCC), squamous non-small cell lung cancer (NSCLC), non-squamous NSCLC, colorectal cancer (CRC), castration-resistant prostate cancer (CRPC), hepatocellular carcinoma (HCC), squamous cell carcinoma of the head and neck, carcinomas of the esophagus, ovary, gastrointestinal tract and breast, or a hematologic malignancy such as multiple myeloma, B-cell lymphoma, T-cell lymphoma, Hodgkin's lymphoma/primary mediastinal B-cell lymphoma, and chronic myelogenous leukemia.

This invention additionally provides a mAb or antigen-binding portion thereof that binds specifically to a cell surface-expressed human PD-L1 antigen in a FFPE tissue sample. In preferred embodiments, the mAb or antigen-binding portion thereof does not bind to a cytoplasmic PD-L1 antigen in the FFPE tissue sample. In other preferred

embodiments, the monoclonal Ab (mAb) is the rabbit mAb designated 28-8, 28-1, 28-12, 29-8 or 20-12, or the mouse mAb designated 5H1.

Other features and advantages of the instant invention will be apparent from the following detailed description and examples which should not be construed as limiting. The contents of all cited references, including scientific articles, newspaper reports, GenBank entries, patents and patent applications cited throughout this application are expressly incorporated herein by reference.

## BRIEF DESCRIPTION OF THE FIGURES

Figures 1A-1C. Cross-competition between 5C4 and other HuMab anti-PD-1 mAbs for binding to human PD-1 (hPD-1) expressed on CHO cells. A, the 5C4 Fab fragment substantially blocked the binding of mAbs 5C4 itself, as well as the binding of 2D3 and 7D3; B, the 5C4 Fab fragment substantially blocked the binding of mAb 4H1; C, the 5C4 mAb substantially blocked the binding of mAb 17D8.

Figures 2A-2F. Cross-competition of FITC-conjugated human anti-hPD-L1 mAbs for binding to human PDF-L1 (hPD-L1) expressed on CHO cells. A, Binding of labeled 10H10 was partially blocked by 10A5, 11E6 and 13G4 and was significantly blocked by itself; B, Binding of labeled 3G10 was significantly blocked by each of the tested anti-PD-L1 Abs except 10H10; C, Binding of labeled 10A5 was significantly blocked by each of the tested anti-PD-L1 Abs except 10H10; D, Binding of labeled 11E6 was significantly blocked by each of the tested anti-PD-L1 Abs except 10H10; and E, Binding of labeled 12A4 was significantly blocked by each of the tested anti-PD-L1 Abs except 10H10; and F, Binding of labeled 13G4 was significantly blocked by each of the tested anti-PD-L1 Abs except 10H10.

Figure 3. Cross-competitive inhibition of binding of biotinylated mAb 12A4 to ES-2 cells by human anti-hPD-L1 mAbs. Fluorescence of bound biotin-12A4 is plotted against the concentration of unlabeled hPD-L1 HuMabs.

Figure 4. Spider plot showing activity of anti-PD-1 mAb in patients with treatment-refractory melanoma (MEL). A representative plot of changes in tumor burden over time demonstrates the time course of change in the sum of the longest diameters of target lesions, compared with baseline, in 27 MEL patients treated with 5C4 at a dose of 1.0 mg/kg. In the majority of patients who achieved an objective response (OR), responses were durable and evident by the end of cycle 3 (6 months) of treatment (vertical dashed line). Tumor regressions followed conventional as well as "immune-

related" patterns of response, such as prolonged reduction in tumor burden in the presence of new lesions.

Figure 5. Activity of anti-PD-1 mAb in patient with metastatic RCC. Partial regression of metastatic RCC in a 57-year-old patient treated with 5C4 at 1 mg/kg is illustrated. This patient had previously undergone radical surgery and had developed progressive disease after receiving sunitinib, temsirolimus, sorafenib, and pazopanib. Arrows show regression of recurrent tumor in the operative field.

Figures 6. Activity of anti-PD-1 mAb in patient with metastatic MEL. A complete response of metastatic MEL is illustrated in a 62-year-old patient treated with 5C4 at 3 mg/kg, associated with vitiligo. (i) Pretreatment CT scan, inguinal lymph node metastasis (arrow); (ii) after 13 months of treatment. Numerous metastases in the subcutaneous tissue and retroperitoneum also regressed completely (not shown). Vitiligo developed after 6 months of treatment; photos taken at 9 months under visible light (iii) and ultraviolet light (iv). Skin biopsies with immunohistochemistry for microphthalmia-associated transcription factor (MITF) show melanocytes (arrows) at the epidermal-dermal junction in normal skin (v), and scarce (vi) or absent (vii) melanocytes in skin partially or fully affected by vitiligo.

Figure 7. Activity of anti-PD-1 mAb in patient with metastatic NSCLC. A partial response is illustrated in a patient with metastatic NSCLC (nonsquamous histology) treated with 5C4 at 10 mg/kg. Arrows show initial progression in pulmonary lesions followed by regression ("immune-related" pattern of response).

Figures 8A and 8B. Correlation between tumor PD-L1 expression and anti-PD-1 clinical response. Pretreatment tumor cell surface expression of PD-L1, as determined by IHC on formalin-fixed paraffin-embedded specimens, correlates with OR to PD-1 blockade. Forty-two subjects with advanced cancers including melanoma, non-small cell lung cancer, colorectal cancer, renal cell cancer, and castration-resistant prostate cancer (n=18, 10, 7, 5, and 2, respectively) were studied. A, There was a significant correlation of tumor cell surface PD-L1 expression with objective clinical response. No patients with PD-L1 negative tumors experienced an OR. B, Examples of IHC analysis with the anti-PD-L1 mAb 5H1 are shown in a melanoma lymph node metastasis (top), a renal cell cancer nephrectomy specimen (middle), and a lung adenocarcinoma brain metastasis (bottom). All 400X original magnification. Arrows indicate one of many tumor cells in each specimen with surface membrane staining for PD-L1. Asterisk indicates a normal

glomerulus in the nephrectomy specimen, which is negative for PD-L1 staining.

Figure 9. Graphical comparison of the binding of mAbs 28-8 and 5H1 to PD-L1 antigen in tumor tissues by histoscore analysis. The rabbit mAb 28-8 showed higher histoscores in 7 out of 10 samples tested.

Figures 10A-10D. Spider plot showing activity of anti-PD-L1 mAb in patients with treatment-refractory MEL and NSCLC. Representative plots demonstrate the time course of target lesion tumor burden over time in patients with MEL treated with BMS-936559 at doses of 1 (A), 3 (B), 10 mg/kg (C) and in patients with NSCLC treated at 10 mg/kg (D). In the majority of patients who achieved ORs, responses were durable and were evident by the end of cycle 2 (3 months) of treatment, irrespective of dose or tumor type. Tumor regressions followed conventional as well as "immune-related" patterns of response.

Figure 11. Complete response in a patient with melanoma treated with BMS-936559 at 3 mg/kg. Circles indicate an initial increase in the size of pulmonary nodules at 6 weeks and 3 months followed by complete regression at 10 months ("immune-related" pattern of response).

Figure 12. Complete response in a patient with melanoma treated with BMS-936559 at 1 mg/kg. This patient developed an isolated brain metastasis 3 months after initiation of treatment that was successfully treated with stereotactic radiosurgery. A partial response in abdominal disease (circled) was noted at 8 months, with no evidence of disease at 15 months.

Figure 13. Partial response in a patient with NSCLC (non-squamous histology) treated with BMS-936559 at 10 mg/kg. Note the response in disease in right lung pleura and liver.

Figures 14A and 14B. Clinical activity of MEL patients who received a concurrent regimen of nivolumab and ipilimumab. A, Representative spider plots show changes from baseline in the tumor burden, measured as the sum of products of perpendicular diameters of all target lesions, in patients who received the concurrent regimen of 1 mg/kg nivolumab + 3 mg/kg ipilimumab, the MTD. Triangles indicate the first occurrence of a new lesion. B, A representative waterfall plot shows maximum percentage response in baseline target lesions in patients who received the concurrent regimen.

Figure 15. Tumor regressions of a 52-year old MEL patient who received a

concurrent regimen of 1 mg/kg nivolumab + 3 mg/kg ipilimumab. This patient presented with extensive neck, mediastinal, axillary, abdominal and pelvic lymphadenopathy, bilateral pulmonary nodules, small bowel metastasis, peritoneal implants and diffuse subcutaneous nodules. Baseline lactate dehydrogenase (LDH) was 2.25 x upper limit of normal, hemoglobin was 9.7 g/dL, and symptoms included nausea and vomiting. Within 4 weeks of treatment the LDH normalized, symptoms improved (appetite increased, nausea decreased), and cutaneous lesions were regressing. At week-12 scans, there was marked reduction in all areas of disease. Arrows denote location of metastatic disease.

Figures 16A-16C. Clinical activity of MEL patients who received various concurrent regimens of nivolumab and ipilimumab. Representative spider plots show changes from baseline in the tumor burden, measured as the sum of products of perpendicular diameters of all target lesions, in patients who received a concurrent regimen of 0.3 mg/kg nivolumab + 3 mg/kg ipilimumab (A), 3 mg/kg nivolumab + 1 mg/kg ipilimumab (B), or 3 mg/kg nivolumab + 3 mg/kg ipilimumab (C). Triangles indicate the first occurrence of a new lesion.

Figure 17. Tumor regressions of a 61-year old MEL patient who received a concurrent regimen of 0.3 mg/kg nivolumab + 3 mg/kg ipilimumab. The patient presented with stage IV (M1c) MEL of an unknown primary site, metastatic to the stomach and the mesentery. Lactate dehydrogenase was 225 and hemoglobin was 9.6 g/dL after a recent transfusion. Twelve weeks after the initiation of study treatment, a CT scan showed an 86% reduction in the bulky disease burden.

Figures 18A-18C. Clinical activity of MEL patients who received a sequenced regimen of nivolumab and ipilimumab. Representative spider plots show changes from baseline in the tumor burden, measured as the sum of products of perpendicular diameters of all target lesions, in patients who received a sequenced regimen of 1 mg/kg nivolumab (A) or 3 mg/kg nivolumab (B) after prior ipilimumab therapy. Triangles indicate the first occurrence of a new lesion. C, A representative waterfall plot shows maximum percentage response in baseline target lesions in patients who received a sequenced regimen; "*" denotes patients who had radiographic progression with prior ipilimumab treatment.

## DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to methods for immunotherapy of a subject afflicted with diseases such as cancer or an infectious disease, which methods comprise

administering to the subject a composition comprising a therapeutically effective amount of a compound or agent that potentiates an endogenous immune response, either stimulating the activation of the endogenous response or inhibiting the suppression of the endogenous response. More specifically, this disclosure provides methods for potentiating an endogenous immune response in a subject afflicted with cancer so as to thereby treat the patient, which method comprises administering to the subject a therapeutically effective amount of an agent, such as an Ab or an antigen-binding portion thereof, that disrupts or inhibits signaling from an inhibitory immunoregulator. In certain embodiments, the inhibitory immunoregulator is a component of the PD-1/PD-L1 signaling pathway. Accordingly, certain embodiments of the invention provide methods for immunotherapy of a subject afflicted with cancer, which methods comprise administering to the subject a therapeutically effective amount of an Ab or an antigen-binding portion thereof that disrupts the interaction between the PD-1 receptor and its ligand, PD-L1. In certain preferred embodiments, the Ab or antigen-binding portion thereof binds specifically to PD-1. In other preferred embodiments, the Ab or antigen-binding portion thereof binds specifically to PD-L1. Certain embodiments comprise the use of an anti-PD-1 Ab is in combination with another anti-cancer agent, preferably an anti-CTLA-4 Ab, to treat cancer. In certain other embodiments, the subject is selected as suitable for immunotherapy in a method comprising measuring the surface expression of PD-L1 in a test tissue sample obtained from a patient with cancer of the tissue, for example, determining the proportion of cells in the test tissue sample that express PD-L1 on the cell surface, and selecting the patient for immunotherapy based on an assessment that PD-L1 is expressed on the surface of cells in the test tissue sample.

*Terms*

In order that the present disclosure may be more readily understood, certain terms are first defined. As used in this application, except as otherwise expressly provided herein, each of the following terms shall have the meaning set forth below. Additional definitions are set forth throughout the application.

"Administering" refers to the physical introduction of a composition comprising a therapeutic agent to a subject, using any of the various methods and delivery systems known to those skilled in the art. Preferred routes of administration for Abs of the invention include intravenous, intramuscular, subcutaneous, intraperitoneal, spinal or other parenteral routes of administration, for example by injection or infusion. The phrase

"parenteral administration" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intralymphatic, intralesional, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion, as well as *in vivo* electroporation. Alternatively, an Ab of the invention can be administered via a non-parenteral route, such as a topical, epidermal or mucosal route of administration, for example, intranasally, orally, vaginally, rectally, sublingually or topically. Administering can also be performed, for example, once, a plurality of times, and/or over one or more extended periods.

An "adverse event" (AE) as used herein is any unfavorable and generally unintended or undesirable sign (including an abnormal laboratory finding), symptom, or disease associated with the use of a medical treatment. For example, an adverse event may be associated with activation of the immune system or expansion of immune system cells (*e.g.*, T cells) in response to a treatment. A medical treatment may have one or more associated AEs and each AE may have the same or different level of severity. Reference to methods capable of "altering adverse events" means a treatment regime that decreases the incidence and/or severity of one or more AEs associated with the use of a different treatment regime.

An "antibody" (Ab) shall include, without limitation, a glycoprotein immunoglobulin which binds specifically to an antigen and comprises at least two heavy (H) chains and two light (L) chains interconnected by disulfide bonds, or an antigen-binding portion thereof. Each H chain comprises a heavy chain variable region (abbreviated herein as $V_H$) and a heavy chain constant region. The heavy chain constant region comprises three constant domains, $C_{H1}$, $C_{H2}$ and $C_{H3}$. Each light chain comprises a light chain variable region (abbreviated herein as $V_L$) and a light chain constant region. The light chain constant region is comprises one constant domain, $C_L$. The $V_H$ and $V_L$ regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FR). Each $V_H$ and $V_L$ comprises three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The constant regions of

the Abs may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (*e.g.*, effector cells) and the first component (C1q) of the classical complement system.

Antibodies typically bind specifically to their cognate antigen with high affinity, reflected by a dissociation constant ($K_D$) of $10^{-5}$ to $10^{-11}$ $M^{-1}$ or less. Any $K_D$ greater than about $10^{-4}$ $M^{-1}$ is generally considered to indicate nonspecific binding. As used herein, an Ab that "binds specifically" to an antigen refers to an Ab that binds to the antigen and substantially identical antigens with high affinity, which means having a $K_D$ of $10^{-7}$ M or less, preferably $10^{-8}$ M or less, even more preferably 5 x $10^{-9}$ M or less, and most preferably between $10^{-8}$ M and $10^{-10}$ M or less, but does not bind with high affinity to unrelated antigens. An antigen is "substantially identical" to a given antigen if it exhibits a high degree of sequence identity to the given antigen, for example, if it exhibits at least 80%, at least 90%, preferably at least 95%, more preferably at least 97%, or even more preferably at least 99% sequence identity to the sequence of the given antigen. By way of example, an Ab that binds specifically to human PD-1 may also have cross-reactivity with PD-1 antigens from certain primate species but may not cross-react with PD-1 antigens from certain rodent species or with an antigen other than PD-1, *e.g.*, a human PD-L1 antigen.

An immunoglobulin may derive from any of the commonly known isotypes, including but not limited to IgA, secretory IgA, IgG and IgM. IgG subclasses are also well known to those in the art and include but are not limited to human IgG1, IgG2, IgG3 and IgG4. "Isotype" refers to the Ab class or subclass (*e.g.*, IgM or IgG1) that is encoded by the heavy chain constant region genes. The term "antibody" includes, by way of example, both naturally occurring and non-naturally occurring Abs; monoclonal and polyclonal Abs; chimeric and humanized Abs; human or nonhuman Abs; wholly synthetic Abs; and single chain Abs. A nonhuman Ab may be humanized by recombinant methods to reduce its immunogenicity in man. Where not expressly stated, and unless the context indicates otherwise, the term "antibody" also includes an antigen-binding fragment or an antigen-binding portion of any of the aforementioned immunoglobulins, and includes a monovalent and a divalent fragment or portion, and a single chain Ab.

An "isolated antibody" refers to an Ab that is substantially free of other Abs having different antigenic specificities (*e.g.*, an isolated Ab that binds specifically to PD-1 is substantially free of Abs that bind specifically to antigens other than PD-1). An isolated

Ab that binds specifically to PD-1 may, however, have cross-reactivity to other antigens, such as PD-1 molecules from different species. Moreover, an isolated Ab may be substantially free of other cellular material and/or chemicals. By comparison, an "isolated" nucleic acid refers to a nucleic acid composition of matter that is markedly different, *i.e.*, has a distinctive chemical identity, nature and utility, from nucleic acids as they exist in nature. For example, an isolated DNA, unlike native DNA, is a free-standing portion of a native DNA and not an integral part of a larger structural complex, the chromosome, found in nature. Further, an isolated DNA, unlike native genomic DNA, can typically be used in applications or methods for which native genomic DNA is unsuited, *e.*g., as a PCR primer or a hybridization probe for, among other things, measuring gene expression and detecting biomarker genes or mutations for diagnosing disease or assessing the efficacy of a therapeutic. An isolated nucleic acid may be purified so as to be substantially free of other cellular components or other contaminants, *e.g.*, other cellular nucleic acids or proteins, using standard techniques well known in the art. Examples of isolated nucleic acids include fragments of genomic DNA, PCR-amplified DNA, cDNA and RNA.

The term "monoclonal antibody" ("mAb") refers to a preparation of Ab molecules of single molecular composition, *i.e.*, Ab molecules whose primary sequences are essentially identical, and which exhibits a single binding specificity and affinity for a particular epitope. A mAb is an example of an isolated Ab. MAbs may be produced by hybridoma, recombinant, transgenic or other techniques known to those skilled in the art.

A "human" antibody (HuMAb) refers to an Ab having variable regions in which both the framework and CDR regions are derived from human germline immunoglobulin sequences. Furthermore, if the Ab contains a constant region, the constant region also is derived from human germline immunoglobulin sequences. The human Abs of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (*e.g.*, mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*). However, the term "human antibody," as used herein, is not intended to include Abs in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences. The terms "human" Abs and "fully human" Abs and are used synonymously.

A "humanized" antibody refers to an Ab in which some, most or all of the amino

acids outside the CDR domains of a non-human Ab are replaced with corresponding amino acids derived from human immunoglobulins. In one embodiment of a humanized form of an Ab, some, most or all of the amino acids outside the CDR domains have been replaced with amino acids from human immunoglobulins, whereas some, most or all amino acids within one or more CDR regions are unchanged. Small additions, deletions, insertions, substitutions or modifications of amino acids are permissible as long as they do not abrogate the ability of the Ab to bind to a particular antigen. A "humanized" Ab retains an antigenic specificity similar to that of the original Ab.

A "chimeric antibody" refers to an Ab in which the variable regions are derived from one species and the constant regions are derived from another species, such as an Ab in which the variable regions are derived from a mouse Ab and the constant regions are derived from a human Ab.

An "antigen-binding portion" of an Ab (also called an "antigen-binding fragment") refers to one or more fragments of an Ab that retain the ability to bind specifically to the antigen bound by the whole Ab.

A "cancer" refers a broad group of various diseases characterized by the uncontrolled growth of abnormal cells in the body. Unregulated cell division and growth divide and grow results in the formation of malignant tumors that invade neighboring tissues and may also metastasize to distant parts of the body through the lymphatic system or bloodstream.

An "immune response" refers to the action of a cell of the immune system (for example, T lymphocytes, B lymphocytes, natural killer (NK) cells, macrophages, eosinophils, mast cells, dendritic cells and neutrophils) and soluble macromolecules produced by any of these cells or the liver (including Abs, cytokines, and complement) that results in selective targeting, binding to, damage to, destruction of, and/or elimination from a vertebrate's body of invading pathogens, cells or tissues infected with pathogens, cancerous or other abnormal cells, or, in cases of autoimmunity or pathological inflammation, normal human cells or tissues.

An "immunoregulator" refers to a substance, an agent, a signaling pathway or a component thereof that regulates an immune response. "Regulating," "modifying" or "modulating" an immune response refers to any alteration in a cell of the immune system or in the activity of such cell. Such regulation includes stimulation or suppression of the immune system which may be manifested by an increase or decrease in the number of

various cell types, an increase or decrease in the activity of these cells, or any other changes which can occur within the immune system. Both inhibitory and stimulatory immunoregulators have been identified, some of which may have enhanced function in the cancer microenvironment.

The term "immunotherapy" refers to the treatment of a subject afflicted with, or at risk of contracting or suffering a recurrence of, a disease by a method comprising inducing, enhancing, suppressing or otherwise modifying an immune response. "Treatment" or "therapy" of a subject refers to any type of intervention or process performed on, or the administration of an active agent to, the subject with the objective of reversing, alleviating, ameliorating, inhibiting, slowing down or preventing the onset, progression, development, severity or recurrence of a symptom, complication, condition or biochemical indicia associated with a disease.

"Potentiating an endogenous immune response" means increasing the effectiveness or potency of an existing immune response in a subject. This increase in effectiveness and potency may be achieved, for example, by overcoming mechanisms that suppress the endogenous host immune response or by stimulating mechanisms that enhance the endogenous host immune response.

A "predetermined threshold value," relating to cell surface PD-L1 expression, refers to the proportion of cells in a test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells above which the sample is scored as being positive for cell surface PD-L1 expression. For cell surface expression assayed by IHC with the mAb 28-8, the predetermined threshold value for cells expressing PD-L1 on the cell surface ranges from at least about 0.01% to at least about 20% of the total number of cells. In preferred embodiments, the predetermined threshold value for cells expressing PD-L1 on the cell surface ranges from at least about 0.1% to at least about 10% of the total number of cells. More preferably, the predetermined threshold value is at least about 5%. Even more preferably, the predetermined threshold value is at least about 1%, or in the range of 1-5%.

The "Programmed Death-1 (PD-1)" receptor refers to an immunoinhibitory receptor belonging to the CD28 family. PD-1 is expressed predominantly on previously activated T cells *in vivo*, and binds to two ligands, PD-L1 and PD-L2. The term "PD-1" as used herein includes human PD-1 (hPD-1), variants, isoforms, and species homologs of hPD-1, and analogs having at least one common epitope with hPD-1. The complete

hPD-1 sequence can be found under GenBank Accession No. U64863.

"Programmed Death Ligand-1 (PD-L1)" is one of two cell surface glycoprotein ligands for PD-1 (the other being PD-L2) that downregulate T cell activation and cytokine secretion upon binding to PD-1. The term "PD-L1" as used herein includes human PD-L1 (hPD-L1), variants, isoforms, and species homologs of hPD-L1, and analogs having at least one common epitope with hPD-L1. The complete hPD-L1 sequence can be found under GenBank Accession No. Q9NZQ7.

A "signal transduction pathway" or "signaling pathway" refers to the biochemical relationship between a variety of signal transduction molecules that play a role in the transmission of a signal from one portion of a cell to another portion of the cell. A "cell surface receptor" includes, for example, molecules and complexes of molecules that are located on the surface of a cell and are capable of receiving a signal and transmitting such a signal across the plasma membrane of a cell. An example of a cell surface receptor of the present invention is the PD-1 receptor, which is located on the surface of activated T cells, activated B cells and myeloid cells, and transmits a signal that results in a decrease in tumor-infiltrating lymphocytes and a decrease in T cell proliferation. An "inhibitor" of signaling refers to a compound or agent that antagonizes or reduces the initiation, reception or transmission of a signal, be that signal stimulatory or inhibitory, by any component of a signaling pathway such as a receptor or its ligand.

A "subject" includes any human or nonhuman animal. The term "nonhuman animal" includes, but is not limited to, vertebrates such as nonhuman primates, sheep, dogs, cats, rabbits and ferrets, rodents such as mice, rats and guinea pigs, avian species such as chickens, amphibians, and reptiles. In preferred embodiments, the subject is a mammal such as a nonhuman primate, sheep, dog, cat, rabbit, ferret or rodent. In more preferred embodiments, the subject is a human. The terms, "subject," "patient" and "individual" are used interchangeably herein.

A "therapeutically effective amount" or "therapeutically effective dosage" of a drug or therapeutic agent, such as an Ab of the invention, is any amount of the drug that, when used alone or in combination with another therapeutic agent, protects a subject against the onset of a disease or promotes disease regression evidenced by a decrease in severity of disease symptoms, an increase in frequency and duration of disease symptom-free periods, or a prevention of impairment or disability due to the disease affliction. The ability of a therapeutic agent to promote disease regression can be

evaluated using a variety of methods known to the skilled practitioner, such as in human subjects during clinical trials, in animal model systems predictive of efficacy in humans, or by assaying the activity of the agent in *in vitro* assays.

By way of example, an anti-cancer agent promotes cancer regression in a subject. In preferred embodiments, a therapeutically effective amount of the drug promotes cancer regression to the point of eliminating the cancer. "Promoting cancer regression" means that administering an effective amount of the drug, alone or in combination with an anti-neoplastic agent, results in a reduction in tumor growth or size, necrosis of the tumor, a decrease in severity of at least one disease symptom, an increase in frequency and duration of disease symptom-free periods, or a prevention of impairment or disability due to the disease affliction. In addition, the terms "effective" and "effectiveness" with regard to a treatment includes both pharmacological effectiveness and physiological safety. Pharmacological effectiveness refers to the ability of the drug to promote cancer regression in the patient. Physiological safety refers to the level of toxicity, or other adverse physiological effects at the cellular, organ and/or organism level (adverse effects) resulting from administration of the drug.

By way of example for the treatment of tumors, a therapeutically effective amount of the drug preferably inhibits cell growth or tumor growth by at least about 20%, more preferably by at least about 40%, even more preferably by at least about 60%, and still more preferably by at least about 80% relative to untreated subjects. In other preferred embodiments of the invention, tumor regression may be observed and continue for a period of at least about 20 days, more preferably at least about 40 days, or even more preferably at least about 60 days. Notwithstanding these ultimate measurements of therapeutic effectiveness, evaluation of immunotherapeutic drugs must also make allowance for "immune-related" response patterns.

An "immune-related" response pattern refers to a clinical response pattern often observed in cancer patients treated with immunotherapeutic agents that produce antitumor effects by inducing cancer-specific immune responses or by modifying native immune processes. This response pattern is characterized by a beneficial therapeutic effect that follows an initial increase in tumor burden or the appearance of new lesions, which in the evaluation of traditional chemotherapeutic agents would be classified as disease progression and would be synonymous with drug failure. Accordingly, proper evaluation of immunotherapeutic agents may require long-term monitoring of the effects of these

agents on the target disease.

A therapeutically effective amount of a drug includes a "prophylactically effective amount," which is any amount of the drug that, when administered alone or in combination with an anti-neoplastic agent to a subject at risk of developing a cancer (*e.g.*, a subject having a pre-malignant condition) or of suffering a recurrence of cancer, inhibits the development or recurrence of the cancer. In preferred embodiments, the prophylactically effective amount prevents the development or recurrence of the cancer entirely. "Inhibiting" the development or recurrence of a cancer means either lessening the likelihood of the cancer's development or recurrence, or preventing the development or recurrence of the cancer entirely.

A "tumor-infiltrating inflammatory cell" is any type of cell that typically participates in an inflammatory response in a subject and which infiltrates tumor tissue. Such cells include tumor-infiltrating lymphocytes (TILs), macrophages, monocytes, eosinophils, histiocytes and dendritic cells.

The use of the alternative (*e.g.*, "or") should be understood to mean either one, both, or any combination thereof of the alternatives. As used herein, the indefinite articles "a" or "an" should be understood to refer to "one or more" of any recited or enumerated component.

The terms "about" or "comprising essentially of" refer to a value or composition that is within an acceptable error range for the particular value or composition as determined by one of ordinary skill in the art, which will depend in part on how the value or composition is measured or determined, *i.e.*, the limitations of the measurement system. For example, "about" or "comprising essentially of" can mean within 1 or more than 1 standard deviation per the practice in the art. Alternatively, "about" or "comprising essentially of" can mean a range of up to 20%. Furthermore, particularly with respect to biological systems or processes, the terms can mean up to an order of magnitude or up to 5-fold of a value. When particular values or compositions are provided in the application and claims, unless otherwise stated, the meaning of "about" or "comprising essentially of" should be assumed to be within an acceptable error range for that particular value or composition.

As described herein, any concentration range, percentage range, ratio range or integer range is to be understood to include the value of any integer within the recited

range and, when appropriate, fractions thereof (such as one tenth and one hundredth of an integer), unless otherwise indicated.

Various aspects of the invention are described in further detail in the following subsections.

*Antibodies of the Invention*

Abs of the present invention include a variety of Abs having structural and functional properties described herein, including high-affinity binding to PD-1 or PD-L1, respectively. These Abs may be used, for example, as therapeutic Abs to treat subjects afflicted with disease or as reagents in diagnostic assays to detect their cognate antigens. Human mAbs (HuMAbs) that bind specifically to PD-1 (*e.g.*, bind to human PD-1 and may cross-react with PD-1 from other species, such as cynomolgus monkey) with high affinity have been disclosed in U.S. Patent No. 8,008,449, and HuMAbs that bind specifically to PD-L1 with high affinity have been disclosed in U.S. Patent No. 7,943,743. The Abs of the invention include, but are not limited to, all of the anti-PD-1 and anti-PD-L1 Abs disclosed in U.S. Patent Nos. 8,008,449 and 7,943,743, respectively. Other anti-PD-1 mAbs have been described in, for example, U.S. Patent Nos. 6,808,710, 7,488,802 and 8,168,757, and PCT Publication No. WO 2012/145493, and anti-PD-L1 mAbs have been described in, for example, U.S. Patent Nos. 7,635,757 and 8,217,149, U.S. Publication No. 2009/0317368, and PCT Publication Nos. WO 2011/066389 and WO 2012/145493. To the extent these anti-PD-1 and anti-PD-L1 mAbs exhibit the structural and functional properties disclosed herein for antibodies of the invention, they too are included as antibodies of the invention.

*Anti-PD-1 Antibodies of the Invention*

Each of the anti-PD-1 HuMAbs disclosed in U.S. Patent No. 8,008,449 has been demonstrated to exhibit one or more of the following characteristics: (a) binds to human PD-1 with a $K_D$ of 1 x $10^{-7}$ M or less, as determined by surface plasmon resonance using a Biacore biosensor system; (b) does not substantially bind to human CD28, CTLA-4 or ICOS; (c) increases T-cell proliferation in a Mixed Lymphocyte Reaction (MLR) assay; (d) increases interferon-γ production in an MLR assay; (e) increases IL-2 secretion in an MLR assay; (f) binds to human PD-1 and cynomolgus monkey PD-1; (g) inhibits the binding of PD-L1 and/or PD-L2 to PD-1; (h) stimulates antigen-specific memory responses; (i) stimulates Ab responses; and (j) inhibits tumor cell growth *in vivo*. Anti-PD-1 Abs of the present invention include mAbs that bind specifically to human PD-1

and exhibit at least one, preferably at least five, of the preceding characteristics.

U.S. Patent No. 8,008,449 exemplifies seven anti-PD-1 HuMAbs: 17D8, 2D3, 4H1, 5C4 (also referred to herein as nivolumab or BMS-936558), 4A11, 7D3 and 5F4. Isolated DNA molecules encoding the heavy and light chain variable regions of these Abs have been sequenced, from which the amino acid sequences of the variable regions were deduced. The $V_H$ amino acid sequences of 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 and 5F4 are provided herein as SEQ ID NOs. 1, 2, 3, 4, 5, 6 and 7, respectively. The $V_L$ amino acid sequences of 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 and 5F4 are provided herein as SEQ ID NOs. 8, 9, 10, 11, 12, 13 and 14, respectively.

Preferred anti-PD-1 Abs of the present invention include the anti-PD-1 HuMAbs 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 and 5F4. These preferred Abs bind specifically to human PD-1 and comprise: (a) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 1 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 8; (b) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 2 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 9; (c) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 3 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 10; (d) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 4 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 11; (e) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 5 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 12; (f) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 6 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 13; or (g) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 7 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 14.

Given that each of these Abs can bind to PD-1, the $V_H$ and $V_L$ sequences can be "mixed and matched" to create other anti-PD-1 Abs of the invention. PD-1 binding of such "mixed and matched" Abs can be tested using binding assays, *e.g.*, enzyme-linked immunosorbent assays (ELISAs), western blots, radioimmunoassays and Biacore analysis that are well known in the art (*see, e.g.*, U.S. Patent No. 8,008,449). Preferably, when $V_H$ and $V_L$ chains are mixed and matched, a $V_H$ sequence from a particular $V_H/V_L$ pairing is replaced with a structurally similar $V_H$ sequence. Likewise, preferably a $V_L$ sequence from a particular $V_H/V_L$ pairing is replaced with a structurally similar $V_L$ sequence. Accordingly, anti-PD-1 Abs of the invention include an isolated mAb or antigen-binding portion thereof comprising: (a) a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs. 1, 2, 3, 4, 5, 6 and 7, and (b) a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs. 8, 9, 10, 11, 12, 13 and 14, wherein the Ab specifically binds PD-1, preferably human PD-1.

The CDR domains of the above Abs have been delineated using the Kabat system, and these Abs may also be defined by combinations of their 3 heavy chain and 3 light chain CDRs (*see* U.S. Patent No. 8,008,449). Since each of these Abs can bind to PD-1 and antigen-binding specificity is provided primarily by the CDR1, CDR2, and CDR3 regions, the $V_H$ CDR1, CDR2, and CDR3 sequences and $V_\kappa$ CDR1, CDR2, and CDR3 sequences can be "mixed and matched" (*i.e.*, CDRs from different Abs can be mixed and match, although each Ab must contain a $V_H$ CDR1, CDR2, and CDR3 and a $V_\kappa$ CDR1, CDR2, and CDR3) to create other anti-PD-1 Abs that also constitute Abs of the invention. PD-1 binding of such "mixed and matched" Abs can be tested using the binding assays described above (*e.g.*, ELISAs, western blots, radioimmunoassays and Biacore analysis).

Abs of the invention also include isolated Abs that bind specifically to PD-1 and comprise a heavy chain variable region derived from a particular germline heavy chain immunoglobulin and/or a light chain variable region derived from a particular germline light chain immunoglobulin. Specifically, in certain embodiments, Abs of the invention include isolated Abs comprising: (a) a heavy chain variable region that comprises consecutively linked amino acids having a sequence derived from a human $V_H$ 3-33 or 4-39 germline sequence, and/or a light chain variable region that comprises consecutively linked amino acids having a sequence derived from a human $V_\kappa$ L6, or L15 germline sequence. The amino acid sequences of the $V_H$ and $V_\kappa$ regions encoded by the $V_H$ 3-33,

$V_H$ 4-39, $V_\kappa$ L6 and $V_\kappa$ L15 germline genes are provided in U.S. Patent No. 8,008,449.

As used herein, an Ab can be identified as comprising a heavy or a light chain variable region that is "derived from" a particular human germline immunoglobulin by comparing the amino acid sequence of the human Ab to the amino acid sequences encoded by human germline immunoglobulin genes, and selecting the human germline immunoglobulin sequence that is closest in sequence (*i.e.*, greatest percentage of sequence identity) to the sequence of the human Ab. A human Ab that is "derived from" a particular human germline immunoglobulin may contain amino acid differences as compared to the germline sequence, due to, for example, naturally-occurring somatic mutations or intentional introduction of site-directed mutation. However, a selected human Ab is generally at least 90% identical in amino acids sequence to an amino acid sequence encoded by a human germline immunoglobulin gene and contains amino acid residues that identify the human Ab as being human when compared to the germline immunoglobulin amino acid sequences of other species (*e.g.*, murine germline sequences). In certain cases, a human Ab may be at least 95%, or even at least 96%, 97%, 98%, or 99% identical in amino acid sequence to the amino acid sequence encoded by the germline immunoglobulin gene.

In certain embodiments, the sequence of a human Ab derived from a particular human germline sequence will display no more than 10 amino acid differences from the amino acid sequence encoded by the human germline immunoglobulin gene. In other embodiments, the human Ab may display no more than 5, or even no more than 4, 3, 2, or 1 amino acid differences from the amino acid sequence encoded by the germline immunoglobulin gene.

Preferred Abs of the invention also include isolated Abs or antigen-binding portions thereof comprising: (a) a heavy chain variable region that comprises consecutively linked amino acids having a sequence derived from a human $V_H$ 3-33 germline sequence, and a light chain variable region that comprises consecutively linked amino acids having a sequence derived from a human $V_\kappa$ L6 germline sequence; or (b) a heavy chain variable region that comprises consecutively linked amino acids having a sequence derived from a human $V_H$ 4-39 germline sequence, and a light chain variable region that comprises consecutively linked amino acids having a sequence derived from a human $V_\kappa$ L15 germline sequence. Examples of Abs having a $V_H$ and a $V_\kappa$ derived from $V_H$ 3-33 and $V_\kappa$ L6 germline sequences, respectively, include 17D8, 2D3, 4H1, 5C4, and

7D3. Examples of Abs having $V_H$ and $V_\kappa$ regions derived from $V_H$ 4-39 and $V_\kappa$ L15 germline sequences, respectively, include 4A11 and 5F4.

In yet other embodiments, anti-PD-1 Abs of the invention comprise heavy and light chain variable regions having amino acid sequences that are highly similar or homologous to the amino acid sequences of the preferred anti-PD-1 Abs described herein, wherein the Ab retains the functional properties of the preferred anti-PD-1 Abs of the invention. For example, Abs of the invention include mAbs comprising a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises consecutively linked amino acids having a sequence that is at least 80% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs. 1, 2, 3, 4, 5, 6 and 7, and the light chain variable region comprises consecutively linked amino acids having a sequence that is at least 80% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs. 8, 9, 10, 11, 12, 13 and 14. In other embodiments, the $V_H$ and/or $V_L$ amino acid sequences may exhibit at least 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to the sequences set forth above.

As used herein, the percent sequence identity (also referred to as the percent sequence homology) between two sequences (amino acid or nucleotide sequences) is a function of the number of identical positions shared by the sequences relative to the length of the sequences compared (*i.e.*, % identity = number of identical positions/total number of positions being compared x 100), taking into account the number of any gaps, and the length of each such gap, introduced to maximize the degree of sequence identity between the two sequences. The comparison of sequences and determination of percent identity between two sequences can be accomplished using mathematical algorithms that are well know to those of ordinary skill in the art (*see, e.g.*, U.S. Patent No. 8,008,449).

Antibodies having very similar amino acid sequences are likely to have essentially the same functional properties where the sequence differences are conservative modifications. As used herein, "conservative sequence modifications" refer to amino acid modifications that do not significantly affect the binding characteristics of the Ab containing the amino acid sequence. Such conservative modifications include amino acid substitutions, additions and deletions. Conservative amino acid substitutions are substitutions in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Thus, for example, one or more amino acid residues within the CDR regions of an Ab of the invention can be replaced with other amino acid residues

from the same side chain family and the altered Ab can be tested for retained function using functional assays that are well known in the art. Accordingly, certain embodiments of the anti-PD-1 Abs of the invention comprise heavy and light chain variable regions each comprising CDR1, CDR2 and CDR3 domains, wherein one or more of these CDR domains comprise consecutively linked amino acids having sequences that are the same as the CDR sequences of the preferred anti-PD-1 Abs described herein (*e.g.*, 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 or 5F4), or conservative modifications thereof, and wherein the Abs retain the desired functional properties of the preferred anti-PD-1 Abs of the invention.

Further, it is well known in the art that the heavy chain CDR3 is the primary determinant of binding specificity and affinity of an Ab, and that multiple Abs can predictably be generated having the same binding characteristics based on a common CDR3 sequence (*see, e.g.*, Klimka *et al.*, 2000; Beiboer *et al.*, 2000; Rader *et al.*, 1998; Barbas *et al.*, 1994; Barbas *et al.*, 1995; Ditzel *et al.*, 1996; Berezov *et al.*, 2001; Igarashi *et al.*, 1995; Bourgeois *et al.*, 1998; Levi *et al.*, 1993; Polymenis and Stoller, 1994; and Xu and Davis, 2000). The foregoing publications demonstrate that, in general, once the heavy chain CDR3 sequence of a given Ab is defined, variability in the other five CDR sequences does not greatly affect the binding specificity of that Ab. Thus, Abs of the invention comprising 6 CDRs can be defined by specifying the sequence of the heavy chain CDR3 domain.

Anti-PD-1 Abs of the invention also include isolated Abs that bind specifically to human PD-1 and cross-compete for binding to human PD-1 with any of HuMAbs 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 and 5F4. Thus, anti-PD-1 Abs of the invention include isolated Abs or antigen-binding portions thereof that cross-compete for binding to PD-1 with a reference Ab or a reference antigen-binding portion thereof comprising: (a) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 1 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 8; (b) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 2 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 9; (c) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 3 and a human light chain

variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 10; (d) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 4 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 11; (e) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 5 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 12; (f) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 6 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 13; or (g) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 7 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEO ID NO: 14.

The ability of Abs to cross-compete for binding to an antigen indicates that these Abs bind to the same epitope region (*i.e.*, the same or an overlapping or adjacent epitope) of the antigen and sterically hinder the binding of other cross-competing Abs to that particular epitope region. Thus, the ability of a test Ab to competitively inhibit the binding of, for example, 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 or 5F4, to human PD-1 demonstrates that the test Ab binds to the same epitope region of human PD-1 as 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 or 5F4, respectively. All isolated Abs that bind to the same epitope region of human PD-1 as does HuMAb 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 or 5F4 are included among the Abs of the invention. These cross-competing Abs are expected to have very similar functional properties by virtue of their binding to the same epitope region of PD-1. For example, cross-competing anti-PD-1 mAbs 5C4, 2D3, 7D3, 4H1 and 17D8 have been shown to have similar functional properties (*see* U.S. Patent No. 8,008,449 at Examples 3-7). The higher the degree of cross-competition, the more similar will the functional properties be. Further, cross-competing Abs can be readily identified based on their ability to cross-compete with 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 or 5F4 in standard PD-1 binding assays. For example, Biacore analysis, ELISA assays or flow cytometry may be used to demonstrate cross-competition with the Abs of the invention (*see, e.g.*, Examples 1 and 2).

In certain embodiments, the Abs that cross-compete for binding to human PD-1

with, or bind to the same epitope region of human PD-1 as, 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 or 5F4 are mAbs. For administration to human patients, these cross-competing Abs are preferably chimeric Abs, or more preferably humanized or human Abs. Such human mAbs can be prepared and isolated as described in U.S. Patent No. 8,008,449. Data provided in Example 1 show that 5C4 or a Fab fragment thereof cross-competes with each of 2D3, 7D3, 4H1 or 17D8 for binding to hPD-1 expressed on the surface of a cell, indicating that all five anti-PD-1 mAbs bind to the same epitope region of hPD-1 (Figures 1A-1C).

An anti-PD-1 Ab of the invention further can be prepared using an Ab having one or more of the $V_H$ and/or $V_L$ sequences disclosed herein as starting material to engineer a modified Ab, which modified Ab may have altered properties from the starting Ab. An Ab can be engineered by modifying one or more residues within one or both variable regions (*i.e.*, $V_H$ and/or $V_L$), for example within one or more CDR regions and/or within one or more framework regions. Additionally or alternatively, an Ab can be engineered by modifying residues within the constant region(s), for example, to alter the effector function(s) of the Ab. Specific modifications to Abs include CDR grafting, site-specific mutation of amino acid residues within the $V_H$ and/or $V_\kappa$ CDR1, CDR2 and/or CDR3 regions to thereby improve one or more binding properties (*e.g.*, affinity) of the Ab, site-specific mutation of amino acid residues within the $V_H$ and/or $V_\kappa$ framework regions to decrease the immunogenicity of the Ab, modifications within the Fc region, typically to alter one or more functional properties of the Ab, such as serum half-life, complement fixation, Fc receptor binding, and/or antigen-dependent cellular cytotoxicity, and chemical modification such as pegylation or alteration in glycosylation patterns to increase or decrease the biological (*e.g.*, serum) half life of the Ab. Specific examples of such modifications and methods of engineering Abs are described in detail in U.S. Patent No. 8,008,449. Anti-PD-1 Abs of the invention include all such engineered Abs that bind specifically to human PD-1 and are obtained by modification of any of the above-described anti-PD-1 Abs.

Anti-PD-1 Abs of the invention also include antigen-binding portions of the above Abs. It has been amply demonstrated that the antigen-binding function of an Ab can be performed by fragments of a full-length Ab. Examples of binding fragments encompassed within the term "antigen-binding portion" of an Ab include (i) a Fab fragment, a monovalent fragment consisting of the $V_L$, $V_H$, $C_L$ and $C_{H1}$ domains; (ii) a

F(ab')$_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the $V_H$ and $C_{H1}$ domains; and (iv) a Fv fragment consisting of the $V_L$ and $V_H$ domains of a single arm of an Ab.

These fragments, obtained initially through proteolysis with enzymes such as papain and pepsin, have been subsequently engineered into monovalent and multivalent antigen-binding fragments. For example, although the two domains of the Fv fragment, $V_L$ and $V_H$, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker peptide that enables them to be made as a single protein chain in which the $V_L$ and $V_H$ regions pair to form monovalent molecules known as single chain variable fragments (scFv). Divalent or bivalent scFvs (di-scFvs or bi-scFvs) can be engineered by linking two scFvs in within a single peptide chain known as a tandem scFv which contains two $V_H$ and two $V_L$ regions. ScFv dimers and higher multimers can also be created using linker peptides of fewer than 10 amino acids that are too short for the two variable regions to fold together, which forces the scFvs to dimerize and produce diabodies or form other multimers. Diabodies have been shown to bind to their cognate antigen with much higher affinity than the corresponding scFvs, having dissociation constants up to 40-fold lower than the $K_D$ values for the scFvs. Very short linkers ($\leq$ 3 amino acids) lead to the formation of trivalent triabodies or tetravalent tetrabodies that exhibit even higher affinities for to their antigens than diabodies. Other variants include minibodies, which are scFv-$C_{H3}$ dimers, and larger scFv-Fc fragments (scFv-$C_{H2}$-$C_{H3}$ dimers), and even an isolated CDR may exhibit antigen-binding function. These Ab fragments are engineered using conventional recombinant techniques known to those of skill in the art, and the fragments are screened for utility in the same manner as are intact Abs. All of the above proteolytic and engineered fragments of Abs and related variants (*see* Hollinger and Hudson, 2005; Olafsen and Wu, 2010, for further details) are intended to be encompassed within the term "antigen-binding portion" of an Ab.

*Anti-PD-L1 Antibodies of the Invention*

Each of the anti-PD-L1 HuMAbs disclosed in U.S. Patent No. 7,943,743 has been demonstrated to exhibit one or more of the following characteristics (a) binds to human PD-L1 with a $K_D$ of 1 x $10^{-7}$ M or less; (b) increases T-cell proliferation in a Mixed Lymphocyte Reaction (MLR) assay; (c) increase interferon-$\gamma$ production in an MLR assay; (d) increase IL-2 secretion in an MLR assay; (e) stimulates Ab responses; (f) inhibits the binding of PD-L1 to PD-1; and (g) reverses the suppressive effect of T

regulatory cells on T cell effector cells and/or dendritic cells. Anti-PD-L1 Abs of the present invention include mAbs that bind specifically to human PD-L1 and exhibit at least one, preferably at least four, of the preceding characteristics.

U.S. Patent No. 7,943,743 exemplifies ten anti-PD-1 HuMAbs: 3G10, 12A4 (also referred to herein as BMS-936559), 10A5, 5F8, 10H10, 1B12, 7H1, 11E6, 12B7, and 13G4. Isolated DNA molecules encoding the heavy and light chain variable regions of these Abs have been sequenced, from which the amino acid sequences of the variable regions were deduced. The $V_H$ amino acid sequences of 3G10, 12A4, 10A5, 5F8, 10H10, 1B12, 7H1, 11E6, 12B7, and 13G4 are shown in SEQ ID NOs. 15, 16, 17, 18, 19, 20, 21, 22, 23 and 24, respectively, whereas their $V_L$ amino acid sequences are shown in SEQ ID NOs. 25, 26, 27, 28, 29, 30, 31, 32, 33 and 34, respectively.

Preferred anti-PD-L1 Abs of the present invention include the anti-PD-L1 HuMAbs 3G10, 12A4, 10A5, 5F8, 10H10, 1B12, 7H1, 11E6, 12B7, and 13G4. These preferred Abs bind specifically to human PD-L1 and comprise: (a) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 15 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 25; (b) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 16 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 26; (c) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 17 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 27; (d) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 18 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 28; (e) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 19 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 29; (f) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 20 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 30; (g) a human heavy chain variable

region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 21 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 31; (h) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 22 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 32; (i) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 23 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEO ID NO: 33; or (j) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 24 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 34.

Given that each of these Abs can bind to PD-L1, the $V_H$ and $V_L$ sequences can be "mixed and matched" to create other anti-PD-L1 Abs of the invention. PD-L1 binding of such "mixed and matched" Abs can be tested using binding assays *e.g.*, ELISAs, western blots, radioimmunoassays and Biacore analysis that are well known in the art (*see, e.g.*, U.S. Patent No. 7,943,743). Preferably, when $V_H$ and $V_L$ chains are mixed and matched, a $V_H$ sequence from a particular $V_H/V_L$ pairing is replaced with a structurally similar $V_H$ sequence. Likewise, preferably a $V_L$ sequence from a particular $V_H/V_L$ pairing is replaced with a structurally similar $V_L$ sequence. Accordingly, Abs of the invention also include a mAb, or antigen binding portion thereof, comprising a heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in any of SEQ ID NOs. 15, 16, 17, 18, 19, 20, 21, 22, 23 or 24, and a light chain variable region comprising consecutively linked amino acids having the sequence set forth in any of SEQ ID NOs. 25, 26, 27, 28, 29, 30, 31, 32, 33 or 34, wherein the Ab binds specifically to PD-L1, preferably human PD-L1.

The CDR domains of the above anti-PD-L1 HuMAbs have been delineated using the Kabat system, and these Abs may also be defined by combinations of their 3 heavy chain and 3 light chain CDRs (*see* U.S. Patent No. 7,943,743). Since each of these Abs can bind to PD-L1 and antigen-binding specificity is provided primarily by the CDR1, CDR2, and CDR3 regions, the $V_H$ CDR1, CDR2, and CDR3 sequences and $V_\kappa$ CDR1, CDR2, and CDR3 sequences can be "mixed and matched" (*i.e.*, CDRs from different Abs

can be mixed and match, although each Ab must contain a $V_H$ CDR1, CDR2, and CDR3 and a $V_\kappa$ CDR1, CDR2, and CDR3) to create other anti-PD-1 Abs that also constitute Abs of the invention. PD-L1 binding of such "mixed and matched" Abs can be tested using, for example, ELISAs, western blots, radioimmunoassays and Biacore analysis.

Antibodies of the invention also include Abs that bind specifically to PD-L1 and comprise a heavy chain variable region derived from a particular germline heavy chain immunoglobulin and/or a light chain variable region derived from a particular germline light chain immunoglobulin. Specifically, in certain embodiments, Abs of the invention include Abs comprising: (a) a heavy chain variable region that comprises consecutively linked amino acids having a sequence derived from a human $V_H$ 1-18, 1-69, 1-3 or 3-9 germline sequence, and/or a light chain variable region that comprises consecutively linked amino acids having a sequence derived from a human $V_\kappa$ L6, L15, A27 or L18 germline sequence. The amino acid sequences of the $V_H$ and $V_\kappa$ regions encoded by the $V_H$ 1-18, $V_H$ 1-3, $V_H$ 1-69, $V_H$ 3-9, $V_\kappa$ L6, $V_\kappa$ L15 and $V_\kappa$ A27 germline genes are provided in U.S. Patent No. 7,943,743.

Preferred Abs of the invention include isolated Abs or antigen-binding portions thereof comprising: (a) a heavy chain variable region that comprises consecutively linked amino acids having a sequence derived from a human $V_H$ 1-18 germline sequence, and a light chain variable region that comprises consecutively linked amino acids having a sequence derived from a human $V_\kappa$ L6 germline sequence; (b) a heavy chain variable region that comprises consecutively linked amino acids having a sequence derived from a human $V_H$ 1-69 germline sequence, and a light chain variable region that comprises consecutively linked amino acids having a sequence derived from a human $V_\kappa$ L6 germline sequence; (c) a heavy chain variable region that comprises consecutively linked amino acids having a sequence derived from a human $V_H$ 1-3 germline sequence, and a light chain variable region that comprises consecutively linked amino acids having a sequence derived from a human $V_\kappa$ L15 germline sequence; (d) a heavy chain variable region that comprises consecutively linked amino acids having a sequence derived from a human $V_H$ 1-69 germline sequence, and a light chain variable region that comprises consecutively linked amino acids having a sequence derived from a human $V_\kappa$ A27 germline sequence; (e) a heavy chain variable region that comprises consecutively linked amino acids having a sequence derived from a human $V_H$ 3-9 germline sequence, and a light chain variable region that comprises consecutively linked amino acids having a

sequence derived from a human $V_\kappa$ L15germline sequence; or (f) a heavy chain variable region that comprises consecutively linked amino acids having a sequence derived from a human $V_H$ 3-9 germline sequence, and a light chain variable region that comprises consecutively linked amino acids having a sequence derived from a human $V_\kappa$ L18germline sequence.

An example of an Ab having a $V_H$ and a $V_\kappa$ derived from $V_H$ 1-18 and $V_\kappa$ L6 germline sequences, respectively, is 3G10. Examples of Abs having $V_H$ and $V_\kappa$ regions derived from $V_H$ 1-69 and $V_\kappa$ L6 germline sequences, respectively, include 12A4, 1B12, 7H1 and 12B7. An example of an Ab having a $V_H$ and a $V_\kappa$ derived from $V_H$ 1-3 and $V_\kappa$ L15 germline sequences, respectively, is 10A5. Examples of Abs having $V_H$ and $V_\kappa$ regions derived from $V_H$ 1-69 and $V_\kappa$ A27 germline sequences, respectively, include 5F8, 11E6 and 11E6a. An example of an Ab having a $V_H$ and a $V_K$ derived from $V_H$ 3-9 and $V_\kappa$ L15 germline sequences, respectively, is 10H10. An example of an Ab having a $V_H$ and a $V_\kappa$ derived from $V_H$ 1-3 and $V_\kappa$ L15 germline sequences, respectively, is 10A5. An example of an Ab having a $V_H$ and a $V_\kappa$ derived from $V_H$ 3-9 and $V_\kappa$ L18 germline sequences, respectively, is 13G4.

In certain embodiments, anti-PD-L1 Abs of the invention comprise heavy and light chain variable regions having amino acid sequences that are highly similar or homologous to the amino acid sequences of the preferred anti-PD-L1 Abs described herein, wherein the Ab retains the functional properties of the aforementioned anti-PD-L1 Abs of the invention. For example, Abs of the invention include mAbs comprising a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises consecutively linked amino acids having a sequence that is at least 80% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs. 15, 16, 17, 18, 19, 20, 21, 22, 23, and 24, and the light chain variable region comprises consecutively linked amino acids having a sequence that is at least 80% identical to an amino acid sequence selected from the group consisting of SEQ ID NOs. 25, 26, 27, 28, 29, 30, 31, 32, 33, and 34. In other embodiments, the $V_H$ and/or $V_L$ amino acid sequences may exhibit at least 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to the sequences set forth above.

Certain embodiments of the anti-PD-L1 Abs of the invention comprise heavy and light chain variable regions each comprising CDR1, CDR2 and CDR3 domains, wherein one or more of these CDR domains comprise consecutively linked amino acids having

sequences that are the same as the CDR sequences of the preferred anti-PD-L1 Abs described herein (*e.g.*, 3G10, 12A4, 10A5, 5F8, 10H10, 1B12, 7H1, 11E6, 12B7 and 13G4), or conservative modifications thereof, and wherein the Abs retain the desired functional properties of the preferred anti-PD-L1 Abs of the invention.

On the basis of the evidence that the heavy chain CDR3 is the primary determinant of binding specificity and affinity of an Ab, it is generally true that once the heavy chain CDR3 sequence of a given Ab is defined, variability in the other five CDR sequences will not greatly affect the binding specificity of that Ab. Accordingly, anti-PD-L1 Abs of the invention include isolated Abs comprising 6 CDRs, wherein the Abs are defined by specifying the sequence of the heavy chain CDR3 domain.

Anti-PD-L1 Abs of the invention also include isolated Abs that bind specifically to human PD-L1 and cross-compete for binding to human PD-L1 with any of HuMAbs 3G10, 12A4, 10A5, 5F8, 10H10, 1B12, 7H1, 11E6, 12B7 and 13G4. Thus, anti-PD-L1 Abs of the invention include isolated Abs or antigen-binding portions thereof that cross-compete for binding to PD-L1 with a reference Ab or a reference antigen-binding portion thereof comprising: (a) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 15 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 25; (b) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 16 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 26; (c) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 17 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 27; (d) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 18 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 28; (e) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 19 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 29; (f) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 20 and a human light chain variable region

comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 30; (g) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 21 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEO ID NO: 31; (h) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 22 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 32; (i) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 23 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEO ID NO: 33; or (j) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 24 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 34.

The ability of an Ab to cross-compete with any of 3G10, 12A4, 10A5, 5F8, 10H10, 1B12, 7H1, 11E6, 12B7 and 13G4 for binding to human PD-L1 demonstrates that such Ab binds to the same epitope region of each of 3G10, 12A4, 10A5, 5F8, 10H10, 1B12, 7H1, 11E6, 12B7 and 13G4, respectively. All isolated Abs that bind to the same epitope region of human PD-L1 as does HuMAb 3G10, 12A4, 10A5, 5F8, 10H10, 1B12, 7H1, 11E6, 12B7 or 13G4 are included among the Abs of the invention. These cross-competing Abs are expected to have very similar functional properties by virtue of their binding to the same epitope region of PD-L1. For example, cross-competing anti-PD-L1 mAbs 3G10, 1B12, 13G4, 12A4 (BMS-936559), 10A5, 12B7, 11E6 and 5F8 have been shown to have similar functional properties (*see* U.S. Patent No. 7,943,743 at Examples 3-11), whereas mAb 10H10, which binds to a different epitope region, behaves differently (U.S. Patent No. 7,943,743 at Example 11). The higher the degree of cross-competition, the more similar will the functional properties be. Further, cross-competing Abs can be identified in standard PD-L1 binding assays, *e.g.*, Biacore analysis, ELISA assays or flow cytometry, that are well known to persons skilled in the art. In preferred embodiments, the Abs that cross-compete for binding to human PD-1 with, or bind to the same epitope region of human PD-L1 as, 3G10, 12A4, 10A5, 5F8, 10H10, 1B12, 7H1, 11E6, 12B7 or 13G4 are mAbs, preferably chimeric Abs, or more preferably humanized or human Abs.. Such human mAbs can be prepared and isolated as described in U.S.

Patent No. 7,943,743.

Data provided in Example 2 show that each of the anti-PD-L1 HuMAbs 5F8, 7H1, 1B12, 3G10, 10A5, 11E6, 12A4, 12B7 and 13G4, *i.e.*, all of the HuMAbs tested except 10H10, substantially blocked binding of mAbs 3G10, 10A5, 11E6, 12A4 and 13G4 to Chinese Hamster Ovary (CHO) cells expressing PD-L1 cells. HuMAb 10H10 substantially blocked the binding only of itself to CHO/PD-L1 cells. These data show that 3G10, 10A5, 11E6, 12A4 and 13G4 cross-compete with all of the HuMAbs tested, except for 10H10, for binding to the same epitope region of human PD-L1 (Figures 2A-F).

Data provided in Example 3 show that the binding of HuMAb 12A4 to ES-2 ovarian carcinoma cells expressing PD-L1 cells was substantially blocked by 12A4 itself and by 1B12 and 12B7, and was moderately to significantly blocked by mAbs 5F8, 10A5, 13G4 and 3G10, but was not blocked by mAb 10H10. These data, largely consistent with the data in Example 2, show that 12A4 itself, and 2 other HuMabs, 12B7 and 1B12, substantially cross-compete with 12A4 for binding to the same epitope region, possibly the same epitope, of human PD-L1; 5F8, 10A5, 13G4 and 3G10, exhibit a significant but lower level of cross-competition with 12A4, suggesting that these mAbs may bind to epitopes that overlap the 12A4 epitope; whereas 10H10 does not cross-compete at all with 12A4 (Figure 3), suggesting that this mAb binds to a different epitope region from 12A4.

Anti-PD-L1 Abs of the invention also include Abs engineered starting from Abs having one or more of the $V_H$ and/or $V_L$ sequences disclosed herein, which engineered Abs may have altered properties from the starting Abs. An anti-PD-L1 Ab can be engineered by a variety of modifications as described above for the engineering of modified anti-PD-1 Abs of the invention.

Anti-PD-L1 Abs of the invention also include isolated Abs selected for their ability to bind to PD-L1 in formalin-fixed, paraffin-embedded (FFPE) tissue specimens. The use of FFPE samples is essential for the long-term follow-up analysis of the correlation between PD-L1 expression in tumors and disease prognosis or progression. Yet, studies on measuring PD-L1 expression have often been conducted on frozen specimens because of the difficulty in isolating anti-human PD-L1 Abs that can be used to stain PD-L1 in FFPE specimens by IHC in general (Hamanishi *et al.*, 2007) and, in particular, Abs that bind specifically to membranous PD-L1 in these tissues. The use of different Abs to stain PD-L1 in frozen versus FFPE tissues, and the ability of certain Abs

to distinguish membranous and/or cytoplasmic forms of PD-L1, may account for some of the disparate data reported in the literature correlating PD-L1 expression with disease prognosis (Hamanishi *et al.*, 2007; Gadiot et al., 2011). This disclosure provides several rabbit mAbs that bind with high affinity specifically to membranous human PD-L1 in FFPE tissue samples comprising tumor cells and tumor-infiltrating inflammatory cells.

Rabbit and mouse anti-hPD-L1 mAbs were produced as described in Example 9. Out of almost 200 Ab multiclones screened, only ten rabbit multiclone Abs were found to specifically detect the membranous form of PD-L1, and the top five multiclones (designated Nos. 13, 20, 28, 29 and 49) were subsequently subcloned. The clone that produced the most robust detection specifically of membranous PD-L1, rabbit clone 28-8, was selected for the IHC assays. The sequences of the variable regions of mAb 28-8 are set forth in SEQ ID NOs. 35 and 36, respectively. The sequences of the heavy and light chain CDR domains of mAb 28-8, as delineated using the Kabat system, are set forth in SEQ ID NOs. 37-42. Rabbit clones 28-1, 28-12, 29-8 and 20-12 were the next best mAbs in terms of robust detection of membranous PD-L1 in FFPE tissues.

Anti-PD-L1 Abs of the invention also include antigen-binding portions of the above Abs, including Fab, F(ab')$_2$ Fd, Fv, and scFv, di-scFv or bi-scFv, and scFv-Fc fragments, diabodies, triabodies, tetrabodies, and isolated CDRs (*see* Hollinger and Hudson, 2005; Olafsen and Wu, 2010, for further details).

*Nucleic Acid Molecules Encoding Antibodies of the Invention*

Another aspect of the present disclosure pertains to isolated nucleic acid molecules that encode any of the Abs of the invention. These nucleic acids may be present in whole cells, in a cell lysate, or in a partially purified or substantially pure form. A nucleic acid of the invention can be, for example, DNA or RNA, and may or may not contain intronic sequences. In a preferred embodiment, the nucleic acid is a cDNA.

Nucleic acids of the invention can be obtained using standard molecular biology techniques. For Abs expressed by hybridomas (*e.g.*, hybridomas prepared from transgenic mice carrying human immunoglobulin genes as described further below), cDNAs encoding the light and heavy chains of the Ab made by the hybridoma can be obtained by standard PCR amplification or cDNA cloning techniques. Nucleic acids encoding Abs obtained from an immunoglobulin gene library (*e.g.*, using phage display techniques) can be recovered from the library.

Preferred nucleic acids molecules of the invention are those encoding the $V_H$ and

$V_\kappa$ sequences of the anti-PD-1 HuMAbs, 17D8, 2D3, 4H1, 5C4, 4A11, 7D3 and 5F4 (disclosed in U.S. Patent No. 8,008,449), and those encoding the $V_H$ and $V_\kappa$ sequences of the anti-PD-L1 HuMAbs, 3G10, 12A4, 10A5, 5F8, 10H10, 1B12, 7H1, 11E6, 12B7, and 13G4 (disclosed in U.S. Patent No. 7,943,743). An isolated DNA encoding the $V_H$ region can be converted to a full-length heavy chain gene by operatively linking the $V_H$-encoding DNA to another DNA molecule encoding heavy chain constant regions ($C_{H1}$, $C_{H2}$ and $C_{H3}$), the sequences of which are known in the art and can be obtained by standard PCR amplification. The heavy chain constant region can be an IgG1, IgG2, IgG3, IgG4, IgA, IgE, IgM or IgD constant region, but is preferably an IgG1 or IgG4 constant region. Similarly, an isolated DNA encoding the $V_L$ region can be converted to a full-length light chain gene by operatively linking the $V_L$-encoding DNA to another DNA molecule encoding the light chain constant region ($C_L$), the sequence of which is known in the art and can be obtained by standard PCR amplification. The light chain constant region can be a kappa or lambda constant region, but most preferably is a kappa constant region.

*Pharmaceutical Compositions*

Antibodies of the present invention may be constituted in a composition, *e.g.*, a pharmaceutical composition, containing one Ab or a combination of Abs, or an antigen-binding portion(s) thereof, and a pharmaceutically acceptable carrier. As used herein, a "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. Preferably, the carrier is suitable for intravenous, intramuscular, subcutaneous, parenteral, spinal or epidermal administration (*e.g.*, by injection or infusion). A pharmaceutical composition of the invention may include one or more pharmaceutically acceptable salts, anti-oxidant, aqueous and nonaqueous carriers, and/or adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents.

Dosage regimens are adjusted to provide the optimum desired response, *e.g.*, a therapeutic response or minimal adverse effects. For administration of an anti-PD-1 or anti-PD-L1 Ab, the dosage ranges from about 0.0001 to about 100 mg/kg, usually from about 0.001 to about 20 mg/kg, and more usually from about 0.01 to about 10 mg/kg, of the subject's body weight. Preferably, the dosage is within the range of 0.1-10 mg/kg body weight. For example, dosages can be 0.1, 0.3, 1, 3, 5 or 10 mg/kg body weight, and

more preferably, 0.3, 1, 3, or 10 mg/kg body weight. The dosing schedule is typically designed to achieve exposures that result in sustained receptor occupancy (RO) based on typical pharmacokinetic properties of an Ab. An exemplary treatment regime entails administration once per week, once every two weeks, once every three weeks, once every four weeks, once a month, once every 3 months or once every three to 6 months. The dosage and scheduling may change during a course of treatment. For example, dosing schedule may comprise administering the Ab: (i) every two weeks in 6-week cycles; (ii) every four weeks for six dosages, then every three months; (iii) every three weeks; (iv) 3-10 mg/kg body weight once followed by 1 mg/kg body weight every 2-3 weeks. Considering that an IgG4 Ab typically has a half-life of 2-3 weeks, a preferred dosage regimen for an anti-PD-1 or anti-PD-L1 Ab of the invention comprises 0.3-10 mg/kg body weight, preferably 3-10 mg/kg body weight, more preferably 3 mg/kg body weight via intravenous administration, with the Ab being given every 14 days in up to 6-week or 12-week cycles until complete response or confirmed progressive disease.

In some methods, two or more mAbs with different binding specificities are administered simultaneously, in which case the dosage of each Ab administered falls within the ranges indicated. Antibody is usually administered on multiple occasions. Intervals between single dosages can be, for example, weekly, every 2 weeks, every 3 weeks, monthly, every three months or yearly. Intervals can also be irregular as indicated by measuring blood levels of Ab to the target antigen in the patient. In some methods, dosage is adjusted to achieve a plasma Ab concentration of about 1-1000 μg/ml and in some methods about 25-300 μg/ml.

Alternatively, the Ab can be administered as a sustained release formulation, in which case less frequent administration is required. Dosage and frequency vary depending on the half-life of the Ab in the patient. In general, human Abs show the longest half-life, followed by humanized Abs, chimeric Abs, and nonhuman Abs. The dosage and frequency of administration can vary depending on whether the treatment is prophylactic or therapeutic. In prophylactic applications, a relatively low dosage is typically administered at relatively infrequent intervals over a long period of time. Some patients continue to receive treatment for the rest of their lives. In therapeutic applications, a relatively high dosage at relatively short intervals is sometimes required until progression of the disease is reduced or terminated, and preferably until the patient shows partial or complete amelioration of symptoms of disease. Thereafter, the patient

can be administered a prophylactic regime.

Actual dosage levels of the active ingredients in the pharmaceutical compositions of the present invention may be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being unduly toxic to the patient. The selected dosage level will depend upon a variety of pharmacokinetic factors including the activity of the particular compositions of the present invention employed, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compositions employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts. A composition of the present invention can be administered via one or more routes of administration using one or more of a variety of methods well known in the art. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results.

*Uses and Methods of the Invention*

The Abs, Ab compositions, nucleic acids and methods of the present invention have numerous *in vitro* and *in vivo* utilities including, for example, methods to determine and quantify the expression of PD-1 or PD-L1 comprising binding of the Abs to the target polypeptides or measuring the amount of nucleic acid encoding these polypeptides, and a method for immunotherapy of a subject afflicted with a disease comprising administering to the subject a composition comprising a therapeutically effective amount of a therapeutic agent that inhibits signaling from an inhibitory immunoregulator. In preferred embodiments of the latter method, the inhibitory immunoregulator is a component of the PD-1/PD-L1 signaling pathway, and the therapeutic agent disrupts signaling of this pathway. More preferably, the therapeutic agent is an Ab that interferes with the interaction between PD-1 and PD-L1. In certain preferred embodiments of this method, the Ab binds specifically to PD-1 and blocks the interaction of PD-1 with PD-L1 and/or PD-L2. In other preferred embodiments, the therapeutic agent is an Ab that binds specifically to PD-L1 and blocks the interaction of PD-L1 with PD-1 and/or B7-1 (CD80). Thus, this disclosure provides methods for potentiating an immune response in a subject comprising administering an anti- PD-1 and/or an anti-PD-L1 Ab in order to disrupt the interaction between PD-1 and PD-L1, and methods of treating diseases

mediated by such a potentiation of the immune response. When Abs to PD-1 and PD-L1 are administered together, the two can be administered sequentially in either order or simultaneously. In certain aspects, this disclosure provides methods of modifying an immune response in a subject comprising administering to the subject an anti-PD-1 and/or an anti-PD-L1 Ab of the invention, or antigen-binding portion thereof, such that the immune response in the subject is modified. Preferably, the immune response is potentiated, enhanced, stimulated or up-regulated. In preferred embodiments, the Abs of the present invention are human Abs.

Preferred subjects include human patients in need of enhancement of an immune response. The immunotherapeutic methods disclosed herein are particularly suitable for treating human patients having a disorder that can be treated by potentiating a T-cell mediated immune response. In certain embodiments, the methods are employed for treatment of subjects afflicted with a disease caused by an infectious agent. In preferred embodiments, the methods are employed for treatment of subjects afflicted with, or at risk of being afflicted with, a cancer.

*Cancer Immunotherapy*

Blockade of PD-1/PD-L1 interaction has been shown to potentiate immune responses *in vitro* (U.S. Patent Nos. 8,008,449 and 7,943,743; Fife *et al.*, 2009) and mediate preclinical antitumor activity (Dong *et al.*, 2002; Iwai *et al.*, 2002). However, the molecular interactions potentially blocked by these two Abs are not identical: anti-PD-1 Abs of the invention disrupt PD-1/PD-L1 and potentially PD-1/PD-L2 interactions; in contrast, whereas anti-PD-L1 Abs of the invention also disrupt PD-1/PD-L1 interactions, they do not block PD-1/PD-L2 interactions but instead may disrupt the PD-1-independent PD-L1/CD80 interaction, which has also been shown to down-modulate T-cell responses *in vitro* and *in vivo* (Park *et al.*, 2010; Paterson *et al.*, 2011; Yang *et al.*, 2011; Butte *et al.*, 2007; Butte *et al.*, 2008). Thus, it is possible that among these varied ligand-receptor pairings, different interactions may dominate in different cancer types, contributing to dissimilar activity profiles for the two Abs.

Disruption of the PD-1/PD-L1 interaction by antagonistic Abs can enhance the immune response to cancerous cells in a patient. PD-L1 is not expressed in normal human cells, but is abundant in a variety of human cancers (Dong *et al.*, 2002). The interaction between PD-1 and PD-L1 impairs T cell responses as manifested by a decrease in tumor-infiltrating lymphocytes (TILs) and a decrease in T-cell receptor-

mediated proliferation, resulting in T cell anergy, exhaustion or apoptosis, and immune evasion by the cancerous cells (Zou and Chen, 2008; Blank *et al.*, 2005; Konishi *et al.*, 2004; Dong *et al.*, 2003; Iwai *et al.*, 2002). Immune suppression can be reversed by inhibiting the local interaction between PD-L1 and PD-1 using an anti-PD-1 and/or an anti-PD-L1 Ab. These Abs may be used alone or in combination to inhibit the growth of cancerous tumors. In addition, either or both of these Abs may be used in conjunction with other immunogenic and/or anti-cancer agents including cytokines, standard cancer chemotherapies, vaccines, radiation, surgery, or other Abs.

*Immunotherapy of Cancer Patients Using an Anti-PD-1 Antibody*

This disclosure provides a method for immunotherapy of a subject afflicted with cancer, which method comprises administering to the subject a composition comprising a therapeutically effective amount of an Ab or an antigen-binding portion thereof that disrupts the interaction of PD-1 with PD-L1 and/or PD-L2. This disclosure also provides a method of inhibiting growth of tumor cells in a subject, comprising administering to the subject an Ab or an antigen-binding portion thereof that disrupts the interaction of PD-1 with PD-L1 and/or PD-L2 in an amount effective to inhibit growth of the tumor cells. In preferred embodiments, the subject is a human. In other preferred embodiments, the Ab or antigen-binding portion thereof is an anti-PD-1 Ab of the invention or an antigen-binding portion thereof. In certain embodiments, the Ab or antigen-binding portion thereof is of an IgG1 or IgG4 isotype. In other embodiments, the Ab or antigen-binding portion thereof is a mAb or an antigen-binding portion thereof. In further embodiments, the Ab or antigen-binding portion thereof is a chimeric, humanized or human Ab or an antigen-binding portion thereof. In preferred embodiments for treating a human patient, the Ab or antigen-binding portion thereof is a human Ab or an antigen-binding portion thereof.

The clinical trials described in the Examples employed the anti-PD-1 HuMAb, nivolumab (designated 5C4 in U.S. Patent No. 8,008,449), to treat cancer. While 5C4 was selected as the lead Ab for entering the clinic, it is notable that several anti-PD-1 Abs of the invention share with 5C4 functional properties that are important to the therapeutic activity of 5C4, including high affinity binding specifically to human PD-1, increasing T-cell proliferation, IL-2 secretion and interferon-γ production in an MLR assay, inhibiting the binding of PD-L1 and/or PD-L2 to PD-1, and inhibiting tumor cell growth *in vivo*. Moreover, certain of the anti-PD-1 Abs of the invention, 17D8, 2D3, 4H1 and 7D3 are

structurally related to 5C4 in comprising $V_H$ and $V_\kappa$ regions that have sequences derived from $V_H$ 3-33 and $V_\kappa$ L6 germline sequences, respectively. In addition, 5C4, 2D3, 7D3, 4H1 and 17D8 all cross-compete for binding to the same epitope region of hPD-1 (Example 1). Thus, the preclinical characterization of nivolumab and other anti-PD-1 HuMabs indicate that the methods of treating cancer provided herein may be performed using different Abs selected from the broad genus of anti-PD-1 Abs of the invention.

Accordingly, certain embodiments of the immunotherapy methods disclosed herein comprise administering to a patient an anti-PD-1 Ab or antigen-binding portion thereof comprising: (a) a heavy chain variable region that comprises consecutively linked amino acids having a sequence derived from a human $V_H$ 3-33 germline sequence, and a light chain variable region that comprises consecutively linked amino acids having a sequence derived from a human $V_\kappa$ L6 germline sequence, or (b) a heavy chain variable region that comprises consecutively linked amino acids having a sequence derived from a human $V_H$ 4-39 germline sequence, and a light chain variable region that comprises consecutively linked amino acids having a sequence derived from a human $V_\kappa$ L15 germline sequence.

In certain other embodiments, the Ab or antigen-binding portion thereof that is administered to the patient cross-competes for binding to PD-1 with a reference Ab or a reference antigen-binding portion thereof comprising: (a) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 1 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 8; (b) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 2 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 9; (c) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 3 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 10; (d) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 4 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 11; (e) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 5 and a human light chain variable region comprising consecutively linked amino

acids having the sequence set forth in SEQ ID NO: 12; (f) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 6 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 13; or (g) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 7 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEO ID NO: 14. In preferred embodiments, the Ab or antigen-binding portion thereof cross-competes for binding to PD-1 with nivolumab.

In certain embodiments of the immunotherapy methods disclosed herein, the anti-PD-1 Ab or antigen-binding portion thereof administered to the patient comprises: (a) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 1 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 8; (b) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 2 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 9; (c) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 3 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 10; (d) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 4 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 11; (e) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 5 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 12; (f) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 6 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 13; or (g) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 7 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEO ID NO: 14. In preferred

embodiments, the anti-PD-1 Ab or antigen-binding portion comprises a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 4 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 11. In other preferred embodiments, the anti-PD-1 Ab is nivolumab.

In certain embodiments of the present methods, the anti-PD-1 Ab is formulated for intravenous administration. In preferred embodiments, the Ab is administered intravenously at a dose of 3 mg/kg over 60 minutes every 2 weeks. Typically, treatment is continued as long as clinical benefit is observed or until unmanageable toxicity or disease progression occurs.

In the clinical trials of anti-PD-1 immunotherapy described in the Examples below, intriguing ORs with durable clinical responses, even in heavily pretreated patients, were observed across multiple tumor types including a substantial proportion of NSCLC, MEL, and RCC patients and in various sites of metastasis including liver, lung, lymph nodes, and bone. MEL and RCC are considered to be immunogenic neoplasms, having previously been demonstrated to be responsive to cancer immunotherapy, *e.g.*, interferon-alfa and interleukin-2 in both MEL and RCC (Eton *et al.*., 2002; Coppin *et al.*, 2005; McDermott and Atkins, 2006) and anti-CTLA-4 Ab in MEL (Hodi *et al.*, 2010). In MEL patients, significant ORRs were observed with nivolumab across different doses of 0.1, 0.3, 1, 3, or 10 mg/kg with an overall response rate of about 31%, and similarly comparable ORRs of about 30% were observed in RCC patients treated with nivolumab doses of 1 or 10 mg/kg (*see* Example 7). In contrast, immunotherapy has historically had minimal success in lung cancer. This lack of success has been attributed to NSCLC being "non-immunogenic" (*see, e.g.*, Holt and Disis, 2008; Holt *et al.*, 2011). The ability of lung cancer to thwart the immune system results from multiple factors, including the secretion of immunosuppressive cytokines, loss of major histocompatibility complex antigen expression, and co-opting of T cell inhibitory pathways (Dasanu *et al.*, 2012; Marincola *et al.*, 2000; Brahmer *et al*, 2012).

Despite the limited efficacy of immunotherapy historically seen in lung cancer and the diverse mechanisms employed by lung cancer cells to evade immune system attack, various tumor cell vaccines (*e.g.*, belagenpumatucel-L, a whole-cell-based vaccine that blocks the action of TGF-β2) and antigen-based vaccines (*e.g.*, a humoral EGF vaccine and vaccines incorporating a MAGE-A3 fusion protein or portions of the tumor-

associated MUC1 antigen) that augment antigen-specific antitumor immunity are being evaluated in clinical trials (Holt *et al.*, 2011; Shepherd *et al.*, 2011; Dasanu *et al.*, 2012; Brahmer *et al*, 2012). However, while such antigen-specific vaccines have shown some promise for the treatment of NSCLC, Holt *et al.* (2011) noted that trials involving nonspecific immunotherapeutic interventions had failed to improve outcomes in NSCLC, which indicated a need to combine them with antigen-specific vaccines. These authors expressed the view that truly efficacious immunotherapy of NSCLC would only result from implementation of strategies to both augment antitumor immunity and counteract tumor-mediated immunosuppression, and concluded that the nonexistent or weak nascent immune responses in NSCLC patients make it unlikely that nonspecific stimulation of the immune system or removal of immunosuppression would create changes in clinical outcomes.

Thus, the results reported herein with NSCLC are particularly striking, unexpected and surprising. In NSCLC patients, ORs were observed at nivolumab doses of 1, 3, or 10 mg/kg with response rates of 3%, 24%, and 20%, respectively (*see* Example 7). ORs were observed across NSCLC histologies: 9 responders of 54 squamous (17%), and 13 of 74 nonsquamous (18%). This level of activity seen with nivolumab in NSCLC patients with significant prior therapy (54% with 3 lines of previous therapy) and across histologies is unprecedented, especially in the squamous histology patients (*cf.* Gridelli *et al.*, 2008; Miller, 2006), and provides a very favorable benefit/risk dynamic regarding efficacy and safety compared to existing standard-of-care.

In certain embodiments of the present immunotherapy methods, the anti-PD-1 Ab is indicated as monotherapy for locally advanced or metastatic squamous or non-squamous NSCLC after platinum based therapy (regardless of maintenance) and one other treatment. In other embodiments, the anti-PD-1 Ab is indicated as monotherapy for locally advanced or metastatic squamous or non-squamous NSCLC after failure of platinum-based therapy and one other prior chemotherapy regimen. In further embodiments, the anti-PD-1 Ab is indicated as monotherapy for locally advanced or metastatic squamous or non-squamous NSCLC after failure of two lines of therapy one of which must include platinum based regimen. In yet further embodiments, the anti-PD-1 Ab is indicated as monotherapy for locally advanced or metastatic squamous or non-squamous NSCLC after at least one prior chemotherapy, after failure of at least one prior platinum based therapy, or after progression on at least one platinum doublet therapy. In

all of the immunotherapy methods disclosed herein, treatment may be continued as long as clinical benefit is observed or until unmanageable toxicity or disease progression occurs.

*Durability of clinical responses to anti-PD-1 in heavily-pretreated cancer patients*

The critical role of the PD-1 pathway in suppressing anti-tumor immunity, first revealed in laboratory models, has now been validated in clinical studies. As described herein, monotherapy with drugs blocking PD-1 (nivolumab) or its major ligand PD-L1 (BMS-936559) can mediate regression in patients with treatment-refractory advanced cancers. The objective response rate (ORR) in heavily pretreated NSCLC patients receiving anti-PD-1 Ab, including patients with squamous histology, is surprising and unexpected, as standard salvage therapies historically show modest benefit in these patients (Scagliotti *et al.*, 2011). As measured by standard RECIST in this study, ORs were long-lasting, with median response durations of about 18 months in 22 of 129 responders ((*see* Example 7). In addition, patterns of tumor regression consistent with immune-related patterns of response were observed.

These findings have established the PD-1 pathway as a new therapeutic focus in oncology (Pardoll, 2012; Topalian *et al.*, 2012c; Hamid and Carvajal, 2013). In the current study, in which 54% of patients had progressive disease following 3 or more prior systemic regimens, preliminary analysis has been conducted up to March 2013. This updated analysis has supported and reinforced the data obtained and conclusions reached from the earlier February 2012 analyses. Thus, conventional ORs or prolonged disease stabilization were documented in patients with NSCLC (17% and 10%, respectively), MEL (31%, 7%) and RCC (29%, 27%), across all doses tested (*see* Example 7). Additionally, 13 patients (4%) manifested unconventional, "immune-related" response patterns as previously described with anti-CTLA-4 therapy, several of which were sustained (Sharma *et al.*, 2011).

The durability of ORs across multiple cancer types in patients treated with the anti-PD-1 Ab is particularly notable. The updated analyses again underscored the durability of clinical activity in nivolumab-treated patients, which has not generally been observed with chemotherapy or small molecule inhibitors to date, but has been observed in some patients with advanced melanoma receiving immunotherapies, including ipilimumab and high-dose interleukin-2 (Topalian *et al.*, 2011; Hodi *et al.*, 2010). The persistence of partial tumor regressions following drug discontinuation suggests that PD-1

blockade has reset the immune equilibrium between tumor and host, and the OS benefit may ultimately prove to be significantly longer than has been measured to date. Further follow-up is needed to determine the ultimate durability of tumor regression and disease stabilization in patients on these clinical trials.

Significantly, the durable objective tumor regression and disease stabilization induced by nivolumab in heavily-pretreated patients with advanced NSCLC, MEL, and RCC translate to survival outcomes that surpass historical data for these patient populations treated with conventional chemotherapy and/or tyrosine-kinase inhibitor (TKI) treatments. In NSCLC, 1- and 2-year survival rates were 42% and 14%, respectively, with median OS of 9.2 and 10.1 months in patients with squamous and non-squamous cancers, respectively (*see* Example 7). This high level of efficacy is especially impressive since 54% of these patients had received 3 or more prior therapies. Moreover, because follow-up of many lung cancer patients was relatively limited, these figures may change as data mature. Historically, 2L chemotherapeutics for lung cancer (*i.e.*, docetaxel and pemetrexed) have achieved a median OS of 7.5-8.3 months, and one-year survival rates of approximately 30% (Shepherd *et al.*, 2000; Hanna *et al.*, 2004). In a 2L/3L population, erlotinib-treated patients had a median survival of 6.7 months, versus 4.7 months in placebo-treated patients (Shepherd *et al.*, 2005). No therapy is currently approved for use in lung cancer beyond the 3L setting, and minimal data exist to benchmark survival in this patient population, except in retrospective reviews with reported median survivals of 5.8-6.5 months and 1-year survivals of 25% (Girard *et al.*, 2009; Scartozi *et al.*, 2010).

In nivolumab-treated MEL patients, median OS of 16.8 months was achieved, with landmark survival rates of 62% (1-year) and 43% (2-year) (*see* Example 7). Survival outcomes in pretreated melanoma patients supported the recent FDA approvals of ipilimumab and vemurafenib. In a recent phase 3 trial enrolling melanoma patients with at least one prior treatment for metastatic disease, ipilimumab increased median OS from 6.4 to 10.1 months, compared to a gp100 peptide vaccine (Hodi *et al.*, 2010). In phase 2 trials of ipilimumab in previously treated patients, 2-year survival rates ranged from 24.2-32.8% (Lebbe *et al.*, 2012). Median OS in previously treated patients with BRAF-mutant melanoma enrolled on a large phase 2 of vemurafenib was 15.9 months, and 1-year survival was 58% (Sosman *et al.*, 2012).

Accordingly, in certain embodiments, immunotherapy with anti-PD-1 Ab is indicated as monotherapy for locally advanced or metastatic MEL after therapy with dacarbazine (regardless of maintenance) and one other treatment. In other embodiments, the anti-PD-1 Ab is indicated as monotherapy for locally advanced or metastatic MEL after failure of dacarbazine-based therapy. In further embodiments, the anti-PD-1 Ab is indicated as monotherapy for locally advanced or metastatic MEL after failure of two lines of therapy one of which must include a dacarbazine-based regimen. In yet further embodiments, the anti-PD-1 Ab is indicated as monotherapy for locally advanced or metastatic MEL after at least one prior chemotherapy, after failure of at least one prior dacarbazine-based therapy, or after progression on at least dacarbazine therapy. In all of the immunotherapy methods disclosed herein, treatment may be continued as long as clinical benefit is observed or until unmanageable toxicity or disease progression occurs.

In nivolumab-treated patients with RCC, among whom 45% received 3 or more prior therapies and 71% received prior anti-angiogenic therapy, median OS was reached at 22 months (as of the March 2013 date of analysis). Landmark survival rates of 70% (1-year) and 50% (2-year) were observed (*see* Example 7). In a recent Phase 3 trial enrolling kidney cancer patients whose disease progressed following anti-angiogenic therapy, everolimus was compared with placebo: median OS was 14.8 versus 14.4 months, respectively (Motzer *et al.*, 2008; Motzer *et al.*, 2010). A recent Phase 3 trial comparing sorafenib to temsirolimus in a sunitinib-refractory kidney cancer population yielded median OS of 16.6 and12.3 months, respectively (Hutson *et al.*, 2012). Thus, as with NSCLC and MEL, treatment of a heavily-pretreated RCC patient population with nivolumab has yielded a considerably longer median OS (> 22 months) than treatment of a less refractory population with standard-of-care therapies. Controlled Phase 3 trials with prospective survival endpoints are underway in NSCLC, MEL and RCC (NCT01673867, NCT01721772, NCT01642004, NCT01668784, and NCT01721746 (*see* Clinical Trials Website, http://www.clinicaltrials.gov). The results from these trials are expected to further demonstrate the high efficacy of, and durability of responses to, nivolumab in these cancers compared standard-of-care therapies.

In certain embodiments, immunotherapy with anti-PD-1 Ab is indicated as monotherapy for locally advanced or metastatic RCC after therapy with an anti-angiogenic TKI or a mTOR inhibitor (regardless of maintenance) and one other treatment. In other embodiments, the anti-PD-1 Ab is indicated as monotherapy for

locally advanced or metastatic RCC after failure of therapy with an anti-angiogenic TKI or a mTOR inhibitor. In further embodiments, the anti-PD-1 Ab is indicated as monotherapy for locally advanced or metastatic RCC after failure of two lines of therapy one of which must include an anti-angiogenic TKI or a mTOR inhibitor. In yet further embodiments, the anti-PD-1 Ab is indicated as monotherapy for locally advanced or metastatic RCC after at least one prior chemotherapy, after failure of at least one prior anti-angiogenic TKI- or mTOR inhibitor-based therapy, or after progression on at least an anti-angiogenic TKI or a mTOR inhibitor therapy. Anti-PD-1 immunotherapy may be continued as long as clinical benefit is observed or until unmanageable toxicity or disease progression occurs.

Notably, OS in lung cancer, melanoma and kidney cancer patients receiving nivolumab was considerably longer than PFS. These results mirror those reported for ipilimumab (Hodi *et al.*, 2010), and reflect the observation that early tumor enlargement or the appearance of new lesions in some patients receiving immune checkpoint blockade can evolve to disease stabilization or regression. These findings suggest that progression-free survival may not be an optimal endpoint for determining the efficacy of nivolumab and other agents in this class.

The data disclosed herein demonstrating the high efficacy, durability and broad applicability of anti-PD-1 immunotherapy for treating cancer has led to nivolumab being tested for additional types of cancer. For example, on the basis that increased PD-L1 expression has been reported with various hematologic malignancies and may prevent the host immune response from exerting a beneficial impact on the malignant cells, a trial to confirm the ability of nivolumab to mediate antitumor activity in patients with hematologic malignancies (multiple myeloma, B-cell lymphoma, T-cell lymphoma, Hodgkin's lymphoma/primary mediastinal B-cell lymphoma, and chronic myelogenous leukemia) has been initiated (NCT01592370). Nivolumab is also being tested as a monotherapy in advanced hepatocellular carcinoma (NCT01658878).

In summary, the results of anti-PD-1 immunotherapy disclosed herein are remarkable in at least the following three respects. First, the efficacy of anti-PD-1 has been shown to surpass historical efficacy data for patients on standard-of-care treatments for cancer. Notably, this efficacy has been demonstrated in patient in heavily pretreated populations in which about half of the patients had progressive disease following 3 or more prior systemic regimens. Such patients, afflicted with advanced, metastatic and/or

refractory cancers, are notoriously difficult to treat. Accordingly, this disclosure provides methods for immunotherapy of a patient afflicted with an advanced, metastatic and/or refractory cancer, which method comprises administering to the patient a therapeutically effective amount of an Ab or an antigen-binding portion thereof that disrupts the interaction of PD-1 with PD-L1 and/or PD-L2. In certain embodiments of any of the therapeutic methods disclosed herein, the subject has been pre-treated for the cancer; for example, the subject had undergone at least one, two, or three prior lines of therapy for cancer.

Second, the present therapeutic methods have been shown to be applicable to a broad genus of different cancers. Based on the surprising discovery that even a "non-immunogenic" cancer such as NSCLC (Holt *et al.*, 2011) and hard-to-treat cancers such as ovarian and gastric cancers (as well as other cancers tested, including MEL, RCC, and CRC) are amendable to treatment with anti-PD-1 and/or anti-PD-L1 (*see* Examples 7 and 14), this disclosure generally provides methods for immunotherapy of a patient afflicted with practically any of a very wide range of cancers.

Third, treatment with an anti-PD-1 or anti-PD-L1 Ab has been shown to produce strikingly durable clinical activity in cancer patients. Accordingly, this disclosure provides immunotherapeutic methods of inducing a durable clinical response in a cancer patient comprising administering to the patient a therapeutically effective amount of an Ab or an antigen-binding portion thereof that disrupts the interaction of PD-1 with PD-L1 and/or PD-L2. In preferred embodiments of any of the therapeutic methods described herein, the clinical response is a durable response.

As used herein, a "durable" response is a therapeutic or clinical response that exceeds the anticipated median OS rate in a patient population. The anticipated median OS rate varies with different cancers and different patient populations. In certain embodiments, a durable response exceeds the anticipated median OS rate in the relevant patient population by at least 10%, preferably by at least 20%, more preferably by at least 30%, and even more preferably by at least 50%. A major benefit of immunotherapeutic approaches based on PD-1 pathway blockade may be the functional restoration of exhausted T cells with long-term generation of memory T cells that may maintain antitumor immune surveillance and inhibit tumor growth for prolonged periods extending to many years, even in the absence of continued therapy (Kim and Ahmed, 2010). Indeed, long-term follow-up studies on patients following cessation of nivolumab therapy have

confirmed that a patient with CRC experienced a complete response which was ongoing after 3 years; a patient with RCC experienced a partial response lasting 3 years off therapy, which converted to a complete response that was ongoing at 12 months; and a patient with melanoma achieved a partial response that was stable for 16 months off therapy, and recurrent disease was successfully treated with reinduction anti-PD-1 therapy (Lipson *et al.*, 2013).

*Immune-related clinical responses*

It has become evident that conventional response criteria may not adequately assess the activity of immunotherapeutic agents because progressive disease (by initial radiographic evaluation) does not necessarily reflect therapeutic failure. For example, treatment with the anti-CTLA-4 Ab, ipilimumab, has been shown to produce four distinct response patterns, all of which were associated with favorable survival: (a) shrinkage in baseline lesions, without new lesions; (b) durable stable disease (in some patients followed by a slow, steady decline in total tumor burden); (c) response after an increase in total tumor burden; and (d) response in the presence of new lesions. Accordingly, to properly evaluate immunotherapeutic agents, long-term effects on the target disease must also be captured. In this regard, systematic immune-related response criteria (irRC) that make allowances for an early increase in tumor burden and/or the appearance of new lesions, and which seek to enhance the characterization of immune-related response patterns, have been proposed (Wolchok *et al.*, 2009). While the full impact of these unconventional response patterns remains to be defined in randomized trials of nivolumab with survival endpoints, the present observations are reminiscent of findings with ipilimumab in which a significant extension of OS was observed in treated patients (Hodi *et al.*, 2010; Robert *et al.*, 2011).

The overall risk/benefit profile of anti-PD-1 immunotherapy is also favorable, with a low incidence of more severe drug-related adverse events (AEs; > grade 3), the specific events observed to date being consistent with other immunotherapeutic agents. This suggests that anti-PD-1 immunotherapy can be delivered in an outpatient setting with minimal supportive care.

*Broad spectrum of cancers treatable by anti-PD-1 immunotherapy*

The clinical data presented herein demonstrate that immunotherapy based on PD-1 blockade is not limited to only "immunogenic" tumor types, such as MEL and RCC, but extends to tumor types not generally considered to be immune-responsive, including

NSCLC. The unexpected successes with treatment-refractory metastatic NSCLC underscore the possibility that any neoplasm can be "immunogenic" in the context of proper immune modulation, and suggest that PD-1 blockade as an immunotherapeutic approach is broadly applicable across a very diverse range of tumor types. Thus, cancers that may be treated using the anti-PD-1 Abs of the invention also include cancers typically responsive to immunotherapy as well as cancers that have traditionally been regarded as non-immunogenic. Non-limiting examples of preferred cancers for treatment include NSCLC, MEL, RCC, CRC, CRPC, HCC, squamous cell carcinoma of the head and neck, carcinomas of the esophagus, ovary, gastrointestinal tract and breast, and a hematologic malignancy. Although NSCLC is not generally considered responsive to immunotherapy, data disclosed herein unexpectedly demonstrate that both squamous and non-squamous NSCLC are responsive to treatment with an anti-PD-1 Ab. Additionally, the disclosure provides for the treatment of refractory or recurrent malignancies whose growth may be inhibited using an anti-PD-1 Ab of the invention.

Examples of cancers that may be treated using an anti-PD-1 Ab in the methods of the present invention, based on the indications of very broad applicability of anti-PD-1 immunotherapy provided herein, include liver cancer, bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, breast cancer, lung cancer, cutaneous or intraocular malignant melanoma, renal cancer, uterine cancer, ovarian cancer, colorectal cancer, colon cancer, rectal cancer, cancer of the anal region, stomach cancer, testicular cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, non-Hodgkin's lymphoma, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, solid tumors of childhood, lymphocytic lymphoma, cancer of the bladder, cancer of the kidney or ureter, carcinoma of the renal pelvis, neoplasm of the central nervous system (CNS), primary CNS lymphoma, tumor angiogenesis, spinal axis tumor, brain stem glioma, pituitary adenoma, Kaposi's sarcoma, epidermoid cancer, squamous cell cancer, environmentally induced cancers including those induced by asbestos, hematologic malignancies including, for example, multiple myeloma, B-cell lymphoma, Hodgkin lymphoma/primary mediastinal B-cell lymphoma, non-Hodgkin's lymphomas, acute myeloid lymphoma, chronic myelogenous leukemia, chronic lymphoid leukemia,

follicular lymphoma, diffuse large B-cell lymphoma, Burkitt's lymphoma, immunoblastic large cell lymphoma, precursor B-lymphoblastic lymphoma, mantle cell lymphoma, acute lymphoblastic leukemia, mycosis fungoides, anaplastic large cell lymphoma, T-cell lymphoma, and precursor T-lymphoblastic lymphoma, and any combinations of said cancers. The present invention is also applicable to treatment of metastatic cancers.

*Medical uses of anti-PD-1 Abs*

One aspect of this invention is the use of any anti-PD-1 Ab or antigen-binding portion thereof of the invention for the preparation of a medicament for inhibiting signaling from the PD-1/PD-L1 pathway so as to thereby potentiate an endogenous immune response in a subject afflicted with cancer. Another aspect is the use of any anti-PD-1 Ab or an antigen-binding portion thereof of the invention for the preparation of a medicament for immunotherapy of a subject afflicted with cancer comprising disrupting the interaction between PD-1 and PD-L1. These uses for the preparation of medicaments are broadly applicable to the full range of cancers disclosed herein. In preferred embodiments of these uses, the cancers include squamous NSCLC, non-squamous NSCLC, MEL, RCC, CRC, CRPC, HCC, squamous cell carcinoma of the head and neck, and carcinomas of the esophagus, ovary, gastrointestinal tract and breast, and a hematologic malignancy. This disclosure also provides medical uses of any anti-PD-1 Ab or antigen-binding portion thereof of the invention corresponding to all the embodiments of the methods of treatment employing an anti-PD-1 Ab described herein.

The disclosure also provides an anti-PD-1 Ab or an antigen-binding portion thereof of the invention for use in treating a subject afflicted with cancer comprising potentiating an endogenous immune response in the subject by inhibiting signaling from the PD-1/PD-L1 pathway. The disclosure further provides an anti-PD-1 Ab or an antigen-binding portion thereof of the invention for use in immunotherapy of a subject afflicted with cancer comprising disrupting the interaction between PD-1 and PD-L1. These Abs may be used in potentiating an endogenous immune response against, or in immunotherapy of, the full range of cancers disclosed herein. In preferred embodiments, the cancers include squamous NSCLC, non-squamous NSCLC, MEL (*e.g.*, metastatic malignant MEL), RCC, CRC, CRPC, HCC, squamous cell carcinoma of the head and neck, and carcinomas of the esophagus, ovary, gastrointestinal tract and breast, and a hematologic malignancy.

*Combination Therapy Including Anti-PD-1 Antibodies*

Whereas monotherapy with anti-PD-1 and anti-PD-L1 Abs has been shown herein to significantly increase the survival of patients with lung cancer, melanoma, kidney cancer, and potentially other malignancies, preclinical data indicate that synergistic treatment combinations based on PD-1 pathway blockade could have even more potent effects. Clinical evaluation of nivolumab combined with ipilimumab (anti-CTLA-4), whose mechanism of action is similar yet distinct from nivolumab's (Parry *et al.*, 2005; Mellman *et al.*, 2011; Topalian *et al.*, 2012c), is ongoing and results from a Phase 1 trial are provided herein (*see, also*, NCT01024231; NCT01844505; NCT01783938; Wolchok *et al.*, 2013a; Wolchok *et al.*, 2013b; Hodi *et al.*, 2013). Clinical studies have also been initiated involving the administration of nivolumab in combination with melanoma vaccines (NCT01176461, NCT01176474; Weber *et al.*, 2013), with lirilumab (BMS-986015), a human IgG4 anti-KIR Ab, in patients with advanced solid tumors (NCT01714739; Sanborn *et al.*, 2013), with cytokines, for example, IL-21, in patients with advanced or metastatic solid tumors (NCT01629758; Chow *et al.*, 2013), with chemotherapeutic drugs, for example, with platinum-based doublet chemotherapy in chemotherapy-naïve NSCLC patients (NCT01454102; Rizvi *et al.*, 2013), and small-molecule targeted therapies in patients with metastatic RCC (NCT01472081; Amin *et al.*, 2013).

In certain aspects, this disclosure relates to the combination of an anti-PD-1 Ab with different cancer treatments, including chemotherapeutic regimes, radiation, surgery, hormone deprivation and angiogenesis inhibitors, for the treatment of various cancers. PD-1 blockade may also be effectively combined with an immunogenic agent, for example, a preparation of cancerous cells, purified tumor antigens (including recombinant proteins, peptides, and carbohydrate molecules), antigen-presenting cells such as dendritic cells bearing tumor-associated antigens, cells transfected with genes encoding immune stimulating cytokines (He *et al.*, 2004), and/or another immunotherapeutic Ab (*e.g.*, an anti-CTLA-4, anti-PD-L1 and/or anti-LAG-3 Ab). Non-limiting examples of tumor vaccines that can be used include peptides of melanoma antigens, such as peptides of gp100, MAGE antigens, Trp-2, MART1 and/or tyrosinase, or tumor cells transfected to express the cytokine GM-CSF.

*Combination of anti-PD-1 and anti-CTLA-4 Abs to treat advanced melanoma*

Given that immunologic checkpoints are non-redundant and can inhibit T-cell activation, proliferation and effector function within lymph nodes and/or the tumor microenvironment, and based on preclinical data that the combination of anti-CTLA-4 and anti-PD-1 had a stronger anti-tumor effect in mouse tumor models than either Ab alone (*see* U.S. Patent No. 8,008,449), the hypothesis that combined blockade of CTLA-4 and PD-1 could produce greater anti-tumor activity than single agents was tested in a clinical trial in MEL patients (Example 15).

Although not formally compared in this study, the concurrent nivolumab/ipilimumab regimen, which comprised achieved ORRs that exceed the rates achieved with either nivolumab (Example 7) or ipilimumab alone (Hodi *et al.*, 2010). Most importantly, rapid and deep responses were achieved in a substantial portion of treated patients, a "deep" tumor response referring to a response in target lesions characterized by a reduction of 80% or more from baseline measurements by radiographic assessment. In the present study, the majority of responding patients, including some with extensive and bulky tumor burden, achieved > 80% tumor regression at the time of the initial tumor assessment. Particularly striking was the observation that 31% of response-evaluable patients treated with concurrent-regimens (which comprise (i) an induction dosing schedule with combined administration of the anti-PD-1 and anti-CTLA-4 Abs followed by administration of the anti-PD-1 Ab alone, and (ii) a maintenance dosing schedule comprising less frequent combined administration of the anti-PD-1 and anti-CTLA-4 Abs) demonstrated > 80% tumor regression by week 12. At the MTD for the concurrent regimen, all 9 responding patients demonstrated > 80% tumor regression with 3 CRs. In contrast, in clinical experience to date, < 3% of MEL patients who received nivolumab or ipilimumab at 3 mg/kg achieved a CR (Example 7; Hodi *et al.*, 2010). Thus, the overall activity of this immunotherapy combination in this preliminary phase 1 trial compares very favorably with that of other agents approved or being developed for advanced melanoma, including the targeted inhibitors of activated kinases (Chapman *et al.*, 2011). An additional advantage of this combination is the durability of response as demonstrated in the present study as well as longer-term immunotherapy trials with nivolumab (as described herein) and ipilimumab (Wolchok *et al.*, 2013d).

These initial data suggest that rapid responses of a greater magnitude may be achieved in patients treated with a nivolumab/ipilimumab combination compared with the

historical experience of either agent alone. Responses were generally durable, and were observed even in patients whose treatment was terminated early because of toxicity. Responding patients included those with elevated LDH, M1c disease and bulky multi-focal tumor burden. Similar to prior reports regarding ipilimumab or nivolumab monotherapy, conventional ORRs may not fully capture the spectrum of clinical activity and potential benefit in patients treated with the concurrent nivolumab/ipilimumab regimen in that a number of patients experienced either long-term SD or unconventional immune-related patterns of response. Indeed, even among the 7 patients in the concurrent regimen with SD $\geq$ 24 weeks or irSD $\geq$ 24 weeks as best response, 6 demonstrated meaningful tumor regression of at least 19%, and the seventh patient has declining tumor burden after prolonged SD. Prior experience with checkpoint blockade monotherapy supports the observation that some patients may survive for extended periods of time with SD as the best OR, lending credence to the hypothesis that re-establishment of the equilibrium phase of immune surveillance is a desirable outcome (Screiber *et al.*, 2011).

The observation that patients can achieve ORs when treated sequentially with nivolumab after prior ipilimumab indicates that lack of response to CTLA-4 blockade does not preclude clinical benefit from PD-1 blockade and further supports the non-redundant nature of these co-inhibitory pathways. Notably, data disclosed herein (Example 8) suggest an association between the occurrence of response and tumor PD-L1 expression in patients receiving nivolumab, and prior data reveal a correlation between OS and increases in peripheral ALC in patients treated with ipilimumab (Berman *et al.*, 2009; Ku *et al.*, 2010; Postow *et al.*, 2012; Delyon *et al.*, 2013). In the present study of the nivolumab/ipilimumab combination, clinical responses were observed in patients irrespective of lymphocyte count or baseline tumor PD-L1 expression (Example 17), suggesting that the immune response generated by combination therapy has unique features compared to either monotherapy, While the data suggest that baseline tumor PD-L1 expression and lymphocyte count may be less relevant in the setting of active combination regimens capable of inducing rapid and pronounced tumor regression, it is also notable that a different anti-PD-L1 Ab (rabbit mAb 28-8 versus mouse 5H1 mAb) was used in a different IHC assay to measure PD-L1 expression in the combination therapy study compared to the nivolumab monotherapy study. In addition to changes in the IHC assay and Ab, the different results may also reflect differences in biopsy samples and tumor heterogeneity. The utility of PD-L1 expression as a biomarker for anti-PD-1

efficacy will be further evaluated prospectively in randomized phase 3 studies (*see, e.g.*, NCT01721772, NCT01668784, and NCT01721746).

The spectrum of adverse events observed among patients treated with the concurrent regimen was qualitatively similar to experience with nivolumab or ipilimumab monotherapy, although the rate of AEs was increased in patients treated with the combination. Grade 3-4 treatment-related AEs were observed in 53% of patients treated with the concurrent nivolumab/ipilimumab regimen, compared with historical rates of 20% in patients treated with ipilimumab monotherapy (Hodi *et al.*, 2010) and 17% in patients treated with nivolumab alone (Example 5) at a dose of 3 mg/kg. In the sequenced-regimen cohorts, 18% of patients experienced grade 3-4 treatment-related AEs. AEs experienced by patients treated with the concurrent and sequenced regimens were manageable and/or generally reversible using existing treatment algorithms.

Collectively, these results suggest that nivolumab and ipilimumab can be administered concurrently with a manageable safety profile and leading to durable clinical responses. More rapid and deeper clinical tumor responses were observed in patients treated with the combination compared with the responses obtained with either single agent.

As of the February 2013 clinical cut-off date for the study described in Example 15, of the 52 subjects on the concurrent regimen evaluable for response, 21 (40%) had an OR by modified World Health Organization (mWHO) criteria (Wolchok *et al.*, 2009). In an additional 2 subjects (4%), there was an unconfirmed OR. In Cohort 1 (0.3 mg/kg nivolumab plus 3 mg/kg ipilimumab), 3 out of 14 evaluable subjects had an OR by mWHO (ORR: 21%, including 1 CR and 2 PRs). In Cohort 2 (1 mg/kg nivolumab plus 3 mg/kg ipilimumab), 9 out of 17 evaluable subjects had an OR by mWHO (ORR: 53%; including 3 CRs and 6 PRs). In Cohort 2a (3 mg/kg nivolumab plus 1 mg/kg ipilimumab), 6 out of 15 response evaluable subjects had an OR by mWHO (ORR: 40%; including 1 CR and 5 PRs). In Cohort 3 (3 mg/kg nivolumab plus 3 mg/kg ipilimumab), 3 out of 6 evaluable subjects had an objective response by mWHO (ORR: 50%, including 3 PRs). Based on these data, the invention disclosed herein includes a method for treating a subject afflicted with a cancer comprising administering to the subject: (a) an Ab or an antigen-binding portion thereof that specifically binds to and inhibits PD-1; and (b) an Ab or an antigen-binding portion thereof that specifically binds to and inhibits CTLA-4; each Ab being administered at a dosage ranging from 0.1 to 20.0 mg/kg body weight in a

concurrent regimen comprising: (i) an induction dosing schedule comprising combined administration of the anti-PD-1 and anti-CTLA-4 Abs at a dosing frequency of at least once every 2, 3 or 4 weeks, or at least once a month, for at least 2, 4, 6, 8 or 10 doses, followed by administration of the anti-PD-1 Ab alone at a dosing frequency of at least once every 2, 3 or 4 weeks, or at least once a month, for at least 2, 4, 6, 8 or 12 doses; followed by (ii) a maintenance dosing schedule comprising combined administration of the anti-PD-1 and anti-CTLA-4 Abs at a dosing frequency of at least once every 8, 12 or 16 weeks, or at least once a quarter, for at least 4, 6, 8, 10, 12 or 16 doses, or for as long as clinical benefit is observed, or until unmanageable toxicity or disease progression occurs.

In certain embodiments of this method, the maintenance dosing schedule comprises combined administration of up to 4, 6, 8, 10, 12 or 16 doses of the anti-PD-1 and anti-CTLA-4 Abs. In other embodiments, the concurrent regimen comprises: (i) an induction dosing schedule comprising combined administration of the anti-PD-1 and anti-CTLA-4 Abs at a dosing frequency of once every 2, 3 or 4 weeks, or once a month, for 2, 4, 6 or 8 doses, followed by administration of the anti-PD-1 Ab alone at a dosing frequency of once every 2, 3 or 4 weeks, or once a month, for 2, 4, 6, 8 or 12 doses; followed by (ii) a maintenance dosing schedule comprising combined administration of the anti-PD-1 and anti-CTLA-4 Abs at a dosing frequency of once every 8, 12 or 16 weeks, or once a quarter, for 4, 6, 8, 10, 12 or 16 doses, or for as long as clinical benefit is observed, or until unmanageable toxicity or disease progression occurs. In certain other embodiments, each of the anti-PD-1 and anti-CTLA-4 Abs is individually administered at a dosage of 0.1, 0.3, 0.5, 1, 3, 5, 10 or 20 mg/kg. In further embodiments, the dosage of each of the anti-PD-1 and anti-CTLA-4 Abs is kept constant during the induction dosing schedule and the maintenance dosing schedule. In yet other embodiments, the anti-PD-1 and anti-CTLA-4 Abs are administered at the following dosages: (a) 0.1 mg/kg anti-PD-1 Ab and 3 mg/kg of anti-CTLA-4 Ab; (b) 0.3 mg/kg anti-PD-1 Ab and 3 mg/kg of anti-CTLA-4 Ab; (c) 1 mg/kg anti-PD-1 Ab and 3 mg/kg of anti-CTLA-4 Ab; (d) 3 mg/kg anti-PD-1 Ab and 3 mg/kg of anti-CTLA-4 Ab; (e) 5 mg/kg anti-PD-1 Ab and 3 mg/kg of anti-CTLA-4 Ab; (f) 10 mg/kg anti-PD-1 Ab and 3 mg/kg of anti-CTLA-4 Ab; (g) 0.1 mg/kg anti-PD-1 Ab and 1 mg/kg of anti-CTLA-4 Ab; (h) 0.3 mg/kg anti-PD-1 Ab and 1 mg/kg of anti-CTLA-4 Ab; (i) 1 mg/kg anti-PD-1 Ab and 1 mg/kg of anti-CTLA-4 Ab; (j) 3 mg/kg anti-PD-1 Ab and 1 mg/kg of anti-CTLA-4 Ab; (k) 5 mg/kg anti-PD-1 Ab and

1 mg/kg of anti-CTLA-4 Ab; or (l) 10 mg/kg anti-PD-1 Ab and 1 mg/kg of anti-CTLA-4 Ab.

In the protocol described in Example 15, the 3 mg/kg nivolumab plus 3 mg/kg ipilimumab dosing regimen exceeded the MTD (though combinations with ipilimumab and other anti-PD-1 Abs may have a higher or lower MTD), whereas both Cohort 2 (1 mg/kg nivolumab plus 3 mg/kg ipilimumab) and Cohort 2a (3 mg/kg nivolumab plus 1 mg/kg ipilimumab) had similar clinical activity. In addition, the majority of responses to the combination of nivolumab and ipilimumab occurred in the first 12 weeks. Given the uncertainty of whether the ipilimumab administered past week 12 contributes to the clinical benefit and the fact that the U.S. Food and Drug Administration (FDA)- and European Medicines Agency (EMA)-approved schedule for ipilimumab is every 3 weeks for a total of four doses, in a preferred embodiment the anti-CTLA-4 Ab is administered during the induction dosing schedule once every 3 weeks for a total of 4 doses. Monotherapy treatment with nivolumab at 3 mg/kg every two weeks until progression has been shown to be associated with durable responses (Examples 4-7), and a maintenance dosing schedule comprising administration of nivolumab every 12 weeks has been shown to be efficacious (Example 15). Thus, starting at week 12, which is after the completion of the four doses of combined nivolumab and ipilimumab, nivolumab at 3 mg/kg may be administered every 2 to at least 12 weeks until progression. Accordingly, in preferred embodiments of the concurrent regimen method, the anti-PD-1 and anti-CTLA-4 Abs are administered at dosages of: (a) 1 mg/kg anti-PD-1 Ab and 3 mg/kg of anti-CTLA-4 Ab; or (b) 3 mg/kg anti-PD-1 Ab and 1 mg/kg of anti-CTLA-4 Ab, and the concurrent regimen further comprises: (i) an induction dosing schedule comprising combined administration of the anti-PD-1 and anti-CTLA-4 Abs at a dosing frequency of once every 3 weeks for 4 doses, followed by administration of the anti-PD-1 alone at a dosing frequency of once every 3 weeks for 4 doses; followed by (ii) a maintenance dosing schedule comprising combined administration of the anti-PD-1 and anti-CTLA-4 antibodies at a dosing frequency of once every 2 to 12 or more weeks for up to 8 doses, or for as long as clinical benefit is observed, or until unmanageable toxicity or disease progression occurs.

Exposure-response analysis of nivolumab monotherapy across dose ranges of 1 mg/kg to 10 mg/kg reveals similar clinical activity (Example 7) while exposure-response analysis of 0.3 mg/kg, 3 mg/kg, and 10 mg/kg of ipilimumab monotherapy has

demonstrated increasing activity with increase in dose in a phase 2 trial (Wolchok *et al.*, 2010). Therefore, a dose of 3 mg/kg of ipilimumab (Cohort 2) may be more clinically impactful than selection of 3 mg/kg of nivolumab (Cohort 2a). Thus, in more preferred embodiments of the concurrent regimen method, the anti-PD-1 and anti-CTLA-4 Abs are administered at dosages of 1 mg/kg anti-PD-1 Ab and 3 mg/kg of anti-CTLA-4 Ab.

In certain embodiments of the present methods, the anti-PD-1 and anti-CTLA-4 Abs are formulated for intravenous administration. In certain other embodiments, when the anti-PD-1 and anti-CTLA-4 Abs are administered in combination, they are administered within 30 minutes of each other. Either Ab may be administered first, that is, in certain embodiments, the anti-PD-1 Ab is administered before the anti-CTLA-4 Ab, whereas in other embodiments, the anti-CTLA-4 Ab is administered before the anti-PD-1 Ab. Typically, each Ab is administered intravenously over a period of 60 minutes. In further embodiments, the anti-PD-1 and anti-CTLA-4 Abs are administered concurrently, either admixed as a single composition in a pharmaceutically acceptable formulation for concurrent administration, or concurrently as separate compositions with each Ab in a pharmaceutically acceptable formulation.

Data disclosed in Example 7 demonstrate that immunotherapy with nivolumab produced significant clinical activity in MEL patients who were unresponsive to prior ipilimumab therapy. Accordingly, this disclosure provides a sequenced regimen method for treating a subject afflicted with a cancer, the subject having previously been treated with an anti-CTLA-4 Ab, which method comprises administering to the subject an Ab or an antigen-binding portion thereof that specifically binds to and inhibits PD-1 at a dosage ranging from 0.1 to 20.0 mg/kg body weight and at a dosing frequency of at least once every week, at least once every 2, 3 or 4 weeks, or at least once a month, for up to 6 to up to 72 doses, or for as long as clinical benefit is observed, or until unmanageable toxicity or disease progression occurs. In certain embodiments of this method, administration of the anti-PD-1 Ab to the subject is initiated within 1-24 weeks after last treatment with the anti-CTLA-4 Ab. In other embodiments, administration of the anti-PD-1 Ab to the subject is initiated within 1, 2, 4, 8, 12, 16, 20 or 24 weeks after last treatment with the anti-CTLA-4 Ab. In preferred embodiments, administration of the anti-PD-1 Ab is initiated within 4, 8 or 12 weeks after last treatment of the subject with the anti-CTLA-4 Ab. Certain embodiments of the method comprise administering the anti-PD-1 Ab at a dosage of 0.1-20 mg/kg, *e.g.*, 0.1, 0.3, 0.5, 1, 3, 5, 10 or 20 mg/kg. In preferred embodiments, the

anti-PD-1 Ab is administered at a dosage of 1 or 3 mg/kg. In certain embodiments, the sequenced regimen comprises administering the anti-PD-1 Ab to the subject at a dosing frequency of once every week, once every 2, 3 or 4 weeks, or once a month for 6 to 72 doses, or for as long as clinical benefit is observed, or until unmanageable toxicity or disease progression occurs. In preferred embodiments, the anti-PD-1 is administered at a dosage of 1 or 3 mg/kg at a dosing frequency of once every 2 weeks for up to 48 doses. In other preferred embodiments, the anti-PD-1 Ab is formulated for intravenous administration.

In certain aspects of any of the present concurrent or sequenced regimen methods, the treatment produces at least one therapeutic effect chosen from a reduction in size and/or growth of a tumor, elimination of the tumor, reduction in number of metastatic lesions over time, complete response, partial response, and stable disease. Based on the broad spectrum of cancers in which nivolumab has shown clinical response, the present combination therapy methods are also applicable to diverse cancers. Examples of cancers that may be treated by these methods include liver cancer, bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, breast cancer, lung cancer, cutaneous or intraocular malignant melanoma, renal cancer, uterine cancer, ovarian cancer, colorectal cancer, colon cancer, rectal cancer, cancer of the anal region, stomach cancer, testicular cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, non-Hodgkin's lymphoma, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, solid tumors of childhood, cancer of the bladder, cancer of the kidney or ureter, carcinoma of the renal pelvis, neoplasm of the CNS, primary CNS lymphoma, tumor angiogenesis, spinal axis tumor, brain stem glioma, pituitary adenoma, Kaposi's sarcoma, epidermoid cancer, squamous cell cancer, environmentally induced cancers including those induced by asbestos, hematologic malignancies including, for example, multiple myeloma, B-cell lymphoma, Hodgkin lymphoma/primary mediastinal B-cell lymphoma, non-Hodgkin's lymphomas, acute myeloid lymphoma, chronic myelogenous leukemia, chronic lymphoid leukemia, lymphocytic lymphoma, follicular lymphoma, diffuse large B-cell lymphoma, Burkitt's lymphoma, immunoblastic large cell lymphoma, precursor B-lymphoblastic lymphoma, mantle cell lymphoma, acute lymphoblastic leukemia, mycosis fungoides,

anaplastic large cell lymphoma, T-cell lymphoma, and precursor T-lymphoblastic lymphoma, and any combinations of said cancers. The present invention is also applicable to treatment of metastatic, refractory or recurring cancers. In preferred embodiments, the cancer to be treated is chosen from MEL, RCC, squamous NSCLC, non-squamous NSCLC, CRC, CRPC, OV, GC, HCC, PC, squamous cell carcinoma of the head and neck, carcinomas of the esophagus, gastrointestinal tract and breast, and a hematological malignancy. In more preferred embodiments, the cancer is MEL.

In certain embodiments, the subject has been pre-treated for the cancer. For example, the patient may have been treated with 1 or 2 or more prior systemic regimens of the type described herein as standard-of-care therapeutics. In certain other embodiments, the cancer is an advanced, recurring, metastatic and/or refractory cancer. In preferred embodiments, the concurrent or sequenced regimen treatment induces a durable clinical response in the subject. In preferred embodiments, the subject is a human, the anti-PD-1 Ab inhibits human PD-1, and the anti-CTLA-4 Ab inhibits human CTLA-4.

The anti-PD-1 Ab used in the present methods may be any therapeutic anti-PD-1 Ab of the invention. In preferred embodiments, the anti-PD-1 Ab is a mAb, which may be a chimeric, humanized or human Ab. In certain embodiments, the anti-PD-1 Ab comprises the CDR1, CDR2 and CDR3 domains in the heavy chain variable region and the CDR1, CDR2 and CDR3 domains in the light chain variable region of 17D8, 2D3, 4Hl, 5C4 (nivolumab), 4A11, 7D3 or 5F4, respectively, as described and characterized in U.S. Patent No. 8,008,449. In further embodiments, the anti-PD-1 Ab comprises the heavy and light chain variable regions of 17D8, 2D3, 4H1, 5C4 (nivolumab), 4A11, 7D3 or 5F4, respectively. In additional embodiments, the anti-PD-1 Ab is 17D8, 2D3, 4H1, 5C4 (nivolumab), 4A11, 7D3 or 5F4. In preferred embodiments, the anti-PD-1 Ab is nivolumab.

Anti-CTLA-4 antibodies of the instant invention bind to human CTLA-4 so as to disrupt the interaction of CTLA-4 with a human B7 receptor. Because the interaction of CTLA-4 with B7 transduces a signal leading to inactivation of T-cells bearing the CTLA-4 receptor, disruption of the interaction effectively induces, enhances or prolongs the activation of such T cells, thereby inducing, enhancing or prolonging an immune response. Anti-CTLA-4 Abs are described in, for example, U.S. Patent Nos. 6,051,227, 7,034,121 in PCT Application Publication Nos. WO 00/37504 and WO 01/14424. An exemplary clinical anti-CTLA-4 Ab is the human mAb 10D1 (now known as ipilimumab

and marketed as YERVOY®) as disclosed in U.S. Patent No. 6,984,720. In certain aspects of any of the present methods, the anti-CTLA-4 Ab is a mAb. In certain other embodiments, the anti-CTLA-4 antibody is a chimeric, humanized or human antibody. In preferred embodiments, the anti-CTLA-4 antibody is ipilimumab.

This disclosure also provides the use of an anti-PD-1 Ab or an antigen-binding portion thereof in combination with an anti-CTLA-4 Ab or an antigen-binding portion thereof for the preparation of a co-administered medicament for treating a subject afflicted with a cancer in a concurrent regimen, wherein the anti-PD-1 and anti-CTLA-4 Abs are each administered at a dosage ranging from 0.1 to 20.0 mg/kg body weight, and further wherein the concurrent regimen comprises: (i) an induction dosing schedule comprising combined administration of the anti-PD-1 and anti-CTLA-4 Abs at a dosing frequency of at least once every 2, 3 or 4 weeks, or at least once a month, for at least 2, 4, 6, 8 or 12 doses, followed by administration of the anti-PD-1 Ab alone at a dosing frequency of at least once every 2, 3 or 4 weeks, or at least once a month, for at least 2, 4, 6, 8 or 10 doses; followed by (ii) a maintenance dosing schedule comprising combined administration of the anti-PD-1 and anti-CTLA-4 Abs at a dosing frequency of at least once every 8, 12 or 16 weeks, or at least once a quarter, for up to 4, 6, 8, 10, 12 or 16 doses, or for as long as clinical benefit is observed, or until unmanageable toxicity or disease progression occurs. The disclosure provides uses of the combination of anti-PD-1 and anti-CTLA-4 Abs for the preparation of co-administered medicaments corresponding to all the embodiments of the methods of treatment employing these Abs described herein, and are broadly applicable to the full range of cancers disclosed herein.

The disclosure also provides an anti-PD-1 Ab or an antigen-binding portion thereof for use in combination with an anti-CTLA-4 Ab or an antigen-binding portion thereof for treating a subject afflicted with a cancer in a concurrent regimen, wherein the anti-PD-1 and anti-CTLA-4 Abs are each administered at a dosage ranging from 0.1 to 20.0 mg/kg body weight, and further wherein the concurrent regimen comprises: (i) an induction dosing schedule comprising combined administration of the anti-PD-1 and anti-CTLA-4 antibodies at a dosing frequency of at least once every 2, 3 or 4 weeks, or at least once a month, for at least 2, 4, 6, 8 or 12 doses, followed by administration of the anti-PD-1 alone at a dosing frequency of at least once every 2, 3 or 4 weeks, or at least once a month, for at least 2, 4, 6, 8 or 10 doses; followed by (ii) a maintenance dosing schedule comprising combined administration of the anti-PD-1 and anti-CTLA-4 antibodies at a

dosing frequency of at least once every 8, 12 or 16 weeks, or at least once a quarter, for up to 4, 6, 8, 10, 12 or 16 doses, or for as long as clinical benefit is observed, or until unmanageable toxicity or disease progression occurs.

Methods for screening a patient population and selecting a patient as suitable for immunotherapy with the combination of anti-PD-1 and anti-CTLA-4 using the concurrent or sequenced regimens, and methods of predicting efficacy of the Ab combination, based on a PD-L1 biomarker assay are performed as described for anti-PD-1 monotherapy, to the extent this biomarker is applicable to combination therapy with anti-PD-1 and anti-CTLA-4 Abs.

The disclosure additionally provides a kit for treating a subject afflicted with a cancer, the kit comprising: (a) a dosage ranging from 0.1 to 20.0 mg/kg body weight of an Ab or an antigen-binding portion thereof that specifically binds to and inhibits PD-1; (b) a dosage ranging from 0.1 to 20.0 mg/kg of an Ab or an antigen-binding portion thereof that specifically binds to and inhibits CTLA-4; and (c) instructions for using the combination of the anti-PD-1 and anti-CTLA-4 antibodies in any of the concurrent regimen methods. In certain embodiments, some of the dosages of the anti-PD-1 and anti-CTLA-4 Abs are admixed within a single pharmaceutical formulation for concurrent administration. In other embodiments, the dosages of the anti-PD-1 and anti-CTLA-4 Abs are formulated as separate compositions with each Ab in a pharmaceutically acceptable formulation.

The disclosure further provides a kit for treating a subject afflicted with a cancer, the kit comprising: (a) a dosage ranging from 0.1 to 20.0 mg/kg body weight of an Ab or an antigen-binding portion thereof that specifically binds to and inhibits PD-1; and (b) instructions for using the anti-PD-1 Ab in any of the sequenced regimen methods.

A combined PD-1 and CTLA-4 blockade may also be further combined with standard cancer treatments. For example, a combined PD-1 and CTLA-4 blockade may be effectively combined with chemotherapeutic regimes, for example, further combination with dacarbazine or IL-2, for the treatment of MEL. In these instances, it may be possible to reduce the dose of the chemotherapeutic reagent. The scientific rationale behind the combined use of PD-1 and CTLA-4 blockade with chemotherapy is that cell death, which is a consequence of the cytotoxic action of most chemotherapeutic compounds, should result in increased levels of tumor antigen in the antigen presentation pathway. Other combination therapies that may result in synergy with a combined PD-1 and CTLA-4

blockade through cell death include radiation, surgery, or hormone deprivation. Each of these protocols creates a source of tumor antigen in the host. Angiogenesis inhibitors may also be combined with a combined PD-1 and CTLA-4 blockade. Inhibition of angiogenesis leads to tumor cell death, which may also be a source of tumor antigen to be fed into host antigen presentation pathways.

*Immunotherapy of Cancer Patients Using an Anti-PD-L1 Antibody*

PD-L1 is the primary PD-1 ligand up-regulated within solid tumors, where it can inhibit cytokine production and the cytolytic activity of PD-1-positive, tumor-infiltrating $CD4^+$ and $CD8^+$ T-cells, respectively (Dong *et al.*, 2002; Hino *et al.*, 2010; Taube *et al.*, 2012). These properties make PD-L1 a promising target for cancer immunotherapy. The clinical trials of anti-PD-L1 immunotherapy described in the Examples demonstrate for the first time that mAb blockade of the immune inhibitory ligand, PD-L1, produces both durable tumor regression and prolonged ($\geq$ 24 weeks) disease stabilization in patients with metastatic NSCLC, MEL, RCC and OV, including those with extensive prior therapy. The human anti-PD-L1 HuMAb, BMS-936559, had a favorable safety profile overall at doses up to and including 10 mg/kg, as is evident from the low (9%) incidence of grade 3-4 drug-related AEs. These findings are consistent with the mild autoimmune phenotype seen in PD-$L1^{-/-}$ mice (Dong *et al.*, 2004) and the more severe hyperproliferation seen in CTLA-$4^{-/-}$ mice relative to PD-$1^{-/-}$ mice (Phan *et al.*, 2003; Tivol *et al.*, 1995; Nishimura *et al.*, 1999). Most of the toxicities associated with anti-PD-L1 administration in patients were immune-related, suggesting on-target effects. The spectrum and frequency of adverse events of special interest (AEOSIs) is somewhat different between anti-PD-L1 and anti-CTLA-4, emphasizing the distinct biology of these pathways (Ribas *et al.*, 2005). Infusion reactions were observed with BMS-936559, although they were mild in most patients. Severe colitis, a drug-related AE observed in ipilimumab-treated patients (Beck *et al.*, 2006), was infrequently noted with anti-PD-L1.

As noted above for anti-PD-1 immunotherapy, another important feature of anti-PD-L1 therapy is the durability of responses across multiple tumor types. This is particularly notable considering the advanced disease and prior treatment of patients on the current study. Although not compared directly, this durability appears greater than that observed with most chemotherapies and kinase inhibitors used to treat these cancers.

Because peripheral blood T-cells express PD-L1, it is possible to assess *in vivo* RO by BMS-963559 as a pharmacodynamic measure. Median RO was 65.8%, 66.2%,

and 72.4% for the doses tested. Whereas these studies provide a direct assessment and evidence of target engagement in patients treated with BMS-936559, relationships between RO in peripheral blood and the tumor microenvironment remain poorly understood.

Based on the clinical data disclosed herein, this disclosure provides a method for immunotherapy of a subject afflicted with cancer, which method comprises administering to the subject a composition comprising a therapeutically effective amount of an anti-PD-L1 Ab of the invention or an antigen-binding portion thereof. The disclosure also provides a method of inhibiting growth of tumor cells in a subject, comprising administering to the subject an anti-PD-L1 Ab of the invention or an antigen-binding portion thereof. In preferred embodiments, the subject is a human. In certain embodiments, the Ab or antigen-binding portion thereof is of an IgG1 or IgG4 isotype. In other embodiments, the Ab or antigen-binding portion thereof is a mAb or an antigen-binding portion thereof. In further embodiments, the Ab or antigen-binding portion thereof is a chimeric, humanized or human Ab or an antigen-binding portion thereof. In preferred embodiments for treating a human patient, the Ab or antigen-binding portion thereof is a human Ab or an antigen-binding portion thereof.

Clinical trials described in the Examples employed the anti-PD-L1 HuMAb BMS-936559 to treat cancer. While BMS-936559 (designated HuMAb 12A4 in U.S. Patent No. 7,943,743) was selected as the lead anti-PD-L1 Ab for entering the clinic, it is notable that several anti-PD-L1 Abs of the invention share with 12A4 functional properties that are important to the therapeutic activity of 12A4, including high affinity binding specifically to human PD-L1, increasing T-cell proliferation, IL-2 secretion and interferon-γ production in an MLR assay, inhibiting the binding of PD-L1 to PD-1, and reversing the suppressive effect of T regulatory cells on T cell effector cells and/or dendritic cells. Moreover, certain of the anti-PD-L1 Abs of the invention, namely 1B12, 7H1 and 12B7 are structurally related to 12A4 in comprising $V_H$ and $V_\kappa$ regions that have sequences derived from $V_H$ 1-69 and $V_\kappa$ L6 germline sequences, respectively. In addition, at least 12B7, 3G10, 1B12 and 13G4 cross-compete with 12A4 for binding to the same epitope region of hPD-L1, whereas 5F8 and 10A5 may bind to the same or an overlapping epitope region as 12A4 (Examples 2 and 3). Thus, the preclinical characterization of 12A4 and other anti-PD-L1 HuMabs indicate that the methods of treating cancer provided herein may be performed using any of the broad genus of anti-

PD-L1 Abs of the invention.

Accordingly, this disclosure provides immunotherapy methods comprising administering to a patient an anti-PD-L1 Ab or antigen-binding portion thereof comprising (a) a heavy chain variable region that comprises consecutively linked amino acids having a sequence derived from a human $V_H$ 1-18 germline sequence, and a light chain variable region that comprises consecutively linked amino acids having a sequence derived from a human $V_\kappa$ L6 germline sequence; (b) a heavy chain variable region that comprises consecutively linked amino acids having a sequence derived from a human $V_H$ 1-69 germline sequence, and a light chain variable region that comprises consecutively linked amino acids having a sequence derived from a human $V_\kappa$ L6 germline sequence; (c) a heavy chain variable region that comprises consecutively linked amino acids having a sequence derived from a human $V_H$ 1-3 germline sequence, and a light chain variable region that comprises consecutively linked amino acids having a sequence derived from a human $V_\kappa$ L15 germline sequence; (d) a heavy chain variable region that comprises consecutively linked amino acids having a sequence derived from a human $V_H$ 1-69 germline sequence, and a light chain variable region that comprises consecutively linked amino acids having a sequence derived from a human $V_\kappa$ A27 germline sequence; (e) a heavy chain variable region that comprises consecutively linked amino acids having a sequence derived from a human $V_H$ 3-9 germline sequence, and a light chain variable region that comprises consecutively linked amino acids having a sequence derived from a human $V_\kappa$ L15germline sequence; or (f) a heavy chain variable region that comprises consecutively linked amino acids having a sequence derived from a human $V_H$ 3-9 germline sequence, and a light chain variable region that comprises consecutively linked amino acids having a sequence derived from a human $V_\kappa$ L18 germline sequence.

In certain embodiments, the anti-PD-L1 Ab or antigen-binding portion thereof administered to the patient cross-competes for binding to PD-L1 with a reference Ab or a reference antigen-binding portion thereof comprising: (a) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 15 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 25; (b) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 16 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 26; (c) a

human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 17 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 27; (d) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 18 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 28; (e) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 19 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 29; (f) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 20 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 30; (g) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 21 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEO ID NO: 31; (h) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 22 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 32; (i) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 23 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEO ID NO: 33; or (j) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 24 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 34. In preferred embodiments, the Ab or antigen-binding portion thereof cross-competes for binding to PD-1 with a reference Ab or reference antigen-binding portion thereof comprising a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 16 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 26.

In certain preferred embodiments of the immunotherapy methods disclosed herein, the anti-PD-L1 Ab or antigen-binding portion thereof administered to the subject

comprises: (a) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 15 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 25; (b) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 16 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 26; (c) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 17 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 27; (d) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 18 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 28; (e) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 19 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 29; (f) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 20 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 30; (g) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 21 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEO ID NO: 31; (h) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 22 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 32; (i) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 23 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEO ID NO: 33; or (j) a human heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 24 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 34. In more preferred embodiments, the anti-PD-L1 Ab or antigen-binding portion comprises a human heavy chain variable

region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 16 and a human light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 26.

*Broad spectrum of cancers treatable by anti-PD-L1 immunotherapy*

The clinical activity of anti-PD-L1 in patients with advanced NSCLC, similar to the activity of anti-PD-1 in these patients, was surprising and unexpected since NSCLC has been considered to be poorly responsive to immune-based therapies (Holt and Disis, 2008; Holt *et al.*, 2011). The present clinical data obtained with BMS-936559, an anti-PD-L1 Ab of the invention, substantiate and extend the evidence obtained using the anti-PD-1 Ab that immunotherapy based on PD-1 blockade is not applicable only to "immunogenic" tumor types, such as MEL and RCC, but is also effective with a broad range of cancers, including treatment-refractory metastatic NSCLC, that are generally not considered to be immune-responsive. Preferred cancers that may be treated using the anti-PD-L1 Abs of the invention include MEL (*e.g.*, metastatic malignant melanoma), RCC, squamous NSCLC, non-squamous NSCLC, CRC, ovarian cancer (OV), gastric cancer (GC), breast cancer (BC), pancreatic carcinoma (PC) and carcinoma of the esophagus. Additionally, the invention includes refractory or recurrent malignancies whose growth may be inhibited using the anti-PD-L1 Abs of the invention.

Accordingly, examples of cancers that may be treated using an anti-PD-L1 Ab in the methods of the invention, based on the indications of very broad applicability of anti-PD-L1 immunotherapy provided herein, include bone cancer, skin cancer, cancer of the head or neck, breast cancer, lung cancer, cutaneous or intraocular malignant melanoma, renal cancer, uterine cancer, castration-resistant prostate cancer, colon cancer, rectal cancer, cancer of the anal region, stomach cancer, testicular cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, carcinomas of the ovary, gastrointestinal tract and breast, Hodgkin's Disease, non-Hodgkin's lymphoma, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, chronic or acute leukemias including acute myeloid leukemia, chronic myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, solid tumors of childhood, lymphocytic lymphoma, cancer of the bladder, cancer of the kidney or ureter, carcinoma of the renal pelvis, neoplasm of the

central nervous system (CNS), primary CNS lymphoma, tumor angiogenesis, spinal axis tumor, brain stem glioma, pituitary adenoma, Kaposi's sarcoma, epidermoid cancer, squamous cell cancer, T-cell lymphoma, multiple myeloma, environmentally induced cancers including those induced by asbestos, metastatic cancers, and any combinations of said cancers. The present invention is also applicable to treatment of metastatic cancers.

*Combination therapy with anti-PD-L1 Abs*

Optionally, Abs to PD-L1 can be combined with an immunogenic agent, for example a preparation of cancerous cells, purified tumor antigens (including recombinant proteins, peptides, and carbohydrate molecules), antigen-presenting cells such as dendritic cells bearing tumor-associated antigens, and cells transfected with genes encoding immune stimulating cytokines (He *et al.*, 2004). Non-limiting examples of tumor vaccines that can be used include peptides of melanoma antigens, such as peptides of gp100, MAGE antigens, Trp-2, MART1 and/or tyrosinase, or tumor cells transfected to express the cytokine GM-CSF. PD-L1 blockade may also be effectively combined with standard cancer treatments, including chemotherapeutic regimes, radiation, surgery, hormone deprivation and angiogenesis inhibitors, as well as another immunotherapeutic Ab (*e.g.*, an anti-PD-1, anti-CTLA-4 or anti-LAG-3 Ab).

*Uses of anti-PD-L1 Abs*

This disclosure provides the use of any anti-PD-L1 Ab or antigen-binding portion thereof of the invention for the preparation of a medicament for inhibiting signaling from the PD-1/PD-L1 pathway so as to thereby potentiate an endogenous immune response in a subject afflicted with cancer. This disclosure also provides the use of any anti-PD-L1 Ab or antigen-binding portion thereof of the invention for the preparation of a medicament for immunotherapy of a subject afflicted with cancer comprising disrupting the interaction between PD-1 and PD-L1. The disclosure provides medical uses of any anti-PD-L1 Ab or antigen-binding portion thereof of the invention corresponding to all the embodiments of the methods of treatment employing an anti-PD-L1 Ab described herein.

This disclosure also provides an anti-PD-L1 Ab or an antigen-binding portion thereof of the invention for use in treating a subject afflicted with cancer comprising potentiating an endogenous immune response in a subject afflicted with cancer by inhibiting signaling from the PD-1/PD-L1 pathway. The disclosure further provides an anti-PD-L1 Ab or an antigen-binding portion thereof of the invention for use in

immunotherapy of a subject afflicted with cancer comprising disrupting the interaction between PD-1 and PD-L1. These Abs may be used in potentiating an endogenous immune response against, or in immunotherapy of, the full range of cancers disclosed herein. In preferred embodiments, the cancers include MEL (*e.g.*, metastatic malignant MEL), RCC, squamous NSCLC, non-squamous NSCLC, CRC, ovarian cancer (OV), gastric cancer (GC), breast cancer (BC), pancreatic carcinoma (PC) and carcinoma of the esophagus.

*Validation of Cancer Immunotherapy by Immune Checkpoint Blockade*

A major implication of the clinical activity of immune checkpoint blockade is that significant endogenous immune responses to tumor antigens are generated and these responses may be harnessed therapeutically to mediate clinical tumor regression upon checkpoint inhibition. In fact, there is evidence that inhibitory ligands such as PD-L1 are induced in response to immune attack, a mechanism termed adaptive resistance (Gajewski *et al.*, 2010; Taube *et al.*, 2012). This potential mechanism of immune resistance by tumors suggests that PD-1/PD-L1-directed therapy might synergize with other treatments that enhance endogenous antitumor immunity. Follow-up studies have verified that patients continue to demonstrate tumor control after cessation of PD-1/PD-L1 pathway blockade (Lipson *et al.*, 2013). Such tumor control may reflect a persistent antitumor immune response and the generation of effective immunologic memory to enable sustained control of tumor growth.

The data disclosed herein on the clinical testing of Abs that block the immunoregulatory receptor, PD-1, and also of Abs that block one of its cognate ligands, PD-L1, are unprecedented. These data constitute the largest clinical experience to date with PD-1 pathway-directed cancer immunotherapy, and the first report specifically describing the safety, tolerability, and initial clinical activity of an anti-PD-L1-directed agent. These findings show that both anti-PD-1 and anti-PD-L1 have favorable overall safety profiles and provide clear evidence of clinical activity across diverse cancers, including NSCLC, a tumor not historically considered responsive to immunotherapy, as well as tumors known to respond to immunotherapy, including MEL, RCC and OV. Thus, these data strongly validate the PD-1/PD-L1 pathway as an important target for therapeutic intervention in cancer.

The remarkable similarities observed between the patterns of clinical activity obtained with the anti-PD-1 and anti-PD-L1 mAbs, and among the tumor types analyzed

to date, validate the general importance of the PD-1/PD-L1 signaling pathway in tumor immune resistance and as a target for therapeutic intervention. Although the molecular interactions blocked by these two Abs are not identical, it has been clearly demonstrated herein that, irrespective of mechanistic details, both anti-PD-1 and anti-PD-L1 Abs of the invention are effective in treating patients afflicted with a wide variety of cancers.

*Infectious Diseases*

Other methods of the invention are used to treat patients that have been exposed to particular toxins or pathogens. For example, another aspect of this disclosure provides a method of treating an infectious disease in a subject comprising administering to the subject an anti-PD1 or an anti-PD-L1 Ab, or antigen-binding portion thereof, of the invention such that the subject is treated for the infectious disease. Preferably, the Ab is a human anti-human PD-1 or PD-L1 Ab (such as any of the human Abs described herein). Alternatively, the Ab is a chimeric or humanized Ab.

Similar to its application to tumors as discussed above, Ab-mediated PD-1 or PD-L1 blockade can be used alone, or as an adjuvant, in combination with vaccines, to potentiate an immune response to pathogens, toxins, and/or self-antigens. Examples of pathogens for which this therapeutic approach may be particularly useful include pathogens for which there is currently no effective vaccine, or pathogens for which conventional vaccines are less than completely effective. These include, but are not limited to HIV, Hepatitis (A, B, and C), Influenza, Herpes, Giardia, Malaria, Leishmania, Staphylococcus aureus, Pseudomonas aeruginosa. PD-1 and/or PD-L1 blockade is particularly useful against established infections by agents such as HIV that present altered antigens over the course of an infection. Novel epitopes on these antigens are recognized as foreign at the time of anti-human PD-1 or PD-L1 administration, thus provoking a strong T cell response that is not dampened by negative signals through the PD-1/PD-L1 pathway.

In the above methods, PD-1 or PD-L1 blockade can be combined with other forms of immunotherapy such as cytokine treatment (*e.g.*, administration of interferons, GM-CSF, G-CSF or IL-2).

*Kits*

Also within the scope of the present invention are kits, including pharmaceutical kits, comprising an anti-PD-1 and/or an anti-PD-L1 Ab of the invention, or a combination of an anti-PD-1 and an anti-CTLA-4 Ab, for therapeutic uses, and diagnostic kits

comprising an anti-PD-L1 Ab of the invention for assaying membranous PD-L1 expression as a biomarker for screening patients for immunotherapy or for predicting the efficacy of an immunotherapeutic agent. Kits typically include a label indicating the intended use of the contents of the kit and instructions for use. The term label includes any writing, or recorded material supplied on or with the kit, or which otherwise accompanies the kit. In certain embodiments of a pharmaceutical kit, the anti-PD-1 and/or anti-PD-L1 Abs may be co-packaged with other therapeutic agents in unit dosage form. In certain embodiments of a diagnostic kit, the anti-PD-L1 Ab may be co-packaged with other reagents for performing an assay to detect and/or quantify PD-L1 expression.

In certain preferred embodiments, the pharmaceutical kit comprises the anti-human PD-1 HuMAb, nivolumab. In other preferred embodiments, the pharmaceutical kit comprises the anti-human PD-L1 HuMAb, BMS-936559. In yet other preferred embodiments, the pharmaceutical kit comprises the anti-human CTLA-4 HuMAb, ipilimumab. In certain preferred embodiments, the diagnostic kit comprises the rabbit anti-human PD-L1 mAb, 28-8, comprising the $V_H$ and $V_\kappa$ regions whose amino acid sequences are set forth in SEQ ID NOs. 35 and 36, respectively. In other preferred embodiments, the diagnostic kit comprises the murine anti-human PD-L1 mAb, 5H1 (Dong *et al.*, 2002).

*PD-L1 Biomarker for Predicting anti-PD-1 Efficacy*

A particular challenge in cancer immunotherapy has been the identification of mechanism-based predictive biomarkers to enable patient selection and guide on-treatment management. Data disclosed in the Examples below indicate that cell surface PD-L1 expression in tumors is a useful molecular marker for predicting the efficacy of, and selecting patients for, immunotherapy with anti-PD-1 and potentially other immune checkpoint inhibitors.

There are conflicting reports in the literature about the clinical implications of PD-L1 being expressed in tumors. Several studies have concluded that PD-L1 expression in tumors correlates with a poor prognosis for the patient. *See, e.g.*, Hino *et al.* (2010) (MEL); Hamanishi *et al.* (2007) (OV); Thompson *et al.* (2006) (RCC). These findings may be rationalized on the basis that the interaction of PD-L1 on tumor cells and PD-1 on T cells helps abrogate immune responses directed against the tumor, resulting in immune evasion from tumor-specific T cells (Blank *et al.*, 2005). However, in contrast to the foregoing studies, Gadiot *et al.* (2011) and Taube *et al.* (2012) have recently reported that

PD-L1 expression in melanoma tumors correlates with a trend toward better survival. These seemingly contradictory data may reflect the relatively small numbers of patients analyzed, different histologic subtypes studied, or different methodologies used, *e.g.*, the use of different Abs to stain PD-L1, the use of frozen versus paraffin-embedded material for IHC, and the detection of membranous and/or cytoplasmic staining of PD-L1. Taube *et al.* (2012) note that PD-L1 is a type I transmembrane molecule, and hypothesize that while the cytoplasmic presence of PD-L1 may represent intracellular stores of this polypeptide that may be deployed to the cell surface upon appropriate stimulation, it is cell surface PD-L1 expression of that is biologically relevant as a potential biomarker for predicting clinical response to PD-1 blockade. *See, also*, Brahmer *et al.* (2010), which describes preliminary evidence, obtained on a small sample size of only 9 patients, of a correlation between membranous PD-L1 expression and anti-PD-1 efficacy. The data described in the Examples below on the use of membranous PD-L1 expression as a biomarker for anti-PD-1 efficacy, which was obtained from analysis of a much larger sample, substantiate the hypothesis that PD-L1 expression may be used as a biomarker for predicting anti-PD-1 clinical response and for screening patients to identify suitable candidates for immunotherapy with an anti-PD-1 Ab. Though the utility of this biomarker was demonstrated for screening patients for, or predicting the clinical response to, anti-PD-1 immunotherapy, PD-L1 expression may also potentially be applicable more broadly as a companion biomarker for other types of inhibitors of inhibitory immunoregulators.

Specifically, membranous PD-L1 expression was assayed using an automated IHC protocol and a rabbit anti-hPD-L1 Ab. Strikingly, in the initial set of data analyzed (*see* Example 8), no patients with cell surface PD-L1-negative tumors (MEL, NSCLC, CRC, RCC and CRPC) experienced an OR following treatment with the anti-PD-1 Ab, nivolumab. In contrast, cell surface expression of PD-L1 on tumor cells in pretreatment biopsies may be associated with an increased rate of OR among patients treated with nivolumab. While tumor cell expression of PD-L1 may be driven by constitutive oncogenic pathways, it may also reflect "adaptive immune resistance" in response to an endogenous antitumor immune response, part of a host inflammatory response, which may remain in check unless unleashed by blockade of the PD-1/PD-L1 pathway (Taube *et al.*, 2012). This emerging concept of adaptive immune resistance in cancer immunology suggests that inhibitory ligands such as PD-L1 are induced in response to immune attack (Gajewski *et al.*, 2010; Taube *et al.*, 2012). A major implication of the clinical activity of

immune checkpoint blockade as described herein is that significant endogenous immune responses to tumor antigens are generated and these responses may be harnessed therapeutically to mediate clinical tumor regression upon checkpoint inhibition. This potential mechanism of immune resistance by tumors suggests that PD-1/PD-L1-directed therapy might synergize with other treatments that enhance endogenous antitumor immunity.

*Assaying Cell-Surface PD-L1 Expression by Automated IHC*

As described in the Examples, an automated IHC method was developed for assaying the expression of PD-L1 on the surface of cells in FFPE tissue specimens. This disclosure provides methods for detecting the presence of human PD-L1 antigen in a test tissue sample, or quantifying the level of human PD-L1 antigen or the proportion of cells in the sample that express the antigen, which methods comprise contacting the test sample, and a negative control sample, with a mAb that specifically binds to human PD-L1, under conditions that allow for formation of a complex between the Ab or portion thereof and human PD-L1. Preferably, the test and control tissue samples are FFPE samples. The formation of a complex is then detected, wherein a difference in complex formation between the test sample and the negative control sample is indicative of the presence of human PD-L1 antigen in the sample. Various methods are used to quantify PD-L1 expression.

In a particular embodiment, the automated IHC method comprises: (a) deparaffinizing and rehydrating mounted tissue sections in an autostainer; (b) retrieving antigen using a decloaking chamber and pH 6 buffer, heated to 110ºC for 10 min; (c) setting up reagents on an autostainer; and (d) running the autostainer to include steps of neutralizing endogenous peroxidase in the tissue specimen; blocking non-specific protein-binding sites on the slides; incubating the slides with primary Ab; incubating with a post-primary blocking agent; incubating with NovoLink Polymer; adding a chromogen substrate and developing; and counterstaining with hematoxylin.

For assessing PD-L1 expression in tumor tissue samples, a pathologist examines the number of membrane PD-L1$^+$ tumor cells in each field under a microscope and mentally estimates the percentage of cells that are positive, then averages them to come to the final percentage. The different staining intensities are defined as 0/negative, 1+/weak, 2+/moderate, and 3+/strong. Typically, percentage values are first assigned to the 0 and 3+ buckets, and then the intermediate 1+ and 2+ intensities are considered. For highly

heterogeneous tissues, the specimen is divided into zones, and each zone is scored separately and then combined into a single set of percentage values. The percentages of negative and positive cells for the different staining intensities are determined from each area and a median value is given to each zone. A final percentage value is given to the tissue for each staining intensity category: negative, 1+, 2+, and 3+. The sum of all staining intensities needs to be 100%.

Staining is also assessed in tumor-infiltrating inflammatory cells such as macrophages and lymphocytes. In most cases macrophages serve as an internal positive control since staining is observed in a large proportion of macrophages. While not required to stain with 3+ intensity, an absence of staining of macrophages should be taken into account to rule out any technical failure. Macrophages and lymphocytes are assessed for plasma membrane staining and only recorded for all samples as being positive or negative for each cell category. Staining is also characterized according to an outside/inside tumor immune cell designation. "Inside" means the immune cell is within the tumor tissue and/or on the boundaries of the tumor region without being physically intercalated among the tumor cells. "Outside" means that there is no physical association with the tumor, the immune cells being found in the periphery associated with connective or any associated adjacent tissue.

In certain embodiments of these scoring methods, the samples are scored by two pathologists operating independently and the scores are subsequently consolidated. In certain other embodiments, the identification of positive and negative cells is scored using appropriate software.

A histoscore is used as a more quantitative measure of the IHC data. The histoscore is calculated as follows:

Histoscore = [(% tumor x 1 (low intensity)) + (% tumor x 2 (medium intensity)) + (% tumor x 3 (high intensity)]

To determine the histoscore, the pathologist estimates the percentage of stained cells in each intensity category within a specimen. Because expression of most biomarkers is heterogeneous the histoscore is a truer representation of the overall expression. The final histoscore range is 0 (no expression) to 300 (maximum expression).

An alternative means of quantifying PD-L1 expression in a test tissue sample IHC is to determine the adjusted inflammation score (AIS) score defined as the density of

inflammation multiplied by the percent PD-L1 expression by tumor-infiltrating inflammatory cells (Taube *et al.*, 2012).

*Cancer Immunotherapy with Anti-PD-1 Comprising a Patient Selection Step*

This disclosure also provides a method for immunotherapy of a subject afflicted with cancer, which method comprises: (a) selecting a subject that is a suitable candidate for immunotherapy, *e.g.*, administration of an anti-PD-1 Ab, the selecting comprising (i) optionally providing a test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells, (ii) assessing the proportion of cells in the test tissue sample that express PD-L1 on the cell surface, and (iii) selecting the subject as a suitable candidate based on an assessment that the proportion of cells in the test tissue sample that express PD-L1 on the cell surface exceeds a predetermined threshold level; and (b) administering to the selected subject a composition comprising a therapeutically effective amount of an agent that inhibits signaling from an inhibitory immunoregulator *e.g.*, an anti-PD-1 Ab.

There is evidence that membranous PD-L1 expression is a surrogate for an endogenous antitumor immune response that is part of a host inflammatory response (Gajewski *et al.*, 2010; Taube *et al.*, 2012). Accordingly, cell surface expression of PD-L1 in tumors and/or inflammatory cells in the tumor microenvironment may be a marker not just for selecting cancer patients who would benefit from treatment with an anti-PD-1 Ab, but also for treatment with an anti-PD-L1 Ab as well as treatments targeting inhibitory immunoregulatory pathways other than the PD-1/PD-L1 pathway. For example, cell surface expression of PD-L1 in tumors and/or tumor-infiltrating inflammatory cells may be used as a marker for identifying or selecting suitable cancer patients who would benefit from immunotherapy with agents, including Abs, that target, and disrupt or inhibit signaling from, immune checkpoints such as PD-L1, Cytotoxic T-Lymphocyte Antigen-4 (CTLA-4), B and T Lymphocyte Attenuator (BTLA), T cell Immunoglobulin and Mucin domain-3 (TIM-3), Lymphocyte Activation Gene-3 (LAG-3), Killer Immunoglobulin-like Receptor (KIR), Killer cell Lectin-like Receptor G1 (KLRG-1), Natural Killer Cell Receptor 2B4 (CD244), and CD160 (Pardoll, 2012; Baitsch et al., 2012). In certain preferred embodiments, the inhibitory immunoregulator is a component of the PD-1/PD-L1 signaling pathway. In other preferred embodiments, the inhibitory immunoregulator is an anti-PD-1 Ab of the invention. In yet other preferred embodiments, the inhibitory immunoregulator is an anti-PD-L1 Ab of the invention.

Where any immunotherapy methods comprising assaying PD-L1 expression, *i.e.*, employing a PD-L1 expression biomarker, are described below as comprising the selection of a patient who is, or is not, suitable for anti-PD-1 immunotherapy, or as comprising the administration of an anti-PD-1 Ab for immunotherapeutic purposes, it should be understood that these methods apply more broadly to the selection of a patient who is, or is not, suitable for immunotherapy with, or to the administration of an inhibitor of, an inhibitory immunoregulator (*e.g.*, CTLA-4, BTLA, TIM3, LAG3 or KIR) or a component or ligand thereof. Further, in any the methods comprising the measurement of PD-L1 expression in a test tissue sample, it should be understood that the step comprising the provision of a test tissue sample obtained from a patient is an optional step. That is, in certain embodiments the method includes this step, and in other embodiments, this step is not included in the method. It should also be understood that in certain preferred embodiments the "assessing" step to identify, or determine the number or proportion of, cells in the test tissue sample that express PD-L1 on the cell surface is performed by a transformative method of assaying for PD-L1 expression, for example by performing a reverse transcriptase-polymerase chain reaction (RT-PCR) assay or an IHC assay. In certain other embodiments, no transformative step is involved and PD-L1 expression is assessed by, for example, reviewing a report of test results from a laboratory. In certain embodiments, the steps of the methods up to, and including, assessing PD-L1 expression provides an intermediate result that may be provided to a physician or other medical practitioner for use in selecting a suitable candidate for immunotherapy and/or administering an immunotherapeutic agent to the patient. In certain embodiments, the steps that provide the intermediate result may be performed by a medical practitioner or someone acting under the direction of a medical practitioner. In other embodiments, these steps are performed by an independent laboratory or by an independent person such as a laboratory technician.

The disclosure also provides a method for treatment of a subject afflicted with cancer, which method comprises: (a) selecting a subject that is not suitable for treatment with an agent that inhibits an inhibitory immunoregulator, *e.g.*, anti-PD-1 Ab immunotherapy, the selecting comprising (i) optionally providing a test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells; (ii) assessing the proportion of cells in the test tissue sample that express PD-L1 on the surface of the cells; and (iii) selecting the

subject as not suitable for immunotherapy with an inhibitor of an inhibitory immunoregulator, *e.g.*, an anti-PD-1 Ab, based on an assessment that the proportion of cells in the test tissue sample that express PD-L1 on the cell surface is less than a predetermined threshold level; and (b) administering a standard-of-care therapeutic other than an inhibitor of an inhibitory immunoregulator, *e.g.*, an anti-PD-1 Ab, to the selected subject.

*Measurement of PD-L1 expression*

In certain embodiments of any of the present methods, the proportion of cells that express PD-L1 is assessed by performing an assay to determine the presence of PD-L1 RNA. In further embodiments, the presence of PD-L1 RNA is determined by RT-PCR, *in situ* hybridization or RNase protection. In other embodiments, the proportion of cells that express PD-L1 is assessed by performing an assay to determine the presence of PD-L1 polypeptide. In further embodiments, the presence of PD-L1 polypeptide is determined by immunohistochemistry (IHC), enzyme-linked immunosorbent assay (ELISA), *in vivo* imaging, or flow cytometry. In preferred embodiments, PD-L1 expression is assayed by IHC. Flow cytometry may be particularly suitable for assaying PD-L1 expression in cells of hematologic tumors. In preferred embodiments of all of these methods, cell surface expression of PD-L1 is assayed using, *e.g.*, IHC or *in vivo* imaging.

Imaging techniques have provided important tools in cancer research and treatment. Recent developments in molecular imaging systems, including positron emission tomography (PET), single-photon emission computed tomography (SPECT), fluorescence reflectance imaging (FRI), fluorescence-mediated tomography (FMT), bioluminescence imaging (BLI), laser-scanning confocal microscopy (LSCM) and multiphoton microscopy (MPM), will likely herald even greater use of these techniques in cancer research. Some of these molecular imaging systems allow clinicians to not only see where a tumor is located in the body, but also to visualize the expression and activity of specific molecules, cells, and biological processes that influence tumor behavior and/or responsiveness to therapeutic drugs (Condeelis and Weissleder, 2010). Ab specificity, coupled with the sensitivity and resolution of PET, makes immunoPET imaging particularly attractive for monitoring and assaying expression of antigens in tissue samples (McCabe and Wu, 2010; Olafsen *et al.*, 2010). In certain embodiments of any of the present methods, PD-L1 expression is assayed by immunoPET imaging.

In certain embodiments of any of the present methods, the proportion of cells in a test tissue sample that express PD-L1 is assessed by performing an assay to determine the presence of PD-L1 polypeptide on the surface of cells in the test tissue sample. In certain embodiments, the test tissue sample is a FFPE tissue sample. In certain preferred embodiments, the presence of PD-L1 polypeptide is determined by IHC assay. In further embodiments, the IHC assay is performed using an automated process. In further embodiments, the IHC assay is performed using an anti-PD-L1 mAb to bind to the PD-L1 polypeptide.

*Abs that bind specifically to cell-surface-expressed PD-L1 in FFPE tissues*

An Ab may bind to an antigen in fresh tissues but completely fail to recognize the antigen in an FFPE tissue sample. This phenomenon, well known in the art, is thought to be due primarily to intra- and inter-molecular cross-linking of polypeptides induced by formalin fixation, which alters the epitope recognized by the Ab (Sompuram *et al.*, 2006). In addition, several factors known to influence staining in FFPE tissue, including variable time to fixation, inadequate fixation period, differences in fixative used, tissue processing, Ab clone and dilution, antigen retrieval, detection system, and interpretation of results using different threshold points are important variables that can affect tissue antigenicity and IHC measurements (Bordeaux *et al.*, 2010). In particular, a lack of anti-human PD-L1 Abs that stain PD-L1 in FFPE specimens has been noted in the art (Hamanishi *et al.*, 2007). Taube *et al.* (2012) and Gadiot *et al.* (2011) have also reported difficulties in identifying anti-PD-L1 Abs that bind specifically to PD-L1 in FFPE tissues, and inconsistent test results on the same Abs. Our own analysis of five commercially available anti-hPD-L1 Abs shows that these Abs failed to distinguish FFPE cells expressing PD-L1 from cells that did not express PD-L1 (see Example 9, Table 7). Thus, the contradictory results reported by different groups on the implications of PD-L1 expression for prognosis of a tumor may, in part, reflect the differential abilities of anti-PD-L1 Abs used to detect PD-L1 polypeptide in FFPE tissue samples. Accordingly, in order to detect hPD-L1 on the surface of cells using an IHC assay on FFPE tissues, there is a need for anti-hPD-L1 Abs that bind specifically to cell surface-expressed PD-L1 in FFPE tissue samples.

This disclosure provides a mAb or an antigen-binding portion thereof that binds specifically to a cell surface-expressed PD-L1 antigen in a FFPE tissue sample. In preferred embodiments, the mAb or antigen-binding portion thereof does not bind to a

cytoplasmic PD-L1 polypeptide in the FFPE tissue sample or exhibits a very low level of background binding. In certain other embodiments, the presence or absence of binding specifically to a cell surface-expressed or a cytoplasmic PD-L1 polypeptide is detected by immunohistochemical staining. In certain preferred aspects of the invention, the mAb or antigen-binding portion is a rabbit Ab or a portion thereof. In other preferred embodiments, the mAb is the rabbit mAb designated 28-8, 28-1, 28-12, 29-8 or 20-12. In more preferred embodiments, the mAb is the rabbit mAb designated 28-8 or an antigen-binding portion thereof. In further embodiments, the mAb is an Ab comprising a heavy chain variable region ($V_H$) comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 35 and a light chain variable region ($V_\kappa$) comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 36. In other embodiments, the mAb comprises the CDR1, CDR2 and CDR3 domains in a $V_H$ having the sequence set forth in SEQ ID NO: 35, and the CDR1, CDR2 and CDR3 domains in a $V_\kappa$ having the sequence set forth in SEQ ID NO: 36.

It is known in the art that rabbit Abs have certain advantages over murine Abs. For example, rabbit Abs generally exhibit more diverse epitope recognition, improved immune response to small-size epitopes, and higher specificity and affinity compared to murine Abs (*see, e.g.*, Fischer *et al.*, 2008; Cheang *et al.*, 2006; Rossi *et al.*, 2005). For example, the rabbit's lower immune dominance and larger B-cell repertoire results in greater epitope recognition compared to murine Abs. Further, the high specificity and novel epitope recognition of rabbit antibodies translates to success with recognition of post-translational modifications (Epitomics, 2013). In addition, many protein targets relevant to signal transduction and disease are highly conserved between mice, rats and humans, and can therefore be recognized as self-antigens by a mouse or rat host, making them less immunogenic. This problem is avoided by generating Abs in rabbits. Moreover, in applications in which two antigen-specific Abs are required, it is more convenient to have the Abs come from two different species. Thus, for example, it is easier to multiplex a rabbit Ab such as 28-8 with other Abs (likely to be murine Abs since the best immune marking Abs are murine Abs) that can mark immune cells that also express PD-L1 (*e.g.*, macrophages and lymphocytes). Accordingly, rabbit anti-hPD-L1 mAbs, *e.g.*, 28-8, are particularly suited to IHC assays for detecting surface-expressed PD-L1 in FFPE tissue samples and potentially have distinct advantages over murine Abs, such as 5H1.

As described in Example 9, a large number (185) of Ab multiclones from both

rabbit and mouse immunizations were screened, and only ten rabbit Ab, but no mouse Ab, multiclones specifically detected the membranous form of hPD-L1. After further extensive screening by multiple rounds of IHC, 15 purified rabbit subclones were selected based on their specificity and intensity of staining (*see* Table 5). Following further characterization of the antibodies to determine their binding affinity and cross-competition by surface plasmon resonance, as well as screening by IHC on FFPE tissues, mAb 28-8 was selected as the Ab with the best combination of binding to membranous PDF-L1 with high affinity and specificity, and low background staining.

In certain aspects of this invention, the mAb or antigen-binding portion cross-competes with mouse mAb 5H1 for binding to PD-L1, which indicates that these antibodies bind to the same epitope region of PD-L1. In certain other aspects, the mAb or antigen-binding portion thereof does not cross-compete with mouse mAb 5H1 for binding to PD-L1, indicating that they do not bind to the same epitope region of PD-L1.

The disclosure also provides nucleic acids encoding all of the rabbit anti-hPD-L1 Abs or portions thereof disclosed herein.

*Immunotherapeutic methods comprising measurement of cell surface PD-L1 expression*

The availability of rabbit Abs that bind with high affinity specifically to membranous PD-L1 in FFPE tissue specimens facilitates methods comprising a step of detecting PD-L1 polypeptide on the surface of cells in FFPE tissue samples. Accordingly, this disclosure also provides a method for immunotherapy of a subject afflicted with cancer, which method comprises: (a) selecting a subject that is a suitable candidate for immunotherapy, the selecting comprising: (i) optionally providing a FFPE test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells; (ii) assessing the proportion of cells in the test tissue sample that express PD-L1 on the cell surface by IHC using a rabbit anti-human PD-L1 Ab, *e.g.*, mAb 28-8, to bind to the PD-L1; and (iii) selecting the subject as a suitable candidate based on an assessment that the proportion of cells in the test tissue sample that express PD-L1 on the cell surface exceeds a predetermined threshold level; and (b) administering a composition comprising a therapeutically effective amount of an agent that inhibits an inhibitory immunoregulator, *e.g.*, an anti-PD-1 Ab, to the selected subject.

In certain embodiments of methods employing IHC to assay PD-L1 expression in FFPE tissues, an automated IHC assay is used. The automated IHC process is performed on an autostainer and comprises: (a) de-paraffinizing the FFPE sample with xylene and rehydrating the sample; (b) retrieving the antigen using a decloaking chamber; (c) blocking nonspecifc protein binding sites by incubation with a Protein Block; (d) incubating the sample with a primary anti-PD-L1 Ab; (e) adding a polymeric horseradish peroxidase (HRP)-conjugated secondary Ab; (f) detecting the bound secondary Ab comprising staining with a 3,3'-diaminobenzidine (DAB) chromogen; and/or (g) counterstaining with hematoxylin. This automated IHC process has been optimized by minimization of the number of steps, optimization of incubation times, and selection of primary Abs, blocking and detection reagents that produce strong specific staining with a low level of background staining. In preferred embodiments of this automated IHC assay, the primary anti-PD-L1 Ab is rabbit mAb 28-8 or murine mAb 5H1. In certain embodiments of this invention, this IHC assay, and any other IHC assay described herein to measure PD-L1 expression, may be used as part of a method of immunotherapy. In other embodiments, any of the IHC methods described herein is used independently of any therapeutic process requiring the administration of a therapeutic, *i.e.*, solely as a diagnostic method to assay PD-L1 expression.

In certain embodiments any of the immunotherapy methods described herein, the Ab administered to the selected subject is any anti-PD-1 or anti-PD-L1 Ab or antigen-binding portion thereof of the invention. In certain preferred embodiments, the subject is a human. In other preferred embodiments, the Ab is a human Ab or antigen-binding portion thereof. In more preferred embodiments, the anti-PD-1 Ab is nivolumab and the anti-PD-L1 Ab is BMS-936559. In certain other embodiments, the anti-PD-1 Ab is an Ab or antigen-binding portion thereof that cross-competes with nivolumab for binding to PD-1, and the anti-PD-L1 Ab is an Ab or antigen-binding portion thereof that cross-competes with BMS-936559 for binding to PD-L1. In certain preferred embodiments, the cancer to be treated is selected from the group consisting MEL, RCC, squamous NSCLC, non-squamous NSCLC, CRC, castration-resistant prostate cancer CRPC, HCC, squamous cell carcinoma of the head and neck, carcinomas of the esophagus, ovary, gastrointestinal tract and breast, and a hematological malignancy.

In certain embodiments of the disclosed methods, the predetermined threshold is based on a proportion of (a) tumor cells, (b) tumor-infiltrating inflammatory cells, (c)

particular tumor-infiltrating inflammatory cells, *e.g.*, TILs or macrophages, or (d) a combination of tumor cells and tumor-infiltrating inflammatory cells, in a test tissue sample that expresses PD-L1 on the cell surface. In certain embodiments, the predetermined threshold is at least 0.001% of tumor cells expressing membranous PD-L1 as determined by IHC. In other embodiments, the predetermined threshold is at least 0.01%, preferably at least 0.1%, more preferably at least 1% of tumor cells expressing membranous PD-L1, as determined by IHC. In certain embodiments, the predetermined threshold is at least 5% of tumor cells expressing membranous PD-L1 as determined by IHC. In certain embodiments, the predetermined threshold is at least 0.01%, at least 0.1%, at least 1%, or at least 5% of tumor cells expressing membranous PD-L1 as determined by IHC, and/or a single tumor-infiltrating inflammatory cell expressing membranous PD-L1 as determined by IHC. In certain other embodiments, the predetermined threshold is at least 0.01%, at least 0.1%, at least 1%, or at least 5% of a tumor-infiltrating inflammatory cell expressing membranous PD-L1 as determined by IHC. In certain other embodiments, the predetermined threshold is at least 0.01%, at least 0.1%, at least 1%, or at least 5% of a tumor-infiltrating lymphocyte expressing membranous PD-L1 as determined by IHC. In certain other embodiments, the predetermined threshold is at least 0.01%, at least 0.1%, at least 1%, or at least 5% of a tumor-infiltrating macrophage expressing membranous PD-L1 as determined by IHC. In yet other embodiments, the predetermined threshold is at least a single tumor cell or a single tumor-infiltrating inflammatory cell expressing membranous PD-L1 as determined by IHC. Preferably, PD-L1 expression is assayed by automated IHC using mAb 28-8 or 5H1 as the primary Ab.

This disclosure also provides a method for treatment of a subject afflicted with cancer, which method comprises: (a) screening a plurality of subjects to identify a subject that is not a suitable candidate for immunotherapy comprising the administration of an agent that inhibits an inhibitory immunoregulator, *e.g.*, an anti-PD-1 Ab, to the subject, the screening comprising: (i) optionally providing test tissue samples from the plurality of subjects, the test tissue samples comprising tumor cells and tumor-infiltrating inflammatory cells; (ii) assessing the proportion of cells in the test tissue samples that express PD-L1 on the surface of the cells; and (iii) selecting the subject as a candidate that is not suitable for immunotherapy based on an assessment that the proportion of cells that express PD-L1 on the surface of cells in the subject's test tissue sample is below a predetermined threshold level; and (b) administering a standard-of-care therapeutic other

than an agent that inhibits an inhibitory immunoregulator, *e.g.*, an anti-PD-1 Ab, to the selected subject.

This disclosure further provides a method for immunotherapy of a subject afflicted with cancer, which method comprises: (a) screening a plurality of subjects to identify a subject that is a suitable candidate for immunotherapy, the screening comprising: (i) optionally providing test tissue samples from the plurality of subjects, the test tissue samples comprising tumor cells and tumor-infiltrating inflammatory cells; (ii) assessing the proportion of cells in the test tissue samples that express PD-L1 on the surface of the cells; and (iii) selecting the subject as a candidate that is suitable for immunotherapy with an agent that inhibits an inhibitory immunoregulator, *e.g.*, an anti-PD-1 Ab, based on an assessment that the proportion of cells in the test tissue sample that express PD-L1 on the cell surface exceeds a predetermined threshold level; and (b) administering a composition comprising a therapeutically effective amount of said agent to the selected subject.

This disclosure additionally provides a method for treatment of a subject afflicted with cancer, which method comprises: (a) screening a plurality of subjects to identify a subject that is a suitable candidate for the treatment, the screening comprising: (i) optionally providing test tissue samples from the plurality of subjects, the test tissue samples comprising tumor cells and tumor-infiltrating inflammatory cells; (ii) assessing the proportion of cells in the test tissue samples that express PD-L1 on the surface of the cells, wherein the subject is identified as a suitable candidate for anti-PD-1 Ab immunotherapy if the proportion of cells in the tissue sample that express PD-L1 on the cell surface exceeds a predetermined threshold level, and the subject is identified as a candidate that is not a suitable candidate for anti-PD-1 Ab immunotherapy if the proportion of cells in the test tissue sample that express PD-L1 on the cell surface is below a predetermined threshold level; and (b) administering a composition comprising a therapeutically effective amount of an agent that inhibits an inhibitory immunoregulator, *e.g.*, an anti-PD-1 Ab, to the subject identified as a suitable candidate for anti-PD-1 Ab immunotherapy, or (c) administering a standard-of-care therapeutic other than said agent to the subject identified as not a suitable candidate for anti-PD-1 Ab immunotherapy.

One aspect of this invention is a method for immunotherapy of a subject afflicted with cancer, which method comprises: (a) optionally providing a test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor

cells and tumor-infiltrating inflammatory cells; (b) determining that the proportion of cells in the test tissue sample that express PD-L1 on the cell surface is above a predetermined threshold level; and (c) based on that determination administering a composition comprising a therapeutically effective amount of an agent that inhibits an inhibitory immunoregulator, *e.g.*, an anti-PD-1 Ab, to the subject. Another aspect of the invention is a method for treatment of a subject afflicted with cancer, which method comprises: (a) optionally providing a test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells; (b) determining that the proportion of cells in the test tissue sample that express PD-L1 on the cell surface is below a predetermined threshold level; and (c) based on that determination administering a standard-of-care therapeutic other than an agent that inhibits an inhibitory immunoregulator, *e.g.*, an anti-PD-1 Ab, to the subject.

Yet another aspect of the invention is a method for immunotherapy of a subject afflicted with cancer, which method comprises: (a) optionally providing a test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells; (b) determining the proportion of cells in the test tissue sample that express PD-L1 on the cell surface; (c) selecting an agent that inhibits an inhibitory immunoregulator, *e.g.*, an anti-PD-1 Ab, as a treatment for the subject based on the recognition that an anti-PD-1 Ab is effective in patients whose test tissue sample contains a proportion of cells above a predetermined threshold level that express PD-L1 on the cell surface; and (d) administering a composition comprising a therapeutically effective amount of said agent to the subject. In another embodiment, this disclosure provides a method for treatment of a subject afflicted with cancer, which method comprises: (a) optionally providing a test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells; (b) determining the proportion of cells in the test tissue sample that express PD-L1 on the cell surface; (c) selecting a standard-of-care therapeutic other than an agent that inhibits an inhibitory immunoregulator, *e.g.*, an anti-PD-1 Ab, as a treatment for the subject based on the recognition that said agent is ineffective in patients in whom the proportion of cells that express PD-L1 on the cell surface in the test tissue sample is below a predetermined threshold level; and (d) administering the standard-of-care therapeutic to the subject.

This disclosure also provides a method of selecting an immunotherapy for a subject afflicted with cancer, which method comprises: (a) assaying cells of a test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells to assess the proportion of cells in the test tissue sample that express PD-L1; and (b) based on an assessment that the proportion of cells that express membranous PD-L1 is above a predetermined threshold level, selecting an immunotherapy comprising a therapeutically effective amount of an agent that inhibits an inhibitory immunoregulator, *e.g.*, an anti-PD-1 Ab, for the subject. The disclosure further provides a method of selecting a treatment for a subject afflicted with cancer, which method comprises: (a) assaying cells of a test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells to assess the proportion of cells in the test tissue sample that express PD-L1; and (b) based on an assessment that the proportion of cells that express membranous PD-L1 is below a predetermined threshold level, selecting a standard-of-care treatment other than an agent that inhibits an inhibitory immunoregulator, *e.g.*, an anti-PD-1 Ab, for the subject.

In addition, this disclosure provides a method for treatment of a subject afflicted with cancer, which method comprises administering to the subject a composition comprising a therapeutically effective amount of an agent that inhibits an inhibitory immunoregulator, *e.g.*, an anti-PD-1 Ab, the subject having been selected on the basis that the proportion of cells in a test tissue sample from the subject that express PD-L1 is determined to exceed a predetermined threshold level, wherein the test tissue sample comprises tumor cells and tumor-infiltrating inflammatory cells. This disclosure also provides a method for treatment of a subject afflicted with cancer, which method comprises administering to the subject a standard-of-care treatment other than an agent that inhibits an inhibitory immunoregulator, *e.g.*, an anti-PD-1 Ab, the subject having been selected on the basis that the proportion of cells in a test tissue sample from the subject that express PD-L1 is determined to be below a predetermined threshold level, wherein the test tissue sample comprises tumor cells and tumor-infiltrating inflammatory cells.

This disclosure further provides a method for selecting a cancer patient for immunotherapy with an agent that inhibits an inhibitory immunoregulator, *e.g.*, an anti-PD-1 Ab, which method comprises: (a) optionally providing a test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells; (b) assaying the test tissue sample to determine the

proportion of cells therein that express PD-L1 on the cell surface; (c) comparing the proportion of cells that express PD-L1 on the cell surface with a predetermined threshold proportion; and (d) selecting the patient for immunotherapy based on an assessment that the proportion of cells in the test tissue sample that express surface PD-L1 is above the predetermined threshold level.

In any of the methods described herein comprising a step for assessing PD-L1 expression, the test tissue sample may be a FFPE tissue sample and PD-L1 polypeptide on the cell surface is detected by IHC using an anti-PD-L1 Ab, *e.g.*, mAb 28-8 or 5H1.

In addition, in any method where an immunotherapy is selected or administered based on an assessment that the proportion of cells in a test tissue sample from the subject expresses PD-L1 at a level above a predetermined threshold level, it follows that a complementary method of treatment may be performed wherein a standard-of-care treatment other than the immunotherapy is selected or administered based on an assessment that the proportion of cells in a test tissue sample from the subject expresses PD-L1 at a level below the predetermined threshold level.

This disclosure further provides a method for predicting the therapeutic effectiveness of an agent that inhibits an inhibitory immunoregulator, *e.g.*, an anti-PD-1 Ab, for treating a cancer patient, which method comprises: (a) optionally providing a test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells; (b) assaying the test tissue sample to determine the proportion of cells therein that express PD-L1 on the cell surface; (c) comparing the proportion of cells that express PD-L1 on the cell surface with a predetermined threshold value; and (d) predicting the therapeutic effectiveness of said agent, wherein if the proportion of cells that express PD-L1 on the cell surface exceeds the threshold proportion the agent is predicted to be effective in treating the patient, and wherein if the proportion of cells that express PD-L1 on the cell surface is below the threshold proportion the agent is predicted to not be effective in treating the patient.

This disclosure also provides a method for determining an immunotherapeutic regimen comprising an agent that inhibits an inhibitory immunoregulator, *e.g.*, an anti-PD-1 Ab, for treating a cancer patient, which method comprises: (a) optionally providing a test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells; (b) assaying the test tissue sample to determine the proportion of cells therein that express PD-L1 on the

cell surface; (c) comparing the proportion of cells that express PD-L1 on the cell surface with a predetermined threshold proportion; and (d) determining an immunotherapeutic regimen comprising said agent based on the determination that the proportion of cells that express PD-L1 on the cell surface exceeds the predetermined threshold proportion.

*Standard-of-Care Therapeutics*

Several of the methods of treatment described herein comprise the administration of a standard-of-care therapeutic to a patient. As used herein, a "standard-of-care therapeutic" is a treatment process, including a drug or combination of drugs, radiation therapy (RT), surgery or other medical intervention that is recognized by medical practitioners as appropriate, accepted, and/or widely used for a certain type of patient, disease or clinical circumstance. Standard-of-care therapies for different types of cancer are well known by persons of skill in the art. For example, the National Comprehensive Cancer Network (NCCN), an alliance of 21 major cancer centers in the USA, publishes the NCCN Clinical Practice Guidelines in Oncology (NCCN GUIDELINES®) that provide detailed up-to-date information on the standard-of-care treatments for a wide variety of cancers (*see* NCCN GUIDELINES®, 2013). By way of example, standard-of-care treatments for MEL, RCC and NSCLC are summarized below.

*Melanoma*

MEL is a malignant tumor of melanocytes, the melanin-producing cells found predominantly in skin. Though less common than other skin cancers, it is the most dangerous of skin cancers if not diagnosed early and causes the majority (75%) of skin cancer deaths. The incidence of MEL is increasing worldwide in Caucasian populations, especially where peoples with low amounts of skin pigmentation receive excessive ultraviolet light exposure from the sun. In Europe, the incidence rate is < 10-20 per 100,000 population; in the USA 20-30 per 100,000; and in Australia, where the highest incidence is observed, 50-60 per 100,000 (Garbe *et al.*, 2012). MEL accounts for about 5% of all new cases of cancer in the United States (U.S.), and the incidence continues to rise by almost 3% per year. This translates to an estimated 76,690 new cases in the U.S. in 2013 with 9,480 associated deaths (Siegel *et al.* (2013).

For *in situ* (stage 0) or early-stage MEL (Stages I-II), surgical excision is the primary treatment. In general, the prognosis is excellent for patients with localized disease and tumors 1.0 mm or less in thickness, with 5-year survival rates of more than 90% (NCCN GUIDELINES®, 2013 – Melanoma). Where surgical excision is not

feasible for *in situ* melanoma due to comorbidity or cosmetically sensitive tumor location, topical imiquimod and radiotherapy are emerging as treatments, especially for lentigo maligna. Chemotherapeutic agents for treating MEL include dacarbazine, temozolomide and imatinib for melanoma with a c-KIT mutation, high-dose interleukin-2, and paclitaxel with or without carboplatin. However, these treatments have modest success, with response rates below 20% in first-line (1L) and second-line (2L) settings.

For patients with localized melanomas more than 1.0 mm in thickness, survival rates range from 50-90%. The likelihood of regional nodal involvement increases with increasing tumor thickness. With Stage III MEL (clinically positive nodes and/or in-transit disease), 5-year survival rates range from 20-70%. By far the most lethal is Stage IV MEL where long-term survival in patients with distant metastatic melanoma is less than 10% (NCCN GUIDELINES®, 2013 – Melanoma).

There is no consensus on the best treatments for metastatic MEL, though a variety of treatments including excision to clear margins, intralesional injections, laser ablation, radiation and biochemotherapy (combination of chemotherapy and biological agents such as interferon-alpha and IL-2) are being investigated. The therapeutic landscape for metastatic MEL has recently seen dramatic improvements with the development of novel drugs such as vemurafenib and ipilimumab. Vemurafenib specifically inhibits signaling by a mutated intracellular kinase, BRAF, that is present in about 50% of patients with metastatic MEL. In clinical trials, vemurafenib delivered an estimated progression-free survival (PFS) of 5.3 months in BRAF mutation-positive patients versus merely 1.6 months for dacarbazine, and a median OS of 15.9 months for vemurafenib versus 5.6-7.8 months for dacarbazine (Chapman *et al.*, 2011; Sosman *et al.*, 2012). Ipilimumab is a HuMAb that inhibits the immune checkpoint receptor, CTLA-4, and thereby stimulates a T cell immune response. Ipilimumab, with or without a glycoprotein 100 (gp100) peptide vaccine, improved median OS in patients with previously treated metastatic MEL to 10.0-10.1 months as compared with 6.4 months among patients receiving gp100 alone (Hodi *et al.*, 2010). Besides these two agents, no other agent has demonstrated an OS benefit in a Phase 3 randomized study. Dacarbazine is approved by the FDA and the EMA for treatment of metastatic MEL with a reported objective response rate of 5-20% and a median OS of about 6.4 months, but these responses are short-lived. Other drugs such as temozolomide and fotemustine have not resulted in significant improvement in survival when compared to dacarbazine. IL-2 has also been approved by the FDA for the

treatment of metastatic MEL as it is associated with a 15-20% response rate including 4-6% complete responses which can be durable, but it is associated with significant toxicities including hypotension, cardiac arrhythmias, and pulmonary edema. Further details on standard-of-care treatments for melanoma are provided by Garbe *et al.* (2012) and the NCCN GUIDELINES®, 2013 – Melanoma. Despite the recent approval of ipilimumab and vemurafenib for advanced MEL, there is still a large unmet need for patients who have progressed on anti-CTLA-4 therapy and a BRAF inhibitor (depending on BRAF status) or patients with previously untreated, unresectable or metastatic BRAF wild-type MEL. The 5-year survival rate for late-stage MEL is currently only 15%.

*Renal Cell Carcinoma*

RCC is the most common type of kidney cancer in adults, responsible for approximately 90% of renal tumors, and 80-90% of these are clear cell tumors (NCCN GUIDELINES®, 2013 – Kidney Cancer). It is also the most lethal of all the genitourinary tumors. An estimated 65,150 patients will be diagnosed with renal cancer and 13,680 will die of the disease in the United States in 2013 (Siegel *et al.* (2013).

For clinically localized RCC (Stage IA and IB), surgical resection, including radical nephrectomy and nephron-sparing surgery, is an effective therapy. Partial nephrectomy is generally not suitable for patients with locally advanced tumors (Stage II and III), in which case radical nephrectomy is preferred. Where the tumor is confined to the renal parenchyma, the 5-year survival rate is 60-70%, but this is lowered considerably in Stage IV disease where metastases have spread. Stage IV RCC is relatively resistant to RT and chemotherapy, although patients may benefit from surgery, and cytoreductive nephrectomy before systemic therapy is recommended for patients with a potentially surgically resectable primary and multiple resectable metastases.

Until recently, the cytokines IL-2 and IFNα were the only active systemic treatments for advanced or metastatic RCC, both providing reported ORRs of 5-27%. However, due to each of these agent's limited clinical benefit and substantial toxicity profile, newer targeted agents have largely replaced cytokines in the treatment of advanced or metastatic renal cell carcinoma. The recognition of the importance of hypoxia inducible factor alpha (HIFα) signaling in the pathogenesis of clear-cell RCC has led to widespread study of two classes of targeted therapies, anti-angiogenic tyrosine kinase inhibitors (TKIs) and mammalian target of rapamycin (mTOR) inhibitors, in 1L and 2L treatments (Mulders, 2009). Targeting of angiogenesis is rational because

constitutive HIFα activation leads to the upregulation or activation of several proteins including vascular endothelial growth factor (VEGF), which can subsequently lead to tumor proliferation and neovasculature formation. Targeting of the mTOR pathway is important because activation of the upstream PI3K/Akt/mTOR signaling pathway is one method by which constitutive HIFα activation or upregulation occurs (Mulders, 2009). Agents that target angiogenesis include VEGF-receptor (VEGFr) TKIs (*e.g.*, sorafenib, sunitinib, pazopanib, axitinib, and tivozanib) and VEGF-binding mAbs (*e.g.*, bevacizumab), while agents that target the mTOR pathway include the mTOR inhibitors (*e.g.*, everolimus and temsirolimus) (Mulders, 2009; NCCN GUIDELINES®, 2013 – Kidney Cancer). However, most patients develop resistance, and OS improvement has only been shown in one phase 3 trial in poor-risk patients: temsirolimus showed a statistically significant benefit for OS in patients with advanced RCC compared to IFNα (10.9 months versus 7.3 months) (Hudes *et al.*, 2007). Everolimus has also demonstrated a 2.1-month improvement in median PFS versus placebo, but with no OS improvement (Motzer et al., 2008). Among the five approved anti-angiogenic agents (sorafenib, sunitinib, bevacizumab, pazopanib, and axitinib) and two approved mTOR inhibitors (temsirolimus, everolimus), only everolimus is approved specifically for use after the failure of treatment with anti-angiogenic therapy. In the U.S., everolimus is indicated for the treatment of advanced RCC after failure of treatment with sunitinib or sorafenib, whereas in the EU, everolimus is more broadly indicated for patients with advanced RCC, whose disease has progressed on or after treatment with VEGF-targeted therapy.

*Non-Small Cell Lung Cancer*

NSCLC is the leading cause of cancer death in the U.S. and worldwide, exceeding breast, colon and prostate cancer combined. In the U.S., an estimated 228,190 new cases of lung and bronchial will be diagnosed in the U.S., and some 159,480 deaths will occur because of the disease (Siegel *et al.*, 2013). The majority of patients (approximately 78%) are diagnosed with advanced/recurrent or metastatic disease. Metastases to the adrenal gland from lung cancer are a common occurrence, with about 33% of patients having such metastases. NSCLC therapies have incrementally improved OS, but benefit has reached a plateau (median OS for late stage patients is just 1 year). Progression after 1L therapy occurred in nearly all of these subjects and the 5-year survival rate is only 3.6% in the refractory setting. From 2005 to 2009, the overall 5-year relative survival rate for

lung cancer in the U.S. was 15.9% (NCCN GUIDELINES®, 2013 – Non-Small Cell Lung Cancer).

Surgery, RT and chemotherapy are the three modalities commonly used to treat NSCLC patients. As a class, NSCLCs are relatively insensitive to chemotherapy and RT, compared to small cell carcinoma. In general, for patients with Stage I or II disease, surgical resection provides the best chance for cure, with chemotherapy increasingly being used both pre-operatively and post-operatively. RT can also be used as adjuvant therapy for patients with resectable NSCLC, the primary local treatment, or as palliative therapy for patients with incurable NSCLC.

Patients with Stage IV disease who have a good performance status (PS) benefit from chemotherapy. Many drugs, including platinum agents (*e.g.*, cisplatin, carboplatin), taxanes agents (*e.g.*, paclitaxel, albumin-bound paclitaxel, docetaxel), vinorelbine, vinblastine, etoposide, pemetrexed and gemcitabine are useful for Stage IV NSCLC. Combinations using many of these drugs produce 1-year survival rates of 30% to 40% and are superior to single agents. Specific targeted therapies have also been developed for the treatment of advanced lung cancer. For example, bevacizumab (AVASTIN®) is a mAb that blocks vascular endothelial growth factor A (VEGF-A). Erlotinib (TARCEVA®) is a small-molecule TKI of epidermal growth factor receptor (EGFR). Crizotinib (XALKORI®) is a small-molecule TKI that targets ALK and MET, and is used to treat NSCLC in patients carrying the mutated *ALK* fusion gene. Cetuximab (ERBITUX®) is a mAb that targets EGFR.

There is a particular unmet need among patients who have squamous cell NSCLC (representing up to 25% of all NSCLC) as there are few treatment options after 1L therapy. Single-agent chemotherapy is standard of care following progression with platinum-based doublet chemotherapy (Pt-doublet), resulting in median OS of approximately 7 months. Docetaxel remains the benchmark treatment in this line of therapy although erlotinib may also be used with less frequency. Pemetrexed has also been shown to produce clinically equivalent efficacy outcomes but with significantly fewer side effects compared with docetaxel in the 2L treatment of patients with advanced NSCLC (Hanna *et al.*, 2004). No therapy is currently approved for use in lung cancer beyond the 3L setting. Pemetrexed and bevacizumab are not approved in squamous NSCLC, and molecularly targeted therapies have limited application. The unmet need in advanced lung cancer has been compounded by the recent failure of Oncothyreon and

Merck KgaA's STIMUVAX® to improve OS in a phase 3 trial, inability of ArQule's and Daiichi Sankyo's c-Met kinase inhibitor, tivantinib, to meet survival endpoints, failure of Eli Lilly's ALIMTA® in combination with Roche's AVASTIN® to improve OS in a late-stage study, and Amgen's and Takeda Pharmaceutical's failure to meet clinical endpoints with the small-molecule VEGF-R antagonist, motesanib, in late-stage trials.

The present invention is further illustrated by the following examples which should not be construed as further limiting. The contents of all references cited throughout this application are expressly incorporated herein by reference.

EXAMPLE 1

*Cross-Competition between Anti-PD-1 HuMAbs for Binding to CHO Cells Expressing Human PD-1*

Chinese Hamster Ovary (CHO) cells transfected to express human PD-1 (CHO/PD-1 cells) were incubated with 10 μg/ml of Fab fragment of the anti-PD-1 HuMAb 5C4 or human IgG1 (hIgG1) isotype control Ab for 30 minutes at 4°C before addition of anti-PD-1 HuMAbs 2D3, 7D3 or 4H1 at a concentration of 0.2 μg/ml. Binding of 4H1, 2D3 or 7D3 to CHO/PD-1 cells were detected by fluorescein isothiocyanate (FITC)-conjugated goat anti-hIgG, Fc-gamma specific Ab. In the case of cross-competition assay with 5C4 and 17D8, CHO/PD-1 cells were incubated with the whole molecule of 5C4 before addition of FITC-labeled 17D8. Binding of 2D3, 7D3, 4H1 or 17D8 to the CHO/PD-1 cells was measured by flow cytometric analysis using a FACScalibur flow cytometer (Becton Dickinson, San Jose, CA).

The results are depicted in Figure 1. The data show that the 5C4 Fab fragment substantially blocked the binding of mAbs 5C4 itself, as well as the binding of 2D3, 7D3 (Figure 1A) and 4H1 (Figure 1B), while the 5C4 whole mAb substantially blocked the binding of 17D8 (Figure 1C) to CHO/PD-1 cells as measured by mean fluorescence intensity (MFI) of staining.

EXAMPLE 2

*Cross-Competition between Anti-PD-L1 HuMAbs for Binding to CHO Cells Expressing Human PD-L1*

CHO cells transfected to express hPD-L1 (CHO/PD-L1 cells) were incubated with 10 μg/ml of each of ten unconjugated human anti-PD-L1 mAbs (5F8, 7H1, 10H10, 1B12, 3G10, 10A5, 11E6, 12A4, 12B7, and 13G4) or human IgG1 (hIgG1) isotype control Ab

for 20 min at 4°C. FITC-conjugated 10H10 (A), 3G10 (B), 10A5 (C), 11E6 (D), 12A4 (E) or 13G4 (F) was added to the cells to a final concentration of 0.09 μg/ml (B, D), 0.27 μg/ml (A, C), 0.91 μg/ml (F), or 2.73 μg/ml (E) for an additional 20 min at 4°C without prior washout of unbound, unconjugated Ab. Different quantities of the various FITC-conjugated HuMAbs were used due to differences in binding efficiency following labeling, and the optimal amounts of these FITC-conjugated HuMAbs were previously determined by dose-titration analysis of binding to CHO/PD-L1 cells. Binding of FITC-conjugated 10H10, 3G10, 10A5, 11E6, 12A4 or 13G4 to the CHO/PD-L1 cells was measured by flow cytometry.

The results are depicted in Figure 2. Binding of labeled 10H10 was partially blocked by 10A5, 11E6 and 13G4, but was substantially blocked only by itself (Figure 2A). Conversely, 10H10 substantially blocked the binding only of itself to CHO/PD-L1 cells. Each of anti-PD-L1 HuMAbs 5F8, 7H1, 1B12, 3G10, 10A5, 11E6, 12A4, 12B7 and 13G4 substantially blocked binding of labeled mAbs 3G10 (Figure 2B), 10A5 (Figure 2C), 11E6 (Figure 2D), 12A4 (Figure 2E) and 13G4 (Figure 2F) to CHO/PD-L1 cells as measured by MFI, though mAbs 5F8 and 13G4 generally blocked binding of the labeled mAbs to a slightly lesser extent.

EXAMPLE 3

*Cross-Competition between Anti-PD-L1 mAbs for Binding to Ovarian Carcinoma Cells Expressing Human PD-L1*

Anti-PD-L1 HuMAbs 5F8, 12B7, 3G10, 1B12, 13G4, 10H10, 10A5 and 12A4, and a human IgG1 (huIgG1) isotype control Ab were serially diluted from 10 μg/ml and incubated with hPD-L1-expressing ES-2 ovarian carcinoma cells for 20 minutes at 4°C. Without washing, biotinylated-12A4 Ab was added to a final concentration of 0.4 μg/ml for an additional 20 minutes at 4°C. After washing, bound biotin-12A4 was detected using fluorescent streptavidin-PE secondary reagent and measured by flow cytometry. Figure 3 shows the fluorescence of bound biotin-12A4 plotted against the concentration of unlabeled hPD-L1 HuMAbs. Binding of biotin-12A4 to ES-2 cells was substantially blocked by 12A4 itself and by 1B12 and 12B7, and was moderately to significantly blocked by mAbs 5F8, 10A5, 13G4 and 3G10, but was not blocked by mAb 10H10.

EXAMPLE 4

*Design of Phase 1 Clinical Study of Anti-PD-1 Ab*

A Phase 1 study was conducted to assess the safety, antitumor activity, and pharmacokinetics of an anti-PD-1 in patients with selected advanced solid tumors. The human anti-PD-1 mAb, BMS-936558 (also referred to herein as nivolumab, and in U.S. Patent No. 8,008,449 as 5C4), was administered as an intravenous infusion every 2 weeks of each 8-week treatment cycle. Tumor status was re-assessed following each cycle. Patients continued treatment for up to 2 years (12 cycles), until they experienced complete remission, unacceptable toxicity, disease progression, or withdrew consent. In patients who were otherwise clinically stable, study treatment was continued beyond apparent initial disease progression until further progression was noted as recommended by proposed immune response criteria (Wolchok *et al.*, 2009). Patients with stable disease (SD) or an ongoing objective response (OR: complete response [CR] or partial response [PR]) at the end of treatment were followed for 1 year and were offered retreatment for 1 additional year in the event of progression.

*Dose escalation*

Patients with advanced melanoma (MEL), non-small cell lung cancer (NSCLC), renal cell carcinoma (RCC), castration-resistant prostate cancer (CRPC) and colorectal cancer (CRC) were eligible to enroll. Cohorts of 3-6 patients per dose level were enrolled sequentially at 1.0, 3.0, and 10.0 mg/kg. Dose escalation proceeded when a minimum of 3 patients had completed the safety evaluation period (56 days) at the given dose level, with dose-limiting toxicity in less than one-third of patients. Intra-patient dose escalation was not permitted.

*Cohort expansion*

A maximum tolerated dose (MTD) was not reached. Initially, 5 expansion cohorts of approximately 16 patients each were enrolled at 10 mg/kg for MEL, NSCLC, RCC, CRPC and CRC. Based on initial signals of activity, and following a 6.5-month hiatus for protocol amendment, additional expansion cohorts of approximately 16 patients each were enrolled for MEL (at 1.0 and 3.0 mg/kg, followed by cohorts randomized to 0.1, 0.3, or 1.0 mg/kg), NSCLC (squamous or nonsquamous histology cohorts randomized to 1, 3, or 10 mg/kg), and RCC (at 1.0 mg/kg). Intra-patient dose escalation to 1 mg/kg was permitted for MEL patients with progressive disease after receiving 0.1 or 0.3 mg/kg.

*Patients*

Eligible patients had documented advanced solid tumors; age > 18 years; life expectancy >12 weeks; Eastern Cooperative Oncology Group performance status of ≤ 2; measurable disease by Response Evaluation Criteria in Solid Tumors (RECIST), v1.0 with modification (*see* Topalian *et al.*, 2012b); adequate hematologic, hepatic, and renal function; and received 1-5 prior systemic treatment regimens. Patients with stable treated brain metastases were enrolled. Exclusion criteria included a history of chronic autoimmune disease, prior therapy with T-cell modulating Abs (*e.g.*, anti-CTLA-4, anti-PD-1, anti-PD-L1), conditions requiring immunosuppressive medications, and chronic infections (*e.g.*, HIV, hepatitis B or C).

A total of 296 patients with advanced solid tumors including MEL (n=104), NSCLC (n=122), RCC (n=34), CRPC (n=17), and CRC (n=19) were treated with BMS-936558 for 40 months up to February 2012. By March 2013, 306 patients including patients with non-small cell lung cancer (n=129), melanoma (n=107), RCC (n=34), CRPC (n=17), and CRC (n=19) had been treated with BMS-936558 from October 2008 through January 2012, all with a minimum of one year of observation. Two patients did not receive a full cycle of treatment and were not considered response-evaluable. Median age was 63 years (range, 29-85). ECOG performance score was 0 or 1 in 98% of patients. The majority of patients were heavily pretreated, with 47% having received at least 3 prior regimens. Notable prior therapies included immunotherapy (64%) and B-RAF inhibitor (8%) in MEL patients; platinum-based chemotherapy (94%) and tyrosine kinase inhibitors (TKIs, 34%) in NSCLC patients; and nephrectomy (94%), immunotherapy (59%), and anti-angiogenic therapy (74%) in RCC patients. Baseline characteristics of the total treated population (N=306) were similar to those of the efficacy population (response evaluable patients, N=270). Details on the patient pre-treatments are provided in Topalian *et al.* (2012b).

*Statistical analysis*

Baseline characteristics and adverse events for all treated patients (N=306), and efficacy results for 270 patients with lung cancer, melanoma, and kidney cancer as of March 2013 are reported. Pharmacokinetic and molecular-marker populations consisted of treated patients with available data as of the February 2012 date of preliminary analysis. The efficacy population consisted of response-evaluable patients commencing treatment at least 8 months before the date of analysis. Tumor measurements were

collected after each treatment cycle (4 doses) by investigators. Individual best objective responses based on the tumor measurements were assessed by the sponsor per modified RECIST v1.0. Objective response was confirmed by at least one sequential tumor assessment. Objective response and stable disease rates were estimated with confidence intervals using the Clopper-Pearson method. Time-to-event endpoints including PFS, OS, survival rates, and duration of response, were estimated using the Kaplan-Meier method. AEs were coded using Medical Dictionary for Regulatory Activities (MedDRA), version 15.1. Select adverse events with potential immunologic etiologies, also referred to as "immune-related adverse events" of "AEs of special interest" (AEOSIs), defined as adverse events that require more frequent monitoring and/or unique intervention, were identified using a pre-defined list of MeDRA terms. Individual best ORs were derived from investigator-reported data per modified RECIST v1.0. OR was confirmed by at least one sequential tumor assessment and OR rate (ORR={[CR + PR] ÷ n} × 100) was calculated.

EXAMPLE 5

*Safety Evaluations on Patients Treated with Anti-PD-1 Antibody*

Safety evaluations, including clinical examination and laboratory assessments, were conducted in all treated patients at baseline and regular intervals up to 100 days following last administration of drug. The severity of AEs was graded based on the NCI Common Terminology Criteria for Adverse Events (NCI CTCAE), v3.0. Computed tomography (CT) or magnetic resonance imaging was performed for tumor assessment at baseline and following each treatment cycle.

A MTD was not defined across the doses of BMS-936558 tested on this study, up to the highest planned dose of 10 mg/kg. A relative BMS-936558 dose intensity of 90% or higher was achieved in 87% of patients (*see* Topalian *et al.*, 2012b; Topalian *et al.*, 2013, for details). AEs were coded using Medical Dictionary for Regulatory Activities (MedDRA), version 14.1. AEOSIs were identified using a pre-defined list of MeDRA terms. Fifteen of 296 (5%) patients discontinued treatment due to BMS-936558-related AEs. As of the February 2012 date of preliminary analysis, 62 (21%) patients had died, and by March 2013, 195 patients (64%) had died, with disease progression being the most common cause of death (Topalian *et al.*, 2012b; Topalian *et al.*, 2013).

The most common adverse events, regardless of causality included fatigue, decreased appetite, diarrhea, nausea, cough, dyspnea, constipation, vomiting, rash,

pyrexia and pruritus (Topalian *et al.*, 2012b; Topalian *et al.*, 2013). Common BMS-936558-related AEs included fatigue, rash, diarrhea, decreased appetite, and nausea. The majority of the events were low grade, with grade 3-4 drug-related AEs observed in 41 of 296 (14%) patients. Treatment-related AEs (any grade) were observed in 230 of 306 patients (75%), the most common being fatigue, rash, diarrhea and pruritus (Topalian *et al.*, 2013). The spectrum, frequency, and severity of BMS-936558-related AEs were generally similar across the dose levels tested, with no clear relationship to treatment duration. Fifty-two of 306 patients (17%) experienced Grade 3-4 treatment-related adverse events, with fatigue (2%), pneumonitis, lymphopenia, diarrhea, abdominal pain, and hypophosphatemia (1% each) being the most common. Treatment-related serious adverse events occurred in 42 of 306 patients (14%) (Topalian *et al.*, 2013).

Drug-related AEOSIs, with potential immune-related etiologies, were categorized by organ involvement to provide a more accurate estimate of frequency and included pneumonitis, vitiligo, colitis, hepatitis, hypophysitis, and thyroiditis among others. Treatment-related select adverse events of any grade were observed in 140 of 306 patients (46%), the most common being rash (15%), diarrhea (13%), and pruritus (11%) (Table 2). Grade 3-4 treatment-related select events were seen in 19 patients (6%).

Fifty-two of 230 patients (23%) with drug-related adverse events required management with systemic glucocorticoids and/or other immunosuppressive agents. Hepatic or gastrointestinal AEOSIs were managed with treatment interruption and, as necessary, with administration of corticosteroids. Among patients treated to-date, these AEs were reversible in all cases. Endocrine AEOSIs were managed with replacement therapy. Thirty-two of 306 patients (11%) discontinued therapy due to treatment-related adverse events. At the discretion of the treating physician, patients successfully reinitiated treatment with BMS-936558. Twenty-one (40%) were able to resume nivolumab therapy after toxicity resolved.

Drug-related pneumonitis (any grade) occurred in 12 of 306 (4%) patients (Topalian *et al.*, 2013). Clinical presentations ranged from radiographic abnormalities in asymptomatic patients, to progressive, diffuse pulmonary infiltrates associated with symptoms (cough, fever, dyspnea). Grade 3-4 pneumonitis developed in 4 patients (1%), of which 3 cases were fatal (2 NSCLC patients, 1 CRC). No clear relationship between the occurrence of pneumonitis and tumor type, dose level, or the number of doses received was noted. In 9 of 12 patients, pneumonitis was reversible with treatment

discontinuation and/or immunosuppression (glucocorticoids, infliximab, mycophenolate). Following the most recent pneumonitis-associated death in November 2011, 79 patients continued to receive nivolumab (median 29 weeks, range 2-69 weeks) with no further mortalities from this or other treatment-related causes.

EXAMPLE 6

*Pharmacokinetics/Pharmacodynamics Analyses on Anti-PD-1 Antibody*

For pharmacokinetic (PK) analyses, serial blood samples were collected and serum concentrations of BMS-936558 were quantified using by ELISA. For pharmacodynamic (PD) analysis, peripheral blood mononuclear cells were isolated from patients at baseline and following cycle 1 to estimate PD-1 receptor occupancy (RO) by BMS-936558 on circulating CD3+ T-cells via flow cytometry (Brahmer *et al.*, 2010).

The maximum concentration of BMS-936558 was observed at a median $T_{max}$ of 1-4 hours after the start of infusion. The PK of BMS-936558 was linear with a dose proportional increase in $C_{max}$ and $AUC_{(0\text{-}14\ d)}$ in the dose range of 0.1-10 mg/kg (n=35). BMS-936558 PD was assessed by PD-1 RO on circulating T-cells. PBMCs from 65 MEL patients treated with one cycle of BMS-936558 at 0.1-10 mg/kg biweekly demonstrated median occupancy of PD-1 molecules on circulating $CD3^+$ T-cells by BMS-936558 ranging from 64%-70% (*see* Topalian *et al.*, 2012b, for details).

EXAMPLE 7

*Antitumor Efficacy Exhibited by Anti-PD-1 Antibody*

*Data analyzed as of February 2012*

Clinical antitumor activity was observed at all BMS-936558 doses tested. ORs (confirmed CR or PR) were observed in a substantial portion of patients with NSCLC, MEL, and RCC (Tables 1 and 2; Figure 4), and in various sites of metastatic disease including liver, lung, lymph nodes, and bone (Figures 5-7 and not shown). Tumor regressions followed conventional as well as "immune-related" patterns of response, such as prolonged reduction in tumor burden in the presence of new lesions. Individual best overall responses were derived from investigator-reported data according to modified RECIST v1.0. OR was confirmed by at least one sequential tumor assessment. At the time of data analysis, 2 patients with NSCLC who were treated with 10 mg/kg had unconfirmed responses, and 8 additional patients (with MEL, NSCLC, or RCC) had a persistent reduction in baseline target lesions in the presence of new lesions, (*i.e.*, an

"immune-related" response pattern). None of these patients was categorized as a responder for the purpose of calculating OR rates. Antitumor responses and/or prolonged disease stabilization were observed in patients irrespective of prior therapies received (*see* summary of progression free interval for patients with OR and SD in Supplementary Appendix 4 of Topalian *et al.*, 2012b).

In NSCLC patients, 14 ORs were observed at BMS-936558 doses of 1, 3, or 10 mg/kg with response rates of 6%, 32%, and 18%, respectively. ORs were observed across NSCLC histologies: 6 responders of 18 squamous (33%), 7 responders of 56 nonsquamous (13%), and 1 of 2 unknown. All 14 patients with ORs started treatment ≥ 24 weeks before data analysis, and of these, 8 had response duration ≥ 24 weeks (Table 1). Stable disease (SD) lasting ≥ 24 weeks was observed in 5 (7%) NSCLC patients, all with nonsquamous histology. Among MEL patients, 26 ORs were observed at doses ranging from 0.1-10 mg/kg, with response rates ranging from 19%-41% per dose level. At the 3 mg/kg dose level, ORs were noted in 7 of 17 (41%) patients. Of 26 MEL patients who achieved an OR, 17 started treatment ≥ 1 year before data analysis, and of these, 13 patients had an OR duration ≥ 1 yr. The remaining 8 patients with OR were on study < 1 year and 6 had responses ranging from 1.9-5.6 months. SD lasting ≥ 24 weeks was observed in 6 (6%) patients. In RCC patients, ORs occurred in 4 of 17 (24%) patients treated with a BMS-936558 dose of 1 mg/kg and 5 of 16 (31%) patients treated with 10 mg/kg. Among 8 RCC patients with OR who started treatment ≥ 1 year prior to data analysis, 5 (63%) had OR duration ≥ 1 yr. SD lasting ≥ 24 weeks was observed in an additional 9 (27%) patients.

Table 1. Clinical Activity of BMS-936558 in the Efficacy Population* (N=236)[†] as Assessed to February 2012

| Tumor Type | Dose (mg/kg) | n | ORR[‡] No. Patients (%) [95% CI][†] | SD ≥ 24 wk No. Patients (%) [95% CI] | PFSR[§] at 24 wk (%) [95% CI] |
|---|---|---|---|---|---|
| MEL | 0.1 | 14 | 4 (29) [8-58] | 1 (7) [0.2-34] | 40 [13-66] |
| | 1.0 | 27 | 8 (30) [14-50] | 3 (11) [2-29] | 45 [26-65] |
| | 3.0 | 17 | 7 (41) [18-67] | 1 (6) [0.1-29] | 55 [30-80] |
| | 10.0 | 20 | 4 (20) [6-44] | 0 | 30 [9-51] |
| ALL MEL | | 94 | 26 (28) [19-38] | 6 (6) [2-13] | 41 [30-51] |

| Tumor Type | Dose (mg/kg) | n | ORR‡ No. Patients (%) [95% CI]† | SD ≥ 24 wk No. Patients (%) [95% CI] | PFSR§ at 24 wk (%) [95% CI] |
|---|---|---|---|---|---|
| NSCLC** | | | | | |
| All | 1 | 18 | 1 (6) [0.1-27] | 1 (6) [0.1-27] | 16 [0-34] |
| Squamous | 1 | 5 | 0 | 0 | 0 |
| Nonsquamous | 1 | 12 | 0 | 1 (8) [0.2-39] | 14 [0-37] |
| Unknown | 1 | 1 | 1(100) [3-100] | 0 | 1 |
| All | 3 | 19 | 6 (32) [13-57] | 2 (11) [1-33] | 41 [18-64] |
| Squamous | 3 | 6 | 3 (50) [12-88] | 0 | 50 [10-90] |
| Nonsquamous | 3 | 13 | 3 (23) [5-54] | 2 (15) [2-45] | 37 [10-64] |
| All | 10 | 39 | 7 (18) [8-34] | 2 (5) [0.6-17] | 24 [11-38] |
| Squamous | 10 | 7 | 3 (43) [10-82] | 0 | 43 [6-80] |
| Nonsquamous | 10 | 31 | 4 (13) [4-30] | 2 (7) [0.8-21] | 21 [6-36] |
| Unknown | 10 | 1 | 0 | 0 | 0 |
| ALL NSCLC | | 76 | 14 (18) [11-29] | 5 (7) [2-15] | 26 [16-36] |
| All Squamous | | 18 | 6 (33) [13-59] | 0 | 33 [12-55] |
| All Nonsquamous | | 56 | 7 (13) [5-24] | 5 (9) [3-20] | 22 [ 11-34] |
| RCC | 1 | 17 | 4 (24) [7-50] | 4 (24) [7-50] | 47 [23-71] |
| | 10 | 16 | 5 (31) [11-59]‡ | 5 (31) [11-59]¶ | 67 [43-91] |
| ALL RCC | | 33 | 9 (27) [13-46] | 9 (27) [13-46] | 56 [39-73 |

* The efficacy population consists of response-evaluable patients whose treatment was initiated at least 8 months before data analysis in February 2012, and had measurable disease at baseline and one of the following: at least 1 on-treatment scan or clinical evidence of disease progression or death.

† CR denotes complete response, MEL melanoma, NSCLC non-small cell lung cancer, ORR objective response rate, PFSR progression-free survival rate, PR partial response, RCC renal cell cancer, SD stable disease, n number of patients.

‡ Objective response rates ({[CR + PR] ÷ n} × 100) have been calculated based on confirmed responses with confidence intervals calculated using the Clopper-Pearson method. Individual patient responses were adjudicated per RECIST v1.0 with

modification (*see* Topalian *et al.*, 2012b).

§ Progression-free survival rate was the proportion of patients who did not progress and were alive at 24 weeks calculated by the Kaplan-Meier methodology with confidence intervals using the Greenwood method.

¶ One CR.

**One NSCLC patient who was treated at the 3 mg/kg dose level had an initial evaluation of progressive disease, subsequently had a PR, and was classified as a responder.

Table 2. Duration of Objective Responses to BMS-936558* as Assessed to February 2012

| Tumor Type | Dose (mg/kg) | No. of Patients with OR | Duration of Response (months)† |
|---|---|---|---|
| MEL | 0.1 | 4 | 7.5+, 5.6+, 5.6, 5.6 |
| | 0.3 | 3 | 3.8+, 2.1+, 1.9+ |
| | 1 | 8 | 24.9+, 22.9, 20.3+, 19.3+, 18.4+, 7.6+, 5.6+, 5.3+ |
| | 3 | 7 | 22.4+, 18.3+, 15.2+, 12.9, 11.1, 9.3, 9.2+ |
| | 10 | 4 | 24.6+, 23.9+, 18.0+, 17.0 |
| NSCLC§ | 1 | 1 | 9.2+ |
| | 3 | 6 | 30.8+, 7.6+, 5.5+, 3.7+, 1.9+, NA‡ |
| | 10 | 7 | 14.8+, 7.6+, 7.3+, 6.7, 4.2, 3.7+, 3.7 |
| RCC | 1 | 4 | 17.5+, 9.2+, 9.2, 5.6+ |
| | 10 | 5 | 22.3+. 21.7+, 12.9, 12.0, 8.4 |

* MEL denotes melanoma, NA not applicable, NSCLC non-small cell lung cancer, RCC renal cell cancer.

† Time from first response to time of documented progression, death, or for censored data, time to last tumor assessment.

‡ One patient was treated beyond an initial evaluation of progressive disease and subsequently had a PR; this patient was classified as a responder for the purposes of calculating response rates by RECIST v1.0 but was not eligible for calculation of duration of response.

*Data analyzed as of March 2013*

Objective responses were observed in patients with NSCLC (17%), MEL (31%), and RCC (29%), but not with CRC or CRPC. Responses were observed across all nivolumab doses tested; response rates in NSCLC patients receiving 1 mg/kg, versus 3 or 10 mg/kg, appeared to be decreased (3%, versus 24% and 20%, respectively) (Tables 3 and 4). Thirteen of 270 patients (4.8%) with responding histologies had unconventional response patterns that did not meet RECIST criteria (*e.g.*, persistent reduction in target lesions in the presence of new lesions or regression following initial progression) (Wolchok *et al*, 2009). Additional patients manifested SD for 24 weeks or longer (10% NSCLC, 7% MEL, 27% RCC). Sustained survival, reflected by 1- and 2-year landmark OS rates, was noted in each of the responding populations as follows: NSCLC, 42% and 14%; MEL, 62% and 43%; and RCC, 70% and 50% (Table 3). Median OS of 9.6 months for lung cancer (9.2 and 10.1 months for squamous and non-squamous NSCLC histologies, respectively), 16.8 months for MEL, and more than 22 months for RCC, were observed; median PFS was 2.3 months in NSCLC, 3.7 months in MEL, and 7.3 months in RCC; and the median duration of response was 74, 104, and 56 weeks, respectively (Table 4). Among 16 responders who discontinued therapy for reasons other than disease progression and were followed for at least 24 weeks, 13 (81%) remained in response at the time of analysis (Figure 8).

Table 3. Clinical Activity of Nivolumab in the Efficacy Population (N = 306)* as Assessed to March 2013

| Tumor Type | ORRs[†] No. of patients/total no. of patients (%) [95% CI] | Median Duration of Response[‡], wk (range) | Stable Disease No. of patients/total no. of patients (%) [95% CI] | | Median Progression-free Survival, mo (95% CI) | Median Overall Survival, mo (95% CI) | Overall Survival Rate, % (95% CI); patients at risk, n | |
|---|---|---|---|---|---|---|---|---|
| | | | ≥ 24 wk | ≥ 48 wk | | | 1 yr | 2 yr |
| Non-small cell lung cancer** | 22/129 (17.1) [11.0, 24.7] | 74.0 (6.1+, 133.9+) | 13/129 (10.1) [5.5, 16.6] | 6/129 (4.7) [1.7, 9.8] | 2.3 (1.9, 3.7) | 9.6 (7.8, 12.4) | 42 (33, 51); 43 | 14 (4, 24); 5 |
| Squamous | 9/54 (16.7) [7.9, 29.3] | NR[§] (16.1, 133.9+) | 8/54 (14.8) [6.6, 27.1] | 3/54 (5.6) [1.2, 15.4] | 3.7 (1.8, 7.2) | 9.2 (7.3, 12.5) | 39 (25, 53):16 | ¶ |
| Non-squamous | 13/74 (17.6) [9.7, 28.2] | 63.9 (6.1+, 74.0+) | 5/74 (6.8) [2.2, 15.1] | 3/74 (4.1) [0.8, 11.4] | 2.0 (1.8, 3.6) | 10.1 (7.2, 13.7) | 43 (31, 54): 26 | ¶ |
| Melanoma | 33/107 (30.8) [22.3, 40.5] | 104 (18.4, 117.0+) | 7/107 (6.5) [2.7, 13.0] | 4/107 (3.7) [1.0, 9.3] | 3.7 (1.9, 9.1) | 16.8 (12.5, 31.6) | 62 (53, 72); 55 | 43 (32, 53); 26 |
| Kidney cancer | 10/34 (29.4) [15.1, 47.5] | 56.1 (36.6, 126.7+) | 9/34 (26.5) [12.9, 44.4] | 2/34 (5.9) [0.7, 19.7] | 7.3 (3.7, 12.9) | >22[#] (13.6, NE^) | 70 (55, 86); 23 | 50 (31, 70); 8 |

* No objective responses were seen in 19 patients with colorectal cancer or 17 patients with castration-resistant prostate cancer.

† Objective response rates ({[CR + PR] ÷ n} × 100) have been calculated based on confirmed responses with confidence intervals calculated using the Clopper-Pearson method. Individual patient responses were adjudicated per RECIST v1.0 with modification (see Methods S1 and study protocol, NEJM.org).

‡ Time from first response to time of documented progression, death, or for censored data (denoted by "+"), time to last tumor assessment.

** Among 129 patients with non-small-cell lung cancer, 1 had an unknown histology and did not show an objective response. Others had squamous or non-squamous histologies, as indicated.

§ NR, not reached; the time point at which the probability that responders progress drops below 50% has not been reached due to insufficient number of events and/or follow-up.

¶ Insufficient period of follow-up.

‖ 1 CR was noted in melanoma, and 1 CR in kidney cancer.

# The median overall survival was not reached at 22 months, the longest time to death so far in patients with kidney cancer in this trial.

^ NE, not estimable.

Table 4. Clinical Activity of Nivolumab by Dose Level as Assessed to March 2013

| Tumor Type | Dose, mg/kg | ORRs* No. of patients/total no. of patients (%) [95% CI] | Median Duration of Response wk (range)† | Stable Disease No. of patients/total no. of patients (%) [95% CI] | | Median Progression-free Survival, mo (95% CI) | Median Overall Survival, mo (95% CI) |
|---|---|---|---|---|---|---|---|
| | | | | ≥ 24 wk | ≥ 48 wk | | |
| Non-small cell lung cancer | All doses | 22/129 (17.1) [11.0, 24.7] | 74.0 (6.1+, 133.9+) | 13/129 (10.1) [5.5, 16.6] | 6/129 (4.7) [1.7, 9.8] | 2.3 (1.9, 3.7) | 9.6 (7.8, 12.4) |
| | 1 | 1/33 (3.0) [0.1, 15.8] | 63.9 (63.9, 63.9) | 5/33 (15.2) [5.1, 31.9] | 2/33 (6.1) [0.7, 20.2] | 1.9 (1.8, 3.6) | 9.2 (5.6, 11.1) |
| | 3 | 9/37 (24.3) [11.8, 41.2] | NR‡ (16.1+, 133.9+) | 3/37 (8.1) [1.7, 21.9] | 2/37 (5.4) [0.7, 18.2] | 1.9 (1.7, 7.3) | 14.9 (9.5, NE^) |
| | 10 | 12/59 (20.3) [11.0, 32.8] | 83.1 (6.1+, 117.1+) | 5/59 (8.5) [2.8, 18.7] | 2/59 (3.4) [0.4, 11.7] | 3.6 (1.9, 3.8) | 9.2 (5.2, 12.4) |
| Squamous | All doses | 9/54 (16.7) [7.9, 29.3] | NR (16.1, 133.9+) | 8/54 (14.8) [6.6, 27.1] | 3/54 (5.6) [1.2, 15.4] | 3.7 (1.8, 7.2) | 9.2 (7.3, 12.5) |
| | 1 | 0 | 0 | 4/15 (26.7) [7.8, 55.1] | 1/15 (6.7) [0.2, 31.9] | 1.9 (1.8, 7.5) | 8.0 (2.6, 13.3) |
| | 3 | 4/18 (22.2) [6.4, 47.6] | NR (16.1, 133.9+) | 1/18 (5.6) [0.1, 27.3] | 1/18 (5.6) [0.1, 27.3] | 3.8 (1.7, 14.9) | 9.5, (6.7, NE) |

| Tumor Type | Dose, mg/kg | ORRs* No. of patients/total no. of patients (%) [95% CI] | Median Duration of Response wk (range)† | Stable Disease No. of patients/total no. of patients (%) [95% CI] | | Median Progression-free Survival, mo (95% CI) | Median Overall Survival, mo (95% CI) |
|---|---|---|---|---|---|---|---|
| | | | | ≥ 24 wk | ≥ 48 wk | | |
| | 10 | 5/21 (23.8) [8.2, 47.2] | 83.1 (16.1, 117+) | 3/21 (14.3) [3.0, 36.3] | 1/21 (4.8) [0.1, 23.8] | 4.1 (1.8, 7.6) | 10.5 (7.8, 12.5) |
| Non-squamous | All doses | 13/74 (17.6) [9.7, 28.2] | 63.9 (6.1+, 74.0+) | 5/74 (6.8) [2.2, 15.1] | 3/74 (4.1) [0.8, 11.4] | 2.0 (1.8, 3.6) | 10.1 (7.2, 13.7) |
| | 1 | 1/18 (5.6) [0.1, 27.3] | 63.9 (63.9, 63.9) | 1/18 (5.6) [0.1, 27.3] | 1/18 (5.6) [0.1, 27.3] | 1.8 (1.7, 3.6) | 9.9 (5.6, 22.7) |
| | 3 | 5/19 (26.3) [9.1, 51.2] | 74.0 (24.3, 74.0+) | 2/19 (10.5) [1.3, 33.1] | 1/19 (5.3) [0.1, 26.0] | 1.8 (1.7, 12.5) | 18.2 (10.3, 18.2) |
| | 10 | 7/37 (18.9) [8.0, 35.2] | NR (6.1+, 65.7+) | 2/37 (5.4) [0.7, 18.2] | 1/37 (2.7) [0.1, 14.2] | 2.3 (1.9, 3.8) | 7.4 (4.6, 12.4) |
| Melanoma§ | All doses | 33/107 (30.8) [22.3, 40.5] | 104 (18.4, 117.0+) | 7 (6.5) [2.7, 13.0] | 4 (3.7) [1.0, 9.3] | 3.7 (1.9, 9.1) | 16.8 (12.5, 31.6) |
| | 0.1¶ | 6/17 (35.3) [14.2, 61.7] | NR (24.1, 48.7+) | 0 | 0 | 3.6 (1.7, 9.1) | 16.2 (8.6, NE) |
| | 0.3¶ | 5/18 (27.8) [9.7, 53.5] | NR (18.4, 66.3+) | 1 (5.6) [0.1, 27.3] | 0 | 1.9 (1.8, 9.3) | 12.5 (7.7, NE) |

| Tumor Type | Dose, mg/kg | ORRs* No. of patients/total no. of patients (%) [95% CI] | Median Duration of Response wk (range)† | Stable Disease No. of patients/total no. of patients (%) [95% CI] | | Median Progression-free Survival, mo (95% CI) | Median Overall Survival, mo (95% CI) |
|---|---|---|---|---|---|---|---|
| | | | | ≥ 24 wk | ≥ 48 wk | | |
| | 1 | 11/35 (31.4) [16.9, 49.3] | 104 (32.4, 108.1+) | 5/35 (14.3) [4.8, 30.3] | 4/35 (11.4) [3.2, 26.7] | 9.1 (1.8, 24.7) | 25.3 (14.6, NE) |
| | 3 | 7/17 (41.2) [16.9, 67.1] | 75.9 (40.1+, 115.4+) | 1/17 (5.9) [0.1, 28.7] | 0 | 9.7 (1.9, 16.4) | 20.3 (8.2, NE) |
| | 10 | 4/20 (20.0) [5.7, 43.7] | 112 (73.9, 117+) | 0 | 0 | 3.7 (1.7, 20.50) | 11.7 (7.2, 37.8) |
| Kidney cancer§ | All doses | 10/34 (29.4) [15.1, 47.5] | 56.1 (36.6, 126.7+) | 9/34 (26.5) [12.9, 44.4] | 2/34 (5.9) [0.7, 19.7] | 7.3 (3.7, 12.9) | >22# (13.6,NE) |
| | 1 | 5/18 (27.8) [9.7, 53.5] | 56.1 (40.1, 76.1+) | 4/18 (22.2) [6.4, 47.6] | 1/18 (5.6) [0.1, 27.3] | 4.7 (1.9, 10.9) | NR (17.9, NE) |
| | 10 | 5/16 (31.3) [11.0, 58.7] | 56.1 (36.6, 126.7+) | 5/16 (31.3) [11.0, 58.7] | 1/16 (6.3) [0.2, 30.2] | 8.0 (3.7, 14.0) | 18.8 (11.6, NE) |

* Objective response rates ({[CR + PR] ÷ n} × 100) have been calculated based on confirmed responses with confidence intervals calculated using the Clopper-Pearson method. Individual patient responses were adjudicated per RECIST v1.0 with modification (see Methods S1 and study protocol, NEJM.org).

† Time from first response to time of documented progression, death, or for censored data (denoted by "+"), time to last tumor assessment.

‡ NR, not reached; the time point at which the probability that responders progress drops below 50% has not been reached due to insufficient number of events and/or follow-up.

§ 1 CR was noted in melanoma, and 1 CR in kidney cancer.

¶ Five patients with tumor progression were dose-escalated from 0.1 to 1.0 mg/kg, and six from 0.3 to 1.0 mg/kg. None of these patients responded to therapy.

# The median overall survival was not reached at 22 months, the longest time to death so far in patients with kidney cancer in this trial.

^ NE, not estimable.

EXAMPLE 8

*Correlation between Membranous PD-L1 Expression and Anti-PD-1 Response*

IHC staining of PD-L1 was performed on pretreatment formalin-fixed paraffin-embedded (FFPE) tumor specimens using the murine anti-human PD-L1 mAb 5H1 (Dong *et al.*, 2002) in a standard IHC protocol (Taube *et al.*, 2012; Supp. Materials). Briefly, 5μm-FFPE sections mounted on glass slides were deparaffinized in xylene and antigen retrieval was performed using a Tris-EDTA buffer, pH 9.0 at 120°C for 10 min in a Decloaking Chamber (Biocare Medical). Endogenous peroxidase, biotin and proteins were blocked (CAS system K1500, Dako; Avidin/biotin Blocking Kit, SP-2001, Vector Laboratories; Serotec Block ACE), and the primary 5H1 Ab was added at a concentration of 2 μg/ml and allowed to incubate at 4°C for 20 h. Secondary Ab (biotinylated anti-mouse IgG1, 553441 BD) was applied at a concentration of 1 μg/ml for 30 min at room temperature (RT). The signal was then developed with amplification according to the manufacturer's protocol (CAS system K1500, Dako). Sections were counterstained with hematoxylin, dehydrated in ethanol and cleared in xylene, and a coverslip was applied.

The percentage of tumor cells exhibiting cell surface staining for PD-L1 was scored by two independent pathologists who were blinded to treatment outcomes. PD-L1 positivity was defined per specimen by a 5% expression threshold (Taube *et al.*, 2012; Thompson *et al.*, 2006), and in cases with multiple specimens, if any specimen met this criterion. A Fisher's exact test was applied to assess the association between PD-L1 expression and OR, noting, however, that this analysis was based in part on optional biopsies from a non-random subset of the population and testing of a statistical hypothesis was not pre-specified.

Sixty-one pretreatment tumor specimens from 42 patients (18 MEL, 10 NSCLC, 7 CRC, 5 RCC, and 2 CRPC) were analyzed for tumor cell surface PD-L1 expression (Figure 8). Biopsy specimens from 25 of 42 patients were positive for PD-L1 expression by IHC. A Fisher's exact test was applied to assess the association between PD-L1 expression and OR in a post-hoc analysis. Among the 42 surface-PD-L1$^{+}$ patients, 9 (36%) achieved an OR , whereas among 17 patients with PD-L1$^{-}$ tumors, none achieved an OR (Figure 8A). Thus, in a subset of patients cell surface expression of PD-L1 on tumor cells in pretreatment biopsies is associated with an increased rate of OR among patients treated with BMS-936558, while no patients with documented PD-L1-negative tumors experienced an OR. These data indicate that tumor PD-L1 expression is a

molecular marker that can enable patient selection for anti-PD-1 immunotherapy.

EXAMPLE 9

*Isolation of Rabbit mAbs that Detect Membranous hPD-L1 Antigen in FFPE Tissues*

Rabbit Abs against human PD-L1 polypeptide were prepared by Epitomics, Inc. (Burlingame, CA) by immunization of rabbits using a recombinant human PD-L1 fusion protein. Antiserum titers were evaluated using standard direct ELISA with the hPD-L1 antigen, and cell ELISA using transfected cells overexpressing hPD-L1. These Abs were also screened for their ability to bind to PD-L1 by IHC assay of FFPE tissue sections. The rabbit with the highest Ab titer was selected for splenectomy. Lymphocytes isolated from the spleen were fused to myeloma cells in 40 x 96-well plates, and screened by ELISA against the immunizing PD-L1 antigen and by cell ELISA against cells overexpressing hPD-L1. Positive clones were expanded into 24-well plates, and confirmatory screens were conducted by direct ELISA and cell ELISA. The supernatants (sups) of clones that were specific to the screening antigen were re-screened by IHC.

A set of mouse anti-hPD-L1 mAbs were also produced by immunization of mice using a protocol similar to that described above for the rabbit mAbs.

Out of a total of 185 multiclones from both rabbit and mouse immunizations screened, only ten rabbit multiclone Abs specifically detected the membranous form of hPD-L1. None of the purified mouse subclones were found to specifically detect cell surface hPD-L1. Sixty subclones from the top five rabbit multiclones (designated Nos. 13, 20, 28, 29 and 49, each comprising 12 subclones) were screened initially by IHC on FFPE low density tissue microarrays (TMAs), followed by confirmation and specificity verification in narrowed 25 subclones. Rabbit IgG was used as a negative isotype control, and mAb 5H1 (Dong *et al.*, 2002) was used as the positive control. Specificity was further verified by antigen preabsorption assay. Through two rounds of IHC, the following 15 purified subclones were selected as the most promising Abs in terms of specificity and intensity of staining: 13-1, 13-3, 13-7, 13-8; 20-5, 20-7, 20-12, 20-6; 28-1, 28-8, 28-12; 29-8; 49-5, 49-7 and 49-9. Immunoreactivity data on these selected Abs are summarized in Table 5.

Table 5. Immunoreactivity of Rabbit Anti-hPD-L1 mAbs

| | Specific Staining | | Non-Specific staining |
|---|---|---|---|
| Antibody Name | Pos. vs Neg. Staining on PD-L1 Cells* | Intensity Range on Tissues† (Very High, High, Medium, Low) | Background Staining on Tissues (High, Medium, Low) |
| 13-1 | Pos | Low to Very High | None |
| 13-3 | Pos | Low to High | None |
| 13-7 | Pos | Low to High | None |
| 13-8 | Pos | Low to High | None |
| 20-5 | Pos | Low to Very High | High |
| 20-6 | Pos | Low to High | Medium |
| 20-7 | Pos | Low to High | Medium |
| 20-12 | Pos | Low to High | Medium |
| 28-1 | Pos | Low to Very High | None |
| 28-8 | Pos | Medium to Very High | None |
| 28-10 | Pos | Medium to Very High | Low |
| 28-12 | Pos | Low to Very High | None |
| 49-5 | Pos | Low to High | None |
| 49-7 | Pos | Low to High | Low |
| 49-9 | Pos | Low to High | None |
| 5H1 | Pos | High to Very High | None |
| Rb IgG | Neg | Neg | None |

* PD-L1 stably transfected CHO cells vs. CHO-S control;

† PD-L1 positive tissues included placenta and one non-small cell lung cancer; Detection up to "very high" expression suggests better sensitivity at detecting membranous PD-L1.

Additional assays were performed to further characterize the purified Ab clones, including determining binding affinity and cross-competition among the Abs (to identify overlapping versus different epitope regions) by surface plasmon resonance. All the Abs exhibited high binding affinity ($K_D < 10^{-9}$ M). These 15 purified clones were also re-screened by IHC on FFPE low density TMA or regular sections against various cell and

tissue types known to be positive or negative for cell surface expression of PD-L1. Rabbit IgG was used as the isotype control, and mAb 5H1 was used as the positive control. At high concentration (10 μg/ml), clones 28-x and 49-x displayed low to moderate levels of background staining in tissues, while clones 13-x exhibited no background staining, which suggests that the 13-x clones have a wider dynamic range. The 20-x clones displayed various degree of background staining which was primarily cytoplasmic and diffuse. The clone with most robust detection specifically of membranous PD-L1, rabbit clone 28-8 ($K_D$ = 100 p M, as determined by SPR), was selected as the lead Ab for subsequent IHC assays. MAbs 28-1, 28-12, 20-12 and 29-8 had $K_D$ values of 130 pM, 94 pM, 160 pM and 1200 pM, respectively. The sequences of the heavy chain variable ($V_H$) and light (kappa) chain variable ($V_\kappa$) regions of mAb 28-8 are set forth in SEQ ID NOs. 35 and 36, respectively. The 28-8 Ab was shown to recognize a different epitope from mouse mAb 5H1, based on SPR analysis. Clones 28-1, 28-12, 29-8 and 20-12 were the next best Abs in terms of robust detection of membranous PD-L1 in FFPE tissues. Although mAb 13-1 had the best specificity in terms of detection of membranous PD-L1, the maximal detection level was lower than that of the other lead Abs. Western blotting was also performed with plus/minus antigen competition to verify the specificity of the top selected Abs for PD-L1.

The binding of mAbs 5H1 and 28-8 to membranous PD-L1 in FFPE test tissue samples comprising tumor cells and tumor-infiltrating inflammatory cells from different types of tumors was compared. Membranous PD-L1 expression was evaluated using the histoscore method performed by 2 independent pathologists. Four NSCLC, 2 MEL, and 2 RCC tumors were stained with 28-8 at 2 mg/ml and 5H1 at 5 mg/ml. The data are tabulated in Table 6, and shown graphically in Figure 9. The rabbit mAb 28-8 showed better detection (higher histoscores) for 7 out of 10 samples using 2.5-fold less Ab, and in only one sample was the histoscore for 5H1 slightly higher than for mAb 28-8.

Table 6. Comparison of mAbs 28-8 and 5H1 by histoscore analysis

| Tissue | Sample I.D. | Histoscore Average (5H1) | Histoscore Average (28-8) |
|---|---|---|---|
| NSCLC | 1080754B | 245 | 261 |
| NSCLC | 1080766B | 103 | 130 |
| NSCLC | 1080790 | 40 | 37 |

| Tissue | Sample I.D. | Histoscore Average (5H1) | Histoscore Average (28-8) |
|---|---|---|---|
| NSCLC | 1080993B | 12 | 16 |
| Mel | T030668 | 98 | 113 |
| Mel | T980744 | 98 | 123 |
| Mel | 1168657B | 0 | 0 |
| Mel | T980747 | 1 | 9 |
| RCC | 1164619B | 4 | 4 |
| RCC | 1167809B | 108 | 125 |

Taube *et al.* (2012) demonstrated by flow cytometry on cultured cells that mAb 5H1 bound to the cell surface, and the specificity of binding to PD-L1 was confirmed using a PD-L1 fusion protein to competitively block binding of the 5H1 mAb to tissue sections. These authors also compared 5H1 with a rabbit polyclonal anti-hPD-L1 Ab, 4059, previously described by Gadiot *et al.* (2011), and found that whereas 5H1 showed a cell surface staining pattern on FFPE samples, pAb 4059 demonstrated diffuse cytoplasmic staining. Further, when 5H1 was compared to pAb 4059 by western blot analysis, Ab 4059 bound to multiple proteins in lysates of melanoma cells in addition to a 50 kDa protein corresponding to the expected mass of glycosylated PD-L1, in contrast to 5H1 which specifically detected the 50 kDa band of glycosylated PD-L1 (Taube *et al.*, 2012). Contrary to the results of Taube *et al.* (2012) and the results disclosed herein (summarized in Table 7), Gadiot *et al.* (2011) reported that mAb 5H1 produced a high level background staining in FFPE tissue samples, whereas they found that pAb 4059 produced satisfactory specific staining of PD-L1 in FFPE samples. Thirteen other Abs tested by Gadiot *et al.* (2011) either did not stain FFPE tissues, gave a high background staining, or were not blocked by PD-L1 fusion protein competition, highlighting the difficulties in obtaining anti-PD-L1 Abs that bind specifically to PD-L1 in FFPE tissues.

In the present study, an automated IHC assay (*see* Example 10) was used to evaluate the binding of several commercially available anti-PD-L1 Abs and 5H1 (Dong *et al.*, 2002) to FFPE tissue samples containing various cells expressing PD-L1. The results, summarized in Table 7, show that none of the commercially available Abs tested specifically recognized membranous PD-L1 expression in human tissues known to express PD-L1, or to clearly distinguish CHO cells expressing PD-L1 versus the

untransfected parent CHO cells that did not express PD-L1. The inability of the polyclonal Ab (pAb) 4059 to bind specifically recognized membranous PD-L1 is consistent with the findings of Taube *et al.* (2012). The binding of 28-8 was similar to that of 5H1 in this assay, though histoscore analysis suggests that 28-8 performs better than 5H1.

Table 7. Binding of mAbs to FFPE Samples Containing PD-L1-expressing cells

| Source | Antibody Types | Clone No. (mAb) /Catalog No. (pAb) | Pos. vs. Neg. Staining on PD-L1 Cells* | Human Positive Tissues† |
|---|---|---|---|---|
| MBL | mAb | 27A2 | Failed | Failed |
| BioLegend | mAb | 29E.2A3 | Failed | Failed |
| eBiosciences | mAb | M1H1 | No Staining | No staining |
| Collaborator | mAb | 5H1 | Passed | Passed |
| ProSci | pAb | 4059 | Failed | Failed |
| LifeSpan BioSciences | pAb | LS-B480/0604 | Failed | Failed |
| Bristol-Myers Squibb | mAb | 28-8 | Passed | Passed |

*PD-L1 stably transfected CHO cells vs. parent CHO-S negative control;

†PD-L1 positive tissues included tonsil and/or thymus;

mAb, mouse monoclonal Ab; pAb, rabbit polyclonal Ab.

EXAMPLE 10

*Development of Automated IHC Protocol for Assessing PD-L1 Expression*

An automated IHC protocol was developed to assay PD-L1 expression in FFPE specimens. Tissue sections (4 μm) were mounted on slides, deparaffinized in an autostainer (Leica) by soaking twice for 5 min in xylene, and re-hydrated by soaking twice for 2 min each time in 100% EtOH, twice in 95% (v/v) EtOH, once in 70% (v/v) EtOH, and once in de-ionized water ($dH_2O$). Antigen retrieval was performed using a decloaking chamber (Biocare Medical Decloaking Chamber Plus) and Dako pH 6 buffer, heated to 110ºC (P1) for 10 min, then moved to the next step (P2 FAN ON at 98ºC; FAN OFF at 90ºC). The slides were cooled at room temperature (RT) for 15 min and rinsed

with water for about 1 min.

Reagents were set up on an autostainer (BioGenex i6000), and the tissue area was defined using a pap pen. The IHC assay, run using the autostainer in research mode, comprised the following steps: neutralizing endogenous peroxidase using the Peroxidase Block (Leica) for 10 min, followed by rinsing 3 times with IHC wash buffer (Dako); applying Protein Block (Leica) to the slides, and incubating for 10 min at RT, followed by washing 3 times with wash buffer; applying the primary Ab to the slides (2 μg/ml) and incubating for 1 h at RT, followed by washing 6 times with wash buffer; adding Post Primary Block (NovoLink Kit) to the slides and incubating for 30 min, followed by washing 6 times with wash buffer; adding NovoLink Polymer (NovoLink Kit) to the slides and incubating for 30 min, followed by washing 6 times with wash buffer; adding the DAB chromogen substrates (NovoLink Kit) and developing for 3 min, followed by rinsing 5 times with $dH_2O$ at RT; counterstaining with hematoxylin (NovoLink Kit) for 1 min at RT, followed by washing 3 times with $dH_2O$ for 5 times at RT. The primary Ab was selected from the rabbit anti-PD-L1 Abs shown in Table 4; mAb 28-8 was the preferred Ab. As a negative control, rabbit IgG (Dako) was used. The tissue sections were dehydrated using a Leica autostainer by washing once for 2 min in 70% EtOH, twice for 2 min in 95% EtOH, and three times for 2 min in 70% EtOH, then cleared by washing three times for 5 min in xylene. The sections were permanently mounted with permount to the slide, covered with a coverslip, and transferred to a chemical hood to dry.

## EXAMPLE 11

*Design of Phase 1 Clinical Study on Anti-PD-L1 Antibody*

*Study design*

A phase 1 study was conducted to assess the safety and tolerability of BMS-936559 (also referred to herein and in U.S. Patent No. 7,943,743 as 12A4) in patients with selected advanced solid tumors. Secondary objectives included initial assessment of the antitumor activity of BMS-936559 and pharmacokinetic evaluation. Pharmacodynamic measures were included under exploratory objectives. Patients were treated in 6-week cycles of BMS-936559 administered as a 60-minute intravenous infusion every 2 weeks on days 1, 15, and 29 of each cycle. Patients continued treatment for up to 16 cycles unless they experienced unacceptable toxicity, disease progression, or withdrew consent. In some patients who were clinically stable, treatment beyond initial disease progression was permitted until further progression was confirmed.

*Dose escalation*

Patients with advanced NSCLC, MEL, CRC, RCC, ovarian (OV), gastric (GC), breast (BC), and pancreatic (PC) carcinoma were eligible to enroll. Using an accelerated titration design, safety was assessed at doses of 0.3, 1, 3, and 10 mg/kg. One patient was enrolled in each successive cohort until there was a ≥ grade 2 drug-related AE during cycle 1. Two additional patients were then enrolled at that dose level and the study was transitioned to a standard 3+3 design. Intra-patient dose escalation or de-escalation was not permitted. The maximum tolerated dose (MTD) was defined as the highest dose where less than one-third of patients had a dose-limiting toxicity.

*Cohort expansion*

Initially, 5 expansion cohorts (n=16/cohort) were enrolled at 10 mg/kg for patients with NSCLC, MEL, RCC, OV, and CRC. Based on initial signals of activity, additional expansion cohorts (up to n=16/cohort) were enrolled for MEL (at 1.0 and 3.0 mg/kg), NSCLC (squamous or nonsquamous histology cohorts randomized to 1, 3, or 10 mg/kg), and at 10 mg/kg for PC, BC, and GC.

*Patients*

Patients were required to have documented advanced NSCLC, MEL, RCC, OV, CRC, PC, GC, or BC, and have failed at least one prior tumor-appropriate therapy for advanced/metastatic disease (except for PC or GC patients who could be treatment-naïve). Other inclusion criteria included age ≥ 18 years, life expectancy ≥ 12 weeks, Eastern Cooperative Oncology Group performance status of ≤ 2, measurable disease as defined by RECIST v1.0, and adequate hematologic, hepatic, and renal function. Patients with treated brain metastases were allowed, if stable for at least 8 weeks. Major exclusion criteria included a history of autoimmune disease or other diseases requiring systemic steroids or immunosuppressive medication, prior therapy with T cell- modulating Abs (including anti-PD-1, anti-PD-L1 and anti-CTLA-4), history of HIV, or active hepatitis B or C.

In this ongoing study, 207 patients with NSCLC (n=75), MEL (n=55), CRC (n=18), RCC (n=17), OV (n=17), PC (n=14), GC (n=7), or BC (n=4) were treated with BMS-936559 during a 34-month period and are included in the safety data. Efficacy was characterized in 160 response-evaluable patients. The baseline demographic characteristics of the total and response-evaluable patient populations were very similar (Brahmer *et al.*, 2012). Among treated patients, 86% had received prior chemotherapy

and 28% immunologic or biological therapy. Prior therapies by tumor type included immunotherapy (56%) and B-RAF inhibitor (9%) in patients with MEL; platinum-based chemotherapy (95%) and tyrosine kinase inhibitors (TKIs; 41%) in patients with NSCLC; and nephrectomy (94%), anti-angiogenic therapy (82%), and immunotherapy (41%) in patients with RCC (*see* Brahmer *et al.*, 2012, for more details on patient pre-treatments).

*Statistical analysis*

All 207 patients commencing treatment as of the date of analysis were used for summaries of baseline characteristics and AEs. The efficacy population consisted of 160 response-evaluable patients who initiated treatment at least 7 months before the date of analysis. AEs were coded using MedDRA v14.1. Individual best overall responses were derived from radiographic scan measurements according to modified RECIST v1.0. ORs were confirmed by at least one sequential tumor assessment. Additional details regarding statistical methods are provided in Brahmer *et al.* (2012).

EXAMPLE 12

*Safety Evaluations on Patients Treated with Anti-PD-L1 Antibody*

Safety evaluations (clinical examination and laboratory assessments) were conducted on all treated patients at baseline and regular intervals (weekly during cycle 1 and biweekly thereafter). AE severity was graded based on the NCI CTCAE, v3.0. Disease assessment via computed tomography (CT) scans or magnetic resonance imaging was performed at baseline and prior to each treatment cycle.

A MTD was not reached up to the highest tested dose of 10 mg/kg of BMS-936559. The median duration of therapy was 12 weeks (range 2.0-111.1 weeks). A relative dose intensity of $\geq$ 90% was achieved in 86% of patients. Twelve of 207 patients (6%) discontinued treatment due to a BMS-936559-related AE (*see* Brahmer *et al.*, 2012, for details).

AEs regardless of causality (any grade) were reported in 188 of 207 patients. Investigator-assessed BMS-936559-related AEs were noted in 126 of 207 (61%) patients. The most common drug-related AEs were fatigue, infusion reactions, diarrhea, arthralgia, rash, nausea, pruritus, and headache. Most events were low grade with BMS-936559-related grade 3-4 events noted in 19 of 207 (9%) patients (Brahmer *et al.*, 2012). The spectrum, frequency, and severity of BMS-936559-related AEs were similar across the dose levels, with the exception of infusion reactions. Drug-related AEOSIs, with potential

immune-related etiologies, were observed in 81 of 207 (39%) of the patients and included rash, hypothyroidism, hepatitis, and single cases each of sarcoidosis, endophthalmitis, diabetes mellitus, and myasthenia gravis (Brahmer *et al.*, 2012). These AEs were predominantly grade 1-2 and generally reversible with treatment interruption or discontinuation. Notably, 9 patients were treated with corticosteroids for the management of AEs. AEs improved or resolved in all patients. Furthermore, 4 of these 9 patients maintained disease control despite treatment with corticosteroids. Endocrine AEs were managed with replacement therapy and patients reinitiated treatment with BMS-936559 at the discretion of the treating physician. Infusion reactions were observed in 21 of 207 (10%) patients, predominantly at 10 mg/kg. They were grade 1-2 with the exception of one grade 3 event at 10 mg/kg. Infusion reactions were generally rapidly reversible with antihistamines and antipyretics and, in some cases, corticosteroids. A prophylactic regimen with antihistamines and antipyretics was implemented during the study. Patients with grade 1-2 infusion reactions were able to continue treatment with BMS-936559 with prophylactic antihistamines and antipyretics and at a reduced infusion rate. BMS-936559-related serious AEs occurred in 11 of 207 (5%) patients. As of the data analysis date, 45 patients (22%) had died (Brahmer *et al.*, 2012); no drug-related deaths were observed.

EXAMPLE 13

*Pharmacokinetics/Pharmacodynamics Analyses on Anti-PD-L1 Antibody*

For PK analyses, serial blood samples were collected and serum concentrations of BMS-936559 were quantified by ELISA. Peripheral blood mononuclear cells were isolated from patients at baseline and following one treatment cycle to assay PD-L1 RO by BMS-936559 on circulating CD3-positive T-cells via flow cytometry (Brahmer *et al.*, 2010).

Serum concentrations of BMS-936559 increased in a dose-dependent manner from 1-10 mg/kg (n=131). Geometric mean area under the curve (0-14 days) for the 1, 3, and 10 mg/kg dose levels were 2210, 7750, and 36620 μg/mL•hr (coefficient of variation [CV] 34-59%), respectively; geometric mean peak concentrations at these dose levels were 27, 83, and 272 μg/mL (CV 30−34%), respectively, after the first dose. The half-life of BMS-936559 was estimated from population pharmacokinetic data as approximately 15 days. PD-L1 RO on CD3-positive peripheral blood lymphocytes was assessed in 29 MEL patients at the end of 1 cycle of treatment, at BMS-936559 doses from 1-10 mg/kg. Median RO exceeded 65% for all groups (Brahmer *et al.*, 2012).

EXAMPLE 14

*Antitumor Efficacy Exhibited by Anti-PD-L1 Antibody*

One-hundred and sixty patients out of the 207 treated were evaluable for response by February 2012 and included those with NSCLC, MEL, CRC, RCC, OV, and PC, but not patients with GC or BC. Clinical activity was observed at all doses ≥ 1 mg/kg (Brahmer *et al.*, 2012). ORs (confirmed complete [CR] or partial [PR] responses) were observed in patients with MEL, NSCLC, RCC, and OV (Table 8), as illustrated by representative spider plots and CT scans (Figures 10-13), and many ORs were also durable (Table 9). Four additional patients had a persistent reduction in target lesions in the presence of new lesions, consistent with an "immune-related" pattern of response; however, for the purpose of calculating response rates, they were not categorized as responders. Antitumor responses and/or prolonged stable disease (SD) were observed in patients with a variety of prior therapies received. ORs were observed even in patients with an extensive burden of metastatic disease.

In patients with MEL, there were 9 ORs across the 1, 3, and 10 mg/kg dose levels, with response rates of 6%, 29%, and 19%, respectively. Three MEL patients achieved a CR. All 9 MEL patients who experienced an OR started treatment ≥ 1 year prior to data analysis; of these 5 had a response duration ≥ 1 year. Additionally 14 MEL patients (27%) had SD lasting ≥ 24 weeks. In patients with NSCLC, there were 5 ORs amongst the 3 and 10 mg/kg dose levels, with response rates of 8% and 16%, respectively. There were ORs in patients with non-squamous (n=4) or squamous histology (n=1). All 5 NSCLC responders started treatment ≥ 24 weeks prior to data analysis; of these, 3 had responses lasting ≥ 24 weeks. Six additional NSCLC patients had SD lasting ≥ 24 weeks. There was 1 PR out of 17 patients with OV (6% response rate) and 3 patients (18%) with SD lasting ≥ 24 weeks, all at the 10 mg/kg dose. In patients with RCC, there were ORs in 2 of 17 (12%) patients treated at 10 mg/kg with responses lasting 4 and 18 months, respectively. Seven additional RCC patients had SD lasting ≥ 24 weeks.

Table 8. Clinical Activity of BMS-936559 in 160 Patients, Response Evaluable*

| Tumor Type | Dose (mg/kg) | n | ORR[§] No. patients (%) [95% CI] | SD ≥ 24 wk No. patients (%) [95% CI] | PFSR** at 24 wk (%) [95% CI] |
|---|---|---|---|---|---|
| MEL | 0.3 | 1 | 0 [0-98] | 0 [0-98] | N/A |
| | 1 | 18 | 1 (6) [0.1-27] | 6 (33) [13-59] | 39 [16-61] |
| | 3 | 17 | 5 (29)[†] [10-56] | 3 (18) [4-43] | 47 [21-72] |
| | 10 | 16 | 3 (19)[††] [4-46] | 5 (31) [11-59] | 44 [19-68] |
| ALL MEL | | 52 | 9 (17) (8-30) | 14 (27) [16-41] | 42 [28-56] |
| NSCLC[§] | 1 | 11 | 0 [0-29] | 0 [0-29] | N/A |
| Squamous | 1 | 1 | 0 [0-98] | 0 [0-98] | N/A |
| Non-Squamous | 1 | 10 | 0 [0-31] | 0 [0-31] | N/A |
| | 3 | 13 | 1 (8) [0.2-36] | 1 (8) [0.2-36] | 34 [7-60] |
| Squamous | 3 | 4 | 0 [0-60] | 1 (25) [0.6-81] | 50 [1-99] |
| Non-Squamous | 3 | 9 | 1 (11) [0.3-48] | 0 [0-34] | 25 [0-55] |
| | 10 | 25 | 4 (16) [5-36] | 5 (20) [7-41] | 46 [25-67] |
| Squamous | 10 | 8 | 1 (13) [0.3-53] | 2 (25) [3-65] | 47 [10-83] |
| Non-Squamous | 10 | 17 | 3 (18) [4-43] | 3 (18) [4-43] | 46 [20-72] |
| ALL NCSLC | | 49 | 5 (10) [3-22] | 6 (12) [5-25] | 31 [17-45] |
| ALL Squamous | | 13 | 1 (8) [0.2-36] | 3 (23.1) [5-54] | 43 [15-71] |
| ALL Non-Squamous | | 36 | 4 (11) [3-26] | 3 (8) [2-23] | 26 [10-42] |
| OV | 3 | 1 | 0 [0-98] | 0 [0-98] | N/A |
| | 10 | 16 | 1 (6) [0.2-30] | 3 (19) [4-46] | 25 [4-46] |
| ALL OV | | 17 | 1 (6) [0.1-29] | 3 (18) [4-43] | 22 [2-43] |
| RCC | 10 | 17 | 2 (12) [2-36] | 7 (41) [18-67] | 53 [29-77] |

CI denotes Confidence intervals, MEL melanoma, RCC renal cell carcinoma, NSCLC non-small cell lung cancer, OV ovarian cancer, RCC renal cell carcinoma, N/A not applicable, ORR objective response rate (complete response + partial response), SD stable disease, and PFSR progression-free survival rate.

* Efficacy population consists of response-evaluable patients who initiated treatment at least 7 months prior to the date of analysis and had measurable disease at a baseline tumor assessment and at least one of the following: an on-study tumor assessment,

clinical progression, or death.

† Includes two CRs.

†† Includes one CR.

§ Objective response rates ({[CR + PR] ÷ n} × 100) are based on confirmed responses only, with confidence intervals calculated using the Clopper-Pearson method.

** Progression-free survival rate was the proportion of patients who did not progress, and were alive at 24 weeks, calculated by the Kaplan-Meier methodology, with confidence intervals using the Greenwood method.

Individual patient responses were adjudicated per RECIST v1.0 with modification (see study protocol in Brahmer *et al.* (2012) *N Engl J Med* (submitted) for additional information).

Table 9. Duration of Objective Responses to BMS-936559*

| Tumor Type | Dose (mg/kg) | No. of Patients with OR | Duration of Response (months)† |
|---|---|---|---|
| MEL | 1 | 1 | 6.9 |
| | 3 | 5 | 23.5+, 22.9+, 16.2+, 4.1+, 3.5 |
| | 10 | 4 | 24.6+, 23.9+, 18.0+, 17.0 |
| NSCLC | 1 | 0 | 9.2+ |
| | 3 | 1 | 2.3+ |
| | 10 | 4 | 16.6+, 12.6+, 9.8, 3.5 |
| RCC | 10 | 2 | 18, 4 |
| OV | 10 | 1 | 1.3+ |

* MEL denotes melanoma, NSCLC non-small cell lung cancer, RCC renal cell cancer, OV ovarian cancer.

† Time from first response to time of documented progression, death, or for censored data (denoted by +), time to last tumor assessment.

EXAMPLE 15

*Design of Phase 1 Clinical Study on Anti-PD-1 plus Anti-CTLA-4 in Advanced MEL*

*Study Design*

In this phase 1 study, successive cohorts of patients were treated with escalating

doses of intravenous nivolumab and ipilimumab administered concurrently (concurrent regimen), and separately, two cohorts of patients treated previously with ipilimumab received nivolumab alone (sequenced regimen).

For the concurrent regimen, patients received during the induction period nivolumab and ipilimumab every 3 weeks for 4 doses, followed by nivolumab alone every 3 weeks for 4 doses. Combined treatment was subsequently continued during the maintenance period every 12 weeks for up to 8 doses. When both drugs were administered together, nivolumab was administered first. Within a cohort, nivolumab and ipilimumab doses were kept constant during the induction and maintenance periods. The dose-limiting-toxicity evaluation period was through week 9. Tumor assessments were at weeks 12, 18, 24, and 36, and then every 12 weeks thereafter.

In the sequenced regimen, patients previously treated with ipilimumab prior to study entry received nivolumab every 2 weeks for up to 48 doses. Nivolumab therapy was initiated within 4-12 weeks after ipilimumab monotherapy. Tumor assessments were at week 8 and then every 8 weeks thereafter. Tumor responses were adjudicated using mWHO and immune-related mWHO criteria for both regimens.

After completion of therapy, patients without confirmed disease progression were followed for up to 2.5 years. Patients with CR, PR, or SD $\geq$ 24 weeks and subsequent disease progression could be retreated with the original regimen. Safety evaluation was performed per protocol. The severity of AEs was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 3.0. Disease assessment, using CT and/or MRI as appropriate, was performed per protocol.

*Dose escalation and cohort expansion*

The study was initially planned to evaluate the concurrent regimen using a standard 3 + 3 design for the dose escalation phase, followed by cohort expansion to a total of up to 16 patients at the maximum tolerated dose or the maximum administered dose. The dose-limiting toxicity (DLT) evaluation period for dose escalation was 9 weeks. No intra-patient dose escalation was allowed and patients who experienced DLT were discontinued from therapy. Patients who withdrew from study during the DLT evaluation period for reasons other than drug-related toxicity could be replaced. The protocol was amended to permit expansion of any concurrent regimen cohort during dose escalation to N = up to 12 patients. Two sequenced-regimen cohorts (6 to 16 patients each) were later added; patients were treated with nivolumab (1 mg/kg or 3 mg/kg) after

having received prior ipilimumab.

*Patients*

Eligible patients were 18 years of age and had a diagnosis of measurable, unresectable stage III or IV melanoma; Eastern Cooperative Oncology Group performance status of 0-1, where 0 is asymptomatic and 1 is mildly symptomatic; adequate organ function; and life expectancy of ≥ 4 months. Patients with active, untreated central nervous system metastasis; history of autoimmune disease; prior therapy with T-cell modulating antibodies (excluding ipilimumab for sequenced-regimen cohorts); HIV; or hepatitis B or C were excluded.

In the sequenced-regimen cohorts, patients were required to have received 3 prior doses of ipilimumab, with the last dose administered within 4-12 weeks of initiation of nivolumab. Patients with CR, progression with evidence of clinical deterioration, or a history of high-grade AEs related to ipilimumab were excluded (*see* Wolchok *et al.* 2013a, for details of protocol).

Eighty-six patients were treated between December 2009 and February 2013, 53 with the concurrent regimen and 33 with the sequenced regimen. Baseline patient characteristics are detailed in Wolchok *et al.* (2013a). In the concurrent and sequenced regimens, 38% and 100% of patients, respectively, received prior systemic therapy. The majority of patients had M1c disease and >30% had elevated serum lactate dehydrogenase (LDH). Most patients enrolled to the sequenced-regimen cohorts demonstrated radiographic progression (73%) with prior ipilimumab treatment.

*PD-L1 immunohistochemistry*

Pre-treatment PD-L1 expression was measured by IHC in FFPE tumor specimens using the rabbit anti-PD-L1 mAb, 28-8, and an automated assay developed by Dako (Carpinteria, CA). Ab specificity was assessed by western blotting against recombinant PD-L1 protein and lysates from PD-L1 expressing and non-expressing cell lines. An IHC assay with and without antigen competition and an assessment of staining patterns in normal human tissues was performed. Analytical sensitivity, specificity, repeatability, reproducibility, and robustness of the immunohistochemistry assay were tested and met all pre-specified acceptance criteria. Two pathologists, blinded to outcome, independently read and adjudicated scores for all clinical specimens. A sample was defined as PD-L1-positive if 5% of tumor cells exhibited membrane PD-L1 staining of any intensity in a section with 100 evaluable cells.

*Statistical analysis*

All treated patients (N=86) as of February 2013 were used to describe baseline characteristics, safety, and absolute lymphocyte count (ALC), and analysis of PD-L1 staining. The efficacy population consisted of 82 response-evaluable patients who received at least one dose of study therapy, had measurable disease at baseline, and one of the following: > 1 on-treatment tumor evaluation, clinical progression, or death before the first on-treatment tumor evaluation. AEs were coded using MedDRA, version 15.1. Select AEs with potential immunologic etiologies were identified using a pre-defined list of MedDRA terms. Best overall responses were derived programmatically from tumor-measurements provided by the study-site radiologist and investigators per modified WHO (mWHO) or immune-related response criteria (Wolchok *et al.* 2009). Complete and partial responses were confirmed by at least one subsequent tumor assessment. An analysis also was performed to assess the magnitude of reduction in target lesions by radiographic assessment. A response was characterized as deep if a reduction of 80% from baseline measurements was noted. Unconfirmed responses as of the date of this analysis were also included in an estimate of aggregate clinical activity.

EXAMPLE 16

*Safety Evaluations on MEL Patients Treated with Anti-PD-1 plus Anti-CTLA-4*

For the concurrent regimen (n=53), AEs of any grade, irrespective of attribution, were observed in 98% of patients. Treatment-related AEs were observed in 93% of patients with the most common being rash (55%), pruritus (47%), fatigue (38%), and diarrhea (34%). Grade 3-4 AEs, irrespective of attribution, were observed in 72% of patients, while grade 3-4 treatment-related events were noted in 53%, with the most common being elevations of lipase (13%), aspartate aminotransferase (13%) and alanine aminotransferase (11%). Six of 28 (21%) patients had grade 3-4 dose- limiting treatment-related events. Treatment-related serious AEs were reported in 49% of patients. Common grade 3-4 treatment-related select AEs included hepatic (15%), gastrointestinal (9%), and renal (6%) events. Isolated cases of pneumonitis and uveitis were seen, consistent with historical monotherapy experiences. Eleven (21%) patients discontinued due to treatment-related AEs.

Cohort 3 (3 mg/kg nivolumab + 3 mg/kg ipilimumab) exceeded the MTD (3 of 6 patients experienced asymptomatic grade 3-4 elevated lipase that persisted for 3 weeks).

Cohort 2 (1 mg/kg nivolumab + 3 mg/kg ipilimumab) was identified as the MTD (grade 3 uveitis, grade 3 elevated AST/ALT in 1 patient each).

For the sequenced regimen (n=33), AEs of any grade, irrespective of attribution, were observed in 29 (88%) patients. Treatment-related AEs were observed in 24 (73%) patients, with the most common including pruritus (18%) and lipase elevation (12%). Grade 3-4 AEs, irrespective of attribution, were observed in 11 (33%) patients, while grade 3-4 treatment-related AEs were observed in 6 (18%) patients, with lipase elevation as the most common event (6%). Treatment-related serious AEs were reported in 7 (21%) patients. Grade 3-4 endocrine events were noted as treatment-related select AEs in 2 patients. One patient had grade-2 pneumonitis. Three (9%) patients discontinued due to treatment-related AEs.

For both the concurrent and sequenced regimens, treatment-related AEs were manageable and generally reversible with immunosuppressants and/or replacement therapy (for endocrinopathies) per previously algorithms previously established for ipilimumab (*see* YERVOY® package insert). Amongst the 86 patients treated on the study, 28 of 73 patients (38%) with drug-related adverse events required management with systemic glucocorticoids. Three patients required additional immunosuppressive therapy with infliximab (2 patients) or mycophenolate mofetil (1 patient). No treatment-related deaths were reported. Additional details of AEs and their management are provided in (Wolchok *et al.* 2013a).

## EXAMPLE 17

*Efficacy Exhibited by Combination of Anti-PD-1 and Anti-CTLA-4 in MEL Patients*

Clinical activity was observed with both the concurrent and sequenced regimens (Tables 10 and 11). In the concurrent-regimen cohorts, confirmed objective responses (OR) by mWHO criteria were observed in 21 of 52 (40%; 95% CI: 27-55) response-evaluable patients across all doses. After noting several patients who demonstrated major responses (approaching CR), the number of patients with at least 80% tumor reduction, an empirical threshold chosen because it represents a level of tumor regression that approaches complete response, was assessed. This depth of response was uncommon in published studies of checkpoint blockade (Hodi *et al.*, 2010; Topalian *et al.*, 2012b). Sixteen patients had ≥ 80% tumor reduction at 12 weeks, including 5 CRs (Table 10, Figures 14A and 15-17). In addition to the 21 patients with OR by mWHO criteria, 4 patients experienced an objective response by immune-related response criteria and 2 patients had

unconfirmed responses. These patients were not included in the calculation of ORRs. For the concurrent regimen, overall evidence of clinical activity (conventional, unconfirmed, or immune-related response or SD ≥ 24 weeks) was observed in 65% (95% CI: 51-78; Table 10) of patients. The profound impact of the concurrent combination can best be appreciated in the waterfall plot (Figure 14B). Responses were ongoing among 19 of 21 responders, with durations ranging from 6.1+ to 72.1+ weeks at the time of data analysis (Table 12). For patients treated at the MTD (cohort 2, 1 mg/kg nivolumab + 3 mg/kg ipilimumab), OR occurred in 9 of 17 (53%; 95% CI: 28-77) patients, including 3 CRs. All 9 responders achieved ≥ 80% tumor reduction at their first scheduled on-treatment assessment (Table 10 and Figure 14A).

For patients in the sequenced-regimen cohorts, 6 of 30 patients achieved OR (20%; 95% CI: 8-39) including 1 CR. Four (13%) patients achieved 80% tumor reduction at 8 weeks (Table 11 and Figure 18). Additional patients had immune-related (n=3) or unconfirmed (n=3) responses. When objective, immune-related or unconfirmed responses or SD ≥ 24 weeks are considered, evidence of clinical activity for the sequenced regimen was observed in 43% (95% CI: 26-63). The waterfall plot reveals that patients who did not respond to prior ipilimumab can respond to subsequent nivolumab (Figure 18C).

Table 10. Clinical Activity of Patients who Received the Concurrent Regimen of Nivolumab and Ipilimumab*

| Cohort | Dose | Response-Evaluable Patients† n | CR n | PR n | uPR‡ n | irPR§ n | SD ≥24 wk n | irSD§ ≥24 wk n | Objective Response Rate¶ % [95% CI] | Aggregate Clinical Activity Rate‖ % [95% CI] | 80% Tumor Reduction at 12 wk n (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.3 mg/kg nivolumab + 3 mg/kg ipilimumab | 14 | 1 | 2 | 0 | 2 | 2 (14) | 0 | 21 [5-51] | 50 [23-77] | 4 (29) |
| 2 | 1 mg/kg nivolumab + 3 mg/kg ipilimumab | 17 | 3 | 6 | 0 | 0 | 0 | 2 (12) | 53 [28-77] | 65 [38-86] | 7 (41)** |
| 2a | 3 mg/kg nivolumab + 1 mg/kg ipilimumab | 15 | 1 | 5 | 2 | 1 | 2 (13) | 0 | 40 [16-68] | 73 [45-92] | 5 (33) |
| 3 | 3 mg/kg nivolumab + 3 mg/kg ipilimumab | 6 | 0 | 3 | 0 | 1 | 0 | 1 (17) | 50 [12-88] | 83 [36-100] | 0 |
| All | Concurrent treatment | 52 | 5 | 16 | 2 | 4 | 4 (8) | 3 (6) | 40 [27-55] | 65 [51-78] | 16 (31) |

* CR denotes complete response, PR partial response, uPR unconfirmed partial response, irPR immune-related partial response, SD stable disease, irSD immune-related stable disease.

† Response-evaluable patients were those who received at least one dose of study therapy, had measurable disease at baseline, and one of the following: 1) at least one on-treatment tumor evaluation, 2) clinical progression, or 3) death prior to the first on-treatment tumor evaluation.

‡ Patients who had a PR after one tumor assessment but did not have sufficient follow-up time for confirmation of the initial PR.

§ Patients who had target tumor-lesion reduction in the presence of new lesions, consistent with immune-related PR or SD.

¶ [(CR + PR) / no. response-evaluable patients] × 100. Confidence intervals were estimated by the Clopper-Pearson method.

‖ [(CR + PR + uCR + uPR + irPR + SD ≥24 wk + irSD ≥24 wk) / no. response-evaluable patients] × 100.

** Two additional patients in cohort 2 achieved ≥80% tumor reduction at their first scheduled assessment, which was conducted after week 12.

Table 11. Clinical Activity of Patients who Received the Sequenced Regimen of Nivolumab and Ipilimumab*

| Cohort | Dose | Response-Evaluable Patients† n | CR n | PR n | uPR‡ n | irPR§ n | SD ≥ 24 wk n | irSD§ ≥ 24 wk n | Objective Response Rate¶ % [95% CI] | Aggregate Clinical Activity Rate‖ % [95% CI] | 80% Tumor Reduction at 8 wk n (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 6 | 1 mg/kg nivolumab + prior ipilimumab | 16 | 1 | 5 | 2 | 2 | 1 (6) | 0 | 38 [15-65] | 69 [41-89] | 4 (25) |
| 7 | 3 mg/kg nivolumab + prior ipilimumab | 14 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 14 [2-43] | 0 |
| All | Sequenced treatment | 30 | 1 | 5 | 3 | 3 | 1 (3) | 0 | 20 [8-39] | 43 [26-63] | 4 (13) |

* CR denotes complete response, PR partial response, uPR unconfirmed partial response, irPR immune-related partial response, SD stable disease, irSD immune-related stable disease.

† Response-evaluable patients were those who received at least one dose of study therapy, had measurable disease at baseline, and one of the

following: 1) at least one on-treatment tumor evaluation, 2) clinical progression, or 3) death prior to the first on-treatment tumor evaluation.

‡ Patients who had a PR after one tumor assessment but did not have sufficient follow-up time for confirmation of the initial PR.

Table 12. Duration of Confirmed Objective Responses of Individual Patients

| Cohort | Dose | Response | Duration of Response, weeks |
|---|---|---|---|
| 1 | 0.3 mg/kg nivolumab + 3 mg/kg ipilimumab | CR | 48.0+ |
| | | PR | 72.1+, 65.4+ |
| 2 | 1 mg/kg nivolumab + 3 mg/kg ipilimumab | CR | 36.1+, 28.9+, 24.7+ |
| | | PR | 54.4+, 52.6, 23+, 18.4+, 14.0 +, 11.1+ |
| 2a | 3 mg/kg nivolumab + 1 mg/kg ipilimumab | CR | 7.7+ |
| | | PR | 18.1+, 12.3, 6.6+, 6.4+, 6.1+ |
| 3 | 3 mg/kg nivolumab + 3 mg/kg ipilimumab | PR | 38.7+, 31.3+, 12.1+ |
| 6 | 1 mg/kg nivolumab + prior ipilimumab | CR | 24.1+ |
| | | PR | 16.1+, 12.1+, 8.7+, 8.4+, 8.1+ |
| 7 | 3 mg/kg nivolumab + prior ipilimumab | CR | NA* |
| | | PR | NA* |

* Not available; as of the date of this analysis, no confirmed objective responses had been reported.

*Assessment of tumor PD-L1 expression and absolute lymphocyte count*

Tumor PD-L1 expression and alterations in the peripheral blood ALC have been explored as biomarkers for nivolumab and ipilimumab monotherapy, respectively (Topalian *et al.*, 2012b; Berman *et al.*, 2009; Ku *et al.*, 2010; Postow *et al.*, 2012; Delyon *et al.*, 2013). Tumor PD-L1 expression was characterized via IHC staining and pharmacodynamic changes in peripheral blood ALC was analyzed. Using a $\geq$ 5% cut off to define PD-L1 positivity, tumor specimens from 21 of 56 (38%) patients were PD-L1-positive. ORs were seen in patients with either PD-L1-positive (6/13) or PD-L1-negative (9/22) tumors amongst patients treated with the concurrent regimen (post-hoc P-value > 0.99; Fisher's exact test). In sequenced-regimen cohorts, a numerically higher number of overall responses were seen in patients with PD-L1-positive tumor samples (4/8) compared with patients with PD-L1-negative tumors (1/13), but the numbers are small.

In contrast to observations with ipilimumab monotherapy, a consistent rise in ALC from baseline was not detected in patients treated with the concurrent combination or in patients treated with nivolumab following ipilimumab therapy. In the concurrent-regimen cohorts, patients with a low ALC at weeks 5 to 7 (< 1000 cells/μL) (Ku *et al.*, 2010) had similar OR (43%) compared with patients with a normal ALC at weeks 5 to 7 (40%). Likewise, in the sequenced-regimen cohorts, 17% of patients with low ALC had OR and 23% of patients with normal or high ALC had OR.

Sequence Listing Summary

| SEQ ID NO: | Description |
|---|---|
| 1 | $V_H$ amino acid sequence of 17D8 |
| 2 | $V_H$ amino acid sequence of 2D3 |
| 3 | $V_H$ amino acid sequence of 4H1 |
| 4 | $V_H$ amino acid sequence of 5C4 |
| 5 | $V_H$ amino acid sequence of 4A11 |
| 6 | $V_H$ amino acid sequence of 7D3 |
| 7 | $V_H$ amino acid sequence of 5F4 |
| 8 | $V_L$ amino acid sequence of 17D8 |
| 9 | $V_L$ amino acid sequence of 2D3 |
| 10 | $V_L$ amino acid sequence of 4H1 |
| 11 | $V_L$ amino acid sequence of 5C4 |

| SEQ ID NO: | Description |
|---|---|
| 12 | $V_L$ amino acid sequence of 4A11 |
| 13 | $V_L$ amino acid sequence of 7D3 |
| 14 | $V_L$ amino acid sequence of 5F4 |
| 15 | $V_H$ amino acid sequence of 3G10 |
| 16 | $V_H$ amino acid sequence of 12A4 |
| 17 | $V_H$ amino acid sequence of 10A5 |
| 18 | $V_H$ amino acid sequence of 5F8 |
| 19 | $V_H$ amino acid sequence of 10H10 |
| 20 | $V_H$ amino acid sequence of 1B12 |
| 21 | $V_H$ amino acid sequence of 7H1 |
| 22 | $V_H$ amino acid sequence of 11E6 |
| 23 | $V_H$ amino acid sequence of 12B7 |
| 24 | $V_H$ amino acid sequence of 13G4 |
| 25 | $V_L$ amino acid sequence of 3G10 |
| 26 | $V_L$ amino acid sequence of 12A4 |
| 27 | $V_L$ amino acid sequence of 10A5 |
| 28 | $V_L$ amino acid sequence of 5F8 |
| 29 | $V_L$ amino acid sequence of 10H10 |
| 30 | $V_L$ amino acid sequence of 1B12 |
| 31 | $V_L$ amino acid sequence of 7H1 |
| 32 | $V_L$ amino acid sequence of 11E6 |
| 33 | $V_L$ amino acid sequence of 12B7 |
| 34 | $V_L$ amino acid sequence of 13G4 |
| 35 | $V_H$ amino acid sequence of 28-8 |
| 36 | $V_L$ amino acid sequence of 28-8 |
| 37 | Heavy chain CDR1 sequence of 28-8 |
| 38 | Heavy chain CDR2 sequence of 28-8 |
| 39 | Heavy chain CDR3 sequence of 28-8 |
| 40 | Light chain CDR1 sequence of 28-8 |
| 41 | Light chain CDR2 sequence of 28-8 |
| 42 | Light chain CDR1 sequence of 28-8 |

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CLAIMS

What is claimed is:

1. A method of treating a subject afflicted with a cancer comprising administering to the subject a therapeutically effective amount of an antibody or an antigen-binding portion thereof that disrupts the interaction between Programmed Death-1 (PD-1) and Programmed Death Ligand-1 (PD-L1, wherein the antibody or antigen-binding portion thereof binds specifically to PD-1 or to PD-L1.

2. The method of claim 1, wherein the cancer is selected from the group consisting of melanoma, renal cell carcinoma, squamous non-small cell lung cancer (NSCLC), non-squamous NSCLC, colorectal cancer, castration-resistant prostate cancer, ovarian cancer, gastric cancer, hepatocellular carcinoma, pancreatic carcinoma, squamous cell carcinoma of the head and neck, carcinomas of the esophagus, gastrointestinal tract and breast, and a hematological malignancy.

3. The method of claim 1, wherein the therapeutically effective amount of the antibody or antigen-binding portion thereof comprises a dose ranging from 0.1 to 10.0 mg/kg body weight which is administered at a dosing schedule of once per week, once every two weeks, or once a month.

4. A method for immunotherapy of a subject afflicted with cancer, which method comprises:

(a) selecting a subject that is a suitable candidate for immunotherapy, the selecting comprising:

(i) providing a test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells;

(ii) assessing the proportion of cells in the test tissue sample that express PD-L1 on the cell surface; and

(iii) selecting the subject as a suitable candidate based on an assessment that the proportion of cells in the test tissue sample that express PD-L1 on the cell surface exceeds a predetermined threshold level; and

(b) administering a composition comprising a therapeutically effective amount of an anti-PD-1 antibody to the selected subject.

5. The method of claim 4, wherein the proportion of cells that express PD-L1 is assessed by performing an assay to determine the presence of PD-L1 polypeptide on the surface of cells in the test tissue sample.

6. The method of claim 5, wherein the test tissue sample is a formalin-fixed paraffin-embedded (FFPE) tissue sample.

7. The method of claim 6, wherein the presence of PD-L1 polypeptide is determined using an automated IHC assay.

8. The method of claim 12, wherein the IHC assay is performed using an anti-PD-L1 monoclonal antibody to bind to the PD-L1 polypeptide, wherein the anti-PD-L1 monoclonal antibody is selected from 28-8, 28-1, 28-12, 29-8, and 5H1.

9. The method of claim 4, wherein the predetermined threshold level is 1% of tumor cells or a single tumor-infiltrating inflammatory cell expressing cell surface PD-L1 as determined by automated IHC using mAb 28-8.

10. A method for treatment of a subject afflicted with cancer, which method comprises:

(a) selecting a subject that is not suitable for anti-PD-1 antibody immunotherapy, the selecting comprising:

(i) providing a test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor cells and tumor-infiltrating inflammatory cells;

(ii) assessing the proportion of cells in the test tissue sample that express PD-L1 on the cell surface; and

(iii) selecting the subject as not suitable for anti-PD-1 antibody immunotherapy based on an assessment that the proportion of cells in the test tissue sample that express PD-L1 on the cell surface is less than a predetermined threshold level; and

(b) administering a standard-of-care therapeutic other than an anti-PD-1 antibody to the selected subject.

11. A monoclonal antibody or an antigen-binding portion thereof that binds specifically to a cell surface-expressed PD-L1 polypeptide in a formalin-fixed, paraffin-embedded (FFPE) tissue sample.

12. The monoclonal antibody or antigen-binding portion thereof of claim 16, which antibody or portion thereof comprises the CDR1, CDR2 and CDR3 regions in a heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 35, and the CDR1, CDR2 and CDR3 regions in a light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 36.

13. The monoclonal antibody or antigen-binding portion thereof of claim 16, which antibody or portion thereof comprises a heavy chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 35, and a light chain variable region comprising consecutively linked amino acids having the sequence set forth in SEQ ID NO: 36.

14. A method of treating a subject afflicted with a cancer comprising administering to the subject:

(a) an antibody or an antigen-binding portion thereof that specifically binds to and inhibits Programmed Death-1 (PD-1); and

(b) an antibody or an antigen-binding portion thereof that specifically binds to and inhibits Cytotoxic T-Lymphocyte Antigen-4 (CTLA-4);

each antibody being administered at a dosage ranging from 0.1 to 20.0 mg/kg body weight in a concurrent regimen comprising:

(i) an induction dosing schedule comprising combined administration of the anti-PD-1 and anti-CTLA-4 antibodies at a dosing frequency of at least once every 2, 3 or 4 weeks, or at least once a month, for at least 2, 4, 6, 8 or 10 doses, followed by administration of the anti-PD-1 alone at a dosing frequency of at least once every 2, 3 or 4 weeks, or at least once a month, for at least 2, 4, 6, 8 or 12 doses; followed by

(ii) a maintenance dosing schedule comprising combined administration of the anti-PD-1 and anti-CTLA-4 antibodies at a

dosing frequency of at least once every 8, 12 or 16 weeks, or at least once a quarter, for at least 4, 6, 8, 10, 12 or 16 doses.

15. The method of claim 14, wherein the anti-PD-1 and anti-CTLA-4 antibodies are administered at the following dosages:

(a) 0.1 mg/kg anti-PD-1 antibody and 3 mg/kg of anti-CTLA-4 antibody;
(b) 0.3 mg/kg anti-PD-1 antibody and 3 mg/kg of anti-CTLA-4 antibody;
(c) 1 mg/kg anti-PD-1 antibody and 3 mg/kg of anti-CTLA-4 antibody;
(d) 3 mg/kg anti-PD-1 antibody and 3 mg/kg of anti-CTLA-4 antibody;
(e) 5 mg/kg anti-PD-1 antibody and 3 mg/kg of anti-CTLA-4 antibody;
(f) 10 mg/kg anti-PD-1 antibody and 3 mg/kg of anti-CTLA-4 antibody;
(g) 0.1 mg/kg anti-PD-1 antibody and 1 mg/kg of anti-CTLA-4 antibody;
(h) 0.3 mg/kg anti-PD-1 antibody and 1 mg/kg of anti-CTLA-4 antibody;
(i) 1 mg/kg anti-PD-1 antibody and 1 mg/kg of anti-CTLA-4 antibody;
(j) 3 mg/kg anti-PD-1 antibody and 1 mg/kg of anti-CTLA-4 antibody;
(k) 5 mg/kg anti-PD-1 antibody and 1 mg/kg of anti-CTLA-4 antibody; or
(l) 10 mg/kg anti-PD-1 antibody and 1 mg/kg of anti-CTLA-4 antibody.

16. A method of treating a subject afflicted with a cancer, the subject having previously been treated with an anti-CTLA-4 antibody, which method comprises administering to the subject in a sequenced regimen an antibody or an antigen-binding portion thereof that specifically binds to and inhibits PD-1 at a dosage ranging from 0.1 to 20.0 mg/kg body weight and at a dosing frequency of at least once every week, at least once every 2, 3 or 4 weeks, or at least once a month, for up to 6 to up to 72 doses.

17. The method of claim 15 or 16, wherein the anti-PD-1 antibody is nivolumab.

18. The method of 15 or 16, wherein the anti-CTLA-4 antibody is ipilimumab.

19. An anti-PD-1 antibody or an antigen-binding portion thereof for use in treating a subject afflicted with a cancer in a concurrent regimen comprising combined administration with an anti-CTLA-4 antibody or an antigen-binding portion thereof, wherein the anti-PD-1 and anti-CTLA-4 antibodies are each administered

at a dosage ranging from 0.1 to 20.0 mg/kg body weight, and further wherein the concurrent regimen comprises:

(i) an induction dosing schedule comprising combined administration of the anti-PD-1 and anti-CTLA-4 antibodies at a dosing frequency of at least once every 2, 3 or 4 weeks, or at least once a month, for at least 2, 4, 6, 8 or 12 doses, followed by administration of the anti-PD-1 alone at a dosing frequency of at least once every 2, 3 or 4 weeks, or at least once a month, for at least 2, 4, 6, 8 or 10 doses; followed by

(ii) a maintenance dosing schedule comprising combined administration of the anti-PD-1 and anti-CTLA-4 antibodies at a dosing frequency of at least once every 8, 12 or 16 weeks, or at least once a quarter, for up to 4, 6, 8, 10, 12 or 16 doses.

20. A kit for treating a subject afflicted with a cancer, the kit comprising:

(a) a dosage ranging from 0.1 to 20.0 mg/kg body weight of an antibody or an antigen-binding portion thereof that specifically binds to and inhibits PD-1;

(b) a dosage ranging from 0.1 to 20.0 mg/kg body weight of an antibody or an antigen-binding portion thereof that specifically binds to and inhibits CTLA-4; and

(c) instructions for using the anti-PD-1 and anti-CTLA-4 antibodies the concurrent regimen method of claim 14.

21. A kit for treating a subject afflicted with a cancer, the kit comprising:

(a) a dosage ranging from 0.1 to 20.0 mg/kg body weight of an antibody or an antigen-binding portion thereof that specifically binds to and inhibits PD-1; and

(b) instructions for using the anti-PD-1 antibody in the sequenced regimen method of claim 16.

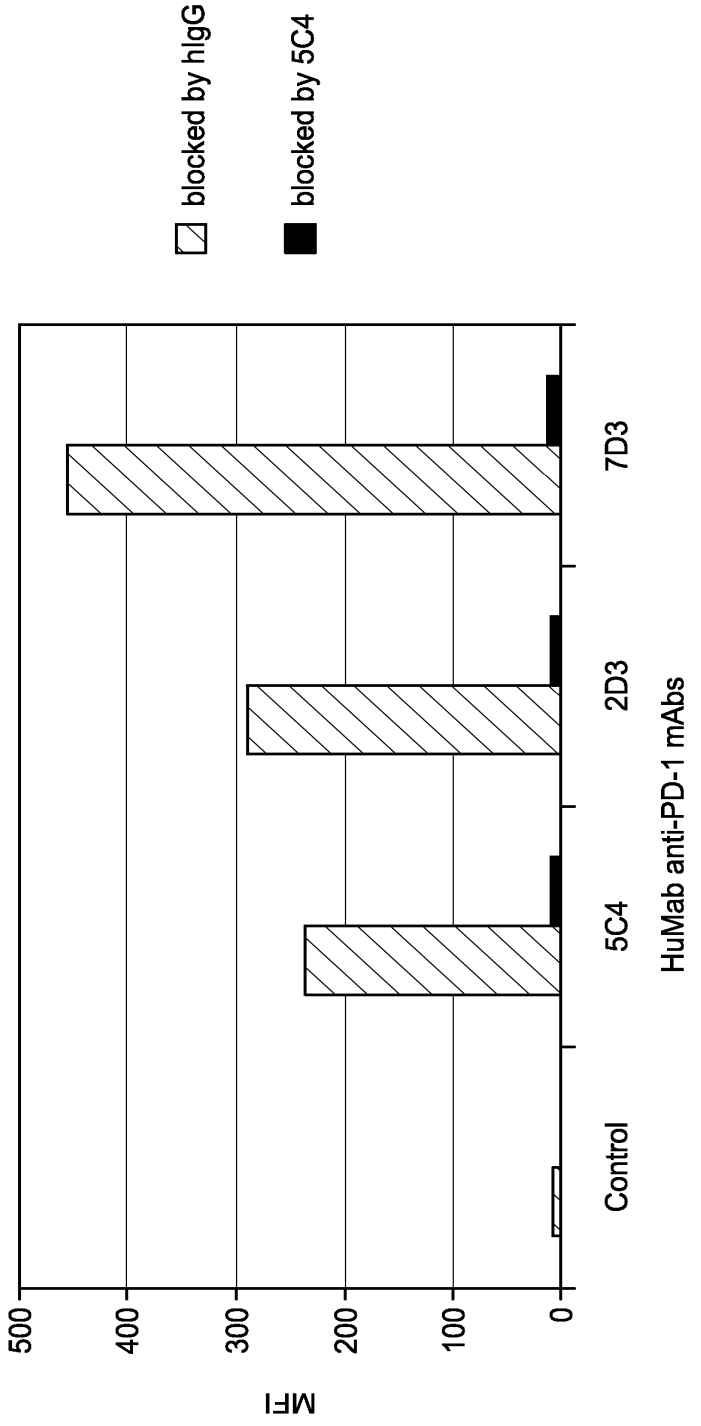

blocked by hIgG
blocked by 5C4
MFI
500
400
300
200
100
0
Control
5C4
2D3
7D3
HuMab anti-PD-1 mAbs

FIG. 1A

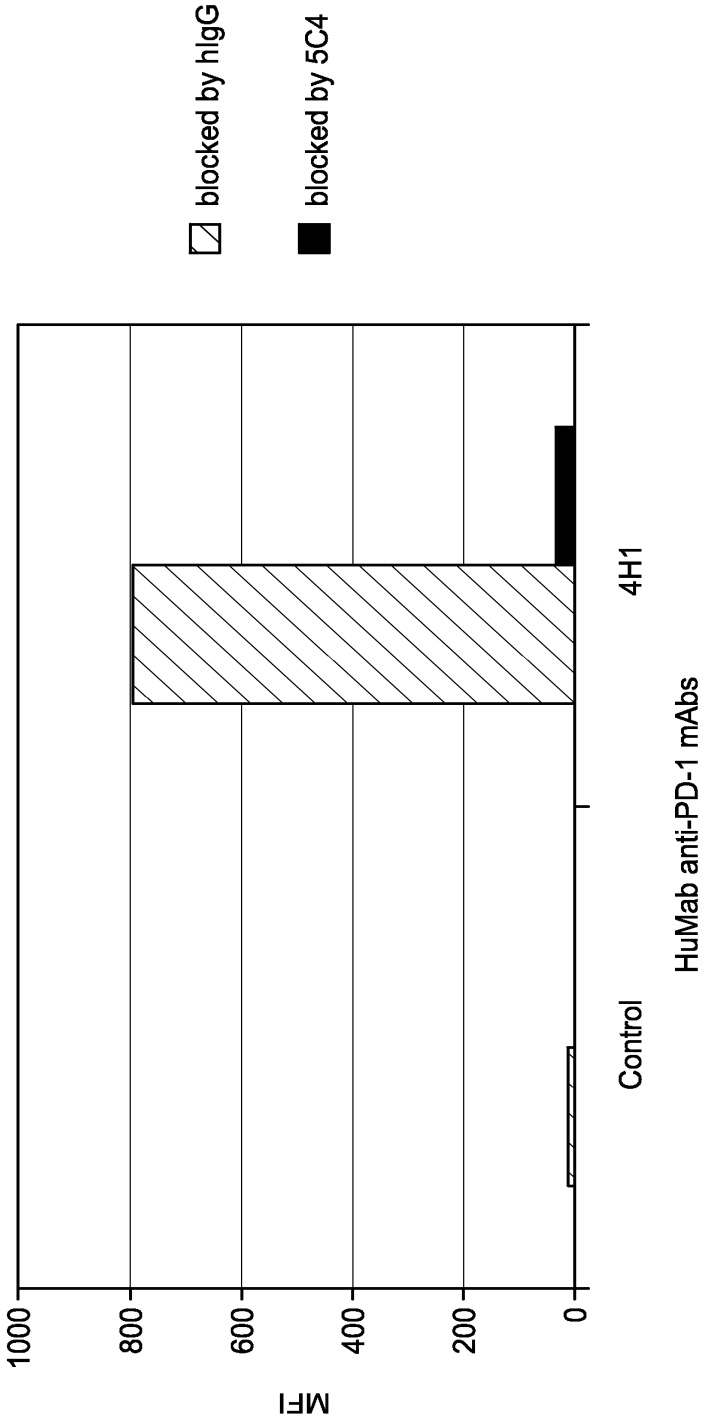

blocked by hIgG
blocked by 5C4
MFI
1000
800
600
400
200
0
Control
4H1
HuMab anti-PD-1 mAbs

FIG. 1B

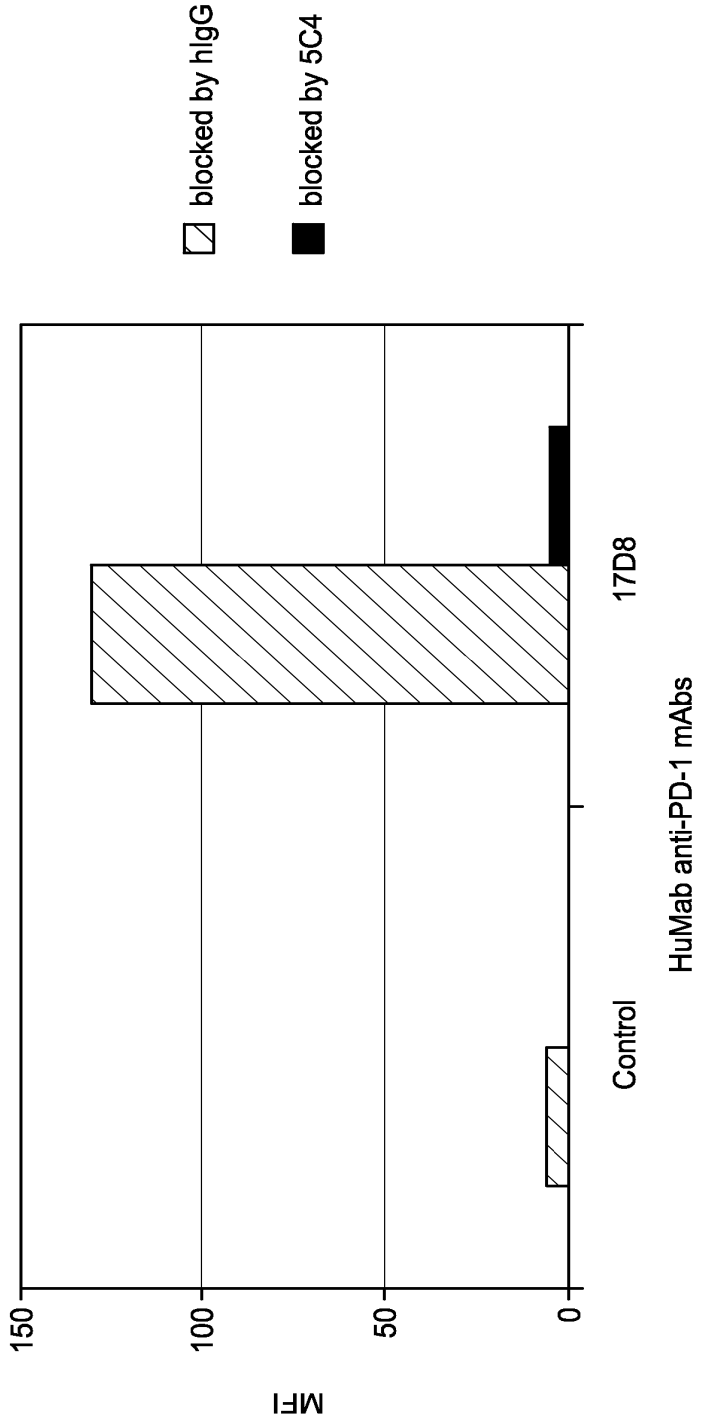

blocked by hIgG
blocked by 5C4
MFI
150
100
50
0
Control
17D8
HuMab anti-PD-1 mAbs

FIG. 1C

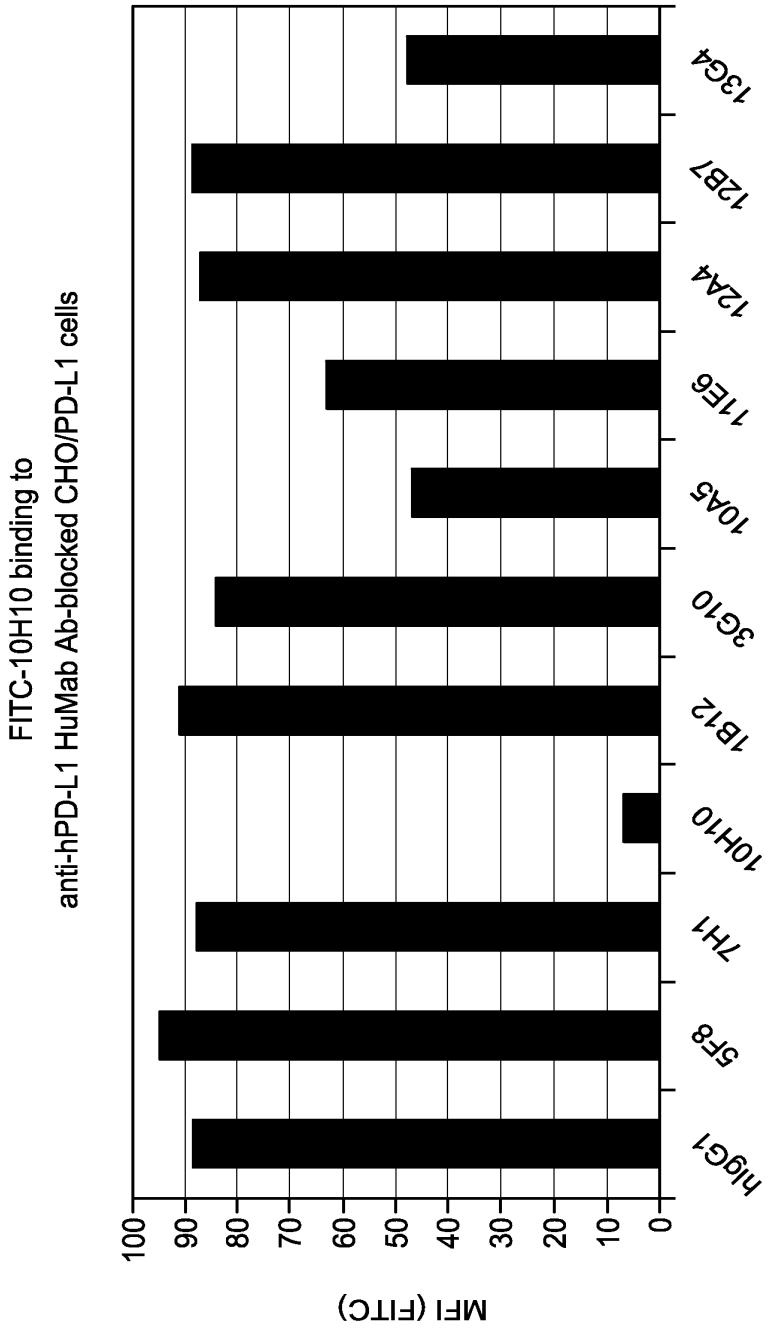

FITC-10H10 binding to
anti-hPD-L1 HuMab Ab-blocked CHO/PD-L1 cells
MFI (FITC)
100
90
80
70
60
50
40
30
20
10
0
hIgG1
5F8
7H1
10H10
1B12
3G10
10A5
11E6
12A4
12B7
13G4
Blocking Antibodies

FIG. 2A

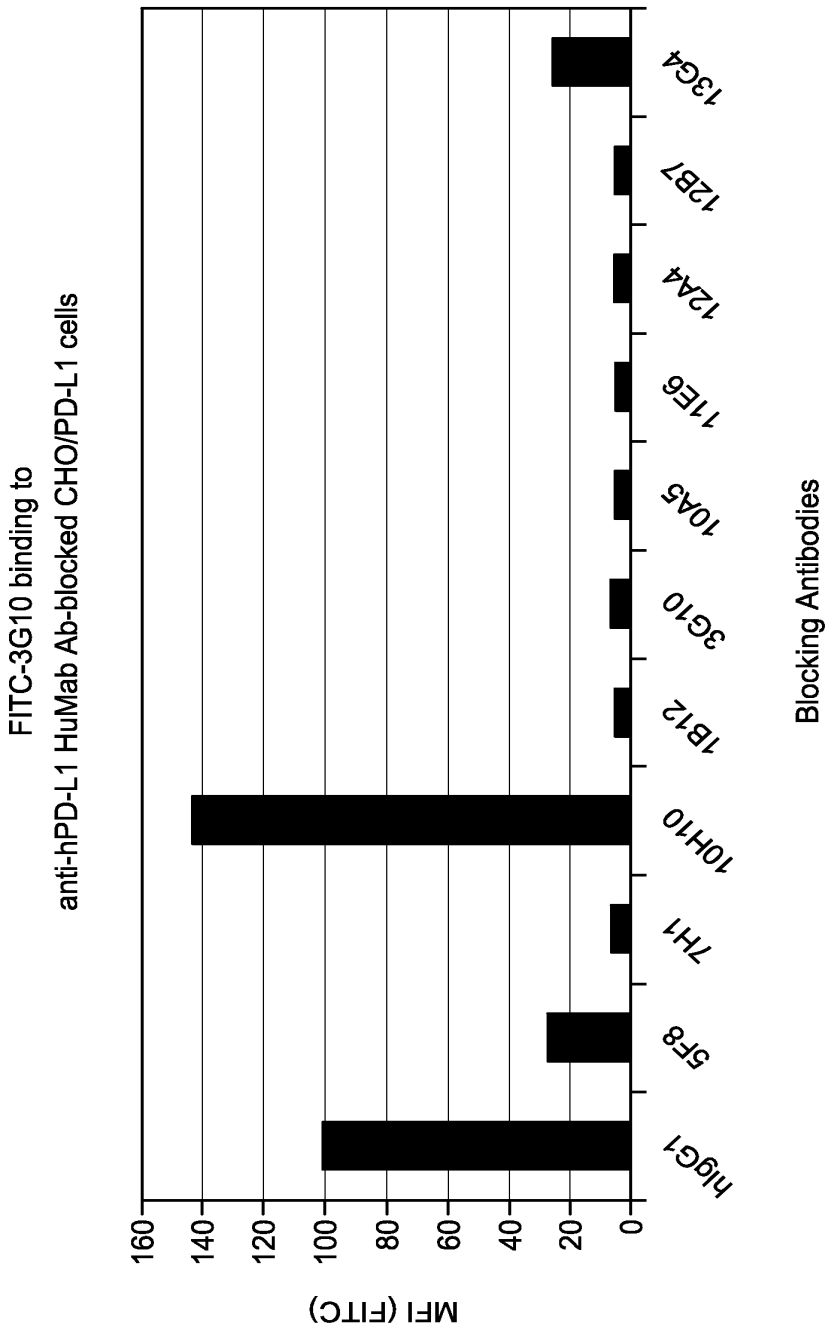

FITC-3G10 binding to
anti-hPD-L1 HuMab Ab-blocked CHO/PD-L1 cells
MFI (FITC)
0
20
40
60
80
100
120
140
160
hIgG1
5F8
7H1
10H10
1B12
3G10
10A5
11E6
12A4
12B7
13G4
Blocking Antibodies

FIG. 2B

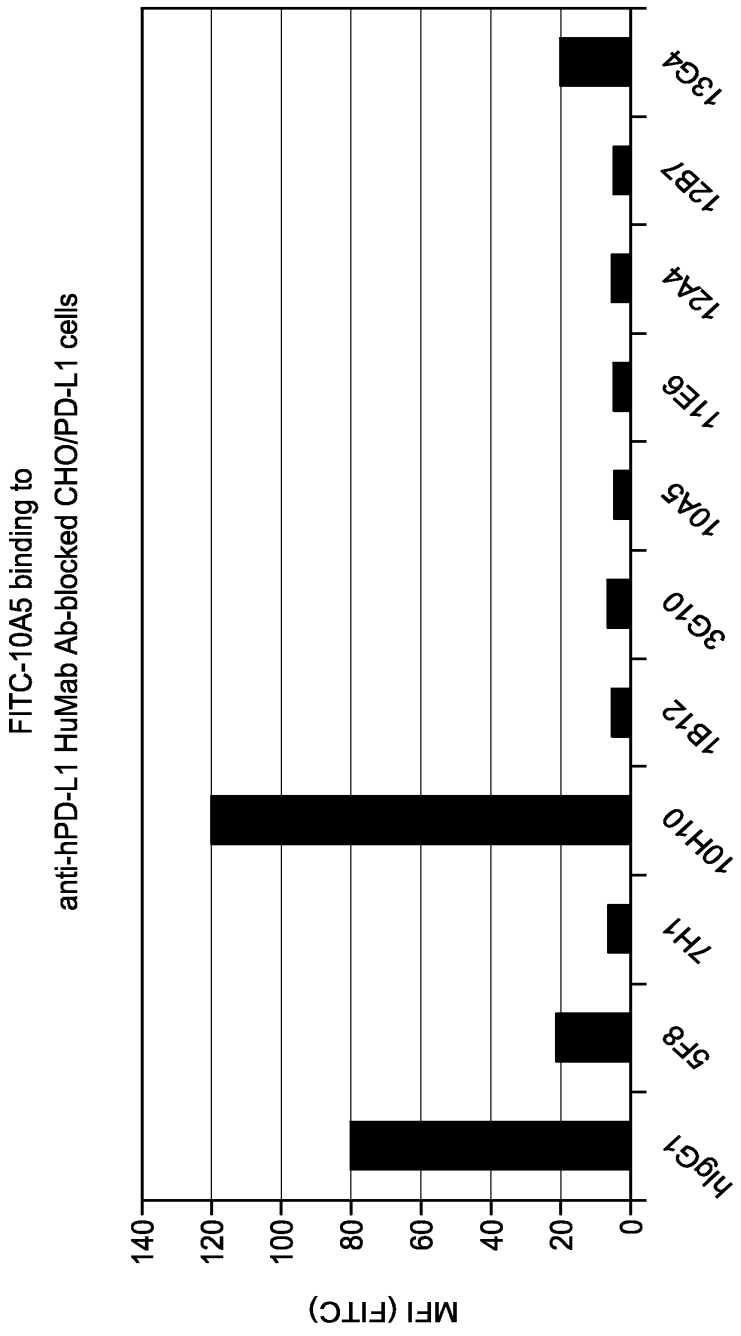

FITC-10A5 binding to
anti-hPD-L1 HuMab Ab-blocked CHO/PD-L1 cells
MFI (FITC)
0
20
40
60
80
100
120
140
hIgG1
5F8
7H1
10H10
1B12
3G10
10A5
11E6
12A4
12B7
13G4
Blocking Antibodies

FIG. 2C

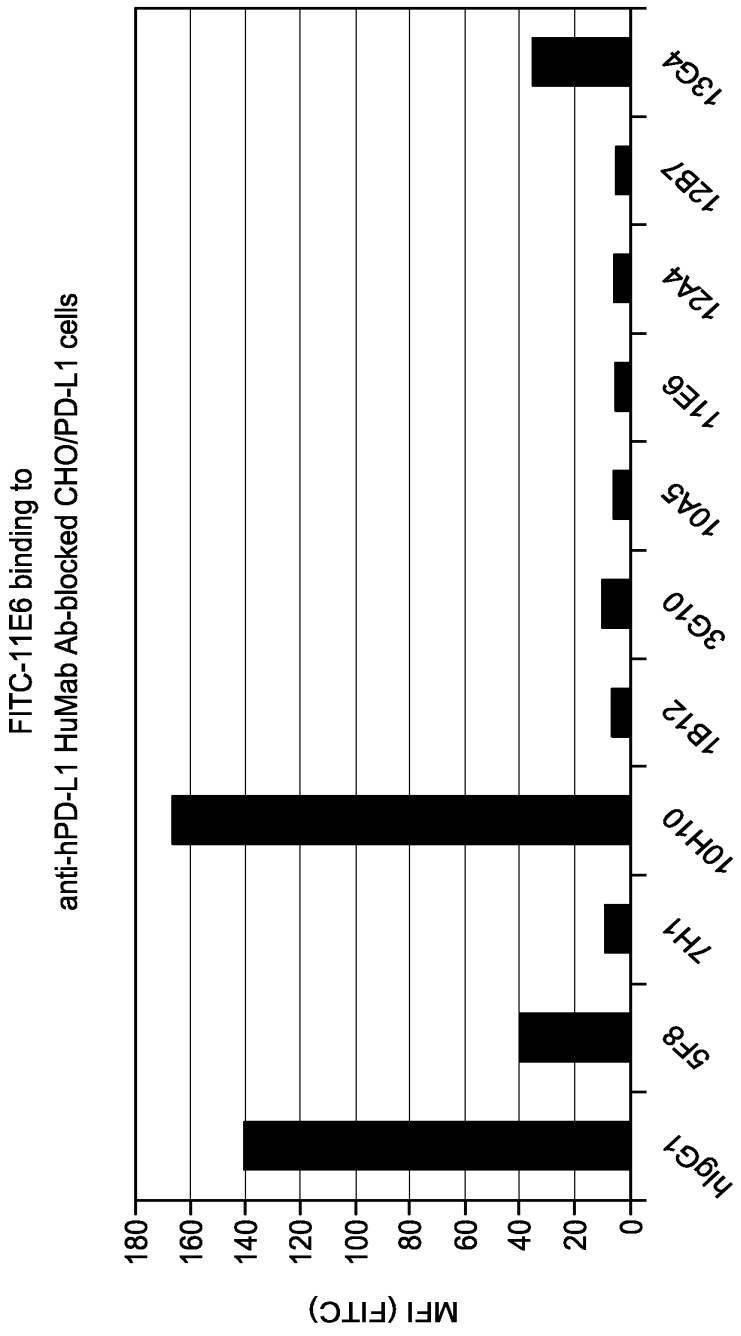

FITC-11E6 binding to
anti-hPD-L1 HuMab Ab-blocked CHO/PD-L1 cells
MFI (FITC)
0
20
40
60
80
100
120
140
160
180
hIgG1
5F8
7H1
10H10
1B12
3G10
10A5
11E6
12A4
12B7
13G4
Blocking Antibodies

FIG. 2D

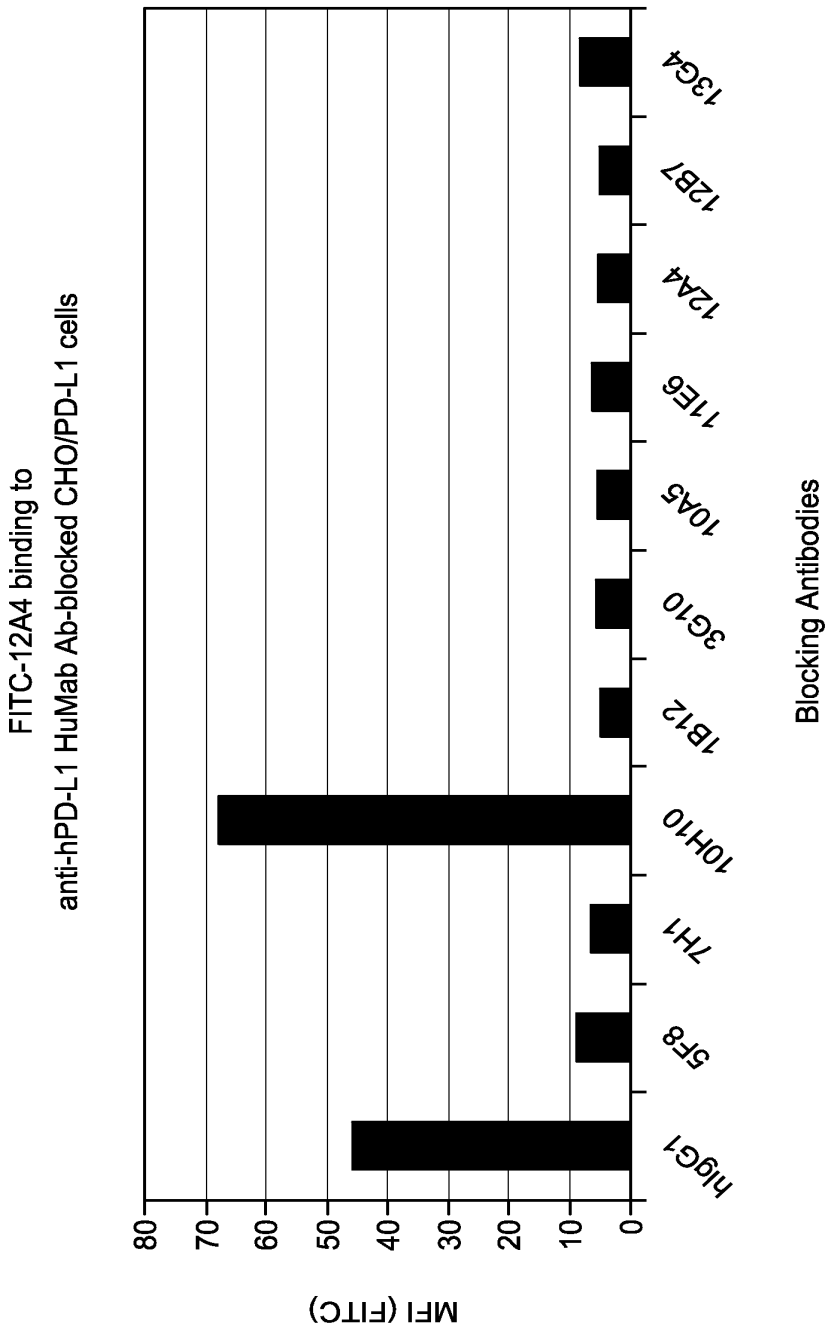

FITC-12A4 binding to
anti-hPD-L1 HuMab Ab-blocked CHO/PD-L1 cells
MFI (FITC)
0
10
20
30
40
50
60
70
80
hIgG1
5F8
7H1
10H10
1B12
3G10
10A5
11E6
12A4
12B7
13G4
Blocking Antibodies

FIG. 2E

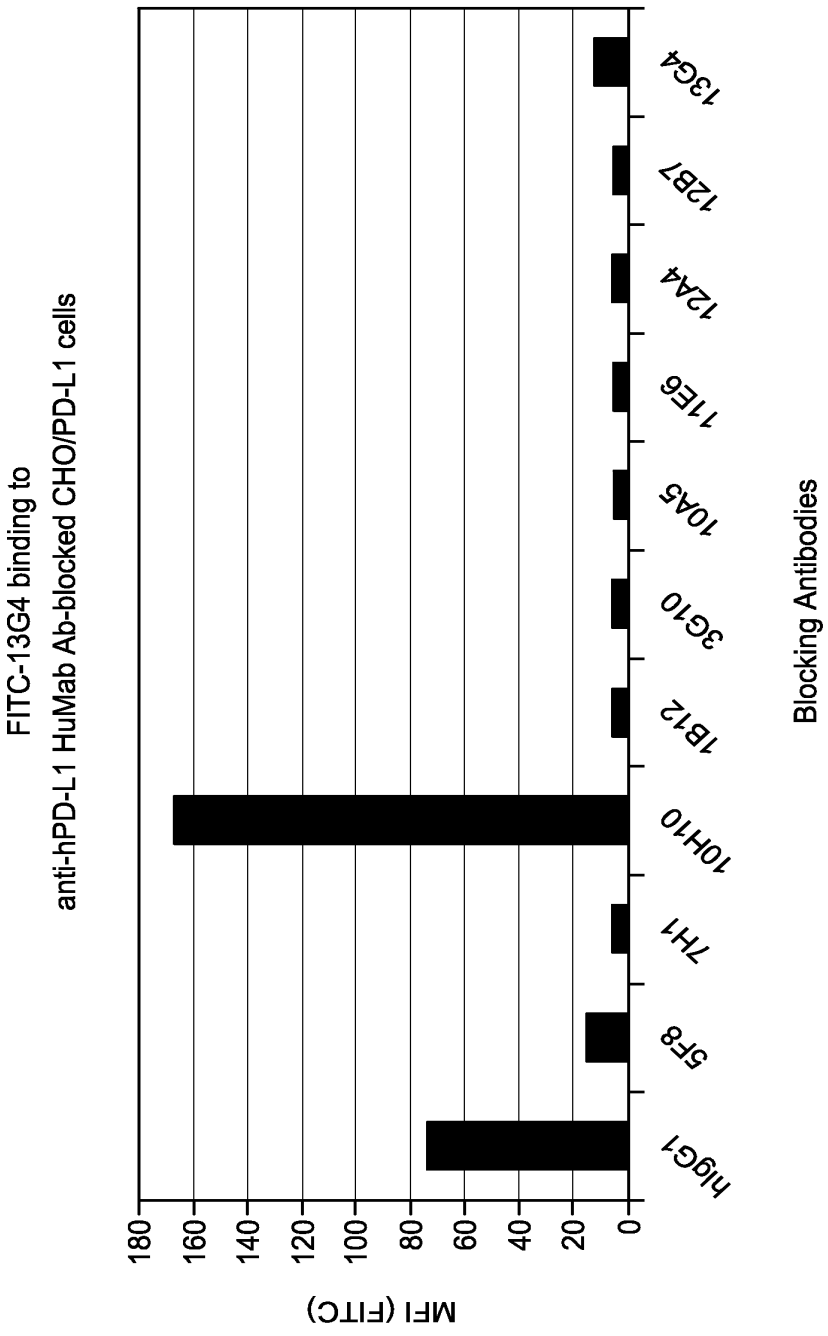

FITC-13G4 binding to
anti-hPD-L1 HuMab Ab-blocked CHO/PD-L1 cells
MFI (FITC)
0
20
40
60
80
100
120
140
160
180
hIgG1
5F8
7H1
10H10
1B12
3G10
10A5
11E6
12A4
12B7
13G4
Blocking Antibodies

FIG. 2F

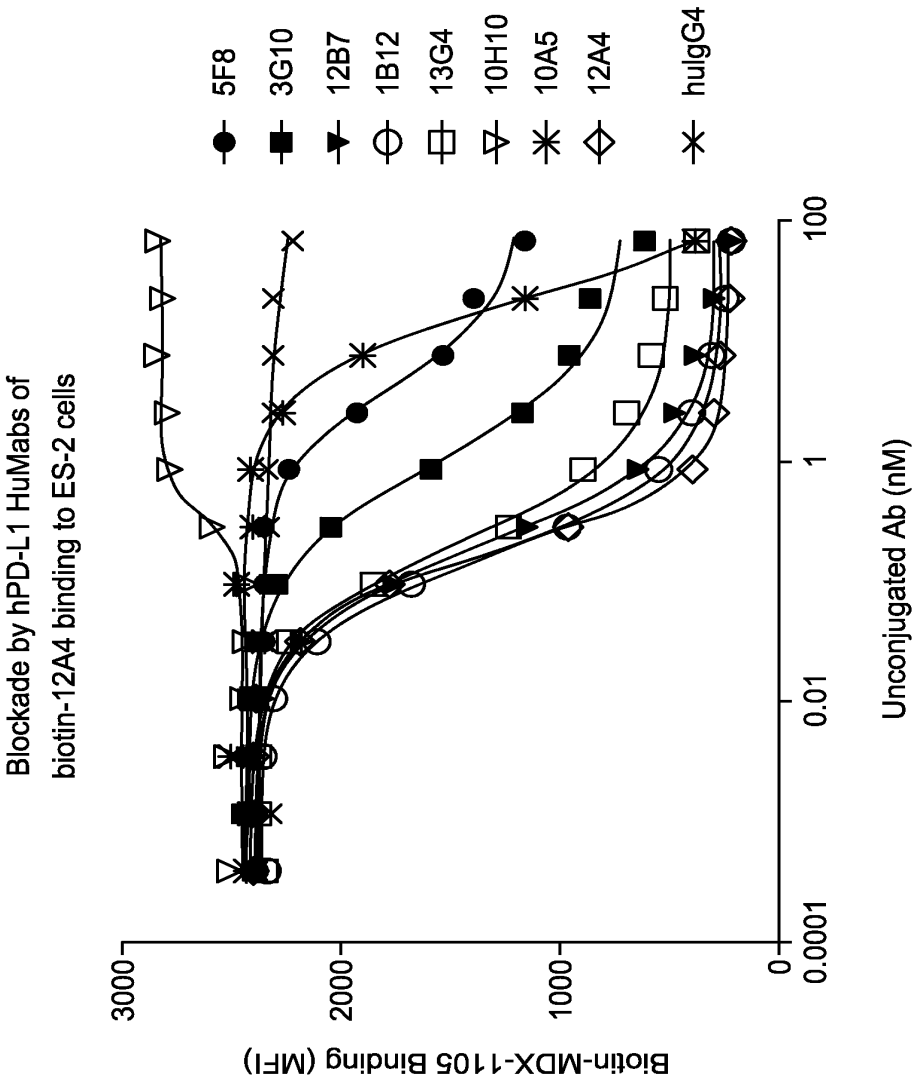

Blockade by hPD-L1 HuMabs of
biotin-12A4 binding to ES-2 cells
Biotin-MDX-1105 Binding (MFI)
3000
2000
1000
0
0.0001
0.01
1
100
Unconjugated Ab (nM)
5F8
3G10
12B7
1B12
13G4
10H10
10A5
12A4
hulgG4

FIG. 3

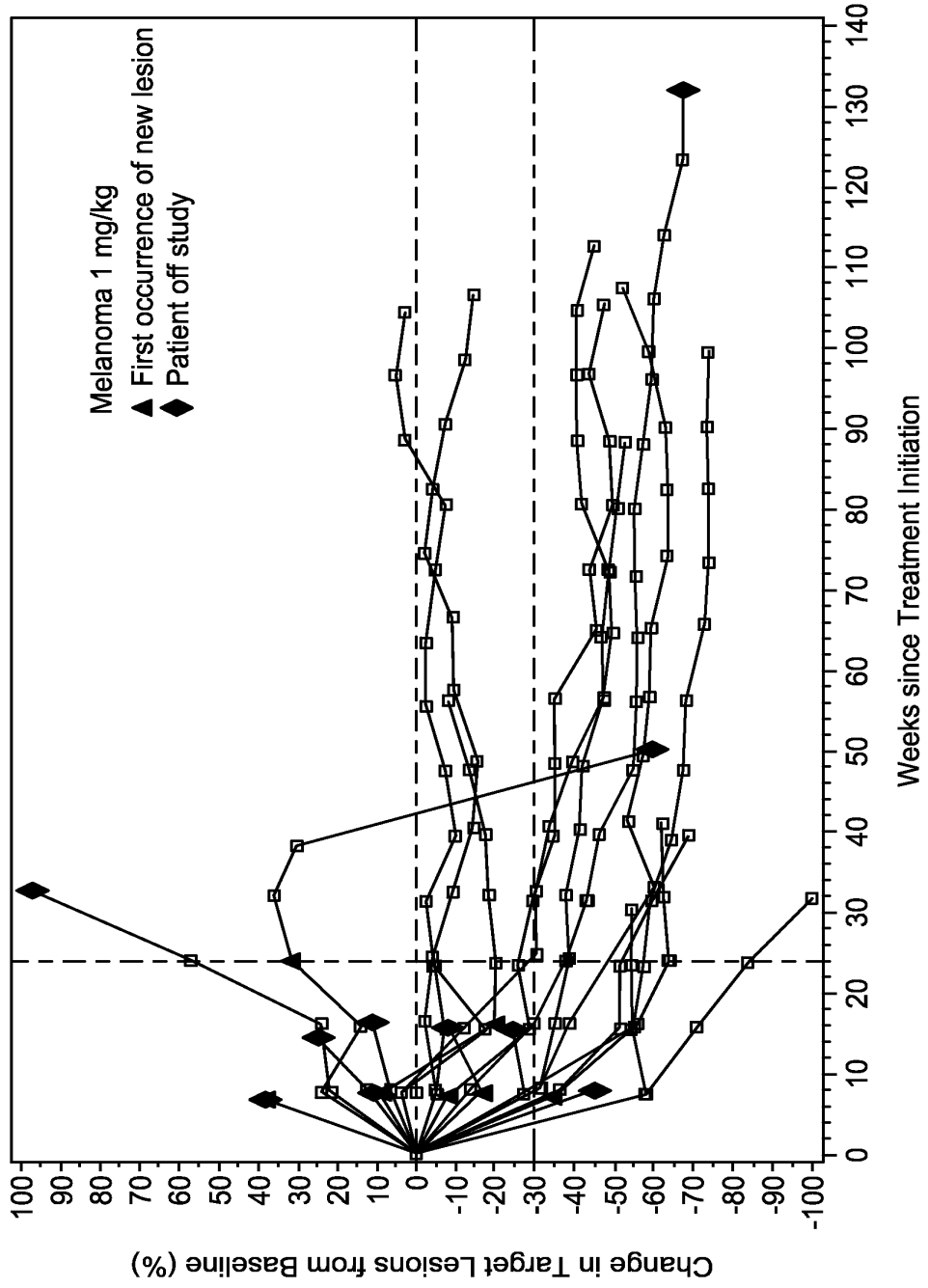

Melanoma 1 mg/kg
First occurrence of new lesion
Patient off study
Change in Target Lesions from Baseline (%)
Weeks since Treatment Initiation
100
90
80
70
60
50
40
30
20
10
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140

FIG. 4

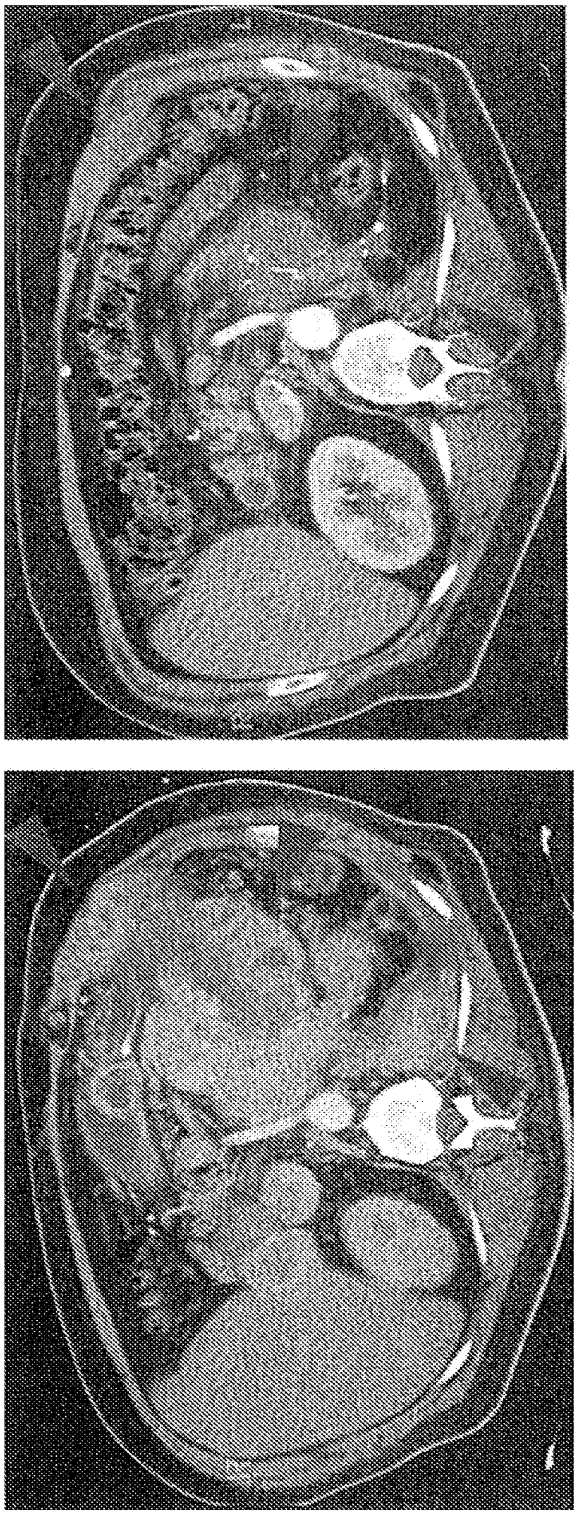


FIG. 5

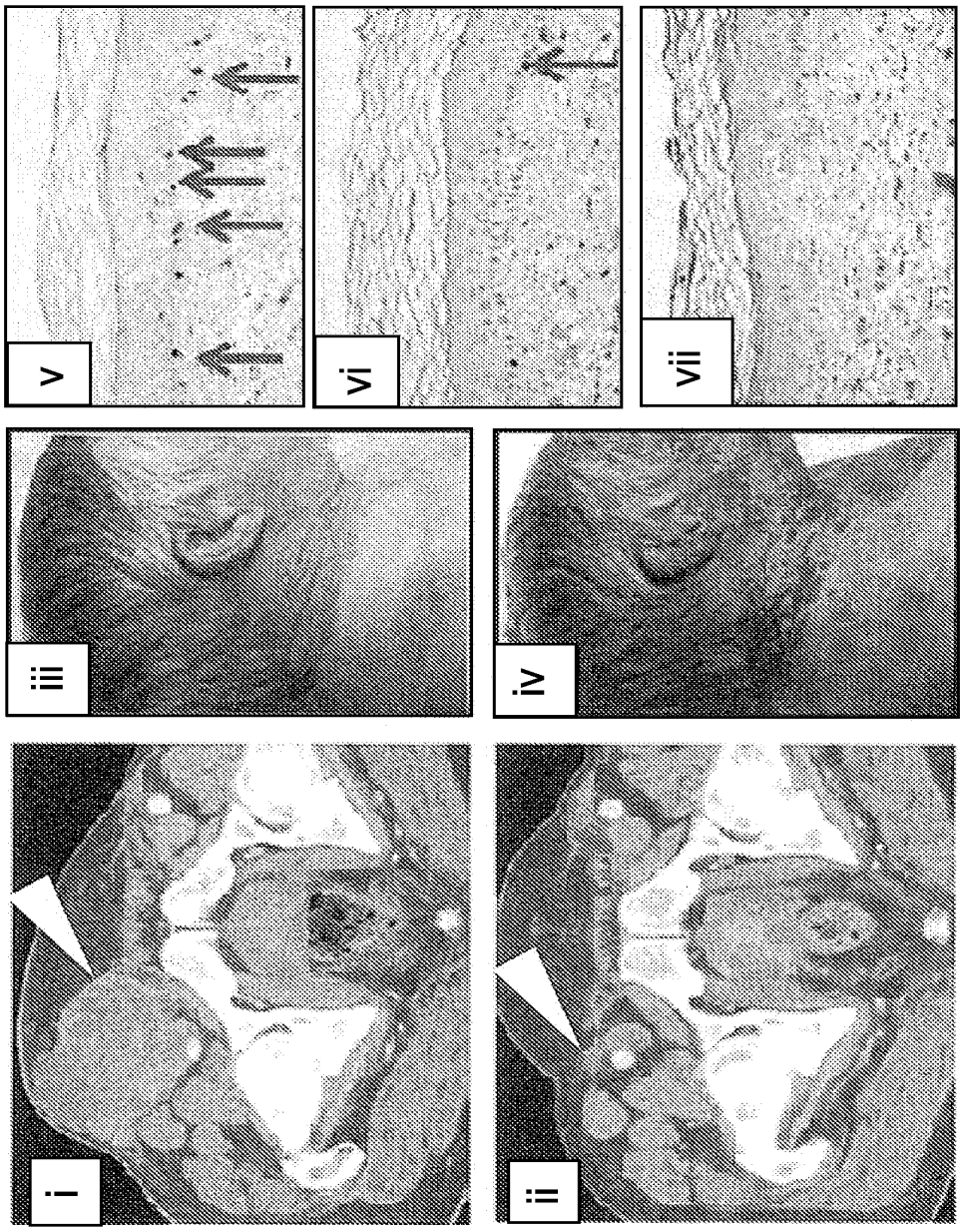



FIG. 6

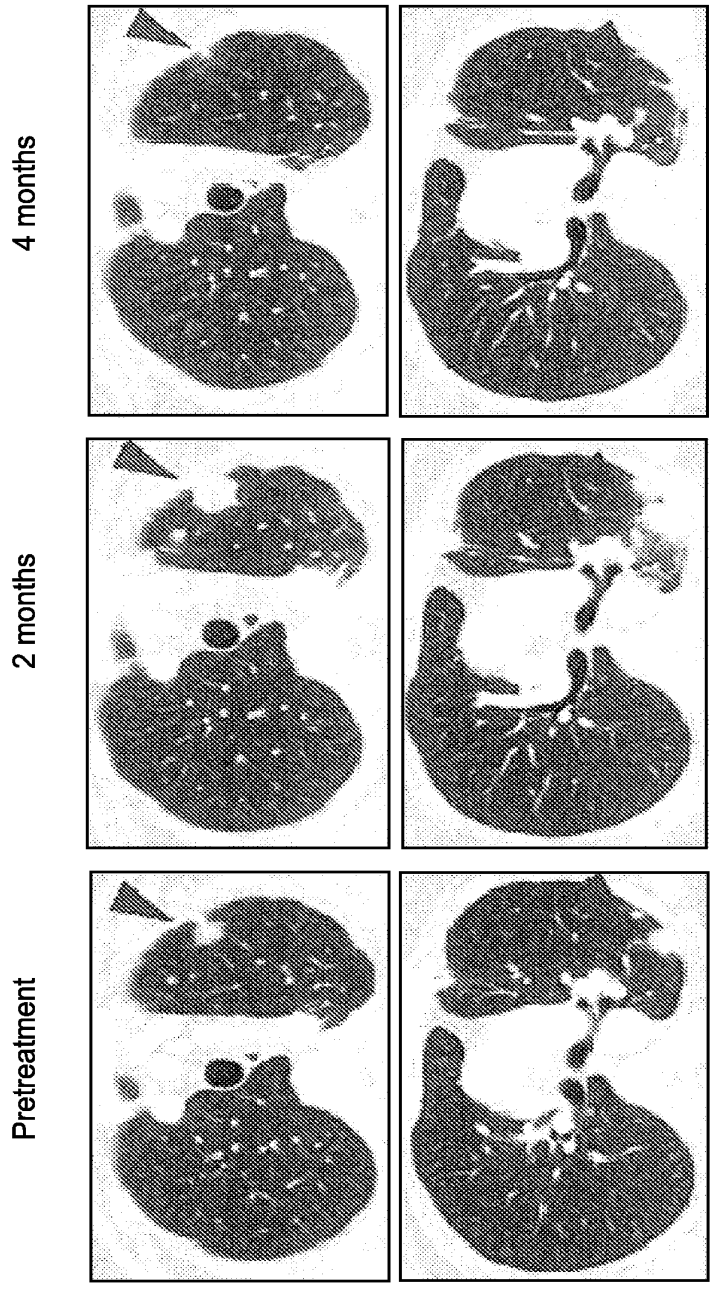
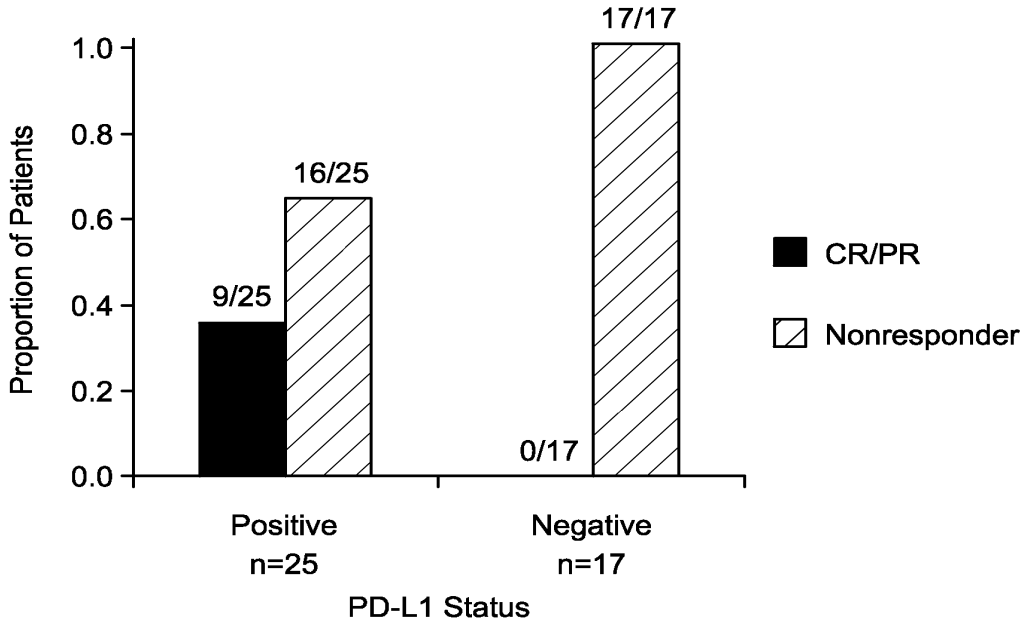



FIG. 7




| Association Between Pretreatment Tumor PD-L1 Expression and Clinical Response | | | |
|---|---|---|---|
| Response Status | PD-L1-Positive no. (%) | PD-L1-Negative no. (%) | Total no. (%) |
| CR/PR | 9(36) | 0 | 9(21) |
| Nonresponder | 16*(64) | 17(100) | 33(79) |
| All Patients | 25 | 17 | 42 |

Fisher's exact test for association P=0.006.

*Two patients categorized as nonresponders at the time of data analysis are still under evaluation.

**FIG. 8A**

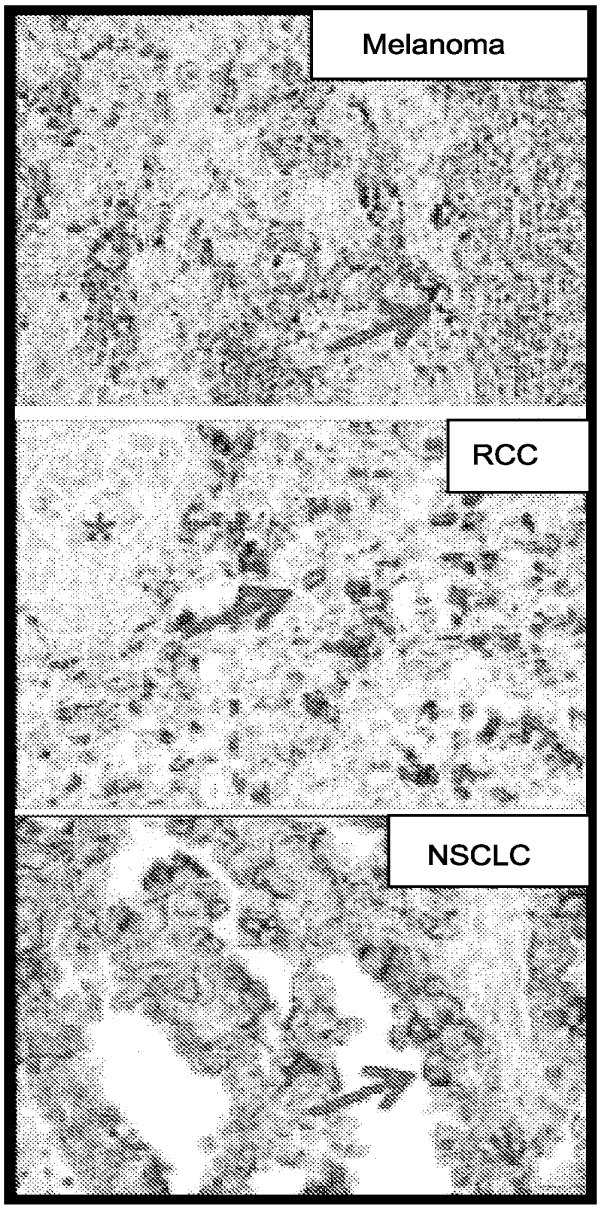



FIG. 8B

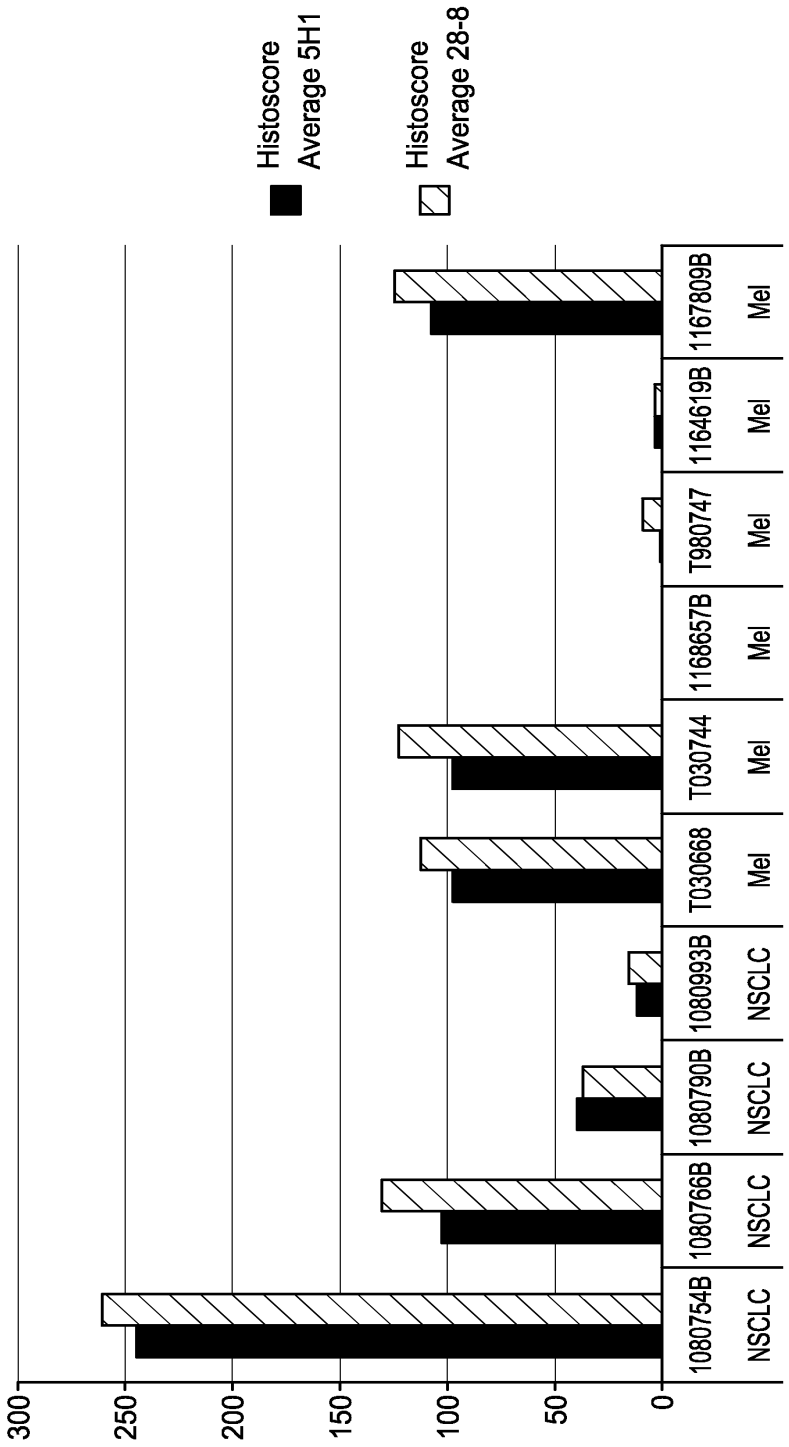



FIG. 9



EX1002 - Page 1345

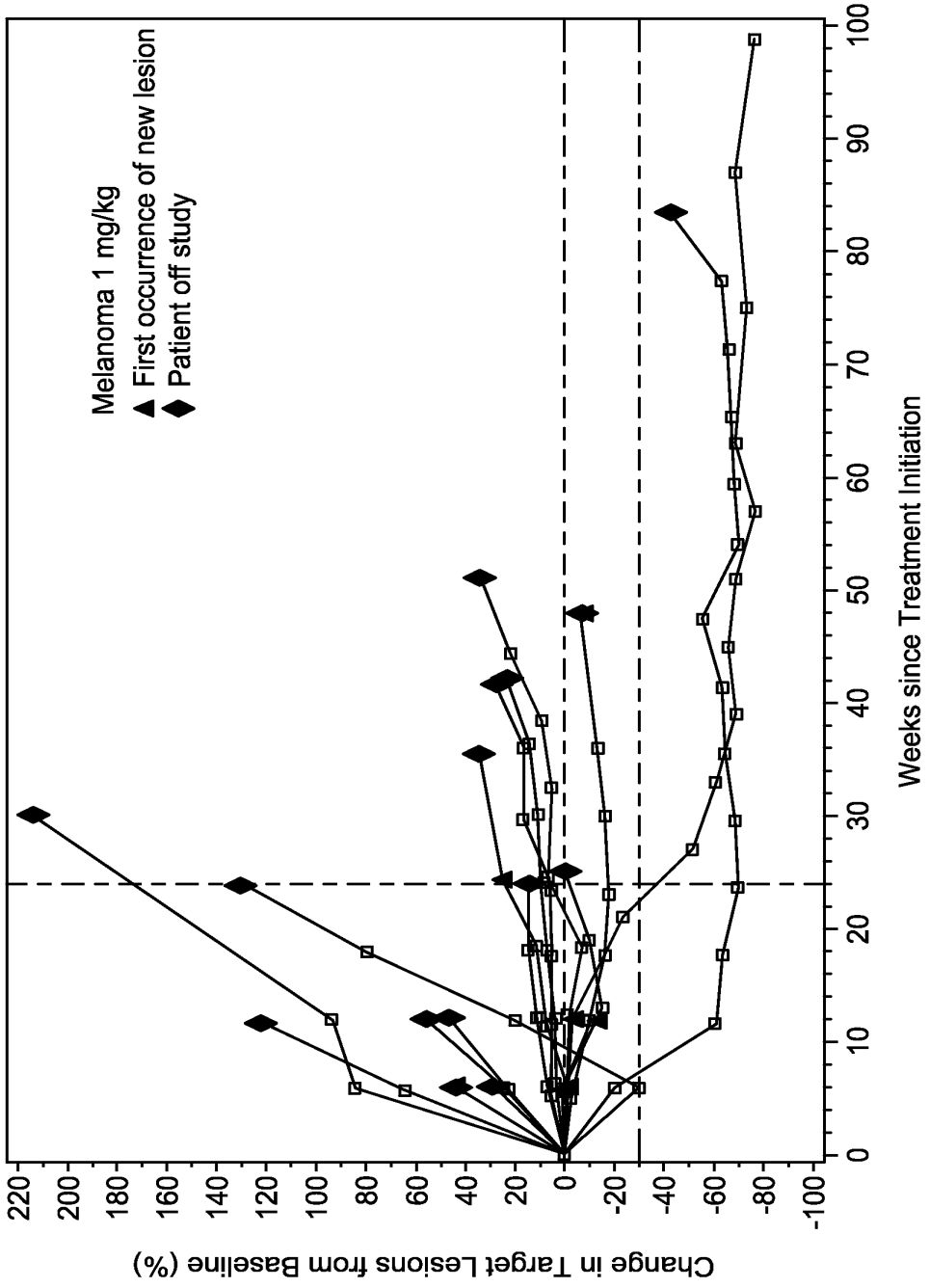

Melanoma 1 mg/kg
First occurrence of new lesion
Patient off study
Change in Target Lesions from Baseline (%)
220
200
180
160
140
120
100
80
60
40
20
0
-20
-40
-60
-80
-100
0
10
20
30
40
50
60
70
80
90
100
Weeks since Treatment Initiation

FIG. 10A

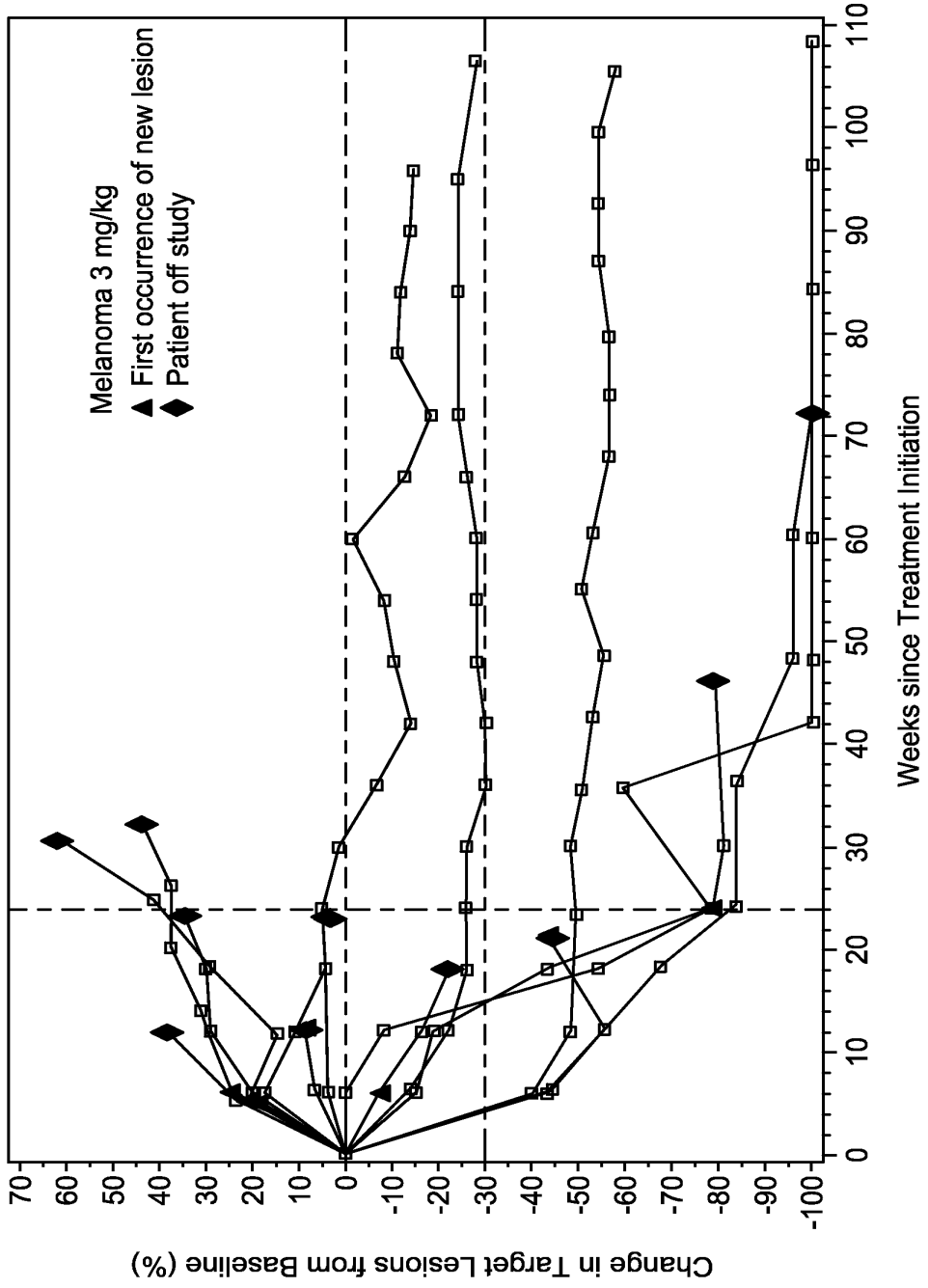

Melanoma 3 mg/kg
First occurrence of new lesion
Patient off study
Change in Target Lesions from Baseline (%)
Weeks since Treatment Initiation
70
60
50
40
30
20
10
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
0
10
20
30
40
50
60
70
80
90
100
110

FIG. 10B

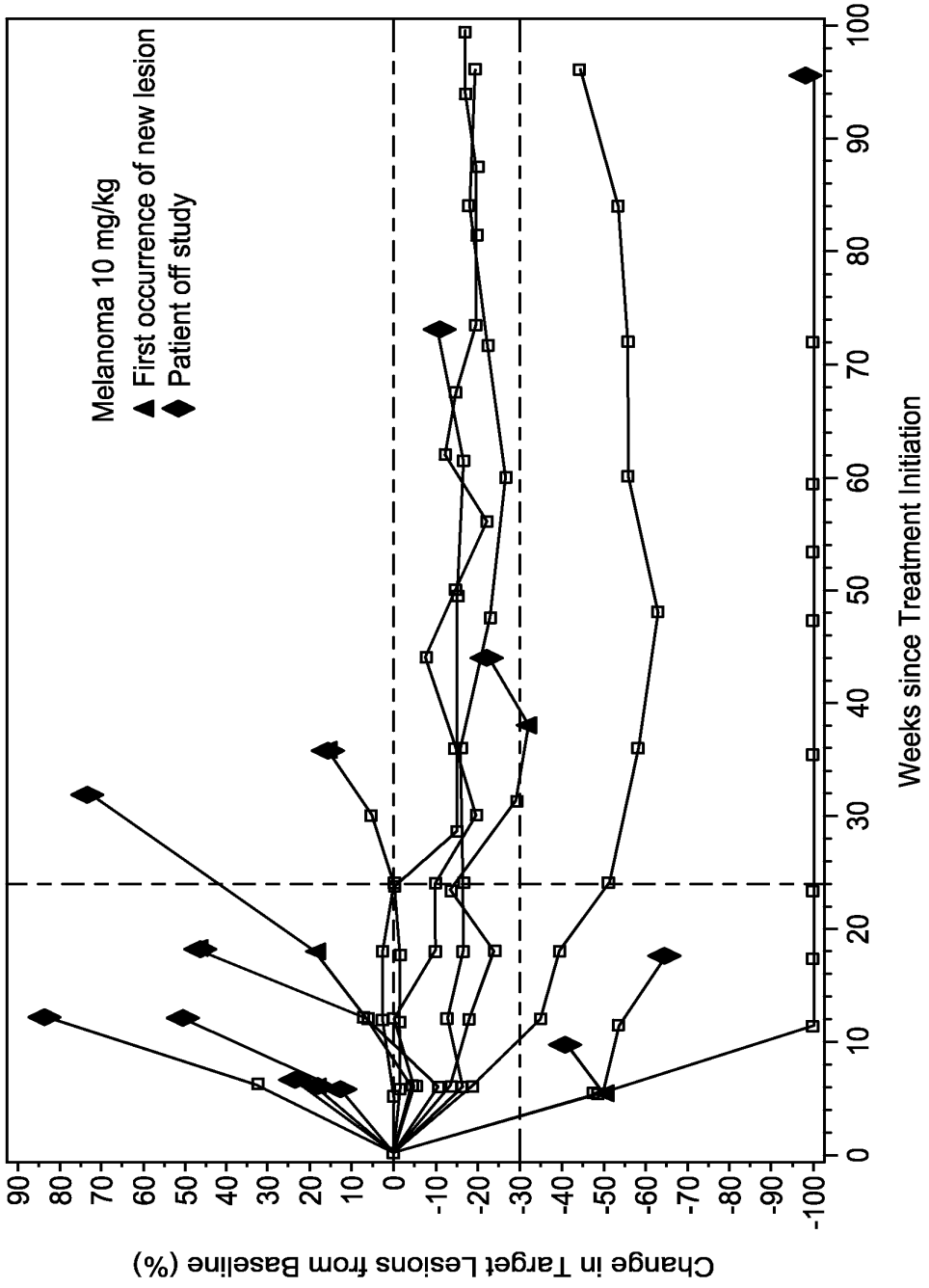

Melanoma 10 mg/kg
First occurrence of new lesion
Patient off study
Change in Target Lesions from Baseline (%)
Weeks since Treatment Initiation
90
80
70
60
50
40
30
20
10
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
0
10
20
30
40
50
60
70
80
90
100

FIG. 10C

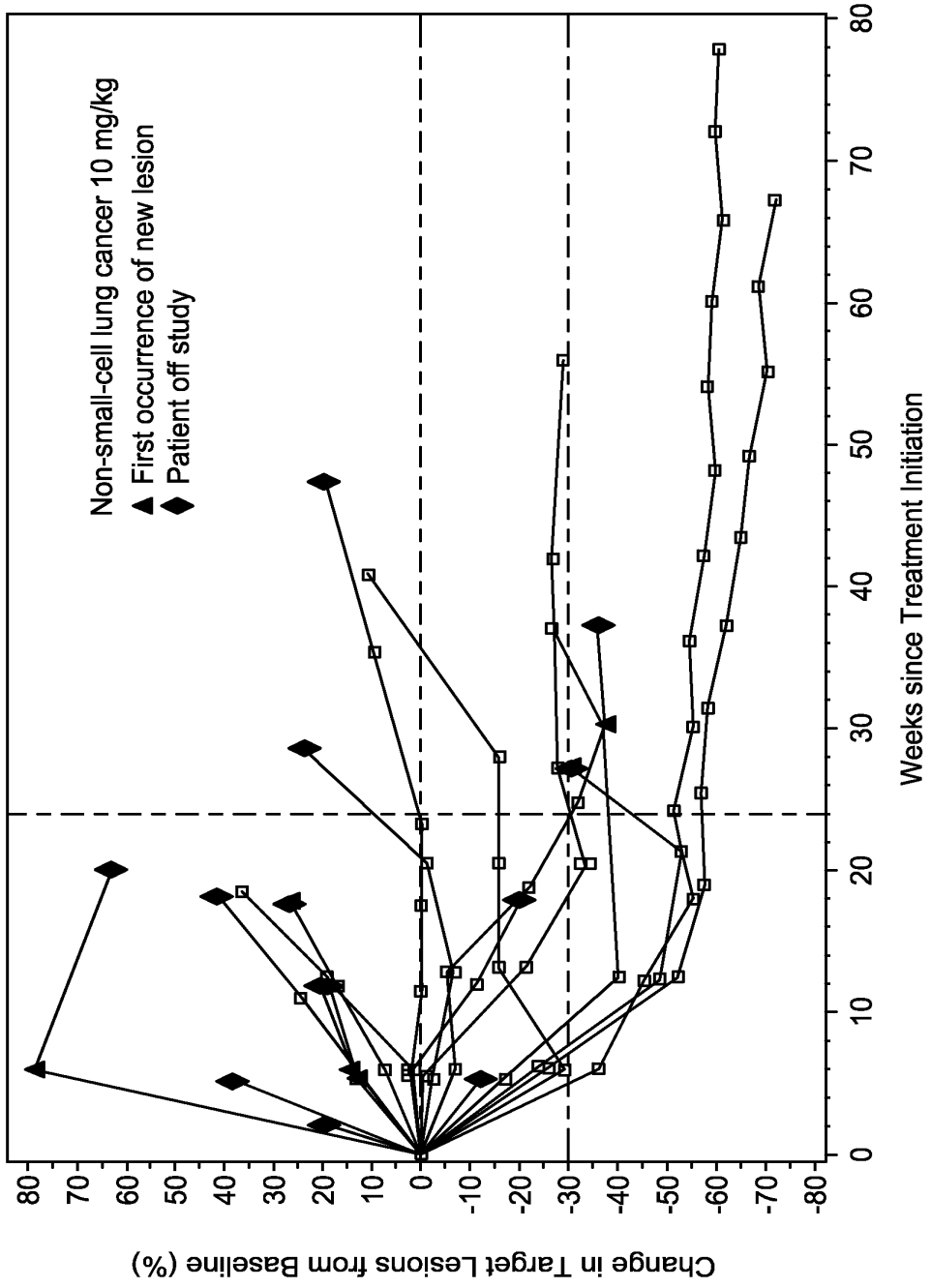

Non-small-cell lung cancer 10 mg/kg
First occurrence of new lesion
Patient off study
Change in Target Lesions from Baseline (%)
80
70
60
50
40
30
20
10
0
-10
-20
-30
-40
-50
-60
-70
-80
0
10
20
30
40
50
60
70
80
Weeks since Treatment Initiation

FIG. 10D

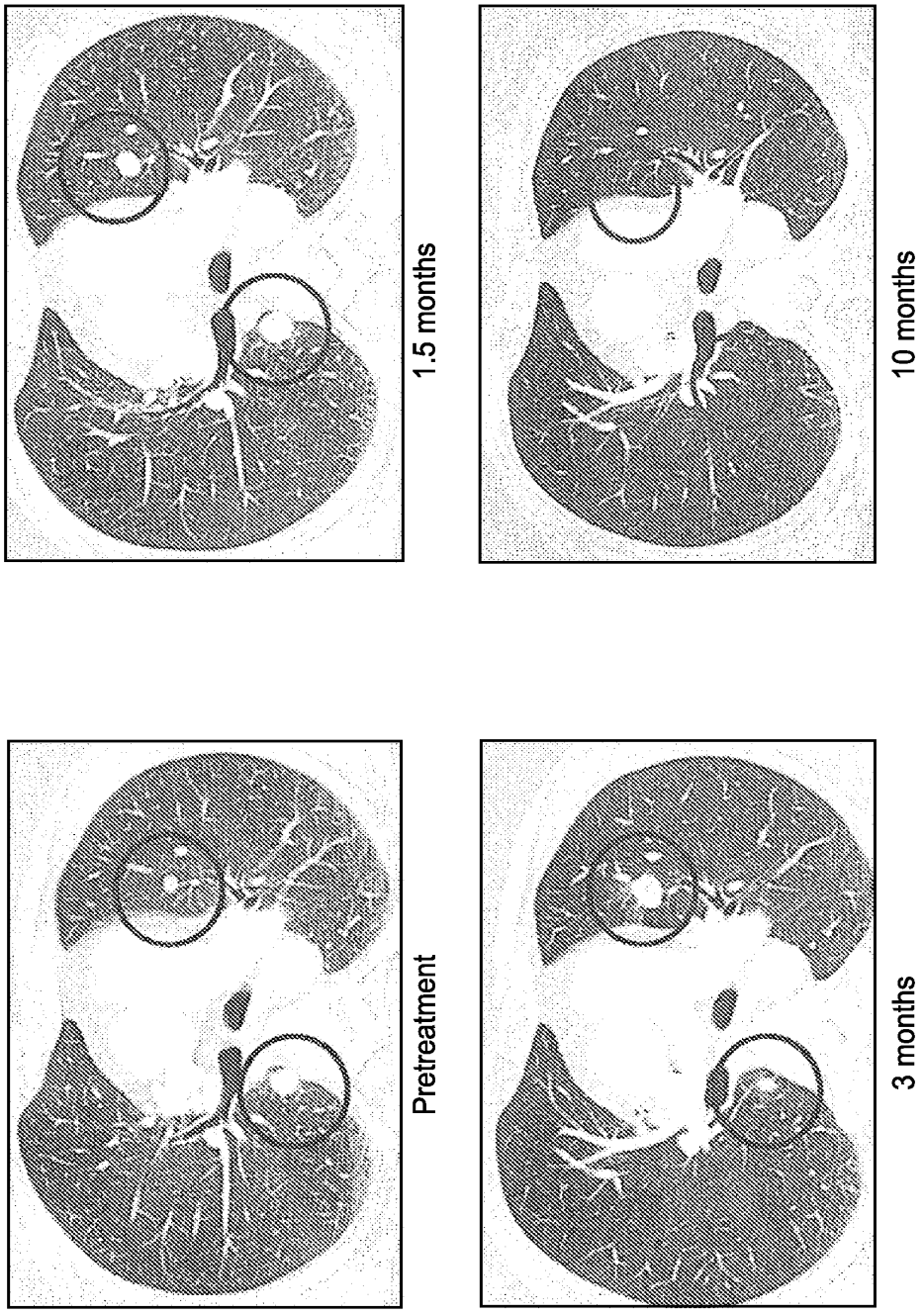



FIG. 11

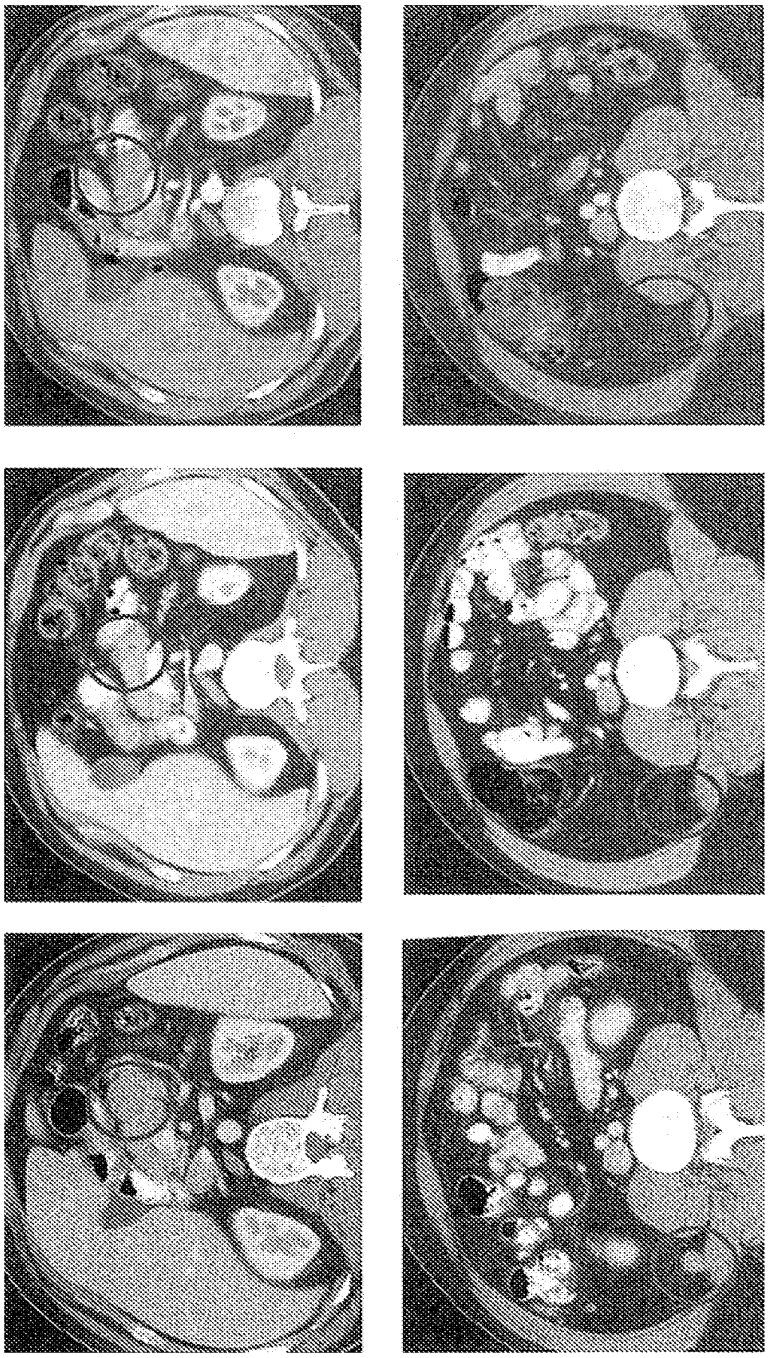



FIG. 12

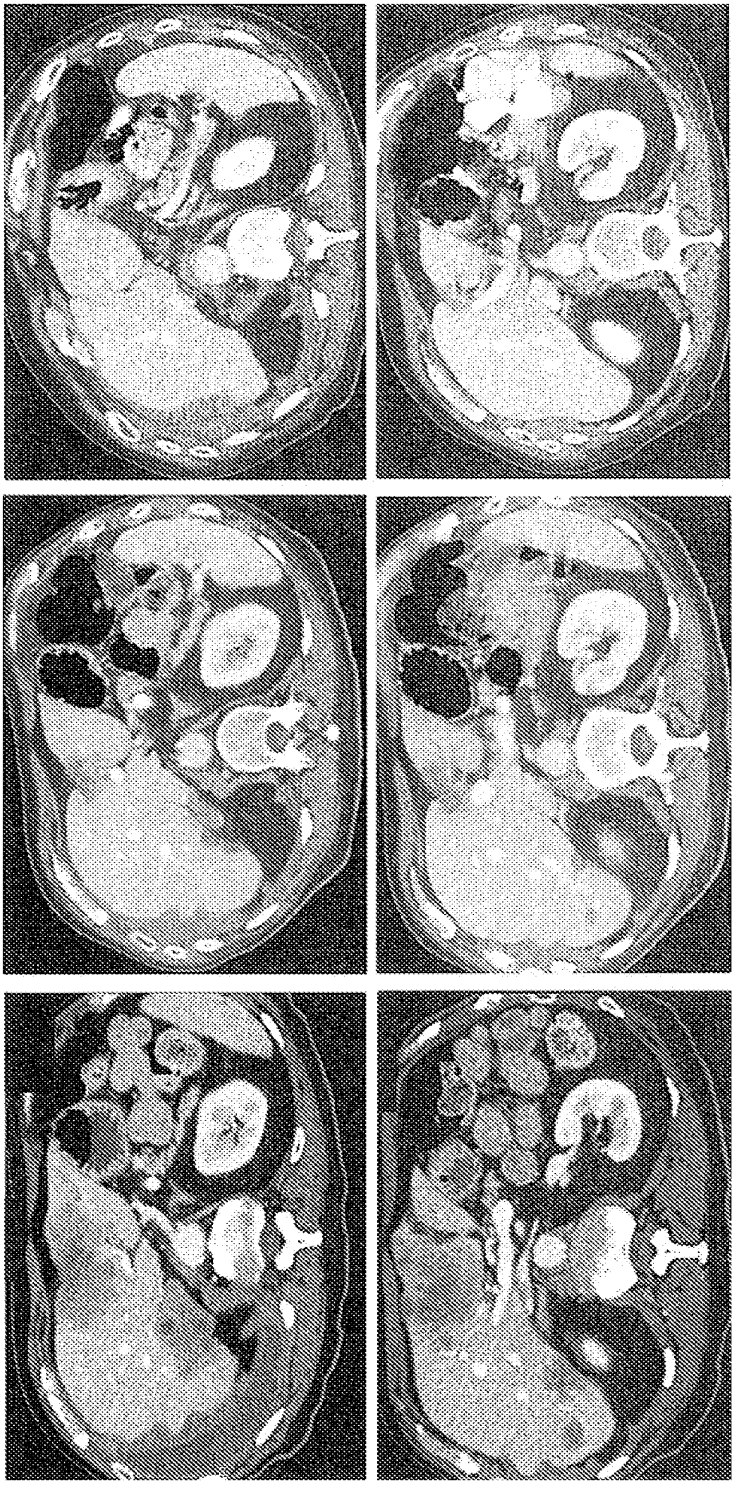



FIG. 13

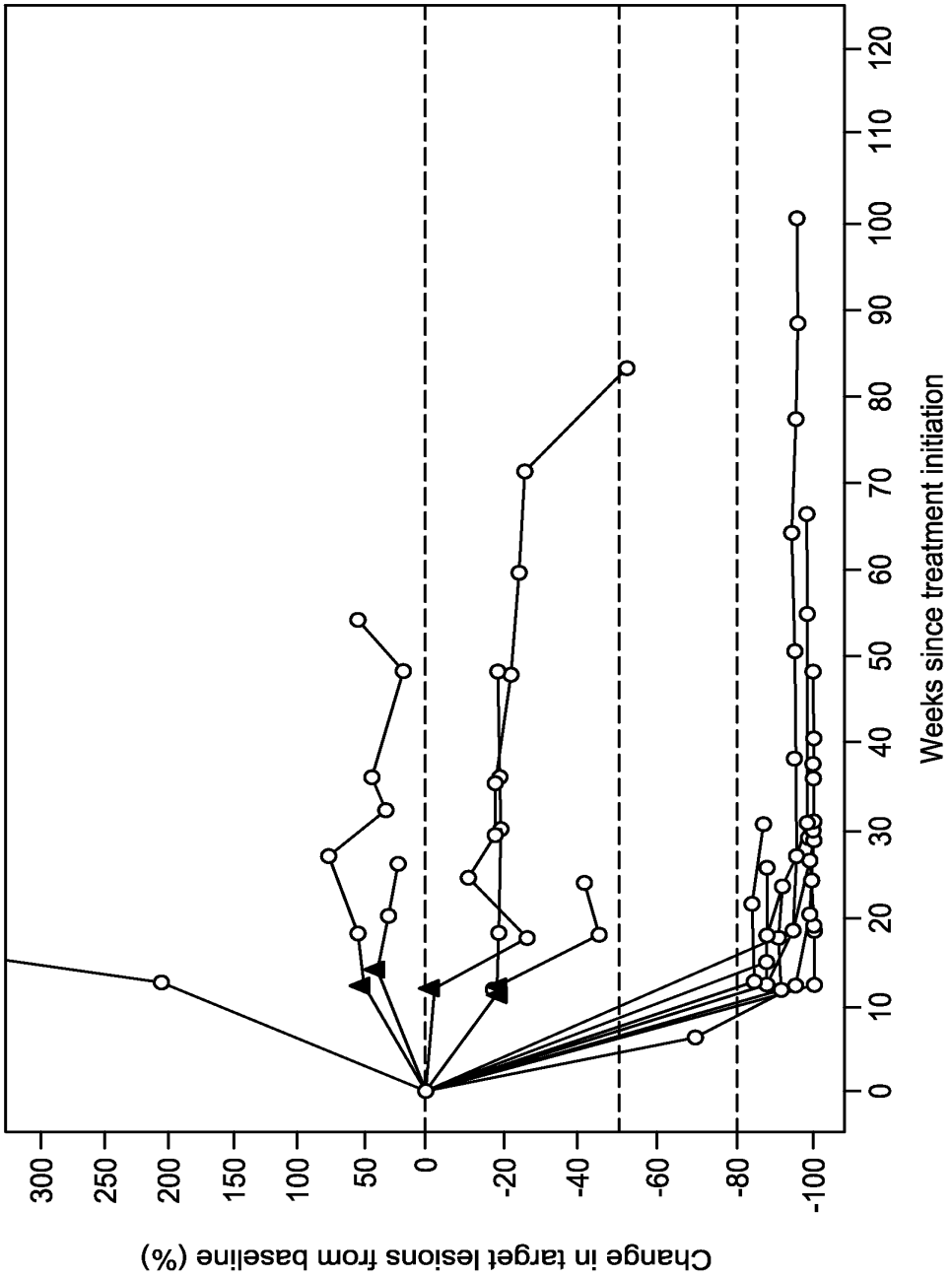

Change in target lesions from baseline (%)
300
250
200
150
100
50
0
-20
-40
-60
-80
-100
0
10
20
30
40
50
60
70
80
90
100
110
120
Weeks since treatment initiation

FIG. 14A

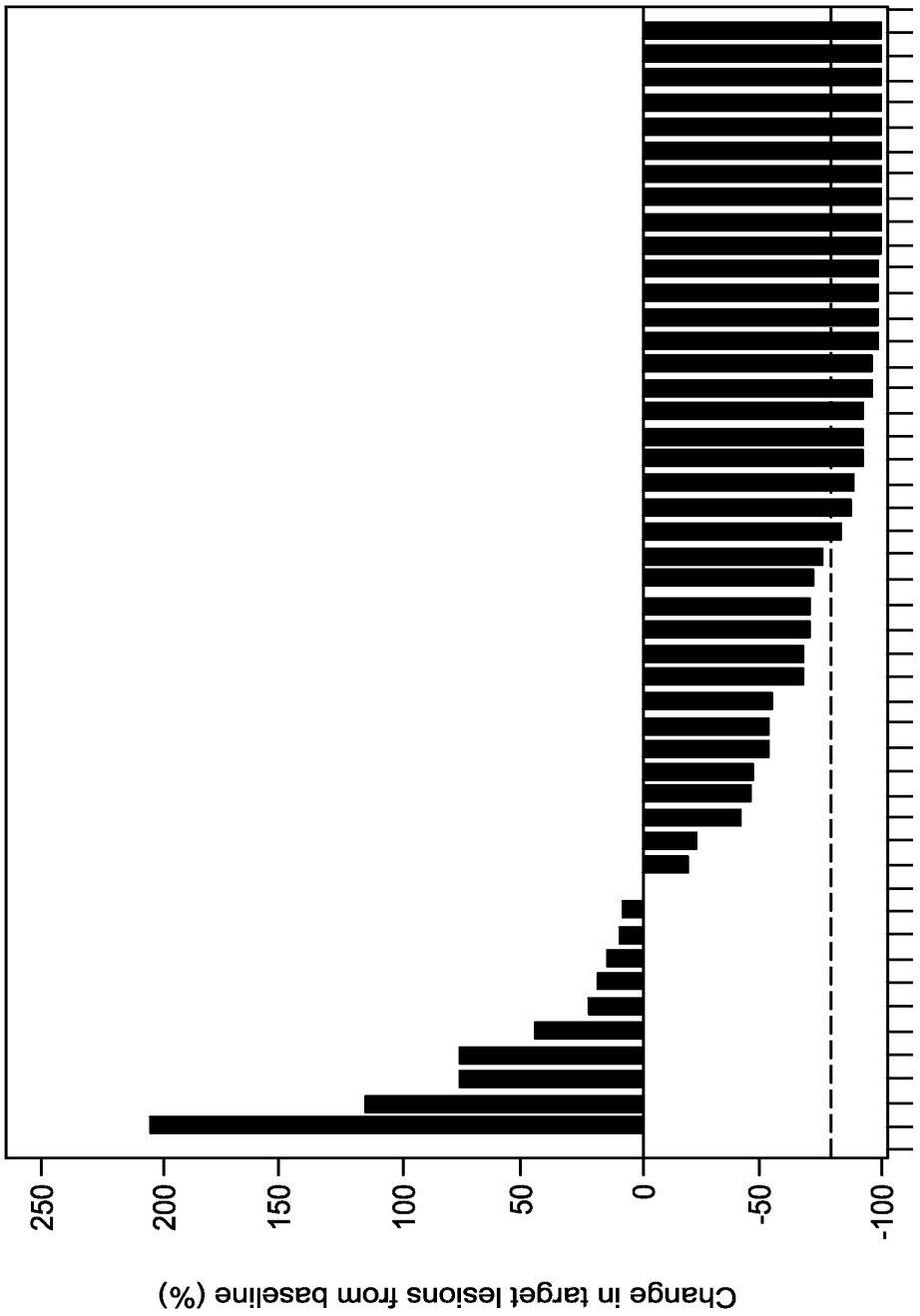

250
200
150
100
50
0
-50
-100
Change in target lesions from baseline (%)
Patients

FIG. 14B

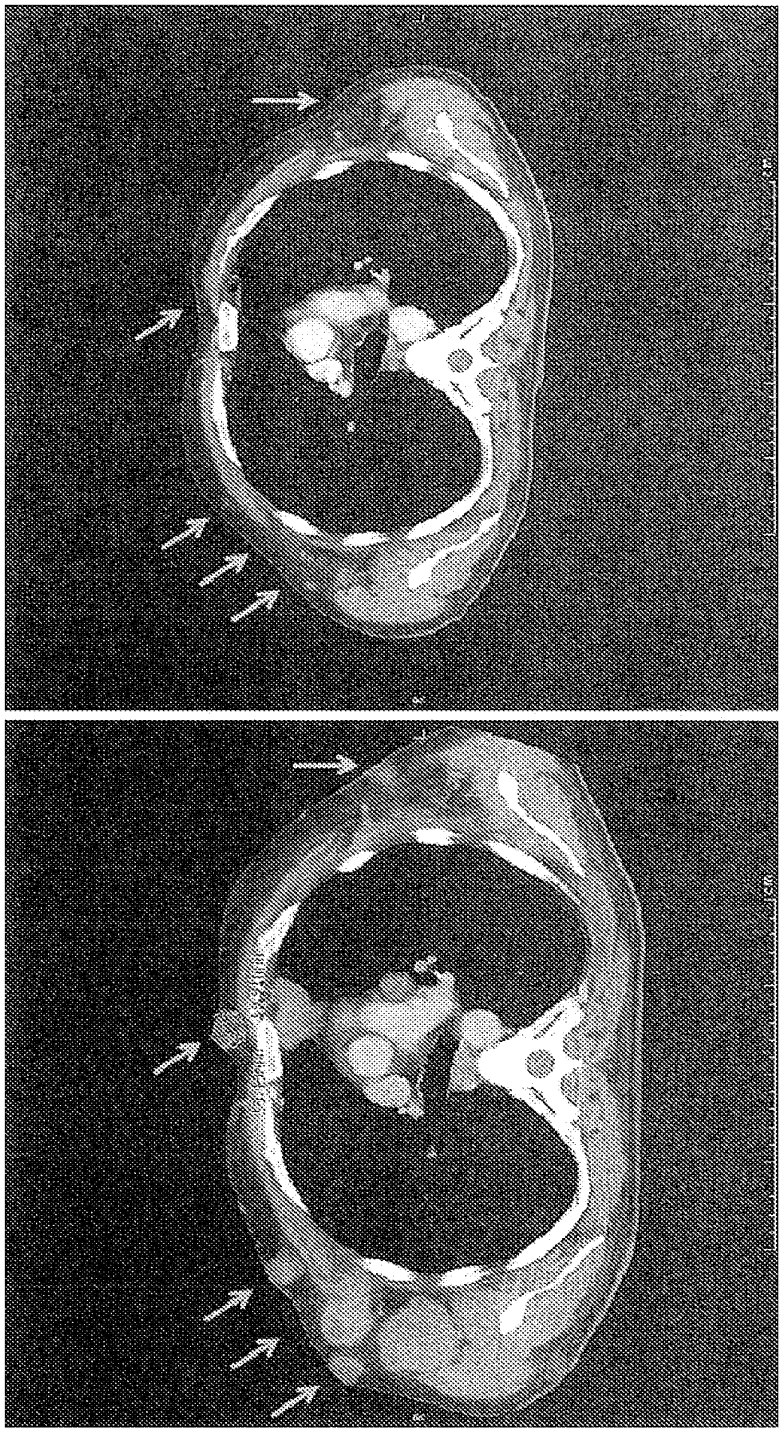



FIG. 15

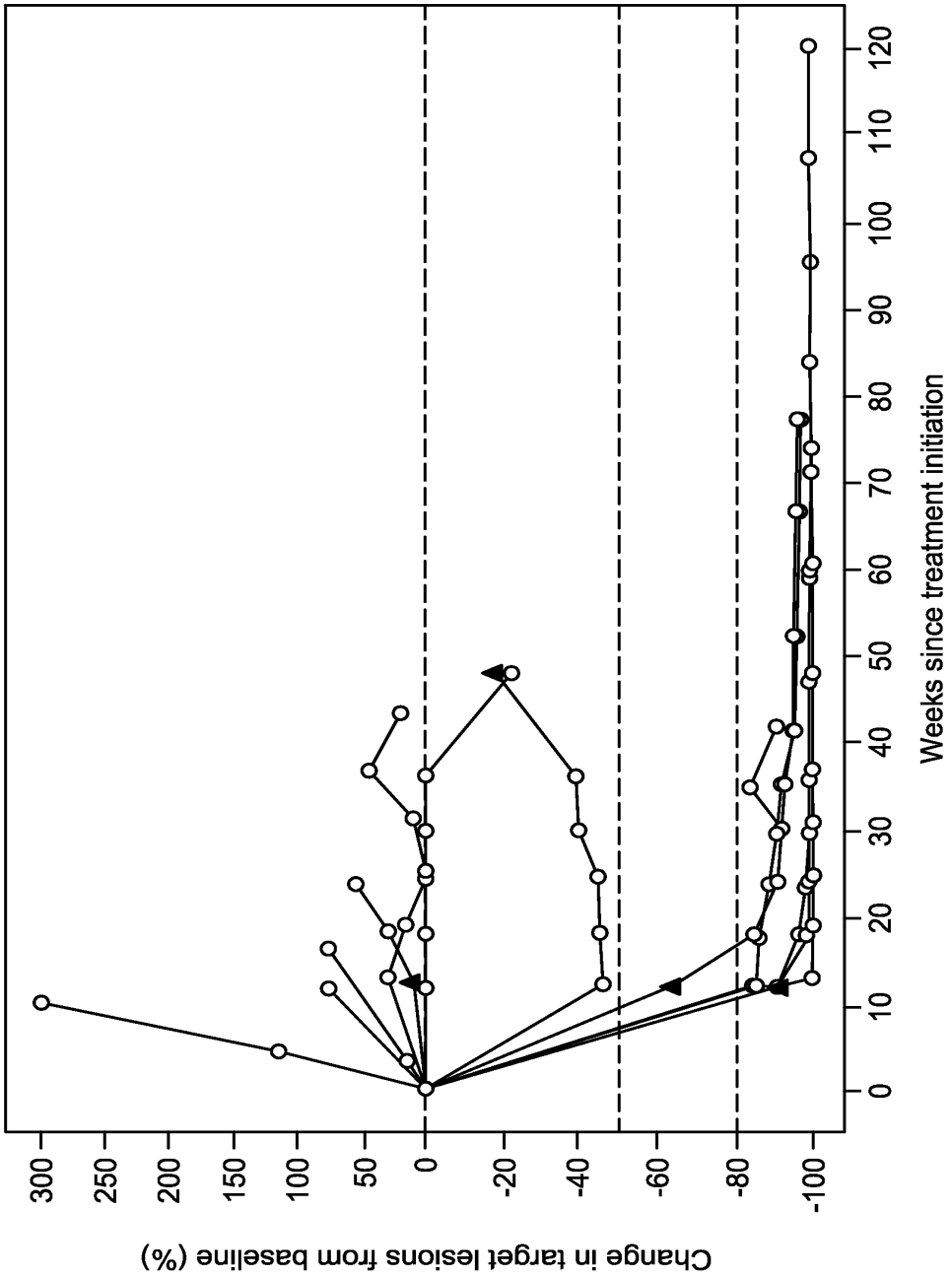

Change in target lesions from baseline (%)
300
250
200
150
100
50
0
-20
-40
-60
-80
-100
0
10
20
30
40
50
60
70
80
90
100
110
120
Weeks since treatment initiation

FIG. 16A

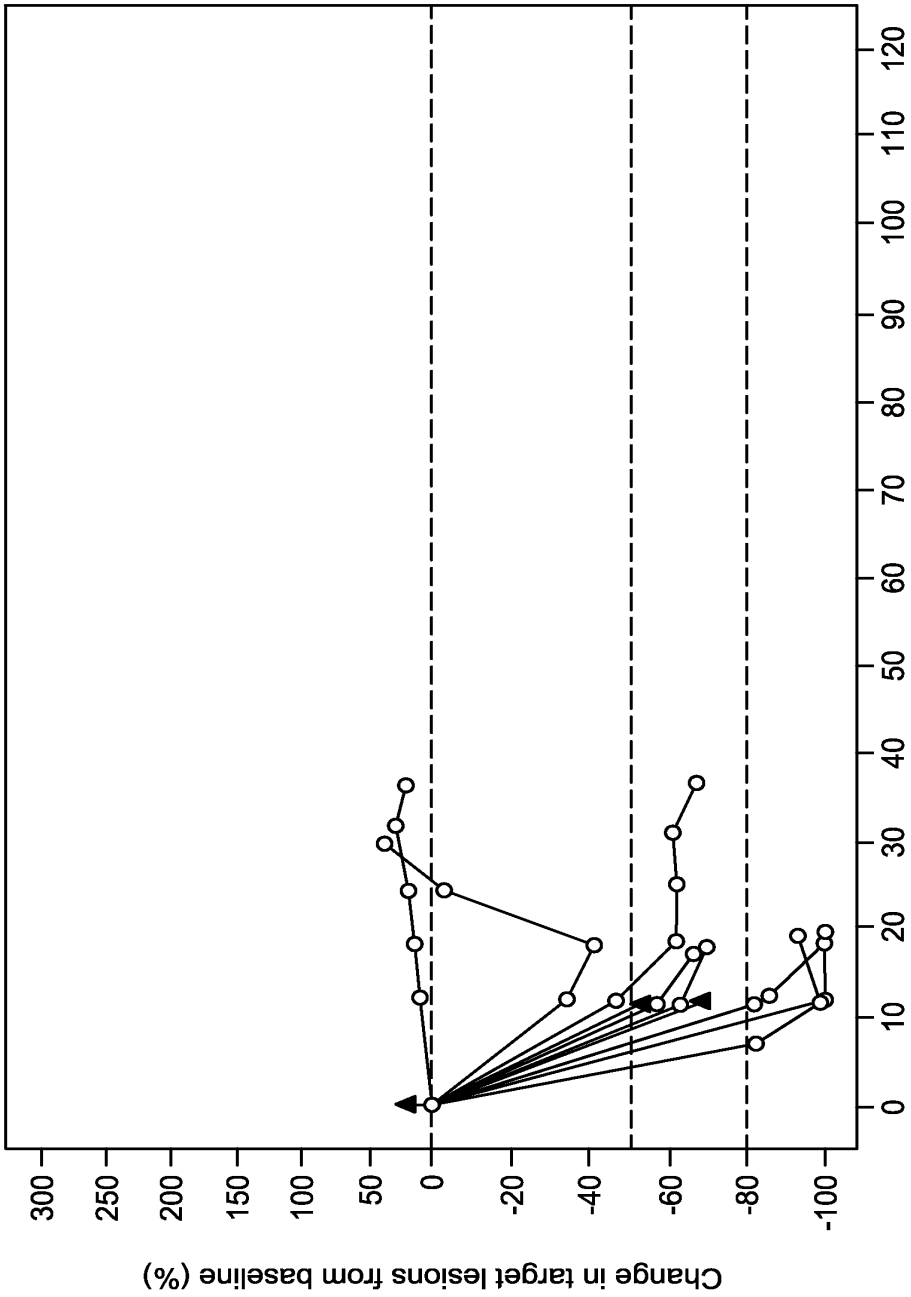

Change in target lesions from baseline (%)
300
250
200
150
100
50
0
-20
-40
-60
-80
-100
0
10
20
30
40
50
60
70
80
90
100
110
120
Weeks since treatment initiation

FIG. 16B

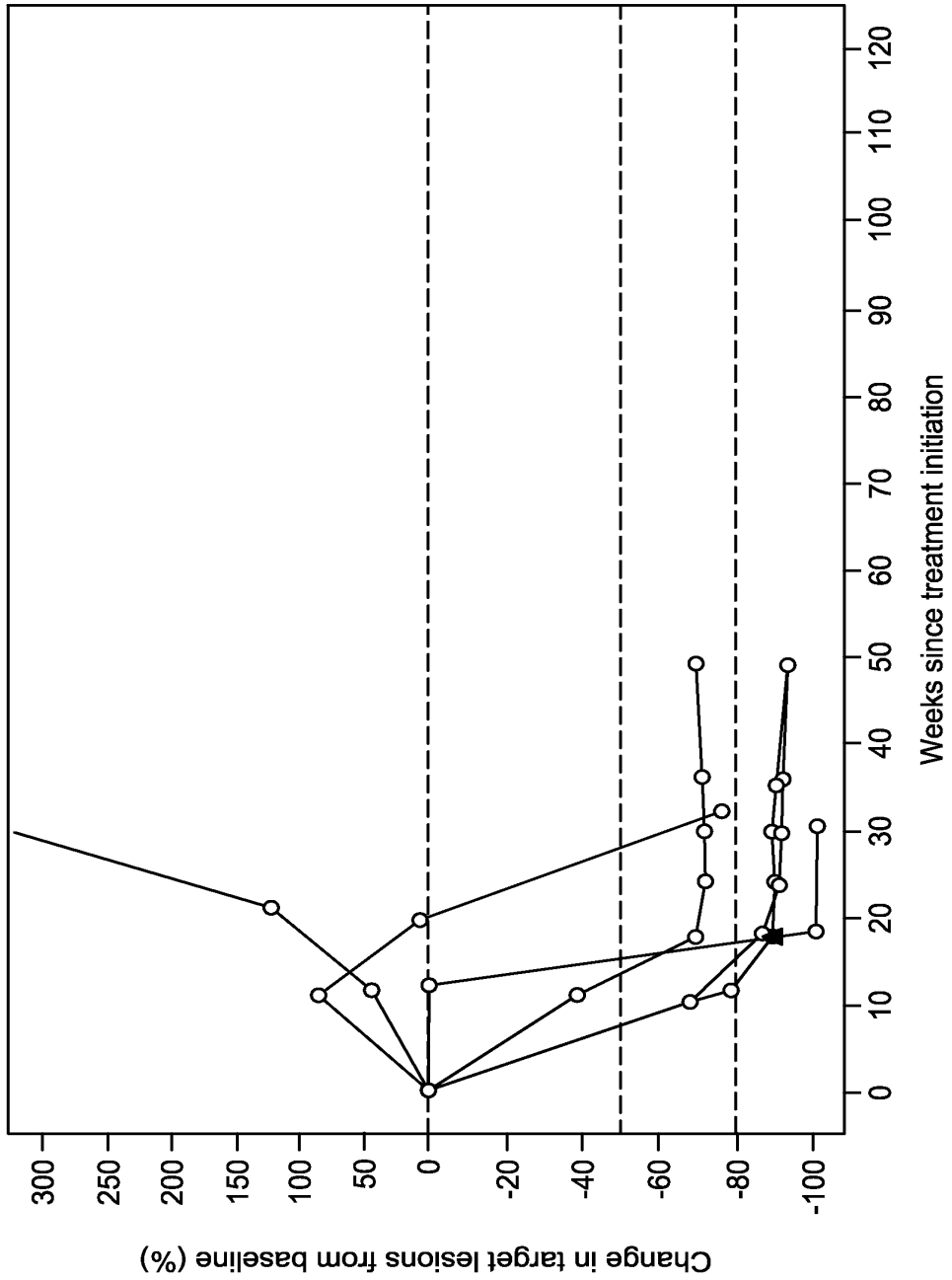

Change in target lesions from baseline (%)
300
250
200
150
100
50
0
-20
-40
-60
-80
-100
0
10
20
30
40
50
60
70
80
90
100
110
120
Weeks since treatment initiation

FIG. 16C

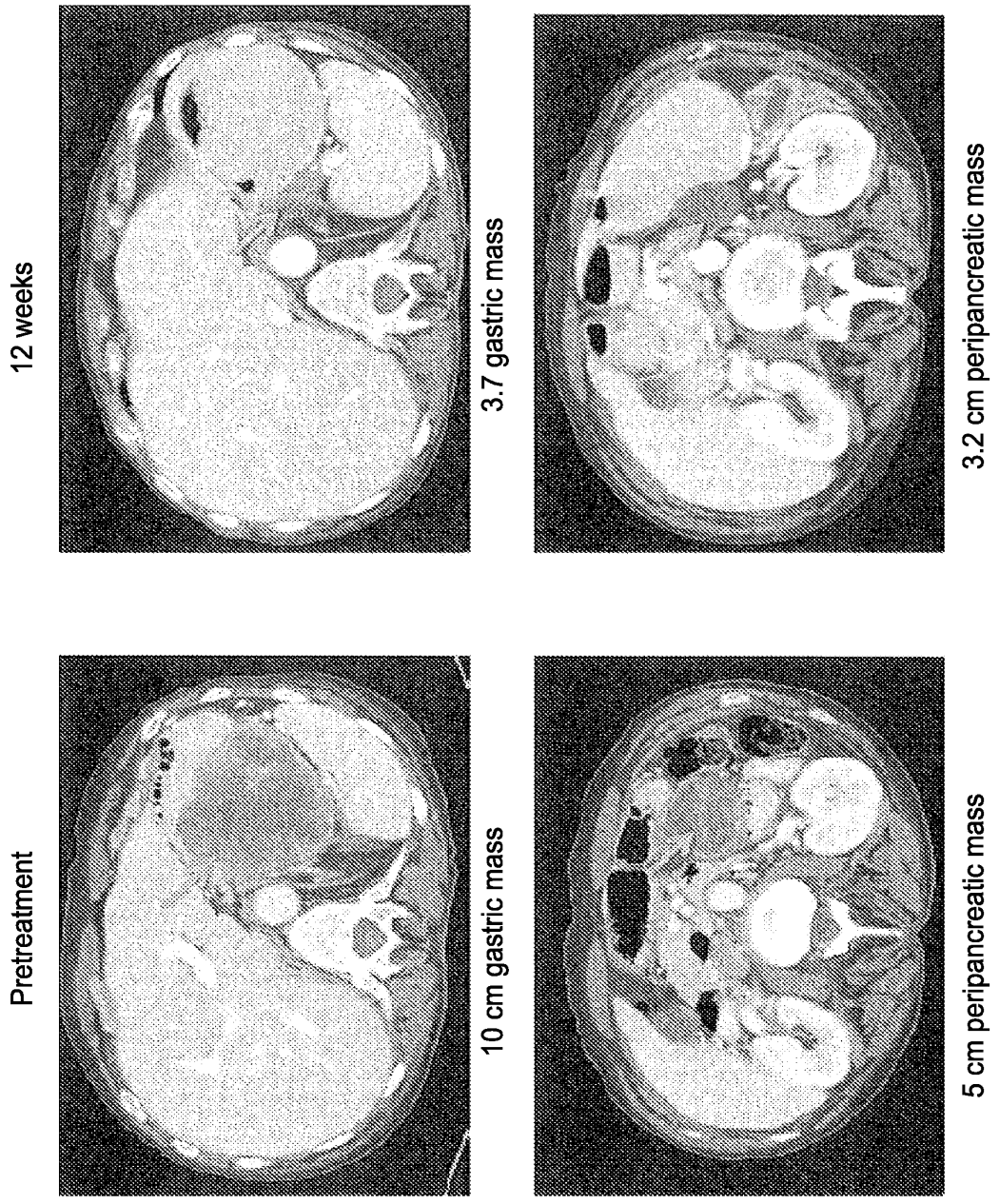

12 weeks
3.7 gastric mass
3.2 cm peripancreatic mass
Pretreatment
10 cm gastric mass
5 cm peripancreatic mass

FIG. 17

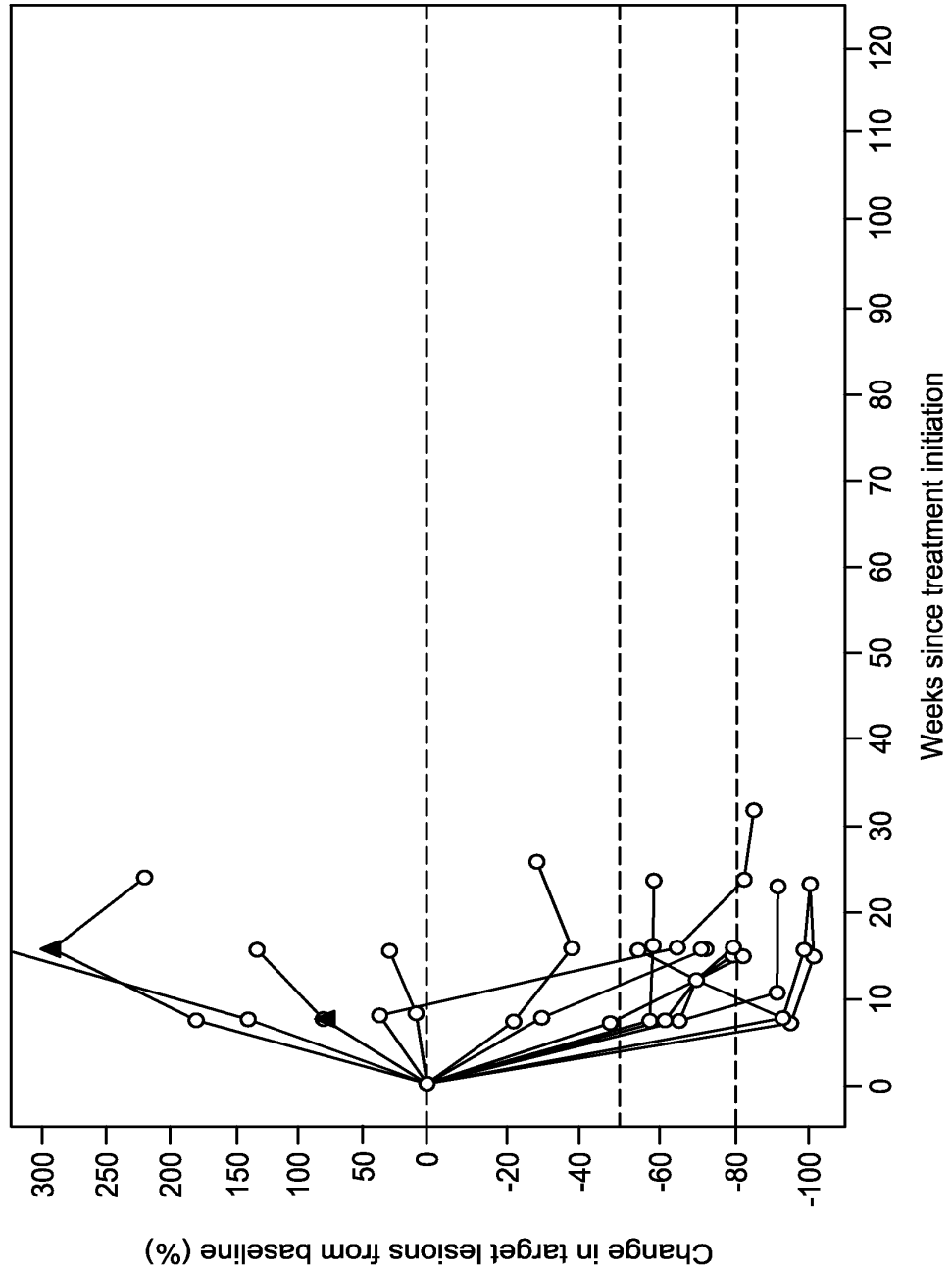

Change in target lesions from baseline (%)
300
250
200
150
100
50
0
-20
-40
-60
-80
-100
0
10
20
30
40
50
60
70
80
90
100
110
120
Weeks since treatment initiation

FIG. 18A

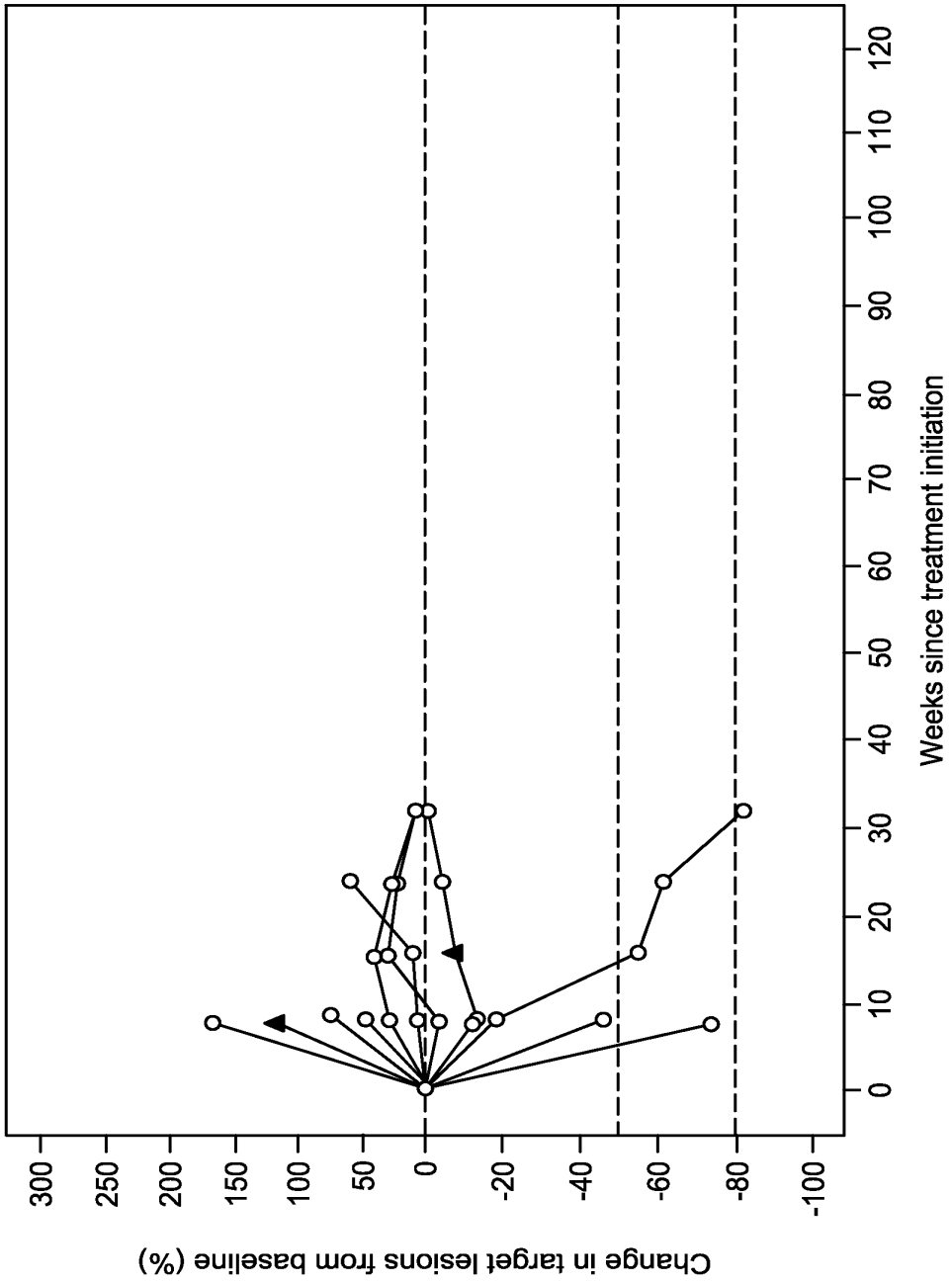

Change in target lesions from baseline (%)
300
250
200
150
100
50
0
-20
-40
-60
-80
-100
0
10
20
30
40
50
60
70
80
90
100
110
120
Weeks since treatment initiation

FIG. 18B

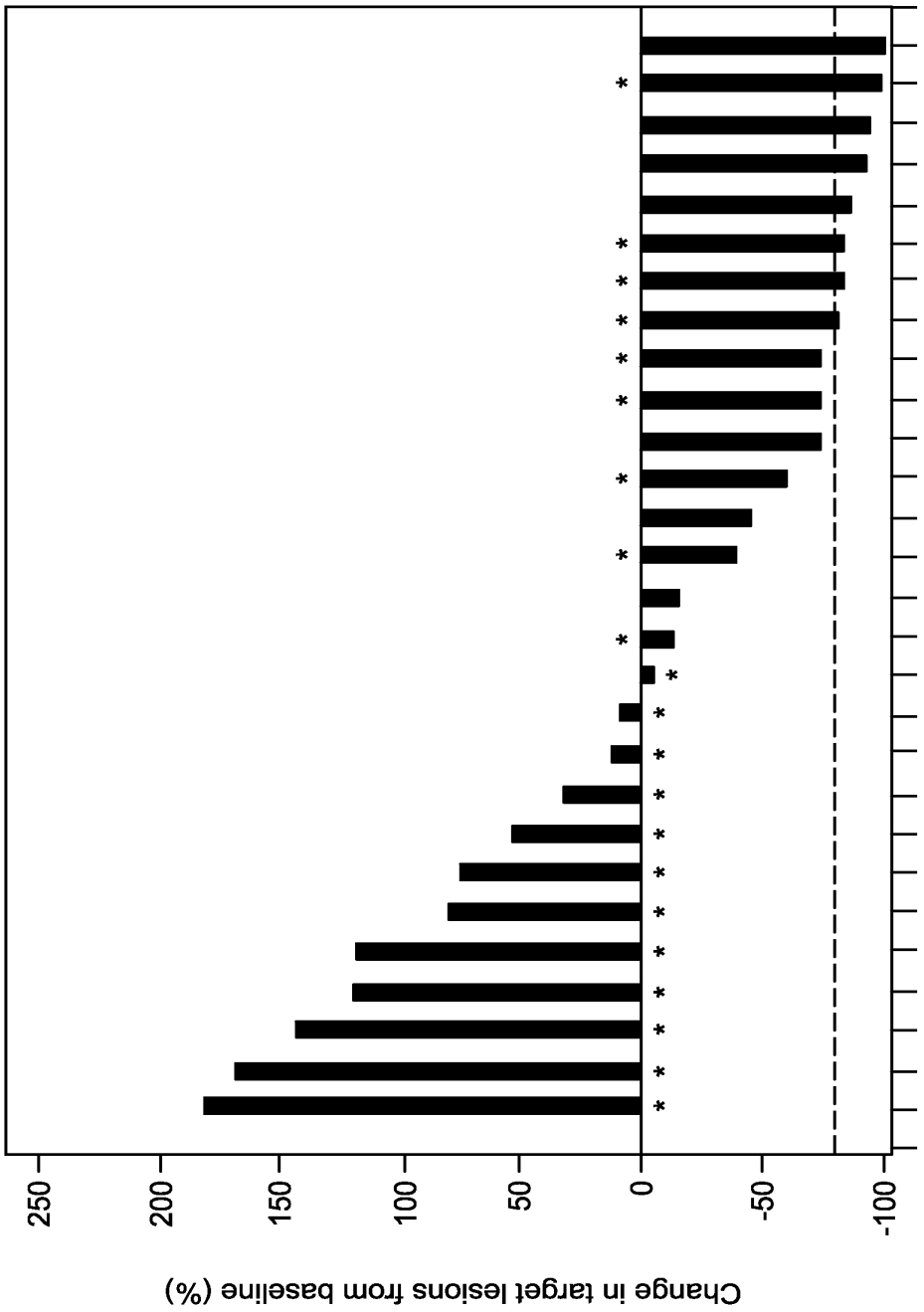

250
200
150
100
50
0
-50
-100
Change in target lesions from baseline (%)
Patients

FIG. 18C

# INTERNATIONAL SEARCH REPORT

International application No
PCT/US2013/040764

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/28
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | WO 2007/005874 A2 (MEDAREX INC [US]; KORMAN ALAN J [US]; SELBY MARK J [US]; WANG CHANGYU) 11 January 2007 (2007-01-11) pages 79,80,75; example all | 1-3 |
| X | WO 2006/121168 A1 (ONO PHARMACEUTICAL CO [JP]; MEDAREX INC [US]; KORMAN ALAN J; SRINIVASA) 16 November 2006 (2006-11-16) pages 58,59,55; example all | 1-3 |
| X | WO 2004/056875 A1 (WYETH CORP [US]; CAMBRIDGE ANTIBODY TECH [GB]; COLLINS MARY [US]; WOOD) 8 July 2004 (2004-07-08) pages 4,25,42; example all | 1-3 |

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 18 July 2013 | 31/10/2013 |

| Name and mailing address of the ISA/ | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040,<br>Fax: (+31-70) 340-3016 | Fellows, Edward |

1

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | HIRANO FUMIYA ET AL: "Blockade of B7-H1 and PD-1 by monoclonal antibodies potentiates cancer therapeutic immunity", CANCER RESEARCH, AMERICAN ASSOCIATION FOR CANCER RESEARCH, US, vol. 65, no. 3, 1 February 2005 (2005-02-01), pages 1089-1096, XP002419626, ISSN: 0008-5472 the whole document | 1-3 |
| A | DATABASE BIOSIS [Online] BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; April 2011 (2011-04), DODSON LINDZY F ET AL: "Potential targets for pancreatic cancer immunotherapeutics", XP002703660, Database accession no. PREV201100567322 the whole document & IMMUNOTHERAPY, vol. 3, no. 4, April 2011 (2011-04), pages 517-537, ISSN: 1750-743X, DOI: 10.2217/IMT.11.10 | 1-3 |
| A | DATABASE BIOSIS [Online] BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; July 2011 (2011-07), ANDORSKY DAVID J ET AL: "Programmed Death Ligand 1 Is Expressed by Non-Hodgkin Lymphomas and Inhibits the Activity of Tumor-Associated T Cells", XP002703661, Database accession no. PREV201100493026 the whole document & CLINICAL CANCER RESEARCH, vol. 17, no. 13, July 2011 (2011-07), pages 4232-4244, ISSN: 1078-0432, DOI: 10.1158/1078-0432.CCR-10-2660 | 1-3 |

1

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2013/040764

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
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| | | JP 2010189395 A | 02-09-2010 |

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/040764

**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-3

**Remark on Protest**

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

**FURTHER INFORMATION CONTINUED FROM PCT/ISA/** 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-3

   A method of treating a subject with cancer comprising administering to the subject a therapeutically effective amount of an antibody, or an antigen-binding portion thereof, that disrupts the interaction between PD-1 and PD-L1 wherein the antibody or antigen-binding portion thereof binds specifically to PD-1 or PD-L1 and related subject-matter.

   ---

2. claims: 4-9, 21

   A method for immunotherapy of a subject with cancer comprising the testing of a tissue sample, assessing the portion of cells in the sample that express PD-L1 and administering a composition comprising a therapeutically effective amount of an anti-PD-1 antibody to a selected subject and related subject-matter.

   ---

3. claim: 10

   A method for immunotherapy of a subject with cancer comprising selecting a subject who is not suitable of anti-PD-1 antibody immunotherapy and administering to the subject a standard-of-care therapeutic other than an anti-PD-1 antibody.

   ---

4. claims: 11-13

   A monoclonal antibody, or an antigen-binding portion thereof, that binds specifically to a cell surface-expressed PD-L1 in a sample and related subject-matter.

   ---

5. claims: 14-20

   A method of treating a subject with cancer comprising administering to the subject an antibody, or an antigen-binding portion thereof, that specifically binds and inhibits PD-1 and an antibody, or an antigen-binding portion thereof, that specifically binds and inhibits CTLA-4, each antibody be administered at a specific dosage range in a concurrent regimen and related subject-matter.

   ---

## INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2013/040764

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
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| | | WO 2004056875 A1 | 08-07-2004 |

# PATENT COOPERATION TREATY

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JUL 20 2016
Sterne Kessler Goldstein & Fox P.L.L.C.

PCT

From the INTERNATIONAL SEARCHING AUTHORITY

To:
Kim, Ji-Eun
STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.
1100 New York Avenue, N.W.
Washington, D.C. 20005-3934
ETATS-UNIS D'AMERIQUE

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT AND THE WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY, OR THE DECLARATION

(PCT Rule 44.1)

| | |
|---|---|
| Applicant's or agent's file reference<br>3338033PC02 | Date of mailing<br>*(day/month/year)*<br>13 July 2016 (13-07-2016) |
| International application No.<br>PCT/US2016/029877 | **FOR FURTHER ACTION** See paragraphs 1 and 4 below |
| Applicant<br>BRISTOL-MYERS SQUIBB COMPANY | International filing date<br>*(day/month/year)*<br>28 April 2016 (28-04-2016) |

1. [X] The applicant is hereby notified that the international search report and the written opinion of the International Searching Authority have been established and are transmitted herewith.

   **Filing of amendments and statement under Article 19:**
   The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

   **When?** The time limit for filing such amendments is normally two months from the date of transmittal of the International Search Report.

   **How?** Directly to the International Bureau of WIPO, 34 chemin des Colombettes 1211 Geneva 20, Switzerland, Fascimile No.: (41-22) 338.82.70

   **For more detailed instructions, see** *PCT Applicant's Guide*, International Phase, paragraphs 9.004 - 9.011.

2. [ ] The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect and the written opinion of the International Searching Authority are transmitted herewith.

3. [ ] **With regard to any protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

   [ ] the protest together with the decision thereon has been transmitted to the International Bureau together with any applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

   [ ] no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Reminders**

   The applicant may **submit comments on an informal basis on the written opinion of the International Searching Authority** to the International Bureau. These comments will be made available to the public after international publication. The International Bureau will send a copy of such comments to all designated Offices unless an international preliminary examination report has been or is to be established.

   Shortly after the expiration of **18 months from the priority date, the international application will be published** by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau before the completion of the technical preparations for international publication (Rules 90*bis*.1 and 90*bis*.3).

   Within **19 months** from the priority date, but only in respect of some designated Offices, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase **until 30 months** from the priority date (in some Offices even later); otherwise, the applicant must, **within 20 months** from the priority date, perform the prescribed acts for **entry into the national phase** before those designated Offices. In respect of other designated Offices, the time limit of **30 months** (or later) will apply even if no demand is filed within 19 months. For details about the applicable time limits, Office by Office, see www.wipo.int/pct/en/texts/time_limits.html and the *PCT Applicant's Guide*, National Chapters.

   Within **19 months from the priority date, the applicant may request that a supplementary international search be carried out** by a different International Searching Authority that offers this service (Rule 45*bis*.1). The procedure for requesting supplementary international search is described in the *PCT Applicant's Guide*, International Phase, paragraphs 8.006-8.032.

| | |
|---|---|
| Name and mailing address of the International Searching Authority<br>European Patent Office, P.B. 5818 Patentlaan 2<br>NL-2280 HV Rijswijk<br>Tel. (+31-70) 340-2040<br>Fax: (+31-70) 340-3016 | Authorized officer<br>DI MICELI, Giuseppe<br>Tel: +31 (0)70 340-3231 |

Form PCT/ISA/220 (July 2014)

# PATENT COOPERATION TREATY

# PCT

## INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

| Applicant's or agent's file reference | FOR FURTHER ACTION | see Form PCT/ISA/220 as well as, where applicable, item 5 below. |
|---|---|---|
| 3338033PC02 | | |
| International application No. | International filing date *(day/month/year)* | (Earliest) Priority Date *(day/month/year)* |
| PCT/US2016/029877 | 28 April 2016 (28-04-2016) | 28 April 2015 (28-04-2015) |
| Applicant | | |
| BRISTOL-MYERS SQUIBB COMPANY | | |

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 6 sheets.

[X] it is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language,** the international search was carried out on the basis of:

[X] the international application in the language in which it was filed

[ ] a translation of the international application into _____, which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b))

b. [ ] This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6*bis*(a)).

c. [X] With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. [ ] **Certain claims were found unsearchable** (See Box No. II)

3. [ ] **Unity of invention is lacking** (see Box No III)

4. With regard to the **title,**

[X] the text is approved as submitted by the applicant

[ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

[X] the text is approved as submitted by the applicant

[ ] the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority

6. With regard to the **drawings,**

a. the figure of the **drawings** to be published with the abstract is Figure No. _____

[ ] as suggested by the applicant

[ ] as selected by this Authority, because the applicant failed to suggest a figure

[ ] as selected by this Authority, because this figure better characterizes the invention

b. [X] none of the figures is to be published with the abstract

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/029877

**Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)**

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed:

      [X] in the form of an Annex C/ST.25 text file.

      [ ] on paper or in the form of an image file.

   b. [ ] furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. [ ] furnished subsequent to the international filing date for the purposes of international search only:

      [ ] in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      [ ] on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. [ ] In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

# INTERNATIONAL SEARCH REPORT

International application No
PCT/US2016/029877

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/28 A61P35/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | P. A. OTT ET AL: "CTLA-4 and PD-1/PD-L1 Blockade: New Immunotherapeutic Modalities with Durable Clinical Benefit in Melanoma Patients", CLINICAL CANCER RESEARCH, vol. 19, no. 19, 1 October 2013 (2013-10-01), pages 5300-5309, XP055200528, ISSN: 1078-0432, DOI: 10.1158/1078-0432.CCR-13-0143 page 5304, left-hand column, paragraph 2 - page 5306, right-hand column, paragraph 1 page 5307, left-hand column, paragraph 2 - right-hand column<br>-----<br>-/-- | 1-12, 21-25, 27-32, 34-47 |

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 1 July 2016 | 13/07/2016 |

| Name and mailing address of the ISA/ | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040,<br>Fax: (+31-70) 340-3016 | Le Flao, Katell |

Form PCT/ISA/210 (second sheet) (April 2005)

# INTERNATIONAL SEARCH REPORT

International application No
PCT/US2016/029877

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | G. K. PHILIPS ET AL: "Therapeutic uses of anti-PD-1 and anti-PD-L1 antibodies", INTERNATIONAL IMMUNOLOGY., vol. 27, no. 1, 16 October 2014 (2014-10-16), pages 39-46, XP055217958, GB ISSN: 0953-8178, DOI: 10.1093/intimm/dxu095 page 44, right-hand column, paragraph 2 - page 45, left-hand column, paragraph 2; table 1 page 43, right-hand column, paragraph 2 - page 44, left-hand column, paragraph 2 page 41, right-hand column, paragraph 2 - page 42, left-hand column, paragraph 2; table 1 ----- | 1-12, 21-32, 34-47 |
| X | MAHONEY KATHLEEN M ET AL: "The Next Immune-Checkpoint Inhibitors: PD-1/PD-L1 Blockade in Melanoma.", CLINICAL THERAPEUTICS 1 APR 2015, vol. 37, no. 4, 1 April 2015 (2015-04-01), pages 764-782, XP055285031, ISSN: 1879-114X page 768, right-hand column, paragraph 3 - page 769, left-hand column, paragraph 2; tables 2-4 page 771, left-hand column, paragraph 2 - page 772, right-hand column, line 13 page 774, right-hand column, paragraph 2 - page 775, left-hand column, paragraph 1 page 778, left-hand column, line 6 - line 15 ----- | 1-12, 21-47 |
| X | JEDD D. WOLCHOK ET AL: "Nivolumab plus Ipilimumab in Advanced Melanoma", NEW ENGLAND JOURNAL OF MEDICINE, vol. 369, no. 2, 11 July 2013 (2013-07-11) , pages 122-133, XP055182024, ISSN: 0028-4793, DOI: 10.1056/NEJMoa1302369 page 124, right-hand column, paragraph 2 page 128, right-hand column, paragraph 4 - page 130, right-hand column, paragraph 3 ----- | 1-12, 21-47 |
| X | MARZUKA ALEXANDER ET AL: "Melanoma Treatments: Advances and Mechanisms.", JOURNAL OF CELLULAR PHYSIOLOGY NOV 2015, vol. 230, no. 11, 22 April 2015 (2015-04-22), pages 2626-2633, XP002759396, ISSN: 1097-4652, DOI: 10.1002/jcp.25019 page 2631, left-hand column, paragraph 2 - right-hand column, paragraph 3 ----- | 13-20 |

1 -/--

# INTERNATIONAL SEARCH REPORT

International application No
PCT/US2016/029877

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | PAOLO A ASCIERTO ET AL: "The additional facet of immunoscore: immunoprofiling as a possible predictive tool for cancer treatment", JOURNAL OF TRANSLATIONAL MEDICINE, BIOMED CENTRAL, LONDON, GB, vol. 11, no. 1, 3 March 2013 (2013-03-03), page 54, XP021142097, ISSN: 1479-5876, DOI: 10.1186/1479-5876-11-54 page 3/4, line 10 - line 17 | 13-20 |
| X | US 2013/309250 A1 (COGSWELL JOHN [US] ET AL) 21 November 2013 (2013-11-21) claims 1-17; sequences 35,36 | 13-20 |
| A | P. A. ASCIERTO ET AL: "Biomarkers for Immunostimulatory Monoclonal Antibodies in Combination Strategies for Melanoma and Other Tumor Types", CLINICAL CANCER RESEARCH, vol. 19, no. 5, 1 March 2013 (2013-03-01), pages 1009-1020, XP055124339, ISSN: 1078-0432, DOI: 10.1158/1078-0432.CCR-12-2982 the whole document | 1-47 |

1

Form PCT/ISA/210 (continuation of second sheet) (April 2005)

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2016/029877

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| US 2013309250 A1 | 21-11-2013 | AU 2013263076 A1 | 22-01-2015 |
| | | CA 2873402 A1 | 21-11-2013 |
| | | CN 104470949 A | 25-03-2015 |
| | | EA 201492105 A1 | 30-06-2015 |
| | | EP 2850102 A1 | 25-03-2015 |
| | | HK 1203971 A1 | 06-11-2015 |
| | | JP 2015518826 A | 06-07-2015 |
| | | KR 20150020189 A | 25-02-2015 |
| | | SG 11201407190T A | 30-12-2014 |
| | | US 2013309250 A1 | 21-11-2013 |
| | | US 2015125463 A1 | 07-05-2015 |
| | | US 2016090417 A1 | 31-03-2016 |
| | | WO 2013173223 A1 | 21-11-2013 |

Form PCT/ISA/210 (patent family annex) (April 2005)

**Information on Search Strategy** - Pilot phase (see OJ 2015, A86)
The type of information contained in this sheet may change during the pilot for improving the usefulness of this new service.

Application Number
PCT/US2016/029877

TITLE: TREATMENT OF PD-L1-NEGATIVE MELANOMA USING AN ANTI-PD-1 ANTIBODY AND AN ANTI-CTLA-4 ANTIBODY

APPLICANT: BRISTOL-MYERS SQUIBB COMPANY

IPC CLASSIFICATION: C07K16/28, A61P35/00

EXAMINER: Le Flao, Katell

CONSULTED DATABASES: DOSYS, EPODOC, MEDLINE, NPL, WPI

CLASSIFICATION SYMBOLS DEFINING EXTENT OF THE SEARCH:

IPC:

CPC: C07K16/2818, A61K2039/505, A61K2039/55, A61K2039/507, A61K2039/545

FI/F-TERMS:

KEYWORDS OR OTHER ELEMENTS FEATURING THE INVENTION:

EPO FORM P04A42

# PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

# PCT

## WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY
(PCT Rule 43*bis*.1)

Date of mailing
*(day/month/year)* see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

**FOR FURTHER ACTION**
See paragraph 2 below

| International application No. | International filing date *(day/month/year)* | Priority date *(day/month/year)* |
|---|---|---|
| PCT/US2016/029877 | 28.04.2016 | 28.04.2015 |

International Patent Classification (IPC) or both national classification and IPC
INV. C07K16/28 A61P35/00

Applicant
BRISTOL-MYERS SQUIBB COMPANY

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

| Name and mailing address of the ISA: | Date of completion of this opinion | Authorized Officer |
|---|---|---|
| European Patent Office<br>P.B. 5818 Patentlaan 2<br>NL-2280 HV Rijswijk - Pays Bas<br>Tel. +31 70 340 - 2040<br>Fax: +31 70 340 - 3016 | see form PCT/ISA/210 | Le Flao, Katell<br>Telephone No. +31 70 340-0 |

Form PCT/ISA/237 (Cover Sheet) (January 2015)

**Box No. I Basis of the opinion**

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1 (b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. ☒ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing:

   a. ☒ forming part of the international application as filed:

      ☒ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

      ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

**Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

| | | | |
|---|---|---|---|
| Novelty (N) | Yes: | Claims | 33 |
| | No: | Claims | 1-32, 34-47 |
| Inventive step (IS) | Yes: | Claims | |
| | No: | Claims | 1-47 |
| Industrial applicability (IA) | Yes: | Claims | 1-47 |
| | No: | Claims | |

2. Citations and explanations

see separate sheet

## Item V

**Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1 Claims 1-46 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 39.1(iv) / 67.1(iv) PCT. The patentability can be dependent upon the formulation of the claims. The EPO, for example, does not recognise as patentable claims to the use of a compound in medical treatment, but may allow claims to a product, in particular substances or compositions for use in a first or further medical treatment.

2 Reference is made to the following documents:

D1 P. A. OTT ET AL: "CTLA-4 and PD-1/PD-L1 Blockade: New Immunotherapeutic Modalities with Durable Clinical Benefit in Melanoma Patients", CLINICAL CANCER RESEARCH,vol. 19, no. 19, 1 October 2013, pages 5300-5309, XP055200528,ISSN: 1078-0432, DOI: 10.1158/1078-0432.CCR-13-0143

D2 G. K. PHILIPS ET AL: "Therapeutic uses of anti-PD-1 and anti-PD-L1 antibodies", INTERNATIONAL IMMUNOLOGY.,vol. 27, no. 1, 16 October 2014, pages 39-46, XP55217958,GBISSN: 0953-8178, DOI: 10.1093/intimm/dxu095

D3 MAHONEY KATHLEEN M ET AL: "The Next Immune-Checkpoint Inhibitors: PD-1/PD-L1 Blockade in Melanoma.", CLINICAL THERAPEUTICS,vol. 37, no. 4, 1 April 2015, pages 764-782, XP055285031,ISSN: 1879-114X

D4 JEDD D. WOLCHOK ET AL: "Nivolumab plus Ipilimumab in Advanced Melanoma", NEW ENGLAND JOURNAL OF MEDICINE,vol. 369, no. 2, 11 July 2013, pages 122-133, XP55182024,ISSN: 0028-4793, DOI: 10.1056/NEJMoa1302369

D5 MARZUKA ALEXANDER ET AL: "Melanoma Treatments: Advances and Mechanisms.", JOURNAL OF CELLULAR PHYSIOLOGY,vol. 230, no. 11, 22 April 2015, pages 2626-2633,XP002759396, ISSN: 1097-4652, DOI: 10.1002/jcp.25019

D6 PAOLO A ASCIERTO ET AL: "The additional facet of immunoscore: immunoprofiling as a possible predictive tool for cancer treatment", JOURNAL OF TRANSLATIONAL MEDICINE,vol. 11, no. 1, 3 March 2013, page 54, XP021142097,ISSN: 1479-5876, DOI: 10.1186/1479-5876-11-54

D7 US 2013/309250 A1 (COGSWELL JOHN [US] ET AL) 21 November 2013

D8 P. A. ASCIERTO ET AL: "Biomarkers for Immunostimulatory Monoclonal Antibodies in Combination Strategies for Melanoma and Other Tumor Types", CLINICAL CANCER RESEARCH,vol. 19, no. 5, 1 March 2013, pages 1009-1020, XP55124339,ISSN: 1078-0432, DOI: 10.1158/1078-0432.CCR-12-2982

3 The present application does not meet the criteria of Article 33(2) PCT, because the subject-matter of claims 1-32, 34-47 is not new.

3.1 Claims 1-12, 21-32, and 34-47 relates to a method for treating a melanoma comprising: (i) identifying a patient having a PD-L negative melanoma tumor; and (ii) administering to the patient an anti-PD-1 antibody in combination with an anti-CTLA-4 antibody. They are not novel over the following documents D1 to D4.

3.2 Document D1 identifies PD-L1 as a biomarker suitable to discriminate the patients suffering from melanoma that would respond to the therapy with anti PD-1 antibody from the others (page 5306, left-hand column, para. 2 to right-hand column, para. 1). The authors of D1 refers to combined therapy with anti PD-1 and anti CTLA-4 antibodies as a way to overcome the lack of response observed in some patients with either monotherapy (page 5307, left-hand column, para.2 to right-hand column, para. 3). The mechanisms of action of inhibition of PD-1/PD-L1 and CTLA-4 are explained and the rational for combining the both in order to treat melanoma patients is given (page 5304, left-hand column, para.2 to page 5306, right-hand column, para.1) as well as the first results indeed showing synergistic effects (page 5307, right-hand column, para. 1).

3.3 Document D2 discloses the combined use of nivolumab and ipilimumab in patients having melanoma, (page 43, right-hand column, para. 4 - para.5) as well as the lack of rational for excluding patients with PD-L1 negative tumor before treating them with the combined therapy (page 44, right-hand column, last para. to page 45, para. 1).

3.4 Document D3 reviews the clinical studies of PD-1/PD-L1-targeted therapies in melanoma. The correlation between the expression of PD-L1 and the objective rate response to nivolumab is reported and its relevance is discussed (page 771, left-hand column, para. 2 to page 772, right-hand column, line 13). Combination therapy with nivolumab and ipilimumab in advance melanoma is reported (page 774, right-hand column, para.2 to page

775, left-hand column, para. 1) and the mechanisms involved are discussed (page 778, left-hand column, lines 6-15). The use of PD-L1 expression to sort out the PD-L1$^+$ patients receiving monotherapy and the PD-L1$^-$ patients receiving combined therapy is put forward (page 778, right-hand column, lines 3 to 22). From D3 is known that Pembrolizumab is an IgG4 (Table II at page 772) tested in clinical trials alone (Tables III and IV) and in a combined therapy with ipilimumab (page 768, right-hand column, para. 3 to page 769, left-hand column, para. 2), which anticipates the novelty of claim 26 in which the anti-PD-1 antibody is pembrolizumab.

3.5 Document D4 discloses the combined therapy of patients with melanoma based upon ipilimumab (anti-CTLA-4 antibody) and nivolimumab (anti PD-1 antibody) as well as the immunohistochemical assessment of PD-L1 (page 124, right-hand column, para. 2 and page 128, right-hand column, last para. to page 130, right-hand column, para. 3). The complementarity of the combined therapy is highlighted, whatever the level of PD-L1 is (page 132, left-hand column, para. 2 to right-hand column, para. 2).

3.6 Claims 13-20 relate to a method for selecting a patient suitable for an anti-PD-1 antibody and anti-CTLA-4 antibody combination therapy. The steps related to the detection and assessment of PD-L1 levels are known and disclosed in the cited documents D1-D7. The features related to the treatment (claim 15) and instruction (claim 13 (ii)) are not technical feature in the sense of Rule 6.3 (a) PCT and therefore shall not represent distinguishing features when assessing the patentability.

3.7 Document D5 discusses the advances and mechanisms in melanoma treatments with some focus on the clinical studies of PD-1/PD-L1-targeted therapies in melanoma. The correlation between the expression of PD-L1 and the objective rate response to nivolumab is reported (page 2631, left-hand column, para. 2 to right-hand column, para.3).

3.8 Document D6 discloses the concept of immunoprofiling patients suffering from cancer as "using an individual's immune system signature (or profile) to predict that patient's response to therapy" and refers to PD-L1 as a possible predictive biomarker of therapeutic response to anti PD-1 treatment (abstract and page 3/4, lines 10-17).

3.9 Document D7 discloses the treatment of melanoma with anti-PD-1 antibody following the determination of PD-L1 levels (claims 1-17).

4 The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claim 33 does not involve an inventive step in the sense of Article 33(3) PCT.

4.1 This claim differs from the closest prior art Document D3 disclosing the combined treatment of melanoma with ipilimumab and nivolumab (page 774, right-hand column, para. 2 to page 775, left-hand column, para. 1) by the fact that in claim 33 the anti-CTLA-4 antibody is tremelimumab.

4.2 No particular effect has been shown to be associated with the difference.

4.3 The technical problem is therefore considered to be the provision of an alternative combined treatment for melanoma based upon anti-PD-1 antibody and anti-CTLA-4 antibody.

4.4 The claimed solution is an obvious solution to the problem posed. Tremelimumab is disclosed in Table II of D3 as an anti CTLA-4 monoclonal antibody and therefore the skilled person trying to solve the problem posed would arrive to the claimed solution without exerting inventive skills.

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XS CPRTENFRDE

BNSDOCID: <XS_____2006100103CF_I_>

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| **EFS ID:** | 28432881 |
| **Application Number:** | 15141769 |
| **International Application Number:** | |
| **Confirmation Number:** | 6926 |
| **Title of Invention:** | Treatment of PD-L1-Negative Melanoma Using an Anti-PD-1 Antibody and an Anti-CTLA-4 Antibody |
| **First Named Inventor/Applicant Name:** | Arvin YANG |
| **Customer Number:** | 115018 |
| **Filer:** | Ji Eun Kim/Kisha Cox |
| **Filer Authorized By:** | Ji Eun Kim |
| **Attorney Docket Number:** | 3338.0330001/ELE/C-K |
| **Receipt Date:** | 22-FEB-2017 |
| **Filing Date:** | 28-APR-2016 |
| **Time Stamp:** | 17:20:34 |
| **Application Type:** | Utility under 35 USC 111(a) |

## Payment information:

| | |
|---|---|
| Submitted with Payment | no |

## File Listing:

| Document Number | Document Description | File Name | File Size(Bytes)/ Message Digest | Multi Part /.zip | Pages (if appl.) |
|---|---|---|---|---|---|
| 1 | | IDS.pdf | 8596863<br>5f444405975e86f6d2f772bedbeb82a2a2c468d6 | yes | 11 |

| | Multipart Description/PDF files in .zip description | | |
|---|---|---|---|
| | **Document Description** | **Start** | **End** |
| | Information Disclosure Statement (IDS) Form (SB08) | 4 | 11 |
| | Transmittal Letter | 2 | 3 |
| | Miscellaneous Incoming Letter | 1 | 1 |

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| **Information:** | | | | | |
| 2 | Foreign Reference | FP1_WO2006121168A1_EFS.PDF | 11155832<br>ecf0f3622ce113d0d4c4cfdad7e67fb08149fa73 | no | 199 |
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| 3 | Foreign Reference | FP2_WO2007005874A2_EFS.PDF | 7816132<br>7d5db583472bc70fcaffc5e7041ac2194a436f11 | no | 184 |
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| 4 | Foreign Reference | FP3_WO2007113648A2_EFS.PDF | 7507855<br>37e0636e5cc8e958266bcde22da49e4596a464d6 | no | 127 |
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| 5 | Foreign Reference | FP4_WO2012122444A1_EFS.PDF | 2572556<br>beda460d5515c745cdf54ba9e0c01c58466fc646 | no | 40 |
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| 6 | Foreign Reference | FP5_WO2012145493A1_EFS.PDF | 6441247<br>c061bdecb047e66c13397c9a7df8bffdc050137a | no | 124 |
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| 7 | Foreign Reference | FP6_WO2013014668A1_EFS.PDF | 2830459<br>719aa753339726ab84fdae3a8b464215f58264f4 | no | 52 |
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| 8 | Foreign Reference | FP7_WO2013173223A1_EFS.PDF | 5919172<br>e4f82aa92b2ae33a4d5a6f1813327a76c3c2d394 | no | 185 |
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| **Information:** | | | | | |
| 9 | Non Patent Literature | NPL1_ASCIERTO_CLINCANRES_2013_19_1009_EFS.PDF | 6256837<br>7c7a6928be85ceefaaaf42c311d035a76f98e324 | no | 12 |
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| 10 | Non Patent Literature | NPL2_ASCIERTO_JTRANSMED_2013_11_54_EFS.PDF | 1766672<br>d3ab54067fdc0d317521666bb4ecbf138b2d681b | no | 4 |
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| 11 | Non Patent Literature | NPL3_BRAHMER_NEWENGJMED_2012_366_2455_EFS.PDF | 5214808<br>c13ba639ddcffc8b67c8811a996c703bc5312bff | no | 11 |
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| 12 | Non Patent Literature | NPL4_CONDEELIS_COLDSPRING_2010_2_A003848_EFS.PDF | 10595264<br>b6cc0e75e148b605794e0e92e47c83a7ecac40d8 | no | 24 |
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| 13 | Non Patent Literature | NPL5_DONG_NATUREMEDICINE_2002_8_793_EFS.PDF | 2886466<br>60e8f227ed0f0b01496a68dcc0dbf50510687079 | no | 8 |
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| 14 | Non Patent Literature | NPL6_GADIOT_CANCER_2011_117_2192_EFS.PDF | 6113055<br>0fbfa78d34c16d5f2c28a04e98586213ac7a193b | no | 11 |
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| 16 | Non Patent Literature | NPL8_GENBANK_AAB59385_01NOV1994_EFS.PDF | 579742<br>f923c6feca5c4c332e3a974797cb3b078d20faaf | no | 3 |
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| 17 | Non Patent Literature | NPL9_GENBANK_Q9NZQ7_02NOV2016_EFS.PDF | 1972615<br>080e43f33369656baf66f434412c5ec7da67a7b9 | no | 11 |
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| 18 | Non Patent Literature | NPL10_GENBANK_U64863_12OCT2005_EFS.PDF | 588195<br>b2a36f1e9613b61f56f858a634a3b3a46fa76d04 | no | 3 |
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| 19 | Non Patent Literature | NPL11_HAMANISHI_PNAS_2007_104_3360_EFS.PDF | 4540010<br>62ed0fdfcb7855d421d4b3f6de5e907c466bee0d | no | 6 |
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| 20 | Non Patent Literature | NPL12_HERBST_JCLINONCOLOGY_2013_31_3000_EFS.PDF | 481268<br>52be68a6ce7708a28db809a206e80a46a974f548 | no | 2 |
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| 21 | Non Patent Literature | NPL13_ISR_WO_PCTUS2016029877_13JULY2016_EFS.PDF | 3190604<br>44fcf875d38a46115dca99105b9da4ba869670ba | no | 16 |
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| 22 | Non Patent Literature | NPL14_KHLEIF_MEDI4736_2013_ABS802_EFS.PDF | 187977<br>19e586ee345e04547ccfadc1d01deb039e1a0f01 | no | 1 |
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| 23 | Non Patent Literature | NPL15_MAHONEY_CLITHERAPEUTICS_2015_37_764_EFS.PDF | 10061409<br>fb8a24899eba9dc1f6d4031b5c7b409449820226 | no | 19 |
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| 24 | Non Patent Literature | NPL16_MARZUKA_JCELLULARPHY_2015_230_2626_EFS.PDF | 3327880<br>b2b8737b272c5d56be4dfc8b17eb1f8308a150fd | no | 8 |
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| 25 | Non Patent Literature | NPL17_MCCABE_CANBIORADIOPHARM_2010_25_253_EFS.PDF | 5548078<br>912f974b53a55ee0baa8f85ea7b14183c8dd431b | no | 10 |
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| 26 | Non Patent Literature | NPL18_NCCN_GUIDELINES_2014_4_EFS.PDF | 1103885<br>5d71b82750b47b57bdc1af22515bd0b837636c40 | no | 4 |
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| 27 | Non Patent Literature | NPL19_NCIDRUGDICTIONARY_AMP_224_700595_EFS.PDF | 319368<br>086a32920c8c99dacc19dac54abb444493c99cda | no | 3 |
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| 28 | Non Patent Literature | NPL20_NCIDRUGDICTIONARY_MEDI0680_756047_EFS.PDF | 346575<br>8be7eaf2eaf57dceeb643308b0a8fd462582<br>58be | no | 3 |
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| 29 | Non Patent Literature | NPL21_NCIDRUGDICTIONARY_PEMBROLIZUMAB_695789_EFS.PDF | 477192<br>2392e39750f1869528fb66b87499bf7b951<br>bb65a | no | 3 |
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| 30 | Non Patent Literature | NPL22_OLAFSEN_PROTEINENGDESSELE_2010_23_243_EFS.PDF | 4302856<br>896a061459a5e000feb51ad2a57cd3ee2f3c<br>b3d6 | no | 7 |
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| 31 | Non Patent Literature | NPL23_OTT_CLICANCERRES_2013_19_5300_EFS.PDF | 5309725<br>dda7afa8ef30b03fe6577f8e85534b465ba2<br>ed53 | no | 10 |
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| 33 | Non Patent Literature | NPL25_SIEGEL_CANJCLINICIANS_2013_63_11_EFS.PDF | 10591386<br>b99e3837fe398680cdaa3544f5861248b9c3<br>77d0 | no | 20 |
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| 34 | Non Patent Literature | NPL26_SJOBLOM_SCIENCE_2006_314_268_EFS.PDF | 6558222<br>ce5bedad0b92a4cd91b9426cf1af45e6381c<br>bebb | no | 8 |
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| 35 | Non Patent Literature | NPL27_TAUBE_SCITRANSMEDI_2012_4_127RA37_EFS.PDF | 10864505<br>1bad8004287ae62232bb85a6d7886f5564a52b93 | no | 22 |
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| 36 | Non Patent Literature | NPL28_TOPALIAN_NENGJMED_2012_366_2443_EFS.PDF | 7439466<br>e1847421ada587475954eaacc3d909abca3fa461 | no | 19 |
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| 37 | Non Patent Literature | NPL29_USAN_COUNCIL_2013_EFS.PDF | 364727<br>732c04252d359a857c67c122d48e8771440126fa | no | 2 |
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| 38 | Non Patent Literature | NPL30_WANG_CANCERIMMUNOLOGYRESEARCH_2014_2_846_EFS.PDF | 5793696<br>e15e6c687963119bd6735f8aef002b5fa0ba5d85 | no | 12 |
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| **Information:** | | | | | |
| 39 | Non Patent Literature | NPL31_WOLCHOK_NEWENGJMED_2013_369_122_EFS.PDF | 1884691<br>430a6b23aa91dd491da4aa5ea6811574ed943fed | no | 12 |
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| **Information:** | | | | | |
| | | **Total Files Size (in bytes):** | 184382150 | | |

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**If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.**

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| APPLICATION NUMBER | FILING OR 371(C) DATE | FIRST NAMED APPLICANT | ATTY. DOCKET NO./TITLE |
|---|---|---|---|
| 15/141,769 | 04/28/2016 | Arvin YANG | 3338.0330001/ELE/C-K |

**CONFIRMATION NO. 6926**

115018
Sterne, Kessler, Goldstein & Fox P.L.L.C.
1100 New York Avenue NW
Washington, DC 20005

**PUBLICATION NOTICE**

*OC000000087447098*

**Title:**Treatment of PD-L1-Negative Melanoma Using an Anti-PD-1 Antibody and an Anti-CTLA-4 Antibody

**Publication No.**US-2016-0340428-A1
**Publication Date:**11/24/2016

## NOTICE OF PUBLICATION OF APPLICATION

The above-identified application will be electronically published as a patent application publication pursuant to 37 CFR 1.211, et seq. The patent application publication number and publication date are set forth above.

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Office of Data Managment, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101

## PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number: 15/141,769

### APPLICATION AS FILED - PART I

| FOR | (Column 1) NUMBER FILED | (Column 2) NUMBER EXTRA | SMALL ENTITY RATE($) | SMALL ENTITY FEE($) | OR | OTHER THAN SMALL ENTITY RATE($) | OTHER THAN SMALL ENTITY FEE($) |
|---|---|---|---|---|---|---|---|
| BASIC FEE (37 CFR 1.16(a), (b), or (c)) | N/A | N/A | N/A | | | N/A | 280 |
| SEARCH FEE (37 CFR 1.16(k), (i), or (m)) | N/A | N/A | N/A | | | N/A | 600 |
| EXAMINATION FEE (37 CFR 1.16(o), (p), or (q)) | N/A | N/A | N/A | | | N/A | 720 |
| TOTAL CLAIMS (37 CFR 1.16(i)) | 20 minus 20 = | * | | | OR | x 80 = | 0.00 |
| INDEPENDENT CLAIMS (37 CFR 1.16(h)) | 3 minus 3 = | * | | | | x 420 = | 0.00 |
| APPLICATION SIZE FEE (37 CFR 1.16(s)) | If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s). | | | | | | 0.00 |
| MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j)) | | | | | | | 0.00 |
| * If the difference in column 1 is less than zero, enter "0" in column 2. | | | TOTAL | | | TOTAL | 1600 |

### APPLICATION AS AMENDED - PART II

| | | (Column 1) CLAIMS REMAINING AFTER AMENDMENT | | (Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR | (Column 3) PRESENT EXTRA | SMALL ENTITY RATE($) | SMALL ENTITY ADDITIONAL FEE($) | OR | OTHER THAN SMALL ENTITY RATE($) | OTHER THAN SMALL ENTITY ADDITIONAL FEE($) |
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| AMENDMENT A | Total (37 CFR 1.16(i)) | * | Minus | ** | = | x = | | OR | x = | |
| | Independent (37 CFR 1.16(h)) | * | Minus | *** | = | x = | | OR | x = | |
| | Application Size Fee (37 CFR 1.16(s)) | | | | | | | | | |
| | FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j)) | | | | | | | OR | | |
| | | | | | | TOTAL ADD'L FEE | | OR | TOTAL ADD'L FEE | |

| | | (Column 1) CLAIMS REMAINING AFTER AMENDMENT | | (Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR | (Column 3) PRESENT EXTRA | RATE($) | ADDITIONAL FEE($) | OR | RATE($) | ADDITIONAL FEE($) |
|---|---|---|---|---|---|---|---|---|---|---|
| AMENDMENT B | Total (37 CFR 1.16(i)) | * | Minus | ** | = | x = | | OR | x = | |
| | Independent (37 CFR 1.16(h)) | * | Minus | *** | = | x = | | OR | x = | |
| | Application Size Fee (37 CFR 1.16(s)) | | | | | | | | | |
| | FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j)) | | | | | | | OR | | |
| | | | | | | TOTAL ADD'L FEE | | OR | TOTAL ADD'L FEE | |

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.

** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".

*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".

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| APPLICATION NUMBER | FILING or 371(c) DATE | GRP ART UNIT | FIL FEE REC'D | ATTY.DOCKET.NO | TOT CLAIMS | IND CLAIMS |
|---|---|---|---|---|---|---|
| 15/141,769 | 04/28/2016 | 1618 | 1740 | 3338.0330001/ELE/C-K | 20 | 3 |

**CONFIRMATION NO. 6926**

115018
Sterne, Kessler, Goldstein & Fox P.L.L.C.
1100 New York Avenue NW
Washington, DC 20005

**UPDATED FILING RECEIPT**

OC000000085143278

Date Mailed: 08/16/2016

Receipt is acknowledged of this non-provisional patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please submit a written request for a Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections**

**Inventor(s)**
Arvin YANG, Princeton, NJ;

**Applicant(s)**
Bristol-Myers Squibb Company, Princeton, NJ

**Assignment For Published Patent Application**
Bristol-Myers Squibb Company, Princeton, NJ

**Power of Attorney:** The patent practitioners associated with Customer Number 115018

**Domestic Priority data as claimed by applicant**
This appln claims benefit of 62/153,973 04/28/2015

**Foreign Applications** for which priority is claimed (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see http://www.uspto.gov for more information.) - None.
*Foreign application information must be provided in an Application Data Sheet in order to constitute a claim to foreign priority. See 37 CFR 1.55 and 1.76.*

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The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 15/141,769**

**Projected Publication Date:** 11/24/2016

**Non-Publication Request:** No

**Early Publication Request:** No
**Title**

Treatment of PD-L1-Negative Melanoma Using an Anti-PD-1 Antibody and an Anti-CTLA-4 Antibody

**Preliminary Class**

424

**Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications:** No

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## Title 37, Code of Federal Regulations, 5.11 & 5.15

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventor: Arvin YANG

Applicant: Bristol-Myers Squibb Co.

Application No.: 15/141,769

Filing Date: April 28, 2016

Title: **TREATMENT OF PD-L1 NEGATIVE MELANOMA USING AN ANTI-PD-1 ANTIBODY AND AN ANTI-CTLA-4 ANTIBODY**

Confirmation No.: 6926

Art Unit: 1618

Examiner: *To Be Assigned*

Atty. Docket: 3338.0330001

# Preliminary Amendment Under 37 C.F.R. § 1.115

***Mail Stop Amendment***

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

Commissioner:

In advance of prosecution, Applicant submits the following amendments and remarks.

Amendments to the Specification begin on page 2 of this paper.

Amendments to the Claims begin on page 3 of this paper.

Amendments to the Drawings begin on page 7 of this paper.

Remarks begin on page 8 of this paper.

It is not believed that extensions of time are required beyond those that may otherwise be provided for in documents accompanying this paper. However, if additional extensions of time are necessary to prevent abandonment of this application, then such extensions of time are hereby petitioned under 37 C.F.R. § 1.136(a), and any additional fees required to continue prosecution or appeal of this application (including issue fee, fees for net addition of claims or forwarding to appeal) are hereby authorized to be charged to our Deposit Account No. 19-0036.

## *Amendments to the Specification*

Please replace the specification with the Substitute Specification filed herewith. Marked-up and clean copies of the Substitute Specification are provided.

## *Amendments to the Claims*

This listing of claims will replace all prior versions, and listings, of claims in the application.

1. (Currently Amended) A method for treating a melanoma tumor in a subject in need thereof comprising:

(i) identifying a patient having a PD-L1-negative melanoma tumor; and

(ii) administering to the patient:

(a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and

(b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4.

2. (Currently Amended) A method for treating a melanoma tumor in a patient in need thereof comprising administering to [[a]]the patient ~~afflicted with a melanoma tumor~~:

(a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and

(b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4,

wherein the patient is identified as having a PD-L1-negative melanoma tumor prior to the administration.

3-7. (Cancelled)

8. (Currently Amended) The method of claim 2 ~~any one of claims 1 to 7~~, wherein the patient is characterized by (i) extended progression-free survival for over 8 months following the administration, (ii) tumor size reduction at least about 10%~~, about 20%, about 30%, about 40%, or about 50%~~ compared to the tumor size prior to the administration, or (iii) both (i) and (ii).

9-12. (Cancelled)

13. (Original) A method for selecting a patient suitable for an anti-PD-1 antibody and anti-CTLA-4 antibody combination therapy comprising:

(i) identifying a patient having a PD-L1-negative melanoma tumor; and

(ii) instructing a healthcare provider to administer to the patient:

(a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and

(b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4.

14. (Currently Amended) The method of claim 2 ~~any one of claims 1, 7, 10, and 13, wherein the identifying comprises~~ further comprising measuring a PD-L1 expression on the melanoma tumor prior to the administration.

15. (Cancelled)

16. (Currently Amended) The method of claim 14 [[or 15]], wherein the measuring comprises providing a test tissue sample obtained from the patient, the test tissue sample comprising tumor cells and/or tumor-infiltrating inflammatory cells.

17. (Original) The method of claim 16, wherein the measuring further comprises assessing the proportion of cells in the test tissue sample that express PD-L1 on the cell surface.

18. (Currently Amended) The method of claim 17, wherein the test tissue sample is a formalin-fixed paraffin-embedded (FFPE) tissue sample, and wherein the presence of PD-L1 is determined using an automated IHC assay.

19. (Cancelled)

20. (Currently Amended) The method of claim 18[[19]], wherein the IHC assay is performed using an anti-PD-L1 monoclonal antibody that specifically binds to the PD-L1 and wherein the anti-PD-L1 monoclonal antibody is selected from the group consisting of 28-8, 28-1, 28-12, 29-8, 5H1, and any combination thereof.

21. (Currently Amended) The method of claim 2 ~~any one of claims 1 to 20~~, wherein the PD-L1-negative melanoma tumor is characterized by having less than about 5%~~, about 4%, about 3%, about 2%, about 1%, or about 0%~~ of tumor cells showing binding to the anti-PD-L1 antibody or an antigen-binding portion thereof.

22. (Currently Amended) The method of claim 2 ~~any one of claims 1 to 21~~, wherein the anti-PD-1 antibody or antigen-binding portion thereof cross-competes with nivolumab for binding to human PD-1.

23-24. (Cancelled)

25. (Currently Amended) The method of claim 2 ~~any one of claims 1 to 24~~, wherein the anti-PD-1 antibody is nivolumab.

26. (Cancelled)

27. (Currently Amended) The method of claim 2 ~~any one of claims 1 to 26~~, wherein the anti-PD-1 antibody or antigen-binding portion thereof is administered at a dose ranging from 0.1 to 10.0 mg/kg body weight once every 2, 3 or 4 weeks.

28. (Original) The method of claim 27, wherein the anti-PD-1 antibody or antigen-binding portion thereof is administered at a dose of 1 or 3 mg/kg body weight once every 3 weeks.

29. (Currently Amended) The method of claim 2 ~~any one of claims 1 to 28~~, wherein the anti-CTLA-4 antibody or antigen-binding portion thereof cross-competes with ipilimumab for binding to human CTLA-4.

30-31. (Cancelled)

32. (Currently Amended) The method of claim 2 ~~any one of claims 1 to 31~~, wherein the anti-CTLA-4 antibody is ipilimumab.

33. (Cancelled)

34. (Currently Amended) The method of claim 2 ~~any one of claims 1 to 33~~, wherein the anti-CTLA-4 antibody or antigen-binding portion thereof is administered at a dose ranging from 0.1 to 10.0 mg/kg body weight once every 2, 3 or 4 weeks.

35. (Cancelled)

36. (Currently Amended) The method of claim 34[[35]], wherein the anti-PD-1 antibody or an antigen-binding portion thereof is administered at a dose of 1 mg/kg body weight once every 3 weeks and the anti-CTLA-4 antibody or an antigen-binding portion thereof is administered at a dose of 3 mg/kg body weight once every 3 weeks.

37-45. (Cancelled)

46. (Currently Amended) The method of claim 2 ~~any one of claims 1 to 45~~, further comprising administering an anti-cancer agent.

47. (Currently Amended) A kit for treating a patient afflicted with a melanoma tumor, the kit comprising:

(a) a dosage ranging from 0.1 to 10 mg/kg body weight of an anti-PD-1 antibody or an antigen-binding portion thereof;

(b) a dosage ranging from 0.1 to 10 mg/kg body weight of an anti-CTLA-4 antibody or an antigen-binding portion thereof; and

(c) instructions for using the anti-PD-1 antibody or the antigen-binding portion thereof and the anti-CTLA-4 antibody or the antigen-binding portion thereof in the method of claim 1 ~~any one of claims 1 to 46~~.

## *Amendments to the Drawings*

Please replace Figs. 3 and 4 with the replacement sheets provided herewith.

## *Remarks*

Upon entry of the foregoing amendment, claims 1, 2, 8, 13, 14, 16-18, 20-22, 25, 27-29, 32, 34, 36, 46, and 47 are pending in the application, with claims 1, 2, and 13 being the independent claims. Claims 3-7, 9-12, 15, 19, 23, 24, 26, 30, 31, 33, 35, and 37-45 are sought to be canceled without prejudice to or disclaimer of the subject matter therein. Applicant reserves the right to pursue the cancelled subject matter in one or more continuing applications.

Claims 1, 2, 8, 14, 16, 18, 20-22, 25, 27, 29, 32, 34, 36, 46, and 47 are sought to be amended. Support for the amendments to the claims can be found throughout the specification as filed. Specifically, support for amended claims 1 and 2 can be found, *inter alia*, at paragraph [0059] and original claim 15. Support for amended claim 18 can be found, *inter alia*, at paragraphs [0014] and [0077] and original claim 19. In addition, claims 1, 2, 8, 14, 16, 20-22, 25, 27, 29, 32, 34, 36, 46, and 47 are amended to update claim dependency, to update antecedent basis, to remove multiple dependency, and/or for clarity.

These changes are believed to introduce no new matter, and their entry is respectfully requested.

***Amendments to the Specification and Replacement Drawings***

In reply to the Notice to File Corrected Application Papers dated May 6, 2016, Applicant submits herewith two sheets of formal replacement drawings. Replacement figures 3A, 3B, 4A, 4B, and 4C have been relabeled in accordance with 37 C.F.R. 1.84(u)(1) so that each figure is labeled with "Fig." and a consecutive Arabic numeral. Identification of the replacement drawing sheets submitted herewith is provided in accordance with 37 C.F.R. §§ 1.84(c) and 1.121(d). Acknowledgement of the receipt, approval, and entry of the replacement drawing sheets into this application is respectfully requested.

In addition, Applicant submits herewith a substitute specification excluding claims. The substitute specification includes amendments to the brief descriptions of figures 1, 2A, 2B, 2C, 3A, 3B, 4A, 4B, and 4C, which are amended to identify the corresponding figures (see paragraphs [0016]-[0019], [0136], [0139], [0140], and [0142]).

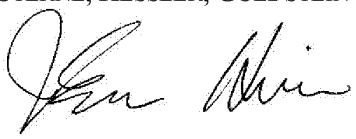
Marked-up and clean copies of the Substitute Specification are provided. Applicant contends that the Substitute Specification includes no new matter, in compliance with 37 C.F.R. §§ 1.121(b)(3) and 1.125.

## *Conclusion*

Prompt and favorable consideration of this Preliminary Amendment is respectfully requested. Applicant believes the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.



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2830286

# TREATMENT OF PD-L1-NEGATIVE MELANOMA USING AN ANTI-PD-1 ANTIBODY AND AN ANTI-CTLA-4 ANTIBODY

## CROSS-REFERENCE TO RELATED APPLICATIONS

**[0001]** The present application claims benefit to U.S. Provisional Application No. 62/153,973 filed April 28, 2015, which is incorporated herein by reference in its entirety.

## REFERENCE TO A SEQUENCE LISTING SUBMITTED ELECTRONICALLY VIA EFS-WEB

**[0002]** The instant application contains a Sequence Listing which has been submitted in ASCII format via EFS-Web, and is hereby incorporated by reference in its entirety. Said ASCII copy, created on April 28, 2016, is named 3338_0330001_SeqListing_ST25.txt and is 3,723 bytes in size.

**[0003]** Throughout this application, various publications are referenced in parentheses by author name and date, or by Patent No. or Patent Publication No. The disclosures of these publications are hereby incorporated in their entireties by reference into this application in order to more fully describe the state of the art as known to those skilled therein as of the date of the invention described and claimed herein. However, the citation of a reference herein should not be construed as an acknowledgement that such reference is prior art to the present invention.

## FIELD OF THE INVENTION

**[0004]** This invention relates to a method of treating PD-L1-negative melanoma comprising administering a combination of an anti-PD-1 antibody and an anti-CTLA-4 antibody.

## BACKGROUND OF THE INVENTION

**[0005]** Human cancers harbor numerous genetic and epigenetic alterations, generating neoantigens potentially recognizable by the immune system (Sjoblom *et al.* (2006)

*Science* 314:268-74). The adaptive immune system, comprised of T and B lymphocytes, has powerful anti-cancer potential, with a broad capacity and exquisite specificity to respond to diverse tumor antigens. Further, the immune system demonstrates considerable plasticity and a memory component. The successful harnessing of all these attributes of the adaptive immune system would make immunotherapy unique among all cancer treatment modalities.

**[0006]** Recently, several immune checkpoint pathway inhibitors have begun to provide new immunotherapeutic approaches for treating cancer, including the development of an antibody (Ab), ipilimumab (YERVOY®), that binds to and inhibits Cytotoxic T-Lymphocyte Antigen-4 (CTLA-4) for the treatment of patients with advanced melanoma and the development of antibodies such as nivolumab and pembrolizumab (formerly lambrolizumab; USAN Council Statement, (2013) Pembrolizumab: Statement on a nonproprietary name adopted by the USAN Council (ZZ-165), November 27, 2013) that bind specifically to the Programmed Death-1 (PD-1) receptor and block the inhibitory PD-1/PD-1 ligand pathway.

**[0007]** The promise of the emerging field of personalized medicine is that advances in pharmacogenomics will increasingly be used to tailor therapeutics to defined sub-populations, and ultimately, individual patients in order to enhance efficacy and minimize adverse effects. Recent successes include, for example, the development of imatinib mesylate (GLEEVEC®), a protein tyrosine kinase inhibitor that inhibits the bcr-abl tyrosine kinase, to treat Philadelphia chromosome-positive chronic myelogenous leukemia (CML); crizotinib (XALKORI®) to treat the 5% of patients with late-stage non-small cell lung cancers who express a mutant anaplastic lymphoma kinase (ALK) gene; and vemurafenib (ZELBORAF®), an inhibitor of mutated B-RAF protein (V600E-BRAF) which is expressed in around half of melanoma tumors. However, unlike the clinical development of small molecule agents that target discrete activating mutations found in select cancer populations, a particular challenge in cancer immunotherapy has been the identification of mechanism-based predictive biomarkers to enable patient selection and guide on-treatment management.

## SUMMARY OF THE INVENTION

**[0008]** The present disclosure provides a method for treating a melanoma comprising (i) identifying a patient having a PD-L1-negative melanoma tumor; and (ii) administering to the patient (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4. The present disclosure also provides a method for treating a melanoma comprising administering to a patient afflicted with a melanoma tumor (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4, wherein the patient is identified as having a PD-L1-negative melanoma tumor prior to the administration.

**[0009]** The present disclosure further provides a method for extending a progression-free survival period for over 8 months in a patient afflicted with a melanoma tumor comprising administering to the patient (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4, wherein the patient is identified as having a PD-L1-negative melanoma tumor prior to the administration and wherein the patient demonstrates progression-free survival for over 8 months. In certain embodiments, the progression-free survival of the patient is extended after the administration for over about 11 months, about 12 months, about 13 months, about 14 months, about 15 months, about 16 months, about 17 months, about 18 months, about 2 years, about 3 years, about 4 years, about 5 years, about 6 years, about 7 years, about 8 years, about 9 years, or about 10 years. In one particular embodiment, the progression-free survival of the patient is extended for over 11 months.

**[0010]** The present disclosure also provides a method for reducing a tumor size at least by 10% in a patient afflicted with a melanoma tumor comprising administering to the patient (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4, wherein the patient is identified as having a PD-L1-negative melanoma tumor prior to the administration and

wherein the administration reduces the tumor size at least about 10%, about 20%, about 30%, about 40%, or about 50% compared to the tumor size prior to the administration.

**[0011]** The present disclosure also provides a method for increasing an objective response rate to be higher than 40% in a patient population, each of whom is afflicted with a melanoma tumor, in a cancer treatment comprising administering to the patient (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4, wherein each patient is identified as having a PD-L1-negative melanoma tumor prior to the administration and wherein the objective response rate is higher than 40%, 45%, 50%, 55%, 60%, 65%, 70%, or 75%.

**[0012]** The present disclosure also provides a method for selecting a patient suitable for an anti-PD-1 antibody and anti-CTLA-4 antibody combination therapy comprising (i) identifying a patient having a PD-L1-negative melanoma tumor; and (ii) instructing a healthcare provider to administer to the patient (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4.

**[0013]** In certain embodiments, the methods disclosed herein further comprise identifying the patient as having a melanoma tumor that does not express PD-L1 prior to the administration. In some embodiments, the patient is characterized by (i) extended progression-free survival for over 8 months, (ii) tumor size reduction at least about 10%, about 20%, about 30%, about 40%, or about 50% compared to the tumor size prior to the administration, or (iii) both.

**[0014]** In some embodiments, the methods disclosed herein further comprise measuring a PD-L1 expression on the melanoma tumor. In certain embodiments, the measuring further comprises assessing the proportion of cells in the test tissue sample that express PD-L1 on the cell surface. In one particular embodiment, the presence of PD-L1 is determined using an automated IHC assay.

**[0015]** The present disclosure further provides a kit for treating a patient afflicted with a melanoma tumor, the kit comprising (a) a dosage ranging from 0.1 to 10 mg/kg body weight of an anti-PD-1 antibody or an antigen-binding portion thereof; (b) a dosage

ranging from 0.1 to 10 mg/kg body weight of an anti-CTLA-4 antibody or an antigen-binding portion thereof; and (c) instructions for using the anti-PD-1 antibody or the antigen-binding portion thereof and the anti-CTLA-4 antibody or the antigen-binding portion thereof in any of the methods disclosed herein.

## BRIEF DESCRIPTION OF THE FIGURES

**[0016]** Fig. 1 shows a patient flow diagram of the randomized, double-blind, multicenter, phase 3 trial.

**[0017]** Figs. 2A-C show progression-free survival data in the intention-to-treat population (Fig. 2A), in patients with positive PD-L1 status (Fig. 2B), and Negative PD-L1 Status (Fig. 2C). Each graph shows the progression free survival for patients treated with nivolumab alone (solid line), ipilimumab alone (dashed line), or the combination of nivolumab and ipilimumab (dotted line) in months (Figs. 2A-C). The number at risk in months for each of nivolumab, nivolumab plus ipilimumab, and ipilimumab is shown below each x-axis (Figs. 2A-C). PD-L1 expression status is based on verified PD-L1 assay data (Figs. 2B-C).

**[0018]** Figs. 3A and 3B show subgroup analyses of progression-free survival among patients treated with nivolumab alone compared to ipilimumab alone (Fig. 3A) and patients treated with nivolumab plus ipilimumab compared to ipilimumab alone (Fig. 3B).

**[0019]** Figs. 4A-4C show the tumor burden change in target lesions in patients treated with nivolumab alone (Fig. 4A), nivolumab plus ipilimumab (Fig. 4B), and ipilimumab alone (Fig. 4C). In each graph, the y-axis shows the best reduction from baseline in target lesions (%) and the x-axis represents each patient (Figs. 4A-C).

## DETAILED DESCRIPTION OF THE INVENTION

**[0020]** The present invention relates to identifying an optimal strategy to treat a patient having a PD-L1-negative melanoma. The present invention shows that in a patient having a PD-L1-negative melanoma tumor, a combination therapy of an anti-PD-1 antibody and an anti-CTLA-4 antibody provides a better response (e.g., progression-free survival) than

a monotherapy of either an anti-PD-1 antibody or an anti-CTLA-4 antibody. Furthermore, the combination therapy when given to a population of patients can increase the objective response rate compared to the monotherapy.

*Definitions*

**[0021]** In order that the present disclosure may be more readily understood, certain terms are first defined. As used in this application, except as otherwise expressly provided herein, each of the following terms shall have the meaning set forth below. Additional definitions are set forth throughout the application.

**[0022]** The term "and/or" where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. Thus, the term "and/or" as used in a phrase such as "A and/or B" herein is intended to include "A and B," "A or B," "A" (alone), and "B" (alone). Likewise, the term "and/or" as used in a phrase such as "A, B, and/or C" is intended to encompass each of the following aspects: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).

**[0023]** It is understood that wherever aspects are described herein with the language "comprising," otherwise analogous aspects described in terms of "consisting of" and/or "consisting essentially of" are also provided.

**[0024]** Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure is related. For example, the Concise Dictionary of Biomedicine and Molecular Biology, Juo, Pei-Show, 2nd ed., 2002, CRC Press; The Dictionary of Cell and Molecular Biology, 3rd ed., 1999, Academic Press; and the Oxford Dictionary Of Biochemistry And Molecular Biology, Revised, 2000, Oxford University Press, provide one of skill with a general dictionary of many of the terms used in this disclosure.

**[0025]** Units, prefixes, and symbols are denoted in their Système International de Unites (SI) accepted form. Numeric ranges are inclusive of the numbers defining the range. The headings provided herein are not limitations of the various aspects of the disclosure, which can be had by reference to the specification as a whole. Accordingly, the terms

defined immediately below are more fully defined by reference to the specification in its entirety.

**[0026]** "Administering" refers to the physical introduction of a composition comprising a therapeutic agent to a subject, using any of the various methods and delivery systems known to those skilled in the art. Routes of administration for the formulations disclosed herein include intravenous, intramuscular, subcutaneous, intraperitoneal, spinal or other parenteral routes of administration, for example by injection or infusion. The phrase "parenteral administration" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intralymphatic, intralesional, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion, as well as *in vivo* electroporation. In some embodiments, the formulation is administered via a non-parenteral route, in some embodiments, orally. Other non-parenteral routes include a topical, epidermal or mucosal route of administration, for example, intranasally, vaginally, rectally, sublingually or topically. Administering can also be performed, for example, once, a plurality of times, and/or over one or more extended periods.

**[0027]** An "adverse event" (AE) as used herein is any unfavorable and generally unintended or undesirable sign (including an abnormal laboratory finding), symptom, or disease associated with the use of a medical treatment. For example, an adverse event may be associated with activation of the immune system or expansion of immune system cells (*e.g.*, T cells) in response to a treatment. A medical treatment may have one or more associated AEs and each AE may have the same or different level of severity. Reference to methods capable of "altering adverse events" means a treatment regime that decreases the incidence and/or severity of one or more AEs associated with the use of a different treatment regime.

**[0028]** An "antibody" (Ab) shall include, without limitation, a glycoprotein immunoglobulin which binds specifically to an antigen and comprises at least two heavy (H) chains and two light (L) chains interconnected by disulfide bonds, or an antigen-binding portion thereof. Each H chain comprises a heavy chain variable region

(abbreviated herein as $V_H$) and a heavy chain constant region. The heavy chain constant region comprises three constant domains, $C_{H1}$, $C_{H2}$ and $C_{H3}$. Each light chain comprises a light chain variable region (abbreviated herein as $V_L$) and a light chain constant region. The light chain constant region is comprises one constant domain, $C_L$. The $V_H$ and $V_L$ regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FR). Each $V_H$ and $V_L$ comprises three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The constant regions of the antibodies may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (*e.g.*, effector cells) and the first component (C1q) of the classical complement system.

**[0029]** An immunoglobulin may derive from any of the commonly known isotypes, including but not limited to IgA, secretory IgA, IgG and IgM. IgG subclasses are also well known to those in the art and include but are not limited to human IgG1, IgG2, IgG3 and IgG4. "Isotype" refers to the antibody class or subclass (*e.g.*, IgM or IgG1) that is encoded by the heavy chain constant region genes. The term "antibody" includes, by way of example, both naturally occurring and non-naturally occurring antibodies; monoclonal and polyclonal antibodies; chimeric and humanized antibodies; human or nonhuman antibodies; wholly synthetic antibodies; and single chain antibodies. A nonhuman antibody may be humanized by recombinant methods to reduce its immunogenicity in man. Where not expressly stated, and unless the context indicates otherwise, the term "antibody" also includes an antigen-binding fragment or an antigen-binding portion of any of the aforementioned immunoglobulins, and includes a monovalent and a divalent fragment or portion, and a single chain antibody.

**[0030]** The term "monoclonal antibody" ("mAb") refers to a non-naturally occurring preparation of antibody molecules of single molecular composition, *i.e.*, antibody molecules whose primary sequences are essentially identical, and which exhibits a single binding specificity and affinity for a particular epitope. A mAb is an example of an

isolated antibody. MAbs may be produced by hybridoma, recombinant, transgenic or other techniques known to those skilled in the art.

**[0031]** A "human" antibody (HuMAb) refers to an antibody having variable regions in which both the framework and CDR regions are derived from human germline immunoglobulin sequences. Furthermore, if the antibody contains a constant region, the constant region is also derived from human germline immunoglobulin sequences. The human antibodies of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (*e.g.*, mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*). However, the term "human antibody," as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences. The terms "human" antibodies and "fully human" antibodies and are used synonymously.

**[0032]** A "humanized antibody" refers to an antibody in which some, most or all of the amino acids outside the CDR domains of a non-human antibody are replaced with corresponding amino acids derived from human immunoglobulins. In one embodiment of a humanized form of an antibody, some, most or all of the amino acids outside the CDR domains have been replaced with amino acids from human immunoglobulins, whereas some, most or all amino acids within one or more CDR regions are unchanged. Small additions, deletions, insertions, substitutions or modifications of amino acids are permissible as long as they do not abrogate the ability of the antibody to bind to a particular antigen. A "humanized" antibody retains an antigenic specificity similar to that of the original antibody.

**[0033]** A "chimeric antibody" refers to an antibody in which the variable regions are derived from one species and the constant regions are derived from another species, such as an antibody in which the variable regions are derived from a mouse antibody and the constant regions are derived from a human antibody.

**[0034]** An "anti-antigen" antibody refers to an antibody that binds specifically to the antigen. For example, an anti-PD-1 antibody binds specifically to PD-1 and an anti-CTLA-4 antibody binds specifically to CTLA-4.

**[0035]** An "antigen-binding portion" of an antibody (also called an "antigen-binding fragment") refers to one or more fragments of an antibody that retain the ability to bind specifically to the antigen bound by the whole antibody.

**[0036]** A "cancer" refers a broad group of various diseases characterized by the uncontrolled growth of abnormal cells in the body. Unregulated cell division and growth results in the formation of malignant tumors that invade neighboring tissues and may also metastasize to distant parts of the body through the lymphatic system or bloodstream. A "cancer" or "cancer tissue" can include a tumor.

**[0037]** "Cytotoxic T-Lymphocyte Antigen-4" (CTLA-4) refers to an immunoinhibitory receptor belonging to the CD28 family. CTLA-4 is expressed exclusively on T cells *in vivo*, and binds to two ligands, CD80 and CD86 (also called B7-1 and B7-2, respectively). The term "CTLA-4" as used herein includes human CTLA-4 (hCTLA-4), variants, isoforms, and species homologs of hCTLA-4, and analogs having at least one common epitope with hCTLA-4. The complete hCTLA-4 sequence can be found under GenBank Accession No. AAB59385.

**[0038]** The term “progression-free survival,” which can be abbreviated as PFS, as used herein refers to the length of time during and after the treatment of a solid tumor (*i.e.*, melanoma) that a patient lives with the disease but it does not get worse.

**[0039]** "Dosing interval," as used herein, means the amount of time that elapses between multiple doses of a formulation disclosed herein being administered to a subject. Dosing interval can thus be indicated as ranges.

**[0040]** The term "dosing frequency" as used herein refers to the frequency of administering doses of a formulation disclosed herein in a given time. Dosing frequency can be indicated as the number of doses per a given time, *e.g.*, once a week or once in two weeks.

**[0041]** The use of the term "fixed dose" with regard to a composition of the invention means that two or more different antibodies in a single composition are present in the composition in particular (fixed) ratios with each other. In some embodiments, the fixed dose is based on the weight (*e.g.*, mg) of the antibodies. In certain embodiments, the fixed dose is based on the concentration (*e.g.*, mg/ml) of the antibodies. In some embodiments, the ratio is at least about 1:1, about 1:2, about 1:3, about 1:4, about 1:5, about 1:6, about

1:7, about 1:8, about 1:9, about 1:10, about 1:15, about 1:20, about 1:30, about 1:40, about 1:50, about 1:60, about 1:70, about 1:80, about 1:90, about 1:100, about 1:120, about 1:140, about 1:160, about 1:180, about 1:200, about 200:1, about 180:1, about 160:1, about 140:1, about 120:1, about 100:1, about 90:1, about 80:1, about 70:1, about 60:1, about 50:1, about 40:1, about 30:1, about 20:1, about 15:1, about 10:1, about 9:1, about 8:1, about 7:1, about 6:1, about 5:1, about 4:1, about 3:1, or about 2:1 mg first antibody to mg second antibody. For example, the 3:1 ratio of a first antibody and a second antibody can mean that a vial can contain about 240 mg of the first antibody and 80 mg of the second antibody or about 3 mg/ml of the first antibody and 1 mg/ml of the second antibody.

**[0042]** The use of the term "flat dose" with regard to the composition of the invention means a dose that is administered to a patient without regard for the weight or body surface area (BSA) of the patient. The flat dose is therefore not provided as a mg/kg dose, but rather as an absolute amount of the agent (*e.g.,* the anti-CTLA-4 antibody and/or anti-PD-1 antibody). For example, a 60 kg person and a 100 kg person would receive the same dose of the composition (*e.g.*, 240 mg of an anti-PD-1 antibody and 80 mg of an anti-CTLA-4 antibody in a single fixed dosing formulation vial containing both 240 mg of an anti-PD-1 antibody and 80 mg of an anti-CTLA-4 antibody (or two fixed dosing formulation vials containing 120 mg of an anti-PD-1 antibody and 40 mg of an anti-CTLA-4 antibody, etc.)).

**[0043]** The term "weight based dose" as referred to herein means that a dose that is administered to a patient is calculated based on the weight of the patient. For example, when a patient with 60 kg body weight requires 3 mg/kg of an anti-PD-1 antibody in combination with 1 mg/kg of an anti-CTLA-4 antibody, one can draw the appropriate amounts of the anti-PD-1 antibody (*i.e.*, 180 mg) and the anti-CTLA-4 antibody (*i.e.*, 60 mg) at once from a 3:1 ratio fixed dosing formulation of an anti-PD1 antibody and an anti-CTLA-4 antibody.

**[0044]** The term "anti-PD-1 antibody monotherapy" as used herein includes a therapy of an anti-PD-1 antibody without an anti-CTLA-4 antibody therapy. The anti-PD-1 antibody monotherapy comprises, consists essentially of, or consists of administering one or more doses of an anti-PD-1 antibody to a patient in need thereof, but does not include

administering an anti-CTLA-4 antibody. In one embodiment, the anti-PD-1 antibody monotherapy comprises administering one or more doses of an anti-PD-1 antibody to a patient in need thereof, but does not include administering an anti-CTLA-4 antibody. In another embodiment, the anti-PD-1 antibody monotherapy comprises administering one or more doses of an anti-PD-1 antibody to a patient in need thereof, but does not include administering an antibody specifically targeting a protein other than PD-1. In other embodiments, the anti-PD-1 antibody monotherapy comprises administering one or more doses of an anti-PD-1 antibody to a patient in need thereof, but does not include administering another anti-cancer agent.

**[0045]** An "immune response" refers to the action of a cell of the immune system (for example, T lymphocytes, B lymphocytes, natural killer (NK) cells, macrophages, eosinophils, mast cells, dendritic cells and neutrophils) and soluble macromolecules produced by any of these cells or the liver (including antibodies, cytokines, and complement) that results in selective targeting, binding to, damage to, destruction of, and/or elimination from a vertebrate's body of invading pathogens, cells or tissues infected with pathogens, cancerous or other abnormal cells, or, in cases of autoimmunity or pathological inflammation, normal human cells or tissues.

**[0046]** "PD-L1 negative" or "PD-L1 expression negative," relating to cell surface PD-L1 expression, refers to the lack of a detectable amount of cell surface PD-L1. For cell surface expression assayed by IHC, e.g., with the mAb 28-8, a PD-L1 negative tumor or PD-L1 expression negative tumor means that less than 0.01% of cells express a detectable level of PD-L1. In some embodiments, a PD-L1 negative tumor or PD-L1 expression negative tumor means that zero (0) cells express a detectable level of PD-L1. In some embodiments, a PD-L1 negative or a PD-L1 expression negative tumor is any tumor other than a PD-L1 positive or a PD-L1 expression positive tumor.

**[0047]** The term "PD-L1 positive" or "PD-L1 expression positive," relating to cell surface PD-L1 expression, refers to the proportion of cells in a test tissue sample comprising tumor cells and tumor- infiltrating inflammatory cells above which the sample is scored as expressing cell surface PD-L1. For cell surface expression assayed by immunohistochemistry (IHC), e.g., with the mAb 28- 8, the PD-L1 positive tumor or PD-L1 expression positive tumor means that at least about 0.01%, at least about 0.5%, at

least about 1%, at least about 2%, at least about 3%, at least about 4%, at least about 5%, at least about 6%, at least about 7%, at least about 8%, at least about 9%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, or at least about 30% of the total number of cells express PD-L1. PD-L1 positive tumor or PD-L1 expression positive tumor can also be expressed herein as tumor expressing PD-L1. In other embodiments, the PD-L1 positive tumor or PD-L1 expression positive tumor means that at least about 0.1% to at least about 20% of the total number of cells express PD-L1. In certain embodiments, the PD-L1 positive tumor or PD-L1 expression positive tumor means that at least about 0.1% to at least about 10% of the total number of cells express PD-L1. In some embodiments, the PD-L1 positive or PD-L1 expression positive tumor means that at least about 1% of the total number of cells express PD-L1 on the cell surface. In other embodiments, the PD-L1 positive or PD-L1 expression positive tumor means that at least about 5% of the total number of cells express PD-L1 on the cell surface. In one particular embodiment, PD-L1 positive or PD-L1 expression positive tumor means that at least about 1%, or in the range of 1- 5% of the total number of cells express PD-L1 on the cell surface.

**[0048]** "Programmed Death-1 (PD-1)" refers to an immunoinhibitory receptor belonging to the CD28 family. PD-1 is expressed predominantly on previously activated T cells *in vivo*, and binds to two ligands, PD-L1 and PD-L2. The term "PD-1" as used herein includes human PD-1 (hPD-1), variants, isoforms, and species homologs of hPD-1, and analogs having at least one common epitope with hPD-1. The complete hPD-1 sequence can be found under GenBank Accession No. U64863.

**[0049]** "Programmed Death Ligand-1 (PD-L1)" is one of two cell surface glycoprotein ligands for PD-1 (the other being PD-L2) that down-regulate T cell activation and cytokine secretion upon binding to PD-1. The term "PD-L1" as used herein includes human PD-L1 (hPD-L1), variants, isoforms, and species homologs of hPD-L1, and analogs having at least one common epitope with hPD-L1. The complete hPD-L1 sequence can be found under GenBank Accession No. Q9NZQ7.

**[0050]** A "patient" as used herein includes any patient who is afflicted with a cancer (*e.g.*, melanoma). The terms "subject" and "patient" are used interchangeably herein.

**[0051]** A "therapeutically effective amount" or "therapeutically effective dosage" of a drug or therapeutic agent is any amount of the drug that, when used alone or in combination with another therapeutic agent, protects a subject against the onset of a disease or promotes disease regression evidenced by a decrease in severity of disease symptoms, an increase in frequency and duration of disease symptom-free periods, or a prevention of impairment or disability due to the disease affliction. The ability of a therapeutic agent to promote disease regression can be evaluated using a variety of methods known to the skilled practitioner, such as in human subjects during clinical trials, in animal model systems predictive of efficacy in humans, or by assaying the activity of the agent in *in vitro* assays.

**[0052]** "Treatment" or "therapy" of a subject refers to any type of intervention or process performed on, or the administration of an active agent to, the subject with the objective of reversing, alleviating, ameliorating, inhibiting, slowing down or preventing the onset, progression, development, severity or recurrence of a symptom, complication or condition, or biochemical indicia associated with a disease.

**[0053]** A "tumor-infiltrating inflammatory cell" is any type of cell that typically participates in an inflammatory response in a subject and which infiltrates tumor tissue. Such cells include tumor-infiltrating lymphocytes (TILs), macrophages, monocytes, eosinophils, histiocytes and dendritic cells.

**[0054]** The use of the alternative (*e.g.*, "or") should be understood to mean either one, both, or any combination thereof of the alternatives. As used herein, the indefinite articles "a" or "an" should be understood to refer to "one or more" of any recited or enumerated component.

**[0055]** The terms "about" or "comprising essentially of" refer to a value or composition that is within an acceptable error range for the particular value or composition as determined by one of ordinary skill in the art, which will depend in part on how the value or composition is measured or determined, *i.e.*, the limitations of the measurement system. For example, "about" or "comprising essentially of" can mean within 1 or more than 1 standard deviation per the practice in the art. Alternatively, "about" or "comprising essentially of" can mean a range of up to 10% or 20% (*i.e.,* ±10% or ±20%). For example, about 3mg can include any number between 2.7 mg and 3.3 mg (for 10%) or

between 2.4 mg and 3.6 mg (for 20%). Furthermore, particularly with respect to biological systems or processes, the terms can mean up to an order of magnitude or up to 5-fold of a value. When particular values or compositions are provided in the application and claims, unless otherwise stated, the meaning of "about" or "comprising essentially of" should be assumed to be within an acceptable error range for that particular value or composition.

**[0056]** The terms "about once a week," "once about every week," "once about every two weeks," or any other similar dosing interval terms as used herein means approximate number, and "about once a week" or "once about every week" can include every seven days ± two days, *i.e.,* every five days to every nine days. The dosing frequency of "once a week" thus can be every five days, every six days, every seven days, every eight days, or every nine days. "Once about every two weeks" can include every fourteen days ± three days, *i.e.*, every eleven days to every seventeen days. Similar approximations apply, for example, to once about every three weeks, once about every four weeks, once about every five weeks, once about every six weeks and once about every twelve weeks. In some embodiments, a dosing interval of once about every six weeks or once about every twelve weeks means that the first dose can be administered any day in the first week, and then the next dose can be administered any day in the sixth or twelfth week, respectively. In other embodiments, a dosing interval of once about every six weeks or once about every twelve weeks means that the first dose is administered on a particular day of the first week (e.g., Monday) and then the next dose is administered on the same day of the sixth or twelfth weeks (i.e., Monday), respectively.

**[0057]** As described herein, any concentration range, percentage range, ratio range or integer range is to be understood to include the value of any integer within the recited range and, when appropriate, fractions thereof (such as one-tenth and one-hundredth of an integer), unless otherwise indicated.

**[0058]** Various aspects of the invention are described in further detail in the following subsections.

*Methods of the Invention*

**[0059]** The present invention is directed to a method for treating a PD-L1-negative melanoma in a subject in need thereof. The present invention shows that for PD-L1 negative tumors, a combination therapy of an anti-PD-1 antibody and an anti-CTLA-4 antibody is more suitable than a monotherapy of either an anti-PD-1 antibody or an anti-CTLA-4 antibody.

**[0060]** Not bound to any theory, the present invention identifies that in patients having a PD-L1-negative tumor progression-free survival and overall response rate are higher following treatment with a combination therapy with an anti-PD-1 antibody and an anti-CTLA-4 antibody than following treatment with either an anti-PD-1 antibody or an anti-CTLA-4 antibody alone. Therefore, in order to increase the response of a patient having a PD-L1-negative tumor, the present invention provides identifying a patient suitable for the combination therapy of an anti-PD-1 antibody and an anti-CTLA-4 antibody.

**[0061]** In one embodiment, the invention includes a method of treating a PD-L1 negative melanoma comprising administering to a patient: (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4, wherein the patient is identified as having a PD-L1-negative melanoma tumor prior to the administration. In another embodiment, the invention includes a method for treating a melanoma in a patient in need thereof comprising: (i) identifying a patient having a PD-L1-negative melanoma tumor; and (ii) administering to the patient: (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4.

**[0062]** In certain embodiments, the invention includes a method for extending a progression-free survival period for over 9 months in a patient afflicted with a PD-L1 negative melanoma tumor comprising administering to the patient: (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4, wherein the patient is identified as having a PD-L1-negative melanoma tumor prior to the administration and wherein the patient

demonstrates progression-free survival for over 9 months. In other embodiments, the invention provides a method for extending a progression-free survival period for over 9 months in a patient afflicted with a melanoma tumor comprising: (i) identifying a patient having a PD-L1-negative melanoma tumor; and (ii) administering to the patient (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4, wherein the patient demonstrates progression-free survival for over 9 months. According to the invention, the progression-free survival of the patient can be extended, after the administration, over about 10 months, over about 11 months, over about 12 months, over about 13 months, about 14 months, about 15 months, about 16 months, about 17 months, about 18 months, about 2 years, about 3 years, about 4 years, about 5 years, about 6 years, about 7 years, about 8 years, about 9 years, or about 10 years. In a particular embodiment, the progression-free survival of the patient is extended for over 10 months.

**[0063]** In still other embodiments, the invention is directed to a method for reducing a tumor size at least by 10% in a patient afflicted with a PD-L1 negative melanoma tumor comprising administering to the patient: (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4, wherein the patient is identified as having a PD-L1-negative melanoma tumor prior to the administration and wherein the administration reduces the tumor size at least about 10%, about 20%, about 30%, about 40%, or about 50% compared to the tumor size prior to the administration. In yet other embodiments, the method comprises (i) identifying a patient having a PD-L1-negative melanoma tumor; and (ii) administering to the patient (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4, wherein the administration reduces the tumor size at least about 10%, about 20%, about 30%, about 40%, or about 50% compared to the tumor size prior to the administration. The tumor size, after the administration, can be reduced at least about 60%, 70%, 80%, 90% or 100%. The tumor can be completely eliminated from the patient's body after the administration.

**[0064]** The invention can also include a method of preventing a relapse and/or induce a remission to a patient having a PD-L1 negative melanoma tumor comprising administering to the patient: (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4, wherein the patient is identified as having a PD-L1-negative melanoma tumor prior to the administration. In some embodiments, the method of the invention comprises (i) identifying a patient having a PD-L1-negative melanoma tumor; and (ii) administering to the patient (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4.

**[0065]** In certain embodiments, the invention includes a method for increasing an objective response rate to be higher than 40% in a patient population, wherein each patient of the patient population is afflicted with a melanoma tumor, in a cancer treatment comprising administering to the patient: (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4, wherein each patient is identified as having a PD-L1-negative melanoma tumor prior to the administration and wherein the objective response rate is higher than 40%, 45%, 50%, 55%, 60%, 65%, 70%, or 75%. The methods can further comprise identifying each patient as having a PD-L1-negative melanoma tumor prior to the administration. In other embodiments, each patient in the methods can further be characterized by (i) extended progression-free survival for over 11 months, (ii) tumor size reduction at least about 10%, about 20%, about 30%, about 40%, or about 50% compared to the tumor size prior to the administration, or (iii) both. In some embodiments, the patient population can be at least 100 patients having a PD-L1-negative melanoma tumor. In some embodiments, the patient population can be at least 200, 300, 400, 500, 600, 700, 800, 900, or 1000 patients having a PD-L1-negative melanoma tumor.

**[0066]** In further embodiments, the invention provides a method for selecting a suitable cancer therapy course in a patient having a PD-L1 negative melanoma tumor comprising: (i) identifying a patient having a PD-L1-negative melanoma tumor; and (ii) instructing a

healthcare provider to administer to the patient: (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4. The method further comprises administering the antibodies to the patient.

**[0067]** The methods of the invention as a result of the combination therapy can treat the melanoma tumor, reduce the tumor size, prevent growth of the tumor, eliminate the tumor from the patient, prevent a relapse of a tumor, induce a remission in a patient, or any combination thereof. In certain embodiments, the combination therapy induces a complete response. In other embodiments, the combination therapy induces a partial response.

*Melanoma*

**[0068]** Melanoma (MEL) is a malignant tumor of melanocytes, the melanin-producing cells found predominantly in skin. Though less common than other skin cancers, it is the most dangerous of skin cancers if not diagnosed early and causes the majority (75%) of skin cancer deaths. The incidence of MEL is increasing worldwide in Caucasian populations, especially where peoples with low amounts of skin pigmentation receive excessive ultraviolet light exposure from the sun. In Europe, the incidence rate is < 10-20 per 100,000 population; in the USA 20-30 per 100,000; and in Australia, where the highest incidence is observed, 50-60 per 100,000 (Garbe *et al.*, *Eur. J. Cancer. 48(15)*:2375-90 (2012)). MEL accounts for about 5% of all new cases of cancer in the United States (U.S.), and the incidence continues to rise by almost 3% per year. This translates to an estimated 76,690 new cases in the U.S. in 2013 with 9,480 associated deaths (Siegel *et al.*, *CA Cancer J. Clin. 63(1)*:11-30 (2013)).

**[0069]** For in situ (stage 0) or early-stage MEL (Stages I-II), surgical excision is the primary treatment. In general, the prognosis is excellent for patients with localized disease and tumors 1.0 mm or less in thickness, with 5-year survival rates of more than 90% (NCCN GUIDELINES®, 2013- Melanoma). Where surgical excision is not feasible for in situ melanoma due to comorbidity or cosmetically sensitive tumor location, topical imiquimod (INN) and radiotherapy are emerging as treatments, especially for *lentigo maligna*. Chemotherapeutic agents for treating MEL include dacarbazine, temozolomide and imatinib for melanoma with a c-KIT mutation, high-dose interleukin-2, and

paclitaxel with or without carboplatin. However, these treatments have modest success, with response rates below 20% in first-line (1L) and second-line (2L) settings.

**[0070]** For patients with localized melanomas more than 1.0 mm in thickness, survival rates range from 50-90%. The likelihood of regional nodal involvement increases with increasing tumor thickness. With Stage III MEL (clinically positive nodes and/or in-transit disease), 5-year survival rates range from 20-70%. By far the most lethal is Stage IV MEL where long-term survival in patients with distant metastatic melanoma is less than 10% (NCCN GUIDELINES®, 2013 - melanoma).

**[0071]** The types of melanoma that can be treated with the present methods include, but are not limited to, lentigo maligna, lentigo maligna melanoma, superficial spreading melanoma, acral lentiginous melanoma, nucosal melanoma, nodular melanoma, polypoid melanoma, desmoplastic melanoma, amelanotic melanoma, soft-tissue melanoma, melanoma with small nevus-like cells, melanoma with features of a Spitz nevus, or uveal melanoma. The stages of melanoma that can be treated with the present methods include, but are not limited to, (i) Stage I / II (invasive melanoma): T1a characterized by less than 1.0 mm primary tumor thickness, without ulceration, and mitosis $< 1/mm^2$; T1b characterized by less than 1.0 mm primary tumor thickness, with ulceration or mitoses $\geq 1/mm^2$; T2a characterized by 1.01–2.0 mm primary tumor thickness, without ulceration; (ii) Stage II (high risk melanoma): T2b characterized by 1.01–2.0 mm primary tumor thickness, with ulceration; T3a characterized by 2.01–4.0 mm primary tumor thickness, without ulceration; T3b characterized by 2.01–4.0 mm primary tumor thickness, with ulceration; T4a characterized by greater than 4.0 mm primary tumor thickness, without ulceration; or T4b characterized by greater than 4.0 mm primary tumor thickness, with ulceration; (iii) Stage III (regional metastasis): N1 characterized by single positive lymph node; N2 characterized by two to three positive lymph nodes or regional skin/in-transit metastasis; or N3 characterized by four positive lymph nodes or one lymph node and regional skin/in-transit metastases; and (iv) Stage IV (distant metastasis): M1a characterized by distant skin metastasis, normal LDH; M1b characterized by Lung metastasis, normal LDH; or M1c characterized by other distant metastasis or any distant metastasis with elevated LDH. PD-L1-negative tumors that are treatable by the present

methods can lack PD-L1 expression on the surface of tumor cells and/or tumor infiltrating inflammatory cells.

*Measurement of PD-L1 expression*

**[0072]** In certain embodiments, identifying a patient suitable for a combination therapy for the present methods includes measuring or assessing a PD-L1 expression on the surface of the melanoma tumor cells or tumor infiltrating inflammatory cells. The phrases "tumors expressing PD-L1," "PD-L1 expressing tumor," "PD-L1 positive tumor," and "PD-L1 expression positive tumor" are used interchangeably herein. The meaning of the phrases is provided elsewhere herein. The methods of measuring or assessing the PD-L1 expression can be achieved by any methods applicable.

**[0073]** In order to assess the PD-L1 expression, in one embodiment, a test tissue sample is obtained from the patient who is in need of the therapy. In another embodiment, the assessment of PD-L1 expression can be achieved without obtaining a test tissue sample. In some embodiments, selecting a suitable patient includes (i) optionally providing a test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor cells and/or tumor-infiltrating inflammatory cells; and (ii) assessing the proportion of cells in the test tissue sample that express PD-L1 on the surface of the cells based on an assessment that the proportion of cells in the test tissue sample that express PD-L1 on the cell surface is lower than a predetermined threshold level. A test tissue sample can be considered to be PD-L1-negative if less than about 5% of the cells in the test tissue express PD-L1 on the cell surface. In other embodiments, a test tissue sample is considered to be PD-L1-negative if less than about 4%, less than about 3%, less than about 2%, less than about 1%, less than about 0.5%, less than about 0.1%, less than about 0.01% or 0% of the cells in the test tissue express PD-L1 on the cell surface. In one particular example, the test tissue sample can be considered to be PD-L1-negative if less than about 5% of the cells in the test tissue express PD-L1 on the cell surface.

**[0074]** In any of the methods comprising the measurement of PD-L1 expression in a test tissue sample, however, it should be understood that the step comprising the provision of a test tissue sample obtained from a patient is an optional step. That is, in certain embodiments the method includes this step, and in other embodiments, this step is not included in the method. It should also be understood that in certain embodiments the

"measuring" or "assessing" step to identify, or determine the number or proportion of, cells in the test tissue sample that express PD-L1 on the cell surface is performed by a transformative method of assaying for PD-L1 expression, for example by performing a reverse transcriptase-polymerase chain reaction (RT-PCR) assay or an IHC assay. In certain other embodiments, no transformative step is involved and PD-L1 expression is assessed by, for example, reviewing a report of test results from a laboratory. In certain embodiments, the steps of the methods up to, and including, assessing PD-L1 expression provides an intermediate result that may be provided to a physician or other healthcare provider for use in selecting a suitable candidate for the combination therapy of an anti-PD-1 antibody and an anti-CTLA-4 antibody. In certain embodiments, the steps that provide the intermediate result is performed by a medical practitioner or someone acting under the direction of a medical practitioner. In other embodiments, these steps are performed by an independent laboratory or by an independent person such as a laboratory technician.

**[0075]** In certain embodiments of any of the present methods, the proportion of cells that express PD-L1 is assessed by performing an assay to determine the presence of PD-L1 RNA. In further embodiments, the presence of PD-L1 RNA is determined by RT-PCR, *in situ* hybridization or RNase protection. In other embodiments, the proportion of cells that express PD-L1 is assessed by performing an assay to determine the presence of PD-L1 polypeptide. In further embodiments, the presence of PD-L1 polypeptide is determined by immunohistochemistry (IHC), enzyme-linked immunosorbent assay (ELISA), *in vivo* imaging, or flow cytometry. In some embodiments, PD-L1 expression is assayed by IHC. In other embodiments of all of these methods, cell surface expression of PD-L1 is assayed using, *e.g.*, IHC or *in vivo* imaging.

**[0076]** Imaging techniques have provided important tools in cancer research and treatment. Recent developments in molecular imaging systems, including positron emission tomography (PET), single-photon emission computed tomography (SPECT), fluorescence reflectance imaging (FRI), fluorescence-mediated tomography (FMT), bioluminescence imaging (BLI), laser-scanning confocal microscopy (LSCM) and multiphoton microscopy (MPM), will likely herald even greater use of these techniques in cancer research. Some of these molecular imaging systems allow clinicians to not only

see where a tumor is located in the body, but also to visualize the expression and activity of specific molecules, cells, and biological processes that influence tumor behavior and/or responsiveness to therapeutic drugs (Condeelis and Weissleder, *Cold Spring Harb. Perspect. Biol. 2(12)*:a003848 (2010)). Antibody specificity, coupled with the sensitivity and resolution of PET, makes immunoPET imaging particularly attractive for monitoring and assaying expression of antigens in tissue samples (McCabe and Wu, *Cancer Biother. Radiopharm. 25(3)*:253-61 (2010); Olafsen *et al.*, *Protein Eng. Des. Sel. 23(4)*:243-9 (2010)). In certain embodiments of any of the present methods, PD-L1 expression is assayed by immunoPET imaging. In certain embodiments of any of the present methods, the proportion of cells in a test tissue sample that express PD-L1 is assessed by performing an assay to determine the presence of PD-L1 polypeptide on the surface of cells in the test tissue sample. In certain embodiments, the test tissue sample is a FFPE tissue sample. In other embodiments, the presence of PD-L1 polypeptide is determined by IHC assay. In further embodiments, the IHC assay is performed using an automated process. In some embodiments, the IHC assay is performed using an anti-PD-L1 mAb to bind to the PD-L1 polypeptide, if present.

*Assaying Cell-Surface PD-L1 Expression by Automated IHC*

**[0077]** In one embodiment of the present methods, an automated IHC method is used to assay the expression of PD-L1 on the surface of cells in FFPE tissue specimens. This disclosure provides methods for detecting the presence of human PD-L1 antigen in a test tissue sample, or quantifying the level of human PD-L1 antigen or the proportion of cells in the sample that express the antigen, which methods comprise contacting the test sample, and a negative control sample, with a mAb that specifically binds to human PD-L1, under conditions that allow for formation of a complex between the antibody or portion thereof and human PD-L1. In certain embodiments, the test and control tissue samples are FFPE samples. The formation of a complex can then be detected, wherein a difference in complex formation between the test sample and the negative control sample is indicative of the presence of human PD-L1 antigen in the sample. Various methods are used to quantify PD-L1 expression.

**[0078]** In a particular embodiment, the automated IHC method comprises: (a) deparaffinizing and rehydrating mounted tissue sections in an autostainer; (b) retrieving

antigen using a decloaking chamber and pH 6 buffer, heated to 110°C for 10 min; (c) setting up reagents on an autostainer; and (d) running the autostainer to include steps of neutralizing endogenous peroxidase in the tissue specimen; blocking non-specific protein-binding sites on the slides; incubating the slides with primary Ab; incubating with a postprimary blocking agent; incubating with NovoLink Polymer; adding a chromogen substrate and developing; and counterstaining with hematoxylin.

**[0079]** For assessing PD-L1 expression in tumor tissue samples, a pathologist examines the number of membrane PD-L1$^+$ tumor cells in each field under a microscope and mentally estimates the percentage of cells that are positive, then averages them to come to the final percentage. The different staining intensities are defined as 0/negative, 1+/weak, 2+/moderate, and 3+/strong. Typically, percentage values are first assigned to the 0 and 3+ buckets, and then the intermediate 1+ and 2+ intensities are considered. For highly heterogeneous tissues, the specimen is divided into zones, and each zone is scored separately and then combined into a single set of percentage values. The percentages of negative and positive cells for the different staining intensities are determined from each area and a median value is given to each zone. A final percentage value is given to the tissue for each staining intensity category: negative, 1+, 2+, and 3+. The sum of all staining intensities needs to be 100%.

**[0080]** Staining is also assessed in tumor-infiltrating inflammatory cells such as macrophages and lymphocytes. In most cases macrophages serve as an internal positive control since staining is observed in a large proportion of macrophages. While not required to stain with 3+ intensity, an absence of staining of macrophages should be taken into account to rule out any technical failure. Macrophages and lymphocytes are assessed for plasma membrane staining and only recorded for all samples as being positive or negative for each cell category. Staining is also characterized according to an outside/inside tumor immune cell designation. "Inside" means the immune cell is within the tumor tissue and/or on the boundaries of the tumor region without being physically intercalated among the tumor cells. "Outside" means that there is no physical association with the tumor, the immune cells being found in the periphery associated with connective or any associated adjacent tissue.

**[0081]** In certain embodiments of these scoring methods, the samples are scored by two pathologists operating independently, and the scores are subsequently consolidated. In certain other embodiments, the identification of positive and negative cells is scored using appropriate software.

**[0082]** A histoscore is used as a more quantitative measure of the IHC data. The histoscore is calculated as follows:

Histoscore = [(% tumor x 1 (low intensity)) + (% tumor x 2 (medium intensity))
+ (% tumor x 3 (high intensity)]

**[0083]** To determine the histoscore, the pathologist estimates the percentage of stained cells in each intensity category within a specimen. Because expression of most biomarkers is heterogeneous the histoscore is a truer representation of the overall expression. The final histoscore range is 0 (no expression) to 300 (maximum expression). A test sample can be designated as PD-L1-negative even though some level of PD-L1 expression is detected. For example, a designation of PD-L1-negative can be assigned to a specimen having a final histoscore of about 15 or less, of about 10 or less, about 9 or less, about 8 or less, about 7 or less, about 6 or less, about 5 or less, about 4 or less, about 3 or less, about 2 or less, about 1 or less, or of 0.

**[0084]** An alternative means of quantifying PD-L1 expression in a test tissue sample IHC is to determine the adjusted inflammation score (AIS) score defined as the density of inflammation multiplied by the percent PD-L1 expression by tumor-infiltrating inflammatory cells (Taube *et al.*, *Sci. Transl. Med. 4(127)*:127ra37 (2012)).

*Anti-PD-1 antibodies*

**[0085]** PD-1 is a key immune checkpoint receptor expressed by activated T and B cells and mediates immunosuppression. PD-1 is a member of the CD28 family of receptors, which includes CD28, CTLA-4, ICOS, PD-1, and BTLA. Two cell surface glycoprotein ligands for PD-1 have been identified, Programmed Death Ligand-1 (PD-L1) and Programmed Death Ligand-2 (PD-L2), that are expressed on antigen-presenting cells as well as many human cancers and have been shown to down regulate T cell activation and cytokine secretion upon binding to PD-1. Inhibition of the PD-1/PD-L1 interaction mediates potent antitumor activity in preclinical models.

**[0086]** HuMAbs that bind specifically to PD-1 with high affinity have been disclosed in U.S. Patent Nos. 8,008,449 and 8,779,105. Other anti-PD-1 mAbs have been described in, for example, U.S. Patent Nos. 6,808,710, 7,488,802, 8,168,757 and 8,354,509, and PCT Publication No. WO 2012/145493. Each of the anti-PD-1 HuMAbs disclosed in U.S. Patent No. 8,008,449 has been demonstrated to exhibit one or more of the following characteristics: (a) binds to human PD-1 with a $K_D$ of 1 x $10^{-7}$ M or less, as determined by surface plasmon resonance using a Biacore biosensor system; (b) does not substantially bind to human CD28, CTLA-4 or ICOS; (c) increases T-cell proliferation in a Mixed Lymphocyte Reaction (MLR) assay; (d) increases interferon-γ production in an MLR assay; (e) increases IL-2 secretion in an MLR assay; (f) binds to human PD-1 and cynomolgus monkey PD-1; (g) inhibits the binding of PD-L1 and/or PD-L2 to PD-1; (h) stimulates antigen-specific memory responses; (i) stimulates antibody responses; and (j) inhibits tumor cell growth *in vivo*. Anti-PD-1 antibodies useful for the present invention include mAbs that bind specifically to human PD-1 and exhibit at least one, at least two, at least three, at least four, or at least five, of the preceding characteristics.

**[0087]** In one embodiment, the anti-PD-1 antibody is nivolumab. Nivolumab (also known as "OPDIVO®"; formerly designated 5C4, BMS-936558, MDX-1106, or ONO-4538) is a fully human IgG4 (S228P) PD-1 immune checkpoint inhibitor antibody that selectively prevents interaction with PD-1 ligands (PD-L1 and PD-L2), thereby blocking the down-regulation of antitumor T-cell functions (U.S. Patent No. 8,008,449; Wang *et al.*, *Cancer Immunol Res*. 2(9):846-56 (2014)). In another embodiment, the anti-PD-1 antibody or fragment thereof cross-competes with nivolumab. In other embodiments, the anti-PD-1 antibody or fragment thereof binds to the same epitope as nivolumab. In certain embodiments, the anti-PD-1 antibody has the same CDRs as nivolumab.

**[0088]** In another embodiment, the anti-PD-1 antibody or fragment thereof cross-competes with pembrolizumab. In some embodiments, the anti-PD-1 antibody or fragment thereof binds to the same epitope as pembrolizumab. In certain embodiments, the anti-PD-1 antibody has the same CDRs as pembrolizumab. In another embodiment, the anti-PD-1 antibody is pembrolizumab. Pembrolizumab (also known as "KEYTRUDA®", lambrolizumab, and MK-3475) is a humanized monoclonal IgG4 antibody directed against human cell surface receptor PD-1 (programmed death-1 or

programmed cell death-1). Pembrolizumab is described, for example, in U.S. Patent Nos. 8,354,509 and 8,900,587; *see also* http://www.cancer.gov/drugdictionary?cdrid=695789 (last accessed: December 14, 2014). Pembrolizumab has been approved by the FDA for the treatment of relapsed or refractory melanoma.

**[0089]** In other embodiments, the anti-PD-1 antibody or fragment thereof cross-competes with MEDI0608. In still other embodiments, the anti-PD-1 antibody or fragment thereof binds to the same epitope as MEDI0608. In certain embodiments, the anti-PD-1 antibody has the same CDRs as MEDI0608. In other embodiments, the anti-PD-1 antibody is MEDI0608 (formerly AMP-514), which is a monoclonal antibody. MEDI0608 is described, for example, in US Pat. No. 8,609,089B2 or in http://www.cancer.gov/drugdictionary?cdrid=756047 (last accessed December 14, 2014).

**[0090]** In certain embodiments, the first antibody is an anti-PD-1 antagonist. One example of the anti-PD-1 antagonist is AMP-224, which is a B7-DC Fc fusion protein. AMP-224 is discussed in U.S. Publ. No. 2013/0017199 or in http://www.cancer.gov/publications/dictionaries/cancer-drug?cdrid=700595 (last accessed July 8, 2015).

**[0091]** In other embodiments, the anti-PD-1 antibody or fragment thereof cross-competes with BGB-A317. In some embodiments, the anti-PD-1 antibody or fragment thereof binds the same epitope as BGB-A317. In certain embodiments, the anti-PD-1 antibody has the same CDRs as BGB-A317. In certain embodiments, the anti-PD-1 antibody is BGB-A317, which is a humanized monoclonal antibody. BGB-A317 is described in U.S. Publ. No. 2015/0079109.

**[0092]** In some embodiments, the antibody is Pidilizumab (CT-011), which is an antibody previously reported to bind to PD-1 but which is believed to bind to a different target. Pidilizumab is described in US Pat. No. 8,686,119 B2 or WO 2013/014668 A1.

**[0093]** Anti-PD-1 antibodies useful for the disclosed compositions also include isolated antibodies that bind specifically to human PD-1 and cross-compete for binding to human PD-1 with nivolumab (*see, e.g.*, U.S. Patent Nos. 8,008,449 and 8,779,105; WO 2013/173223). The ability of antibodies to cross-compete for binding to an antigen indicates that these antibodies bind to the same epitope region of the antigen and sterically hinder the binding of other cross-competing antibodies to that particular epitope

region. These cross-competing antibodies are expected to have functional properties very similar to those of nivolumab by virtue of their binding to the same epitope region of PD-1. Cross-competing antibodies can be readily identified based on their ability to cross-compete with nivolumab in standard PD-1 binding assays such as Biacore analysis, ELISA assays or flow cytometry (*see, e.g.*, WO 2013/173223).

**[0094]** In certain embodiments, the antibodies that cross-compete for binding to human PD-1 with, or bind to the same epitope region of human PD-1 as, nivolumab are mAbs. For administration to human subjects, these cross-competing antibodies can be chimeric antibodies, or humanized or human antibodies. Such chimeric, humanized or human mAbs can be prepared and isolated by methods well known in the art.

**[0095]** Anti-PD-1 antibodies useful for the compositions of the disclosed invention also include antigen-binding portions of the above antibodies. It has been amply demonstrated that the antigen-binding function of an antibody can be performed by fragments of a full-length antibody. Examples of binding fragments encompassed within the term "antigen-binding portion" of an antibody include (i) a Fab fragment, a monovalent fragment consisting of the $V_L$, $V_H$, $C_L$ and $C_{H1}$ domains; (ii) a F(ab')$_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the $V_H$ and $C_{H1}$ domains; and (iv) a Fv fragment consisting of the $V_L$ and $V_H$ domains of a single arm of an antibody.

**[0096]** Anti-PD-1 antibodies suitable for use in the disclosed compositions are antibodies that bind to PD-1 with high specificity and affinity, block the binding of PD-L1 and or PD-L2, and inhibit the immunosuppressive effect of the PD-1 signaling pathway. In any of the compositions or methods disclosed herein, an anti-PD-1 "antibody" includes an antigen-binding portion or fragment that binds to the PD-1 receptor and exhibits the functional properties similar to those of whole antibodies in inhibiting ligand binding and upregulating the immune system. In certain embodiments, the anti-PD-1 antibody or antigen-binding portion thereof cross-competes with nivolumab for binding to human PD-1. In other embodiments, the anti-PD-1 antibody or antigen-binding portion thereof is a chimeric, humanized or human monoclonal antibody or a portion thereof. In certain embodiments, the antibody is a humanized antibody. In other embodiments, the antibody is a human antibody. Antibodies of an IgG1, IgG2, IgG3 or IgG4 isotype can be used.

**[0097]** In certain embodiments, the anti-PD-1 antibody or antigen-binding portion thereof comprises a heavy chain constant region which is of a human IgG1 or IgG4 isotype. In certain other embodiments, the sequence of the IgG4 heavy chain constant region of the anti-PD-1 antibody or antigen-binding portion thereof contains an S228P mutation which replaces a serine residue in the hinge region with the proline residue normally found at the corresponding position in IgG1 isotype antibodies. This mutation, which is present in nivolumab, prevents Fab arm exchange with endogenous IgG4 antibodies, while retaining the low affinity for activating Fc receptors associated with wild-type IgG4 antibodies (Wang *et al.*, *Cancer Immunol Res.* 2(9):846-56 (2014)). In yet other embodiments, the antibody comprises a light chain constant region which is a human kappa or lambda constant region. In other embodiments, the anti-PD-1 antibody or antigen-binding portion thereof is a mAb or an antigen-binding portion thereof. In certain embodiments of any of the therapeutic methods described herein comprising administration of an anti-PD-1 antibody, the anti-PD-1 antibody is nivolumab. In other embodiments, the anti-PD-1 antibody is pembrolizumab. In other embodiments, the anti-PD-1 antibody is chosen from the human antibodies 17D8, 2D3, 4H1, 4A11, 7D3 and 5F4 described in U.S. Patent No. 8,008,449. In still other embodiments, the anti-PD-1 antibody is MEDI0608 (formerly AMP-514), AMP-224, or BGB-A317.

**[0098]** Because anti-PD-1 and anti-PD-L1 target the same signaling pathway and have been shown in clinical trials to exhibit similar levels of efficacy in a variety of cancers, including RCC (*see* Brahmer *et al.* (2012) *N Engl J Med* 366:2455-65; Topalian *et al.* (2012a) *N Engl J Med* 366:2443-54; WO 2013/173223), an anti-PD-L1 antibody may be substituted for the anti-PD-1 Ab in any of the therapeutic methods disclosed herein. In certain embodiments, the anti-PD-L1 antibody is BMS-936559 (formerly 12A4 or MDX-1105) (*see, e.g.*, U.S. Patent No. 7,943,743; WO 2013/173223). In other embodiments, the anti-PD-L1 antibody is MPDL3280A (also known as RG7446) (*see, e.g.*, Herbst et al. (2013) *J Clin Oncol* 31(suppl):3000. Abstract; U.S. Patent No. 8,217,149) or MEDI4736 (Khleif (2013) In: Proceedings from the European Cancer Congress 2013; September 27-October 1, 2013; Amsterdam, The Netherlands. Abstract 802). In certain embodiments, the antibodies that cross-compete for binding to human PD-L1 with, or bind to the same epitope region of human PD-L1 as the above-references PD-L1 antibodies are mAbs. For

administration to human subjects, these cross-competing antibodies can be chimeric antibodies, or can be humanized or human antibodies. Such chimeric, humanized or human mAbs can be prepared and isolated by methods well known in the art.

*Anti-PD-L1 Antibodies*

**[0099]** In certain embodiments, the present application encompasses use of an anti-PD-L1 antibody in lieu of anti-PD-1 antibody. In one embodiment, the anti-PD-L1 antibody inhibits the binding of PD-L1 receptor, i.e., PD-1, with its ligand PD-L1.

**[0100]** Anti-PD-L1 antibodies useful for the invention include antibodies engineered starting from antibodies having one or more of the $V_H$ and/or $V_L$ sequences disclosed herein, which engineered antibodies can have altered properties from the starting antibodies. An anti-PD-L1 antibody can be engineered by a variety of modifications as described above for the engineering of modified anti-PD-1 antibodies of the invention.

**[0101]** Anti-PD-L1 antibodies of the invention also include isolated antibodies selected for their ability to bind to PD-L1 in formalin-fixed, paraffin-embedded (FFPE) tissue specimens. The use of FFPE samples is essential for the long-term follow-up analysis of the correlation between PD-L1 expression in tumors and disease prognosis or progression. The use of different antibodies to stain PD-L1 in frozen versus FFPE tissues, and the ability of certain antibodies to distinguish membranous and/or cytoplasmic forms of PD-Ll, may account for some of the disparate data reported in the literature correlating PD-Ll expression with disease prognosis (Hamanishi *et al.*, *Proc. Natl. Acad. Sci. USA 104(9)*:3360-3365 (2007); Gadiot *et al.*, *Cancer 117(10)*:2192-2201 (2011)). This disclosure provides several rabbit mAbs that bind with high affinity specifically to membranous human PD-Ll in FFPE tissue samples comprising tumor cells and tumor-infiltrating inflammatory cells.

**[0102]** In some embodiments, an anti-PD-L1 antibody useful for the present methods includes mAb 28-8 set forth in SEQ ID NOs. 1 and 2, respectively. The sequences of the heavy and light chain CDR domains of mAb 28-8, as delineated using the Kabat system, are set forth in SEQ ID NOs. 3-8. In other embodiments, an anti-PD-L1 antibody useful for the invention comprises mAbs 28-1, 28-12, 29-8 and 20-12 or an antigen-binding portion thereof, for example, including Fab, $F(ab')_2$ Fd, Fv, and scFv, di-scFv or bi-scFv, and scFv-Fc fragments, diabodies, triabodies, tetrabodies, and isolated CDRs.

*Anti-CTLA-4 antibodies*

**[0103]** Anti-CTLA-4 antibodies of the instant invention bind to human CTLA-4 so as to disrupt the interaction of CTLA-4 with a human B7 receptor. Because the interaction of CTLA-4 with B7 transduces a signal leading to inactivation of T-cells bearing the CTLA-4 receptor, disruption of the interaction effectively induces, enhances or prolongs the activation of such T cells, thereby inducing, enhancing or prolonging an immune response.

**[0104]** HuMAbs that bind specifically to CTLA-4 with high affinity have been disclosed in U.S. Patent Nos. 6,984,720 and 7,605,238. Other anti-PD-1 mAbs have been described in, for example, U.S. Patent Nos. 5,977,318, 6,051,227, 6,682,736, and 7,034,121. The anti-PD-1 HuMAbs disclosed in U.S. Patent No. Nos. 6,984,720 and 7,605,238 have been demonstrated to exhibit one or more of the following characteristics: (a) binds specifically to human CTLA-4 with a binding affinity reflected by an equilibrium association constant ($K_a$) of at least about $10^7$ $M^{-1}$, or about $10^9$ $M^{-1}$, or about $10^{10}$ $M^{-1}$ to $10^{11}$ $M^{-1}$ or higher, as determined by Biacore analysis; (b) a kinetic association constant ($k_a$) of at least about $10^3$, about $10^4$, or about $10^5$ $m^{-1}$ $s^{-1}$; (c) a kinetic disassociation constant ($k_d$) of at least about $10^3$, about $10^4$, or about $10^5$ $m^{-1}$ $s^{-1}$; and (d) inhibits the binding of CTLA-4 to B7-1 (CD80) and B7-2 (CD86). Anti-CTLA-4 antibodies useful for the present invention include mAbs that bind specifically to human CTLA-4 and exhibit at least one, at least two, or at least three of the preceding characteristics.

**[0105]** An exemplary clinical anti-CTLA-4 antibody is the human mAb 10D1 (now known as ipilimumab and marketed as YERVOY®) as disclosed in U.S. Patent No. 6,984,720. Ipilimumab is an anti-CTLA-4 antibody for use in the methods disclosed herein. Ipilimumab is a fully human, IgG1 monoclonal antibody that blocks the binding of CTLA-4 to its B7 ligands, thereby stimulating T cell activation and improving overall survival (OS) in patients with advanced melanoma.

**[0106]** Another anti-CTLA-4 antibody useful for the present methods is tremelimumab (also known as CP-675,206). Tremelimumab is human IgG2 monoclonal anti-CTLA-4 antibody. Tremelimumab is described in WO/2012/122444, U.S. Publ. No. 2012/263677, or WO Publ. No. 2007/113648 A2.

**[0107]** Anti-CTLA-4 antibodies useful for the disclosed composition also include isolated antibodies that bind specifically to human CTLA-4 and cross-compete for binding to human CTLA-4 with ipilimumab or tremelimumab or bind to the same epitope region of human CTLA-4 as ipilimumab or tremelimumab. In certain embodiments, the antibodies that cross-compete for binding to human CTLA-4 with, or bind to the same epitope region of human CTLA-4 as does ipilimumab or tremelimumab, are antibodies comprising a heavy chain of the human IgG1 isotype. For administration to human subjects, these cross-competing antibodies are chimeric antibodies, or humanized or human antibodies. Useful anti-CTLA-4 antibodies also include antigen-binding portions of the above antibodies such as Fab, $F(ab')_2$ , Fd or Fv fragments.

*Dosages*

**[0108]** The combination of an anti-PD-1 antibody and an anti-CTLA-4 antibody can be administered to a suitable patient in therapeutically effective amounts. For example, each antibody can be administered at a dosage ranging from at least about 0.1 to at least about 20.0 mg/kg body weight. In certain embodiments, each of the anti-PD-1 and anti-CTLA-4 antibodies is individually administered at a dosage of at least about 0.1, at least about 0.3, at least about 0.5, at least about 1, at least about 3, at least about 5, at least about 10 or at least about 20 mg/kg, *e.g.*, at least about 1 to at least about 10 mg/kg, *e.g.*, at least about 1 to at least about 3 mg/kg, *e.g.*, at least about 3 mg/kg, *e.g.*, at least about 1 mg/kg. Each of the anti-PD-1 antibody and anti-CTLA-4 antibody can be administered at a dosing frequency of at least about once every week, at least about once every 2 weeks, at least about once every 3 weeks, or at least about once every 4 weeks, or at least about once a month, for up to 6 to up to 72 doses, or for as long as clinical benefit is observed, or until unmanageable toxicity or disease progression occurs. In some embodiments, the anti-PD-1 antibody is administered at a dosage of about 1 or about 3 mg/kg. In certain embodiments, the sequenced regimen comprises administering the anti-PD-1 antibody to the subject at a dosing frequency of once about every week, once about every 2 weeks, once about every 3 weeks, or once about every 4 weeks, or once a month for 6 to 72 doses, or for as long as clinical benefit is observed, or until unmanageable toxicity or disease progression occurs. In other embodiments, the anti-PD-1 is administered at a

dosage of about 1 mg/kg at a dosing frequency of once about every 3 weeks for up to 48 doses. In some embodiments, the anti-CTLA-4 antibody is administered at a dosage of about 1 or about 3 mg/kg. In certain embodiments, the sequenced regimen comprises administering the anti-CTLA-4 antibody to the subject at a dosing frequency of once about every week, at least about once every 2 weeks, at least about once every 3 weeks, or at least about once every 4 weeks, or about once a month for 6 to 72 doses, or for as long as clinical benefit is observed, or until unmanageable toxicity or disease progression occurs. In other embodiments, the anti-CTLA-4 antibody is administered at a dosage of about 3 mg/kg at a dosing frequency of once about every 3 weeks for up to 48 doses.

**[0109]** In other embodiments, the anti-PD-1 and anti-CTLA-4 antibodies are administered at the following dosages: (a) 0.1 mg/kg anti-PD-1 antibody and 3 mg/kg of anti-CTLA-4 antibody; (b) 0.3 mg/kg anti-PD-1 antibody and 3 mg/kg of anti- CTLA-4 antibody; (c) 1 mg/kg anti-PD-1 antibody and 3 mg/kg of anti-CTLA-4 antibody; (d) 3 mg/kg anti-PD-1 antibody and 3 mg/kg of anti-CTLA-4 antibody; (e) 5 mg/kg anti-PD-1 antibody and 3 mg/kg of anti-CTLA-4 antibody; (f) 10 mg/kg anti-PD-1 antibody and 3 mg/kg of anti-CTLA-4 antibody; (g) 0.1 mg/kg anti-PD-1 antibody and 1 mg/kg of anti-CTLA-4 antibody; (h) 0.3 mg/kg anti-PD-1 antibody and 1 mg/kg of anti-CTLA-4 antibody; (i) 1 mg/kg anti-PD-1 antibody and 1 mg/kg of anti-CTLA-4 antibody; (j) 3 mg/kg anti-PD-1 antibody and 1 mg/kg of anti-CTLA-4 antibody; (k) 5 mg/kg anti-PD-1 antibody and 1 mg/kg of anti-CTLA-4 antibody; or (1) 10 mg/kg anti-PD-1 antibody and 1 mg/kg of anti-CTLA-4 antibody. In a particular embodiment, the methods include administration of 1 mg/kg of an anti-PD-1 antibody and 3 mg/kg of an anti-CTLA-4 antibody.

**[0110]** In some embodiments, the dosage of each of the anti-PD-1 and anti-CTLA-4 antibodies is kept constant during the induction dosing schedule and the maintenance dosing schedule. In certain embodiments, a regimen comprises: (i) an induction dosing schedule comprising combined administration of the anti-PD-1 and anti-CTLA-4 antibodies at a dosing frequency of at least once about every 2 weeks, at least once about every 3 weeks, or at least once about every 4 weeks, or at least about once a month, for at least 2, 4, 6, 8 or 10 doses, followed by administration of the anti-PD-1 antibody alone at a dosing frequency of at least once every 2, 3 or 4 weeks, or at least once a month, for at

least 2, 4, 6, 8 or 12 doses; followed by (ii) a maintenance dosing schedule comprising combined administration of the anti-PD-1 and anti-CTLA-4 antibodies at a dosing frequency of at least about once every 8 weeks, once about every 12 weeks, or once about every 16 weeks, or at least once a quarter, for at least 4, 6, 8, 10, 12 or 16 doses, or for as long as clinical benefit is observed, or until unmanageable toxicity or disease progression occurs. In another embodiment, the dosing schedule comprises administering about 3 mg/kg of an anti-PD-1 antibody every 2 weeks, or about 1 mg/kg of an anti-PD-1 antibody every 3 weeks plus about 3 mg/kg of an anti-CTLA-4 antibody every 3 weeks for 4 doses, followed by about 3 mg/kg of an anti-PD-1 antibody every 2 weeks for cycle 3 and beyond, or 3 mg/kg of an anti-CTLA-4 antibody every 3 weeks for 4 doses. In one particular embodiment, the dosing schedule comprises administering about 1 mg/kg of an anti-PD-1 antibody followed by an anti-CTLA-4 antibody (*e.g.*, about 3 mg/kg anti-CTLA-4 antibody) on the same day every 3 weeks for 4 doses, followed by about 3 mg/kg of an anti-PD-1 antibody every 2 weeks.

**[0111]** In certain embodiments of this method, the maintenance dosing schedule comprises combined administration of up to 4, 6, 8, 10, 12 or 16 doses of the anti-PD-1 and anti-CTLA-4 antibodies. In other embodiments, the concurrent regimen comprises: (i) an induction dosing schedule comprising combined administration of the anti-PD-1 and anti- CTLA-4 antibodies at a dosing frequency of once about every 2 weeks, at least once about every 3 weeks, or at least once about every 4 weeks, or about once a month, for 2, 4, 6 or 8 doses, followed by administration of the anti-PD-1 antibody alone at a dosing frequency of once about every 2 weeks, at least once about every 3 weeks, or at least once about every 4 weeks, or about once a month, for 2, 4, 6, 8 or 12 doses; followed by (ii) a maintenance dosing schedule comprising combined administration of the anti-PD-1 and anti-CTLA-4 antibodies at a dosing frequency of once every once about every 8, once about every 12 or once about every 16 weeks, or once a quarter, for 4, 6, 8, 10, 12 or 16 doses, or for as long as clinical benefit is observed, or until unmanageable toxicity or disease progression occurs.

**[0112]** In a particular embodiment, the anti-PD-1 and anti-CTLA-4 antibodies are administered at dosages of about 1 mg/kg anti-PD-1 antibody every three weeks and about 3 mg/kg of anti-CTLA-4 antibody every three weeks. In another embodiment, the

patient is administered at a dose of about 1 mg/kg of an anti-PD-1 antibody, followed by an anti-CTLA-4 antibody on the same day, every 3 weeks for 4 doses, then 3 mg/kg of an anti-PD-1 antibody every 2 weeks.

**[0113]** In certain embodiments of the present methods, the anti-PD-1 and anti-CTLA-4 antibodies are formulated for parenteral administration, *e.g.*, intravenous administration. In certain other embodiments, when the anti-PD-1 and anti-CTLA-4 antibodies are administered in combination, they are administered within about 30 minutes of each other. Either antibody may be administered first, that is, in certain embodiments, the anti-PD-1 antibody is administered before the anti-CTLA-4 antibody, whereas in other embodiments, the anti-CTLA-4 antibody is administered before the anti-PD-1 antibody. Typically, each antibody is administered intravenously over a period of about 60 minutes. In further embodiments, the anti-PD-1 and anti-CTLA-4 antibodies are administered concurrently, either admixed as a single composition in a pharmaceutically acceptable formulation for concurrent administration, or concurrently as separate compositions with each antibody in a pharmaceutically acceptable formulation.

**[0114]** In some embodiments, the anti-PD-1 antibody and the anti-CTLA-4 antibody are formulated in a single composition. The ratio of the amount of the anti-PD-1 antibody and the amount of the anti-CTLA-4 antibody in the single composition can be 10:1 to 1:10. In other embodiments, the ratio of the amount of the anti-PD-1 antibody and the amount of the anti-CTLA-4 antibody in the single composition is 5:1, 4:1, 3:1, 2:1, 1:1, 1:2, 1:3, 1:4, or 1:5. In a particular embodiment, the ratio of the amount of the anti-PD-1 antibody and the amount of the anti-CTLA-4 antibody in the single composition is 1:3.

**[0115]** In some embodiments, the composition is administered at a flat dose regardless of the weight of the patient. For example, each of the anti-PD-1 antibody with the anti-CTLA-4 antibody may be administered at a flat dose of 20, 50, 75, 80, 160, 200, 240, 300, 360, 400, 480, 500, 750 or 1500 mg, without regard to the patient's weight. In some embodiments the composition is administered at a weight-based dose at any dose disclosed herein. In some embodiments, the amount of the anti-PD-1 antibody and the amount of the anti-CTLA-4 antibody administered to the patient at a single dose are identical.

**[0116]** In certain embodiments of the present methods, the therapeutically effective dosage of the anti-PD-1 antibody or antigen-binding portion thereof is a flat dosing (not a bodyweight based dosing) and comprises about 60 mg, about 80 mg, about 100 mg, about 120 mg, about 140 mg, about 160 mg, about 180 mg, about 200 mg, about 220 mg, about 240 mg, about 260 mg, about 280 mg, or about 300 mg. In other embodiments, the therapeutically effective dosage of the anti-PD-1 antibody or antigen-binding portion thereof comprises about 320 mg, 360mg, 400 mg, 420mg, 480 mg, 500mg, 540 mg, 550mg, 600mg, 620 mg, 650mg, 680mg, 700 mg, 720mg, 780 mg, 800mg, 840mg, or 900mg.. In some embodiments, the dose of the anti-PD-1 antibody in the composition is between about 60 mg and about 300 mg, between about 60 mg and about 100 mg, between about 100 mg and about 200 mg, or between about 200 mg and about 300 mg. In some embodiments, the amount of the anti-PD-1 antibody in the composition is at least about 80 mg, about 160 mg, or about 240mg. In some embodiments, the dose of the anti-PD-1 antibody in the composition is at least about 240 mg or at least about 80 mg.

**[0117]** In other embodiments, the dosages are given every week, every two weeks, every three weeks, every four weeks, every five weeks, every six weeks, every seven weeks, every eight weeks, every nine weeks, or every 10 weeks.

**[0118]** In some embodiments, the anti-PD-1 antibody or antigen-binding portion thereof is administered at a dose of about 240 mg. In embodiments, the anti-PD-1 antibody or antigen-binding portion thereof is administered at a dose of about 360 mg. In embodiments, the anti-PD-1 antibody or antigen-binding portion thereof is administered at a dose of about 480 mg. In one embodiment, 360mg of the anti-PD-1 antibody or antigen binding fragment is administered once every 3 weeks. In another embodiment, 480mg of the anti-PD-1 antibody or antigen binding fragment is administered once every 4 weeks.

**[0119]** In other embodiments, the therapeutically effective dosage of the anti-CTLA-4 antibody or antigen-binding portion thereof is a flat dosing (not a bodyweight based dosing) and comprises about 60 mg, about 80 mg, about 100 mg, about 120 mg, about 140 mg, about 160 mg, about 180 mg, about 200 mg, about 220 mg, about 240 mg, about 260 mg, about 280 mg, or about 300 mg. In other embodiments, the therapeutically effective dosage of the anti-CTLA-4 antibody or antigen-binding portion thereof

comprises about 320 mg, 360mg, 400 mg, 420mg, 480 mg, 500mg, 540 mg, 550mg, 600mg, 620 mg, 650mg, 680mg, 700 mg, 720mg, 780 mg, 800mg, 840mg, or 900mg. In some embodiments, the dose of the anti-CTLA-4 antibody in the composition is between about 60 mg and about 300 mg, between about 60 mg and about 100 mg, between about 100 mg and about 200 mg, or between about 200 mg and about 300 mg. In some embodiments, the amount of the anti-CTLA-4 antibody in the composition is at least about 80 mg, about 160 mg, or about 240 mg. In some embodiments, the dose of the anti-CTLA-4 antibody in the composition is at least about 240 mg or at least about 80 mg.

**[0120]** In some embodiments, a flat dose of an anti-PD-1 antibody is 80 mg, and a flat dose of an anti-CTLA-4 antibody is 240 mg.

**[0121]** In some embodiments, the anti-PD-1 antibody is administered at a subtherapeutic dose, *i.e.*, a dose of the therapeutic agent that is significantly lower than the usual or FDA-approved dose when administered as monotherapy for the treatment of the cancer. The quantity of the second antibody in the composition is calculated based on the desired ratio.

**[0122]** In some embodiments, the composition is administered by intravenous infusion about once per week, about once every 2 weeks, about once every 3 weeks, or about once a month. In certain embodiments, the composition is administered once every 3 weeks. In some embodiments, the infusion occurs over at least about 10 minutes, about 20 minutes, about 30 minutes, about 45 minutes, about 60 minutes, about 90 minutes, about 2 hours, about 3 hours, about 4 hours or about 5 hours.

**[0123]** Actual dosage levels of the active ingredients in the pharmaceutical compositions of the present invention can be flat or varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being unduly toxic to the patient. The selected dosage level will depend upon a variety of pharmacokinetic factors including the activity of the particular compositions of the present invention employed, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compositions employed, the age, sex, weight, condition, general health and prior medical history of the

patient being treated, and like factors well known in the medical arts. A composition of the present invention can be administered via one or more routes of administration using one or more of a variety of methods well known in the art. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results.

*Kits*

**[0124]** Also within the scope of the present invention are kits, including pharmaceutical kits, comprising a combination of an anti-PD-1 antibody and an anti-CTLA-4 antibody, for therapeutic uses, and diagnostic kits comprising an anti-PD-Ll antibody for assaying membranous PD-L1 expression as a biomarker for screening patients for the combination therapy or for predicting the efficacy of the combination therapy. Kits typically include a label indicating the intended use of the contents of the kit and instructions for use. The term "label" includes any writing, or recorded material supplied on or with the kit, or which otherwise accompanies the kit. In certain embodiments of a pharmaceutical kit, the anti-PD-1 antibody is co-packaged with an anti-CTLA-4 antibody in unit dosage form. In certain embodiments of a diagnostic kit, the anti-PD-Ll antibody is co-packaged with an anti-PD-1 antibody and an anti-CTLA-4 antibody for performing an assay to detect and/or quantify PD-L1 expression.

**[0125]** In certain embodiments, the pharmaceutical kit comprises the anti-human PD-1 HuMAb, nivolumab. In other embodiments, the pharmaceutical kit comprises the anti-human PD-L1 HuMAb, BMS-936559. In yet other embodiments, the pharmaceutical kit comprises the anti-human CTLA-4 HuMAb, ipilimumab. In certain embodiments, the diagnostic kit comprises the rabbit anti-human PD-L1 mAb, 28-8, comprising the $V_H$ and $V_L$ regions whose amino acid sequences are set forth in SEQ ID NOs. 1 and 2, respectively. In other embodiments, the diagnostic kit comprises the murine anti-human PD-L1 mAb, 5H1 (Dong *et al.*, *Nature Med. 8(8)*:793-800 (2002)).

**[0126]** In some embodiments, the invention provides a kit for treating a patient afflicted with a melanoma tumor, the kit comprising:

(a) a dosage ranging from at least about 0.1 to at least about 10 mg/kg body weight of an anti-PD-1 antibody or an antigen-binding portion thereof;

(b) a dosage ranging from at least about 0.1 to at least about 10 mg/kg body weight of an anti-CTLA-4 antibody or an antigen-binding portion thereof; and

(c) instructions for using the anti-PD-1 antibody or the antigen-binding portion thereof and the anti-CTLA-4 antibody or the antigen-binding portion thereof in the methods disclosed herein.

**[0127]** The present invention is further illustrated by the following examples which should not be construed as further limiting. The contents of all references cited throughout this application are expressly incorporated herein by reference.

## EXAMPLES

### EXAMPLE 1

**[0128]** A randomized, double-blind, multicenter, phase 3 trial was conducted to evaluate the safety and efficacy of nivolumab alone or nivolumab combined with ipilimumab in comparison with ipilimumab alone in previously untreated metastatic melanoma.

*Patients*

**[0129]** Eligible patients had histologically confirmed stage III (unresectable) or stage IV melanoma, and no prior systemic treatment for unresectable or metastatic melanoma. Other eligibility criteria included an age of at least 18 years, an Eastern Cooperative Oncology Group (ECOG) performance status score of 0 (indicating no symptoms) or 1 (indicating mild symptoms), measurable disease by computed tomography or magnetic resonance imaging per RECIST v1.1, availability of tissue collected from metastatic or unresectable tumors for the assessment of PD-L1 status, and known BRAF V600 mutation status (or consent to BRAF V600 mutation testing per local standards). Key exclusion criteria were presence of active brain metastases, ocular melanoma, or autoimmune disease, and any prior treatment with an anti-PD-1, anti-PD-L1, anti-PD-L2, or anti-CTLA-4 antibody. Patients who required systemic corticosteroid treatment or other immunosuppressive medications within 14 days of study drug administration were excluded.

*Study Design and Treatment*

**[0130]** In the double-blind, phase 3 study, enrolled patients were randomly assigned in a 1:1:1 ratio to receive 3 mg of nivolumab per kilogram of body weight every 2 weeks (plus ipilimumab-matched placebo), or 1 mg of nivolumab per kilogram every 3 weeks plus 3 mg of ipilimumab per kilogram every 3 weeks for 4 doses (plus nivolumab-matched placebo), followed by 3 mg of nivolumab per kilogram every 2 weeks for cycle 3 and beyond, or 3 mg of ipilimumab per kilogram every 3 weeks for 4 doses (plus nivolumab-matched placebo).

**[0131]** Both nivolumab and ipilimumab were administered by intravenous infusion. Randomization was stratified according to tumor PD-L1 status (positive vs. negative or indeterminate), BRAF mutation status (V600 mutation positive vs. wild-type), and American Joint Committee on Cancer metastasis stage (M0, M1a, or M1b vs. M1c). Treatment continued until RECIST v1.1-defined disease progression, unacceptable toxicity, or withdrawal of consent. Patients could be treated beyond progression provided they had a clinical benefit without clinical deterioration, and did not have substantial adverse effects, as assessed by the investigator.

**[0132]** Progression-free survival and overall survival were co-primary end points. Secondary end points included objective response rate, tumor PD-L1 expression as a predictive biomarker for progression-free and overall survival, and health-related quality of life. Exploratory end points include duration of objective response and safety/tolerability of study drug therapy.

*Assessments*

**[0133]** Patients were assessed for tumor response, according to RECIST v1.1 at 12 weeks after randomization and continuing every 6 weeks for 49 weeks, and then every 12 weeks until progression or treatment discontinuation, whichever occurred later. Progression-free survival was defined as the time between the date of randomization and the first date of documented progression or death, whichever occurred first. Patients treated beyond progression were considered to have progressive disease at the time of the initial progression event, as assessed by the investigator, regardless of subsequent tumor responses. Expression of PD-L1 on the surface of the tumor cells was assessed in a central laboratory by immunohistochemistry in formalin-fixed, paraffin-embedded tumor

specimens using a rabbit monoclonal anti-human PD-L1 antibody (clone 28-8) and an analytically validated automated assay developed by Dako (Carpinteria, CA). PD-L1 positivity was defined as at least 5% of tumor cells showing cell surface PD-L1 staining of any intensity in a section containing at least 100 tumor cells that could be evaluated. Indeterminate status was attributed to samples for which tumor cell–surface expression could not be discerned because of melanin content or strong cytoplasmic staining.

**[0134]** Any patient who received at least one dose of study drug in each of the 3 treatment groups was included in the assessment of safety. The severity of adverse events was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0.18. Safety assessments were made continuously during the treatment phase, and up to 100 days after the last dose of study drug.

*Statistical Analysis*

**[0135]** A study sample size of approximately 915 patients, randomized to the 3 treatment arms in a 1:1:1 ratio, was planned. For the comparison of progression-free survival, the number of events projected to be observed at a follow-up of at least 9 months provided approximately 83% power to detect an average hazard ratio of 0.71 with a type I error of 0.005 (two-sided). Progression-free survival was compared between nivolumab plus ipilimumab and ipilimumab alone, and between nivolumab alone and ipilimumab alone with the use of a two-sided log-rank test stratified according to PD-L1 status, BRAF mutation status, and metastasis stage (as described above). The study was not designed for a formal statistical comparison between the nivolumab alone and nivolumab plus ipilimumab groups. Hazard ratios and corresponding two-sided 99.5% confidence intervals (CIs) were estimated using a Cox proportional hazards model, with treatment group as a single covariate, stratified by the above factors. Progression-free survival curves, medians with 95% CIs, and progression-free survival rates at 6, 12, and 18 months with 95% CIs were estimated using Kaplan-Meier methodology. Overall survival will be analyzed when all patients have a minimum follow-up of 22 months.

**Results**

*Patients and Treatment*

**[0136]** From July 2013 through March 2014, a total of 1296 patients were enrolled at 137 centers in Australia, Europe, Israel, New Zealand, and North America. A total of 945 patients underwent randomization: 316 patients were assigned to the nivolumab group, 314 to the nivolumab plus ipilimumab group, and 315 to the ipilimumab group (~~Figure~~ Fig. 1). Baseline characteristics were balanced across the three groups. A total of 58.0% had stage M1c disease, 36.1% had an elevated lactate dehydrogenase level, 31.5% had a BRAF mutation, and 73.5% had negative PD-L1 status (Table 1).

**[0137]** All randomized patients had been followed for a minimum of 9 months at the time of database lock (February 17, 2015); 117 of 313 patients (37.4%) in the nivolumab group, 93 of 313 patients (29.7%) in the nivolumab plus ipilimumab group, and 50 of 311 patients (16.1%) in the ipilimumab group were continuing study treatment (Table 2). The most frequent reason for discontinuation was disease progression in the nivolumab and ipilimumab monotherapy groups (154 of 313 patients [49.2%] and 202 of 311 patients [65.0%], respectively), versus study drug toxicity in the nivolumab plus ipilimumab group (120 of 313 patients [38.3%]). The number of patients who had died was 85 (27.2%) in the nivolumab group, 86 (27.5%) in the nivolumab plus ipilimumab group, and 114 (36.7%) in the ipilimumab group.

**[0138]** The median number of doses in patients who received nivolumab alone or ipilimumab alone was 15 (range 1-38) and 4 (1-4), respectively. In the combination group, the median number of doses was 4 (range 1–39) for nivolumab and 4 (range 1–4) for ipilimumab; 147 of 313 patients (47%) received four or more doses of nivolumab monotherapy after combination treatment.

Table 1. Baseline Characteristics of the Patients.

| Characteristic | Nivolumab alone (N=316) | Nivolumab plus Ipilimumab (N=314) | Ipilimumab alone (N=315) | Total (N=945) |
|---|---|---|---|---|
| | | | | |
| Age — yr | | | | |
| Mean | 58.7 | 59.3 | 60.8 | 59.6 |
| Range | 25–90 | 18–88 | 18–89 | 18–90 |
| | | | | |
| Age groups — no. (%) | | | | |
| <65 yr | 198 (62.7) | 185 (58.9) | 182 (57.8) | 565 (59.8) |
| ≥ 65, < 75 yr | 79 (25.0) | 94 (29.9) | 89 (28.3) | 262 (27.7) |
| ≥ 75 yr | 39 (12.3) | 35 (11.1) | 44 (14.0) | 118 (12.5) |
| | | | | |
| Sex — no. (%) | | | | |
| Male | 202 (63.9) | 206 (65.6) | 202 (64.1) | 610 (64.6) |
| Female | 114 (36.1) | 108 (34.4) | 113 (35.9) | 335 (35.4) |
| | | | | |
| ECOG performance status — no. (%) | | | | |
| 0 | 238 (75.3) | 230 (73.2) | 224 (71.1) | 692 (73.2) |
| 1 | 77 (24.4) | 83 (26.4) | 91 (28.9) | 251 (26.6) |
| 2 | 1 (0.3) | 0 | 0 | 1 (0.1) |
| Not reported | 0 | 1 (0.3) | 0 | 1 (0.1) |
| | | | | |
| M stage — no. (%) | | | | |
| M1c | 184 (58.2) | 181 (57.6) | 183 (58.1) | 548 (58.0) |
| M0, M1a, or M1b | 132 (41.8) | 133 (42.4) | 132 (41.9) | 397 (42.0) |
| | | | | |
| Lactate dehydrogenase — no. (%)* | | | | |
| ≤ ULN | 196 ( 62.0) | 199 ( 63.4) | 194 ( 61.6) | 589 ( 62.3) |
| >ULN | 112 ( 35.4) | 114 ( 36.3) | 115 ( 36.5) | 341 ( 36.1) |
| ≤2x ULN | 271 ( 85.8) | 276 ( 87.9) | 279 ( 88.6) | 826 ( 87.4) |

| | | | | |
|---|---|---|---|---|
| >2x ULN | 37 ( 11.7) | 37 (11.8) | 30 (9.5) | 104 (11.0) |
| Unknown | 8 (2.5) | 1 (0.3) | 6 (1.9) | 15 (1.6) |
| | | | | |
| Brain metastases at baseline — no. (%) | | | | |
| Yes | 8 (2.5) | 11 (3.5) | 15 (4.8) | 34 (3.6) |
| No | 308 (37.5) | 303 (96.5) | 300 (95.2) | 911 (96.4) |
| | | | | |
| PD-L1 status — no. (%) | | | | |
| Positive | 80 (27.8) | 68 (24.5) | 75 (27.1) | 223(26.4) |
| Negative | 208 (72.2) | 210 (75.5) | 202 (72.9) | 620 (73.5) |
| | | | | |
| BRAF status — no. (%) | | | | |
| Mutation | 100 (31.6) | 101 (32.2) | 97 (30.8) | 298 (31.5) |
| No mutation | 216 (68.4) | 213 (67.8) | 218 (69.2) | 647 (68.5) |

* ULN denotes upper limit of normal.

*Efficacy*

**[0139]** The median progression-free survival was 6.5 months (95% confidence interval [CI], 4.3 to 9.5) in the nivolumab group, 11.5 months (95% CI, 8.9 to 16.5) in the nivolumab plus ipilimumab group, and 2.9 months (95% CI, 2.8 to 3.4) in the ipilimumab group (Fig. 2A). A significant improvement in progression-free survival was observed in the nivolumab plus ipilimumab group as compared with the ipilimumab group (hazard ratio, 0.42; 95% CI, 0.31 to 0.57; P<0.0001) (Fig. 2A). A significant improvement in progression-free survival was also observed in the nivolumab group as compared with the ipilimumab group (hazard ratio, 0.57; 95% CI, 0.43 to 0.76; P<0.00001) (Fig. 2A). The hazard ratio for the comparison between nivolumab plus ipilimumab and nivolumab groups was 0.74 (95% CI, 0.60 to 0.92).

**[0140]** Analyses of progression-free survival among prespecified patient subgroups showed a consistent improvement with nivolumab or nivolumab plus ipilimumab as compared with ipilimumab, including subgroups defined by PD-L1 status, BRAF mutation status, and metastasis stage (Figs. 3A and 3B). In the combination group, median PFS was 11.7 months (95% CI, 8.0 to not reached) among patients with a BRAF mutation and was 11.2 months (95% CI, 8.3 to not reached) in patients with wild-type BRAF (Fig. 3B). For patients with a positive PD-L1 tumor status, median progression-free survival in the nivolumab, nivolumab plus ipilimumab, and ipilimumab groups was 14.0 months (95% CI, 9.1 to not reached), 14.0 months (95% CI, 9.7 to not reached), and 3.9 months (95% CI, 2.8 to 4.2), respectively (Fig. 2B). For patients with a negative PD-L1 tumor status, median progression-free survival in the nivolumab, nivolumab plus ipilimumab, and ipilimumab groups was 5.3 months (95% CI, 2.8 to 7.1 months), 11.2 months (95% CI, 8.0 to not reached) and 2.8 months (95% CI, 2.8 to 3.1), respectively (Fig. 2C).

**[0141]** Investigator-assessed objective response rates were 43.7% (95% CI, 38.1 to 49.3%), 57.6% (95% CI, 52.0 to 63.2), and 19.0% (95% CI, 14.9 to 23.8) in the nivolumab, nivolumab plus ipilimumab, and ipilimumab groups, respectively (Table 3). The percentage of patients with a complete response was higher in the nivolumab plus ipilimumab group than in either the nivolumab or ipilimumab alone groups (11.5% vs.

8.9% and 2.2%) (Table 3). Time to objective response was similar in each group (Table 3), and the median duration of response was not reached in any of the groups.

**[0142]** Median reduction in the sum of the longest diameters of tumor target lesions was -34.5% (interquartile range: -75.4 to 15.4), -51.1% (-75.8 to -10.2), and 5.8% (-28.0 to 33.3) in the nivolumab, nivolumab plus ipilimumab, and ipilimumab groups, respectively (Figs. 4A-4C). Among patients with PD-L1-positive tumors, the objective response rates were 57.5% (95% CI, 45.9 to 68.5), 72.1% (95% CI, 59.9 to 82.3), and 21.3% (95% CI, 12.7 to 32.3) for the nivolumab, nivolumab plus ipilimumab, and ipilimumab groups, respectively; in patients with PD-L1-negative tumors, the objective response rates were 41.3% (95% CI, 34.6 to 48.4), 54.8% (95% CI, 47.8 to 61.6), and 17.8% (95% CI, 12.8 to 23.8) (Table 4).

*Adverse Events*

**[0143]** Treatment-related adverse events of any grade occurred in 82.1%, 95.5%, and 86.2% of patients in the nivolumab, nivolumab plus ipilimumab groups, and ipilimumab groups, respectively (Table 5). The most common adverse events in the nivolumab group were fatigue (in 34.2% of patients), rash (in 21.7%), and diarrhea (in 19.2%). In the nivolumab plus ipilimumab and ipilimumab groups, diarrhea (in 44.1% and 33.1% of patients, respectively), fatigue (in 35.1% and 28.0%), and pruritus (in 33.2% and 35.4%) were most common (Table 5). The incidence of treatment-related adverse events of grade 3 or 4 was also higher in the nivolumab plus ipilimumab group than in either the nivolumab or ipilimumab groups (55.0% vs. 16.3% and 27.3%), with diarrhea being the most common (2.2% , 9.3%, and 6.1% in the nivolumab, nivolumab plus ipilimumab, and ipilimumab groups, respectively) (Table 5). Treatment-related adverse events of any grade leading to discontinuation occurred in 7.7%, 36.4%, and 14.8% of patients in the nivolumab, nivolumab plus ipilimumab, and ipilimumab groups, respectively, with the most common being diarrhea (in 1.9%, 8.3%, and 4.5%, respectively) and colitis (in 0.6%, 8.3%, and 7.7%, respectively) (Table 5). One death due to study-drug toxicity was reported in the nivolumab group (neutropenia) and one in the ipilimumab group (cardiac arrest), although such adverse events have not been associated with these drugs in prior studies. No treatment-related deaths were reported in the combination group.

Table 2. Patient Disposition.*

| | Nivolumab alone (N=313) | Nivolumab plus Ipilimumab (N=313) | Ipilimumab alone (N=311) |
|---|---|---|---|
| | | | |
| **Patients in the treatment period — no. (%)** | | | |
| Continuing | 117 (37.4) | 93 (29.7) | 50 (16.1) |
| Not continuing | 196 (62.6) | 220 (70.3) | 261 (83.9) |
| | | | |
| **Reason for not continuing the treatment — no. (%)** | | | |
| Disease progression | 154 (49.2) | 69 (22.0) | 202 (65.0) |
| Study drug toxicity | 27 (8.6) | 120 (38.3) | 47 (15.1) |
| Adverse event unrelated to study drug | 5 (1.6) | 12 (3.8) | 4 (1.3) |
| Patient request to discontinue treatment | 5 (1.6) | 5 (1.6) | 4 (1.3) |
| Death | 5 (1.6) | 5 (1.6) | 4 (1.3) |
| Maximum clinical benefit | 2 (0.6) | 2 (0.6) | 0 |
| Poor/non-compliance | 1 (0.3) | 1 (0.3) | 1 (0.3) |
| Patient withdrew consent | 0 | 3 (1.0) | 0 |
| Lost to follow-up | 1 (0.3) | 0 | 0 |
| Patient no longer meets study criteria | 0 | 1 (0.3) | 0 |
| Other | 0 | 3 (1.0) | 2 (0.6) |
| | | | |
| **Patients in the study — no. (%)** | | | |
| Continuing | 223 (71.2) | 221 (70.6) | 189 (60.8) |
| Died | 85 (27.2) | 86 (27.5) | 114 (36.7) |
| Not continuing | 5 (1.6) | 6 (1.9) | 8 (2.6) |

****At the time of database lock on February 17, 2015*.

Table 3. Response to Treatment.

| Response | Nivolumab alone (N=316) | Nivolumab plus Ipilimumab (N=314) | Ipilimumab alone (N=315) |
|---|---|---|---|
| | | | |
| **Best overall response — no. (%)[†]** | | | |
| Complete response | 28 (8.9) | 36 (11.5) | 7 (2.2) |
| Partial response | 110 (34.8) | 145 (46.2) | 53 (16.8) |
| Stable disease | 34 (10.8) | 41 (13.1) | 69 (21.9) |
| Progressive disease | 119 (37.7) | 71 (22.6) | 154 (48.9) |
| Could not be determined | 25 (7.9) | 21 (6.7) | 32 (10.2) |
| | | | |
| **Objective response[‡]** | | | |
| No. of patients (% [95% CI]) | 138 (43.7 [38.1–49.3]) | 181 (57.6 [52.0–63.2]) | 60 (19.0 [14.9–23.8]) |
| Estimated odds ratio (95% CI)[§] | 3.40 (2.02–5.72) | 6.11 (3.59–10.38) | --- |
| Two-sided P value | <0.00001 | <0.00001 | --- |
| | | | |
| **Time to objective response — mo** | | | |
| No. of responders | 138 | 181 | 60 |
| Median | 2.78 | 2.76 | 2.79 |
| Range | 2.3–12.5 | 1.1–11.6 | 2.5–12.4 |

[†] ***Best overall response were assessed by the investigators with the use of RECIST v1.1.***

[‡] ***Data include patients with a complete response and those with a partial response.***

[§] ***Relative to ipilimumab alone.***

Table 4. Objective Response Rate by PD-L1 Status.

| **PD-L1 Positive** | | | |
|---|---|---|---|
| | **Nivolumab alone (N=80)** | **Nivolumab plus Ipilimumab (N=68)** | **Ipilimumab alone (N=75)** |
| | | | |
| **Objective response**[†] | | | |
| No. of patients (% [95% CI]) | 46 (57.5 [45.9–68.5]) | 49 (72.1 [59.9–82.3]) | 16 (21.3 [12.7–32.3]) |
| Estimated odds ratio (95% CI)[§] | 5.03 (2.44–10.37) | 10.41 (4.63–23.40) | --- |
| | | | |
| **PD-L1 Negative** | | | |
| | **Nivolumab alone (N=208)** | **Nivolumab plus Ipilimumab (N=210)** | **Ipilimumab alone (N=202)** |
| **Objective response**[†] | | | |
| No. of patients (% [95% CI]) | 86 (41.3 [34.6–48.4]) | 115 (54.8 [47.8–61.6]) | 36 (17.8 [12.8–23.8]) |
| Estimated odds ratio (95% CI)[§] | 3.25 (2.05–5.13) | 5.90 (3.71–9.38) | --- |

[†] ***Best overall response was assessed by the investigators with the use of RECIST v1.1.***

[§] Relative to ipilimumab alone.

**[0144]** The most frequent grade 3 or 4 treatment-related select adverse events were diarrhea (2.2%, 9.3%, and 6.1% of patients in the nivolumab, nivolumab plus ipilimumab, and ipilimumab groups, respectively), colitis (in 0.6%, 7.7%, and 8.7%, respectively), increased alanine aminotransferase (in 1.3%, 8.3%, and 1.6%, respectively), and increased aspartate aminotransferase (in 1.0%, 6.1%, and 0.6%, respectively) (Table 6). With the use of immune modulatory agents, resolution rates for grade 3 or 4 select adverse events were generally similar across treatment groups, and were between 85-100% across organ categories in the nivolumab plus ipilimumab group. As observed in prior studies, most endocrine events in all treatment groups did not resolve (Table 7).

**Discussion**

**[0145]** In this randomized, double-blind, phase 3 study, both nivolumab alone and the combination of nivolumab and ipilimumab significantly increased progression-free survival and objective response rates, as compared with ipilimumab alone, in previously untreated advanced melanoma. These results were observed independently of PD-L1 tumor status, BRAF mutation status, or metastasis stage. Baseline characteristics of study participants were typical of patients with advanced melanoma, although the BRAF mutation rate (31.5%) was lower than the 40-50% generally reported for advanced disease. While not a primary end point of the study, the combination of nivolumab and ipilimumab resulted in numerically longer progression-free survival and a higher response rate as compared with nivolumab alone in the overall study population. While time to response was similar between groups, the first tumor assessment was done at week 12 and thus the possibility that responses may have occurred earlier with the combination remains unknown.

Table 5. Adverse Events (Safety Population).*

| Event | Nivolumab alone (N=313) | | Nivolumab plus Ipilimumab (N=313) | | Ipilimumab alone (N=311) | |
|---|---|---|---|---|---|---|
| | Total | Grade 3 or 4 | Total | Grade 3 or 4 | Total | Grade 3 or 4 |
| | *no. of patients with event (%)* | | | | | |
| Any adverse event | 311 (99.4) | 136 (43.5) | 312 (99.7) | 215 (68.7) | 308 (99.0) | 173 (55.6) |
| Treatment-related adverse event† | 257 (82.1) | 51 (16.3) | 299 (95.5) | 172 (55.0) | 268 (86.2) | 85 (27.3) |
| Diarrhea | 60 (19.2) | 7 (2.2) | 138 (44.1) | 29 (9.3) | 103 (33.1) | 19 (6.1) |
| Fatigue | 107 (34.2) | 4 (1.3) | 110 (35.1) | 13 (4.2) | 87 (28.0) | 3 (1.0) |
| Pruritus | 59 (18.8) | 0 | 104 (33.2) | 6 (1.9) | 110 (35.4) | 1 (0.3) |
| Rash | 81 (25.9) | 2 (0.6) | 126 (40.3) | 15 (4.8) | 102 (32.8) | 6 (1.9) |
| Nausea | 41 (13.1) | 0 | 81 (25.9) | 7 (2.2) | 50 (16.1) | 2 (0.6) |
| Pyrexia | 18 (5.8) | 0 | 58 (18.5) | 2 (0.6) | 21 (6.8) | 1 (0.3) |
| Decreased appetite | 34 (10.9) | 0 | 56 (17.9) | 4 (1.3) | 39 (12.5) | 1 (0.3) |
| Increase in alanine aminotransferase | 12 (3.8) | 4 (1.3) | 55 (17.6) | 26 (8.3) | 12 (3.9) | 5 (1.6) |
| Vomiting | 20 (6.4) | 1 (0.3) | 48 (15.3) | 8 (2.6) | 23 (7.4) | 1 (0.3) |
| Increase in aspartate aminotransferase | 12 (3.8) | 3 (1.0) | 48 (15.3) | 19 (6.1) | 11 (3.5) | 2 (0.6) |
| Hypothyroidism | 27 (8.6) | 0 | 47 (15.0) | 1 (0.3) | 13 (4.2) | 0 |
| Colitis | 4 (1.3) | 2 (0.6) | 37 (11.8) | 24 (7.7) | 36 (11.6) | 27 (8.7) |
| Arthralgia | 24 (7.7) | 0 | 33 (10.5) | 1 (0.3) | 19 (6.1) | 0 |
| Headache | 23 (7.3) | 0 | 32 (10.2) | 1 (0.3) | 24 (7.7) | 1 (0.3) |
| Dyspnea | 14 (4.5) | 1 (0.3) | 32 (10.2) | 2 (0.6) | 13 (4.2) | 0 |
| Treatment-related adverse event leading to discontinuation | 24 (7.7) | 16 (5.1) | 114 (36.4) | 92 (29.4) | 46 (14.8) | 41 (13.2) |

* The severity of adverse events was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0.
† The treatment-related adverse events listed here were reported in at least 10% of the patients in any of the three study groups.

Table 6. Treatment-related Select Adverse Events (Safety Population).*

| Event | Nivolumab alone (N=313) | | Nivolumab plus Ipilimumab (N=313) | | Ipilimumab alone (N=311) | |
|---|---|---|---|---|---|---|
| | Total | Grade 3 or 4 | Total | Grade 3 or 4 | Total | Grade 3 or 4 |
| | *no. of patients with event (%)* | | | | | |
| Any select adverse event | 194 (62.0) | 24 (7.7) | 275 (87.9) | 124 (39.6) | 229 (73.6) | 58 (18.6) |
| | | | | | | |
| Treatment-related select adverse event† | | | | | | |
| Skin | 131 (41.9) | 5 (1.6) | 185 (59.1) | 18 (5.8) | 168 (54.0) | 9 (2.9) |
| Pruritus | 59 (18.8) | 0 | 104 (33.2) | 6 (1.9) | 110 (35.4) | 1 (0.3) |
| Rash | 68 (21.7) | 1 (0.3) | 89 (28.4) | 9 (2.9) | 65 (20.9) | 5 (1.6) |
| Rash maculo-papular | 13 (4.2) | 1 (0.3) | 37 (11.8) | 6 (1.9) | 37 (11.9) | 1 (0.3) |
| Vitiligo | 23 (7.3) | 1 (0.3) | 21 (6.7) | 0 | 12 (3.9) | 0 |
| Gastrointestinal | 61 (19.5) | 7 (2.2) | 145 (46.3) | 46 (14.7) | 114 (36.7) | 36 (11.6) |
| Diarrhea | 60 (19.2) | 7 (2.2) | 138 (44.1) | 29 (9.3) | 103 (33.1) | 19 (6.1) |
| Colitis | 4 (1.3) | 2 (0.6) | 37 (11.8) | 24 (7.7) | 36 (11.6) | 27 (8.7) |
| Hepatic | 20 (6.4) | 8 (2.6) | 94 (30.0) | 59 (18.8) | 22 (7.1) | 5 (1.6) |
| Increase in alanine aminotransferase | 12 (3.8) | 4 (1.3) | 55 (17.6) | 26 (8.3) | 12 (3.9) | 5 (1.6) |
| Increase in aspartate aminotransferase | 12 (3.8) | 3 (1.0) | 48 (15.3) | 19 (6.1) | 11 (3.5) | 2 (0.6) |
| Endocrine | 45 (14.4) | 2 (0.6) | 94 (30.0) | 15 (4.8) | 34 (10.9) | 7 (2.3) |
| Hypothyroidism | 27 (8.6) | 0 | 47 (15.0) | 1 (0.3) | 13 (4.2) | 0 |
| Hyperthyroidism | 13 (4.2) | 0 | 31 (9.9) | 3 (1.0) | 3 (1.0) | 0 |
| Hypophysitis | 2 (0.6) | 1 (0.3) | 24 (7.7) | 5 (1.6) | 12 (3.9) | 6 (1.9) |
| Pulmonary | 5 (1.6) | 1 (0.3) | 22 (7.0) | 3 (1.0) | 6 (1.9) | 1 (0.3) |
| Pneumonitis | 4 (1.3) | 1 (0.3) | 20 (6.4) | 3 (1.0) | 5 (1.6) | 1 (0.3) |

* The severity of adverse events was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0.
† The treatment-related select adverse events listed here were reported in at least 5% of the patients in any of the three study groups.

Table 7. Management of Treatment-related Select Adverse Events With Immune Modulatory Medication (IMM).

| | Nivolumab | | | Nivolumab plus Ipilimumab | | | Ipilimumab | | |
|---|---|---|---|---|---|---|---|---|---|
| **Select Adverse Event Organ Category** | **Patients managed with IMM, n (%)** | **Patients with resolution of AE after treatment with IMM, n (%)** | **Median time to resolution, weeks (95% CI)** | **Patients managed with IMM, n (%)** | **Patients with resolution of AE after treatment with IMM, n (%)** | **Median time to resolution, weeks (95% CI)** | **Patients managed with IMM, n (%)** | **Patients with resolution of AE after treatment with IMM, n (%)** | **Median time to resolution, weeks (95% CI)** |
| **Skin**<br>Grade 3-4 | 33/131 (25.2)<br>3/5 (60.0) | 17 (54.8)<br>3 (75.0) | 34.9 (18.0, NE)<br>2.1 (0.9, NE) | 80/185 (43.2)<br>12/18 (66.7) | 55 (75.3)<br>12 (85.7) | 8.6 (7.0, 12.6)<br>3.4 (1.1, 12.4) | 58/168 (34.5)<br>5/9 (55.6) | 41 (74.5)<br>5 (83.3) | 12.4 (8.9, 19.1)<br>6.1 (4.4, NE) |
| **Gastrointestinal**<br>Grade 3-4 | 9/61 (14.8)<br>5/7 (71.4) | 5 (71.4)<br>3 (50.0) | 4.0 (0.8, NE)<br>NE (0.8, NE) | 71/145 (49.0)<br>41/46 (89.1) | 62 (93.9)<br>41 (97.6) | 4.5 (3.5, 5.7)<br>3.0 (1.9, 4.3) | 54/114 (47.4)<br>33/36 (91.7) | 44 (88.0)<br>31 (88.6) | 4.9 (2.9, 7.6)<br>4.7 (1.9, 6.1) |
| **Endocrine**<br>Grade 3-4 | 5/45 (11.1)<br>2/2 (100) | 2 (40.0)<br>0 | 24.3 (7.1, NE)<br>NE (NE, NE) | 36/94 (38.3)<br>10/15 (66.7) | 14 (41.2)<br>5 (45.5) | NE (10.4, NE)<br>NE (4.5, NE) | 15/34 (44.1)<br>7/7 (100.0) | 4 (28.6)<br>3 (42.9) | NE (0.71, NE)<br>NE (0.43, NE) |
| **Hepatic**<br>Grade 3-4 | 8/22 (36.4)<br>7/8 (87.5) | 6 (100.0)<br>6 (100.0) | 7.0 (2.0, 27.1)<br>7.0 (2.0, 27.1) | 46/95 (48.4)<br>37/60 (61.7) | 43 (95.6)<br>38 (100.0) | 5.9 (3.3, 6.6)<br>4.1 (3.0, 6.1) | 3/22 (13.6)<br>2/5 (40.0) | 3 (100.0)<br>2 (100.0) | 4.1 (4.0, 7.7)<br>5.9 (4.0, 7.7) |
| **Pulmonary**<br>Grade 3-4 | 4/5 (80.0)<br>1/1 (100) | 4 (100.0)<br>1 (100.0) | 3.3 (2.3, 9.1)<br>2.3 (NE, NE) | 17/22 (77.3)<br>2/3 (66.7) | 16 (94.1)<br>2 (100.0) | 6.1 (1.1, 8.3)<br>4.2 (1.1, 7.3) | 3/6 (50.0)<br>1/1 (100) | 2 (66.7)<br>1 (100.0) | 6.1 (6.0, 6.3)<br>4.7 (NE, NE) |
| **Renal**<br>Grade 3-4 | 1/3 (33.3)<br>0/1 | 1 (100.0)<br>0 | 0.3 (NE, NE)<br>- | 4/17 (23.5)<br>3/6 (50.0) | 3 (100.0)<br>3 (100.0) | 1.7 (0.4, 3.7)<br>1.7 (0.4, 3.6) | 3/8 (37.5)<br>1/1 (100) | 3 (100.0)<br>1 (100.0) | 4.6 (0.6, 16.1)<br>4.6 (NE, NE) |

**[0146]** The median progression-free survival reported for the combination of nivolumab and ipilimumab in this study (11.7 months in BRAF-mutant patients) is similar to that recently reported for the combination of BRAF and MEK inhibition in BRAF-mutant metastatic melanoma (9.9 months for vemurafenib and cobimetinib; 9.3 to 11.4 months for dabrafenib and trametinib). Resistance to such targeted therapies is almost inevitable when used as monotherapy and in many cases is very rapid. The confirmed rate of objective response for combined nivolumab and ipilimumab (57.6%) is numerically higher than observed with PD-1 blockade alone in advanced melanoma (nivolumab [40%] in treatment-naïve patients with wild-type BRAF or pembrolizumab [37%] in ipilimumab-naïve patients).

**[0147]** The results of subgroup analyses suggest that the greatest benefit for the combination of nivolumab and ipilimumab versus nivolumab alone may occur in the setting of negative PD-L1 tumor expression. In the PD-L1-positive group, both nivolumab alone and nivolumab plus ipilimumab resulted in a similar prolongation of progression-free survival as compared with ipilimumab alone, although objective response rates were numerically higher in the combination group versus either nivolumab or ipilimumab alone. Thus, the use of PD-L1 as a biomarker may allow clinicians to make more informed decisions about the risk-benefit of combination therapy versus monotherapy. Nonetheless, the observation of at least additive activity of the combination of ipilimumab and nivolumab in the setting of negative PD-L1 expression is of interest in melanoma as well as in other tumor types in which PD-1 checkpoint inhibitors are under evaluation.

**[0148]** The incidence of adverse events in this study was, in general, lowest in the nivolumab group and highest in the combination group. The overall incidence of grade 3 or 4 drug-related adverse events was higher in the combination group as compared with ipilimumab alone (39.6% versus 18.6%), as a result of a slightly higher incidence in most adverse events, particularly hepatic toxicity, where the rates of grade 3 or 4 ALT/AST elevations were 6-8% for the combination and approximately 1% for ipilimumab alone. One drug-related death was reported in each of the nivolumab and ipilimumab groups but none in the combination group. Overall, the safety profile of the combination of nivolumab and ipilimumab was consistent with previous experience with nivolumab or ipilimumab alone. No new safety signals were identified, and adverse events were manageable with established treatment guidelines as most

select adverse events resolved with immune modulatory agents. These data suggest that the combination of nivolumab and ipilimumab can be used safely in a broad range of clinical settings.

**[0149]** In summary, the report shows increased progression-free survival and objective response rates for nivolumab alone and the combination of nivolumab and ipilimumab, as compared with ipilimumab alone, in previously untreated advanced melanoma. Adverse events with the combination were managed with established algorithms, with no study drug-related deaths. The combination of nivolumab and ipilimumab may represent a means to improve outcomes with either agent as monotherapy, particularly for patients having PD-L1-negative tumors. Overall, nivolumab alone and the combination of nivolumab and ipilimumab are promising treatment options for previously untreated advanced melanoma.

## SEQUENCES

$V_H$ amino acid sequence of 28-8 (*Oryctolagus cuniculus*) (SEQ ID NO:1)

Met Glu Thr Gly Leu Arg Trp Leu Leu Leu Val Ala Val Leu Lys Gly Val Gln Cys Leu Ser Val Glu Glu Ser Gly Gly Arg Leu Val Thr Pro Gly Thr Pro Leu Thr Leu Thr Cys Thr Ala Ser Gly Phe Thr Ile Thr Asn Tyr His Met Phe Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile Gly Val Ile Thr Ser Ser Gly Ile Gly Ser Ser Ser Thr Thr Tyr Tyr Ala Thr Trp Ala Lys Gly Arg Phe Thr Ile Ser Lys Thr Ser Thr Thr Val Asn Leu Arg Ile Thr Ser Pro Thr Thr Glu Asp Thr Ala Thr Tyr Phe Cys Ala Arg Asp Tyr Phe Thr Asn Thr Tyr Tyr Ala Leu Asp Ile Trp Gly Pro Gly Thr Leu Val Thr Val Ser Ser

$V_L$ amino acid sequence of 28-8 (*Oryctolagus cuniculus*) (SEQ ID NO:2)

Met Asp Thr Arg Ala Pro Thr Gln Leu Leu Gly Leu Leu Leu Leu Trp Leu Pro Gly Ala Arg Cys Ala Leu Val Met Thr Gln Thr Pro Ser Ser Thr Ser Thr Ala Val Gly Gly Thr Val Thr Ile Lys Cys Gln Ala Ser Gln Ser Ile Ser Val Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro Lys Leu Leu Ile Tyr Ser Ala Ser Thr Leu Ala Ser Gly Val Pro Ser Arg Phe Lys Gly Ser Arg Ser Gly Thr Glu Tyr Thr Leu Thr Ile Ser Gly Val Gln Arg Glu Asp Ala Ala Thr Tyr Tyr Cys Leu Gly Ser Ala Gly Ser

Heavy Chain CDR1 sequence of 28-8 (*Oryctolagus cuniculus*) (SEQ ID NO:3)

Asn Tyr His Met Phe

Heavy Chain CDR2 sequence of 28-8 (*Oryctolagus cuniculus*) (SEQ ID NO:4)

Val Ile Thr Ser Ser Gly Ile Gly Ser Ser Ser Thr Thr Tyr Tyr Ala Thr Trp Ala Lys Gly

Heavy Chain CDR3 sequence of 28-8 (*Oryctolagus cuniculus*) (SEQ ID NO:5)

Asp Tyr Phe Thr Asn Thr Tyr Tyr Ala Leu Asp Ile

Light Chain CDR1 sequence of 28-8 (*Oryctolagus cuniculus*) (SEQ ID NO:6)

Gln Ala Ser Gln Ser Ile Ser Val Tyr Leu Ala

Light Chain CDR2 sequence of 28-8 (*Oryctolagus cuniculus*) (SEQ ID NO:7)

Ser Ala Ser Thr Leu Ala Ser

Light Chain CDR3 sequence of 28-8 (*Oryctolagus cuniculus*) (SEQ ID NO:8)

Leu Gly Ser Ala Gly Ser Asp Asp Ala Ala

# ABSTRACT

The invention provides a method of treating a melanoma comprising (i) identifying a patient having a PD-L1-negative melanoma and (ii) administering to the patient a combination of an anti-PD-1 antibody or an antigen-binding portion thereof and an anti-CTLA-4 antibody or an antigen-binding portion thereof. The methods of the invention can extend progression-free survival for over 8 months and/or reduces the tumor size at least about 10%, about 20%, about 30%, about 40%, or about 50% compared to the tumor size prior to the administration.

# TREATMENT OF PD-L1-NEGATIVE MELANOMA USING AN ANTI-PD-1 ANTIBODY AND AN ANTI-CTLA-4 ANTIBODY

## CROSS-REFERENCE TO RELATED APPLICATIONS

**[0001]** The present application claims benefit to U.S. Provisional Application No. 62/153,973 filed April 28, 2015, which is incorporated herein by reference in its entirety.

## REFERENCE TO A SEQUENCE LISTING SUBMITTED ELECTRONICALLY VIA EFS-WEB

**[0002]** The instant application contains a Sequence Listing which has been submitted in ASCII format via EFS-Web, and is hereby incorporated by reference in its entirety. Said ASCII copy, created on April 28, 2016, is named 3338_0330001_SeqListing_ST25.txt and is 3,723 bytes in size.

**[0003]** Throughout this application, various publications are referenced in parentheses by author name and date, or by Patent No. or Patent Publication No. The disclosures of these publications are hereby incorporated in their entireties by reference into this application in order to more fully describe the state of the art as known to those skilled therein as of the date of the invention described and claimed herein. However, the citation of a reference herein should not be construed as an acknowledgement that such reference is prior art to the present invention.

## FIELD OF THE INVENTION

**[0004]** This invention relates to a method of treating PD-L1-negative melanoma comprising administering a combination of an anti-PD-1 antibody and an anti-CTLA-4 antibody.

## BACKGROUND OF THE INVENTION

**[0005]** Human cancers harbor numerous genetic and epigenetic alterations, generating neoantigens potentially recognizable by the immune system (Sjoblom *et al.* (2006)

*Science* 314:268-74). The adaptive immune system, comprised of T and B lymphocytes, has powerful anti-cancer potential, with a broad capacity and exquisite specificity to respond to diverse tumor antigens. Further, the immune system demonstrates considerable plasticity and a memory component. The successful harnessing of all these attributes of the adaptive immune system would make immunotherapy unique among all cancer treatment modalities.

**[0006]** Recently, several immune checkpoint pathway inhibitors have begun to provide new immunotherapeutic approaches for treating cancer, including the development of an antibody (Ab), ipilimumab (YERVOY®), that binds to and inhibits Cytotoxic T-Lymphocyte Antigen-4 (CTLA-4) for the treatment of patients with advanced melanoma and the development of antibodies such as nivolumab and pembrolizumab (formerly lambrolizumab; USAN Council Statement, (2013) Pembrolizumab: Statement on a nonproprietary name adopted by the USAN Council (ZZ-165), November 27, 2013) that bind specifically to the Programmed Death-1 (PD-1) receptor and block the inhibitory PD-1/PD-1 ligand pathway.

**[0007]** The promise of the emerging field of personalized medicine is that advances in pharmacogenomics will increasingly be used to tailor therapeutics to defined sub-populations, and ultimately, individual patients in order to enhance efficacy and minimize adverse effects. Recent successes include, for example, the development of imatinib mesylate (GLEEVEC®), a protein tyrosine kinase inhibitor that inhibits the bcr-abl tyrosine kinase, to treat Philadelphia chromosome-positive chronic myelogenous leukemia (CML); crizotinib (XALKORI®) to treat the 5% of patients with late-stage non-small cell lung cancers who express a mutant anaplastic lymphoma kinase (ALK) gene; and vemurafenib (ZELBORAF®), an inhibitor of mutated B-RAF protein (V600E-BRAF) which is expressed in around half of melanoma tumors. However, unlike the clinical development of small molecule agents that target discrete activating mutations found in select cancer populations, a particular challenge in cancer immunotherapy has been the identification of mechanism-based predictive biomarkers to enable patient selection and guide on-treatment management.

## SUMMARY OF THE INVENTION

**[0008]** The present disclosure provides a method for treating a melanoma comprising (i) identifying a patient having a PD-L1-negative melanoma tumor; and (ii) administering to the patient (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4. The present disclosure also provides a method for treating a melanoma comprising administering to a patient afflicted with a melanoma tumor (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4, wherein the patient is identified as having a PD-L1-negative melanoma tumor prior to the administration.

**[0009]** The present disclosure further provides a method for extending a progression-free survival period for over 8 months in a patient afflicted with a melanoma tumor comprising administering to the patient (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4, wherein the patient is identified as having a PD-L1-negative melanoma tumor prior to the administration and wherein the patient demonstrates progression-free survival for over 8 months. In certain embodiments, the progression-free survival of the patient is extended after the administration for over about 11 months, about 12 months, about 13 months, about 14 months, about 15 months, about 16 months, about 17 months, about 18 months, about 2 years, about 3 years, about 4 years, about 5 years, about 6 years, about 7 years, about 8 years, about 9 years, or about 10 years. In one particular embodiment, the progression-free survival of the patient is extended for over 11 months.

**[0010]** The present disclosure also provides a method for reducing a tumor size at least by 10% in a patient afflicted with a melanoma tumor comprising administering to the patient (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4, wherein the patient is identified as having a PD-L1-negative melanoma tumor prior to the administration and

wherein the administration reduces the tumor size at least about 10%, about 20%, about 30%, about 40%, or about 50% compared to the tumor size prior to the administration.

**[0011]** The present disclosure also provides a method for increasing an objective response rate to be higher than 40% in a patient population, each of whom is afflicted with a melanoma tumor, in a cancer treatment comprising administering to the patient (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4, wherein each patient is identified as having a PD-L1-negative melanoma tumor prior to the administration and wherein the objective response rate is higher than 40%, 45%, 50%, 55%, 60%, 65%, 70%, or 75%.

**[0012]** The present disclosure also provides a method for selecting a patient suitable for an anti-PD-1 antibody and anti-CTLA-4 antibody combination therapy comprising (i) identifying a patient having a PD-L1-negative melanoma tumor; and (ii) instructing a healthcare provider to administer to the patient (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4.

**[0013]** In certain embodiments, the methods disclosed herein further comprise identifying the patient as having a melanoma tumor that does not express PD-L1 prior to the administration. In some embodiments, the patient is characterized by (i) extended progression-free survival for over 8 months, (ii) tumor size reduction at least about 10%, about 20%, about 30%, about 40%, or about 50% compared to the tumor size prior to the administration, or (iii) both.

**[0014]** In some embodiments, the methods disclosed herein further comprise measuring a PD-L1 expression on the melanoma tumor. In certain embodiments, the measuring further comprises assessing the proportion of cells in the test tissue sample that express PD-L1 on the cell surface. In one particular embodiment, the presence of PD-L1 is determined using an automated IHC assay.

**[0015]** The present disclosure further provides a kit for treating a patient afflicted with a melanoma tumor, the kit comprising (a) a dosage ranging from 0.1 to 10 mg/kg body weight of an anti-PD-1 antibody or an antigen-binding portion thereof; (b) a dosage

ranging from 0.1 to 10 mg/kg body weight of an anti-CTLA-4 antibody or an antigen-binding portion thereof; and (c) instructions for using the anti-PD-1 antibody or the antigen-binding portion thereof and the anti-CTLA-4 antibody or the antigen-binding portion thereof in any of the methods disclosed herein.

## BRIEF DESCRIPTION OF THE FIGURES

**[0016]** ~~Figure~~ Fig. 1 shows a patient flow diagram of the randomized, double-blind, multicenter, phase 3 trial.

**[0017]** ~~Figures~~ Figs. 2A-C show progression-free survival data in the intention-to-treat population (~~Figure~~ Fig. 2A), in patients with positive PD-L1 status (~~Figure~~ Fig. 2B), and Negative PD-L1 Status (~~Figure~~ Fig. 2C). Each graph shows the progression free survival for patients treated with nivolumab alone (solid line), ipilimumab alone (dashed line), or the combination of nivolumab and ipilimumab (dotted line) in months (~~Figures~~ Figs. 2A-C). The number at risk in months for each of nivolumab, nivolumab plus ipilimumab, and ipilimumab is shown below each x-axis (~~Figures~~ Figs. 2A-C). PD-L1 expression status is based on verified PD-L1 assay data (~~Figures~~ Figs. 2B-C).

**[0018]** ~~Figures 3A-B~~ Figs. 3A and 3B show subgroup analyses of progression-free survival among patients treated with nivolumab alone compared to ipilimumab alone (~~Figure~~ Fig. 3A) and patients treated with nivolumab plus ipilimumab compared to ipilimumab alone (~~Figure~~ Fig. 3B).

**[0019]** ~~Figures 4A-C~~ Figs. 4A-4C show the tumor burden change in target lesions in patients treated with nivolumab alone (~~Figure~~ Fig. 4A), nivolumab plus ipilimumab (~~Figure~~ Fig. 4B), and ipilimumab alone (~~Figure~~ Fig. 4C). In each graph, the y-axis shows the best reduction from baseline in target lesions (%) and the x-axis represents each patient (~~Figures~~ Figs. 4A-C).

## DETAILED DESCRIPTION OF THE INVENTION

**[0020]** The present invention relates to identifying an optimal strategy to treat a patient having a PD-L1-negative melanoma. The present invention shows that in a patient having a PD-L1-negative melanoma tumor, a combination therapy of an anti-PD-1 antibody and

an anti-CTLA-4 antibody provides a better response (e.g., progression-free survival) than a monotherapy of either an anti-PD-1 antibody or an anti-CTLA-4 antibody. Furthermore, the combination therapy when given to a population of patients can increase the objective response rate compared to the monotherapy.

*Definitions*

**[0021]** In order that the present disclosure may be more readily understood, certain terms are first defined. As used in this application, except as otherwise expressly provided herein, each of the following terms shall have the meaning set forth below. Additional definitions are set forth throughout the application.

**[0022]** The term "and/or" where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. Thus, the term "and/or" as used in a phrase such as "A and/or B" herein is intended to include "A and B," "A or B," "A" (alone), and "B" (alone). Likewise, the term "and/or" as used in a phrase such as "A, B, and/or C" is intended to encompass each of the following aspects: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).

**[0023]** It is understood that wherever aspects are described herein with the language "comprising," otherwise analogous aspects described in terms of "consisting of" and/or "consisting essentially of" are also provided.

**[0024]** Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure is related. For example, the Concise Dictionary of Biomedicine and Molecular Biology, Juo, Pei-Show, 2nd ed., 2002, CRC Press; The Dictionary of Cell and Molecular Biology, 3rd ed., 1999, Academic Press; and the Oxford Dictionary Of Biochemistry And Molecular Biology, Revised, 2000, Oxford University Press, provide one of skill with a general dictionary of many of the terms used in this disclosure.

**[0025]** Units, prefixes, and symbols are denoted in their Système International de Unites (SI) accepted form. Numeric ranges are inclusive of the numbers defining the range. The headings provided herein are not limitations of the various aspects of the disclosure, which can be had by reference to the specification as a whole. Accordingly, the terms

defined immediately below are more fully defined by reference to the specification in its entirety.

**[0026]** "Administering" refers to the physical introduction of a composition comprising a therapeutic agent to a subject, using any of the various methods and delivery systems known to those skilled in the art. Routes of administration for the formulations disclosed herein include intravenous, intramuscular, subcutaneous, intraperitoneal, spinal or other parenteral routes of administration, for example by injection or infusion. The phrase "parenteral administration" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intralymphatic, intralesional, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion, as well as *in vivo* electroporation. In some embodiments, the formulation is administered via a non-parenteral route, in some embodiments, orally. Other non-parenteral routes include a topical, epidermal or mucosal route of administration, for example, intranasally, vaginally, rectally, sublingually or topically. Administering can also be performed, for example, once, a plurality of times, and/or over one or more extended periods.

**[0027]** An "adverse event" (AE) as used herein is any unfavorable and generally unintended or undesirable sign (including an abnormal laboratory finding), symptom, or disease associated with the use of a medical treatment. For example, an adverse event may be associated with activation of the immune system or expansion of immune system cells (*e.g.*, T cells) in response to a treatment. A medical treatment may have one or more associated AEs and each AE may have the same or different level of severity. Reference to methods capable of "altering adverse events" means a treatment regime that decreases the incidence and/or severity of one or more AEs associated with the use of a different treatment regime.

**[0028]** An "antibody" (Ab) shall include, without limitation, a glycoprotein immunoglobulin which binds specifically to an antigen and comprises at least two heavy (H) chains and two light (L) chains interconnected by disulfide bonds, or an antigen-binding portion thereof. Each H chain comprises a heavy chain variable region

(abbreviated herein as $V_H$) and a heavy chain constant region. The heavy chain constant region comprises three constant domains, $C_{H1}$, $C_{H2}$ and $C_{H3}$. Each light chain comprises a light chain variable region (abbreviated herein as $V_L$) and a light chain constant region. The light chain constant region is comprises one constant domain, $C_L$. The $V_H$ and $V_L$ regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FR). Each $V_H$ and $V_L$ comprises three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The constant regions of the antibodies may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (*e.g.*, effector cells) and the first component (C1q) of the classical complement system.

**[0029]** An immunoglobulin may derive from any of the commonly known isotypes, including but not limited to IgA, secretory IgA, IgG and IgM. IgG subclasses are also well known to those in the art and include but are not limited to human IgG1, IgG2, IgG3 and IgG4. "Isotype" refers to the antibody class or subclass (*e.g.*, IgM or IgG1) that is encoded by the heavy chain constant region genes. The term "antibody" includes, by way of example, both naturally occurring and non-naturally occurring antibodies; monoclonal and polyclonal antibodies; chimeric and humanized antibodies; human or nonhuman antibodies; wholly synthetic antibodies; and single chain antibodies. A nonhuman antibody may be humanized by recombinant methods to reduce its immunogenicity in man. Where not expressly stated, and unless the context indicates otherwise, the term "antibody" also includes an antigen-binding fragment or an antigen-binding portion of any of the aforementioned immunoglobulins, and includes a monovalent and a divalent fragment or portion, and a single chain antibody.

**[0030]** The term "monoclonal antibody" ("mAb") refers to a non-naturally occurring preparation of antibody molecules of single molecular composition, *i.e.*, antibody molecules whose primary sequences are essentially identical, and which exhibits a single binding specificity and affinity for a particular epitope. A mAb is an example of an

isolated antibody. MAbs may be produced by hybridoma, recombinant, transgenic or other techniques known to those skilled in the art.

**[0031]** A "human" antibody (HuMAb) refers to an antibody having variable regions in which both the framework and CDR regions are derived from human germline immunoglobulin sequences. Furthermore, if the antibody contains a constant region, the constant region is also derived from human germline immunoglobulin sequences. The human antibodies of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (*e.g.*, mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*). However, the term "human antibody," as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences. The terms "human" antibodies and "fully human" antibodies and are used synonymously.

**[0032]** A "humanized antibody" refers to an antibody in which some, most or all of the amino acids outside the CDR domains of a non-human antibody are replaced with corresponding amino acids derived from human immunoglobulins. In one embodiment of a humanized form of an antibody, some, most or all of the amino acids outside the CDR domains have been replaced with amino acids from human immunoglobulins, whereas some, most or all amino acids within one or more CDR regions are unchanged. Small additions, deletions, insertions, substitutions or modifications of amino acids are permissible as long as they do not abrogate the ability of the antibody to bind to a particular antigen. A "humanized" antibody retains an antigenic specificity similar to that of the original antibody.

**[0033]** A "chimeric antibody" refers to an antibody in which the variable regions are derived from one species and the constant regions are derived from another species, such as an antibody in which the variable regions are derived from a mouse antibody and the constant regions are derived from a human antibody.

**[0034]** An "anti-antigen" antibody refers to an antibody that binds specifically to the antigen. For example, an anti-PD-1 antibody binds specifically to PD-1 and an anti-CTLA-4 antibody binds specifically to CTLA-4.

**[0035]** An "antigen-binding portion" of an antibody (also called an "antigen-binding fragment") refers to one or more fragments of an antibody that retain the ability to bind specifically to the antigen bound by the whole antibody.

**[0036]** A "cancer" refers a broad group of various diseases characterized by the uncontrolled growth of abnormal cells in the body. Unregulated cell division and growth results in the formation of malignant tumors that invade neighboring tissues and may also metastasize to distant parts of the body through the lymphatic system or bloodstream. A "cancer" or "cancer tissue" can include a tumor.

**[0037]** "Cytotoxic T-Lymphocyte Antigen-4" (CTLA-4) refers to an immunoinhibitory receptor belonging to the CD28 family. CTLA-4 is expressed exclusively on T cells *in vivo*, and binds to two ligands, CD80 and CD86 (also called B7-1 and B7-2, respectively). The term "CTLA-4" as used herein includes human CTLA-4 (hCTLA-4), variants, isoforms, and species homologs of hCTLA-4, and analogs having at least one common epitope with hCTLA-4. The complete hCTLA-4 sequence can be found under GenBank Accession No. AAB59385.

**[0038]** The term “progression-free survival,” which can be abbreviated as PFS, as used herein refers to the length of time during and after the treatment of a solid tumor (*i.e.*, melanoma) that a patient lives with the disease but it does not get worse.

**[0039]** "Dosing interval," as used herein, means the amount of time that elapses between multiple doses of a formulation disclosed herein being administered to a subject. Dosing interval can thus be indicated as ranges.

**[0040]** The term "dosing frequency" as used herein refers to the frequency of administering doses of a formulation disclosed herein in a given time. Dosing frequency can be indicated as the number of doses per a given time, *e.g.*, once a week or once in two weeks.

**[0041]** The use of the term "fixed dose" with regard to a composition of the invention means that two or more different antibodies in a single composition are present in the composition in particular (fixed) ratios with each other. In some embodiments, the fixed dose is based on the weight (*e.g.*, mg) of the antibodies. In certain embodiments, the fixed dose is based on the concentration (*e.g.*, mg/ml) of the antibodies. In some embodiments, the ratio is at least about 1:1, about 1:2, about 1:3, about 1:4, about 1:5, about 1:6, about

1:7, about 1:8, about 1:9, about 1:10, about 1:15, about 1:20, about 1:30, about 1:40, about 1:50, about 1:60, about 1:70, about 1:80, about 1:90, about 1:100, about 1:120, about 1:140, about 1:160, about 1:180, about 1:200, about 200:1, about 180:1, about 160:1, about 140:1, about 120:1, about 100:1, about 90:1, about 80:1, about 70:1, about 60:1, about 50:1, about 40:1, about 30:1, about 20:1, about 15:1, about 10:1, about 9:1, about 8:1, about 7:1, about 6:1, about 5:1, about 4:1, about 3:1, or about 2:1 mg first antibody to mg second antibody. For example, the 3:1 ratio of a first antibody and a second antibody can mean that a vial can contain about 240 mg of the first antibody and 80 mg of the second antibody or about 3 mg/ml of the first antibody and 1 mg/ml of the second antibody.

**[0042]** The use of the term "flat dose" with regard to the composition of the invention means a dose that is administered to a patient without regard for the weight or body surface area (BSA) of the patient. The flat dose is therefore not provided as a mg/kg dose, but rather as an absolute amount of the agent (*e.g.,* the anti-CTLA-4 antibody and/or anti-PD-1 antibody). For example, a 60 kg person and a 100 kg person would receive the same dose of the composition (*e.g.*, 240 mg of an anti-PD-1 antibody and 80 mg of an anti-CTLA-4 antibody in a single fixed dosing formulation vial containing both 240 mg of an anti-PD-1 antibody and 80 mg of an anti-CTLA-4 antibody (or two fixed dosing formulation vials containing 120 mg of an anti-PD-1 antibody and 40 mg of an anti-CTLA-4 antibody, etc.)).

**[0043]** The term "weight based dose" as referred to herein means that a dose that is administered to a patient is calculated based on the weight of the patient. For example, when a patient with 60 kg body weight requires 3 mg/kg of an anti-PD-1 antibody in combination with 1 mg/kg of an anti-CTLA-4 antibody, one can draw the appropriate amounts of the anti-PD-1 antibody (*i.e.*, 180 mg) and the anti-CTLA-4 antibody (*i.e.*, 60 mg) at once from a 3:1 ratio fixed dosing formulation of an anti-PD1 antibody and an anti-CTLA-4 antibody.

**[0044]** The term "anti-PD-1 antibody monotherapy" as used herein includes a therapy of an anti-PD-1 antibody without an anti-CTLA-4 antibody therapy. The anti-PD-1 antibody monotherapy comprises, consists essentially of, or consists of administering one or more doses of an anti-PD-1 antibody to a patient in need thereof, but does not include

administering an anti-CTLA-4 antibody. In one embodiment, the anti-PD-1 antibody monotherapy comprises administering one or more doses of an anti-PD-1 antibody to a patient in need thereof, but does not include administering an anti-CTLA-4 antibody. In another embodiment, the anti-PD-1 antibody monotherapy comprises administering one or more doses of an anti-PD-1 antibody to a patient in need thereof, but does not include administering an antibody specifically targeting a protein other than PD-1. In other embodiments, the anti-PD-1 antibody monotherapy comprises administering one or more doses of an anti-PD-1 antibody to a patient in need thereof, but does not include administering another anti-cancer agent.

**[0045]** An "immune response" refers to the action of a cell of the immune system (for example, T lymphocytes, B lymphocytes, natural killer (NK) cells, macrophages, eosinophils, mast cells, dendritic cells and neutrophils) and soluble macromolecules produced by any of these cells or the liver (including antibodies, cytokines, and complement) that results in selective targeting, binding to, damage to, destruction of, and/or elimination from a vertebrate's body of invading pathogens, cells or tissues infected with pathogens, cancerous or other abnormal cells, or, in cases of autoimmunity or pathological inflammation, normal human cells or tissues.

**[0046]** "PD-L1 negative" or "PD-L1 expression negative," relating to cell surface PD-L1 expression, refers to the lack of a detectable amount of cell surface PD-L1. For cell surface expression assayed by IHC, e.g., with the mAb 28-8, a PD-L1 negative tumor or PD-L1 expression negative tumor means that less than 0.01% of cells express a detectable level of PD-L1. In some embodiments, a PD-L1 negative tumor or PD-L1 expression negative tumor means that zero (0) cells express a detectable level of PD-L1. In some embodiments, a PD-L1 negative or a PD-L1 expression negative tumor is any tumor other than a PD-L1 positive or a PD-L1 expression positive tumor.

**[0047]** The term "PD-L1 positive" or "PD-L1 expression positive," relating to cell surface PD-L1 expression, refers to the proportion of cells in a test tissue sample comprising tumor cells and tumor- infiltrating inflammatory cells above which the sample is scored as expressing cell surface PD-L1. For cell surface expression assayed by immunohistochemistry (IHC), e.g., with the mAb 28- 8, the PD-L1 positive tumor or PD-L1 expression positive tumor means that at least about 0.01%, at least about 0.5%, at

least about 1%, at least about 2%, at least about 3%, at least about 4%, at least about 5%, at least about 6%, at least about 7%, at least about 8%, at least about 9%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, or at least about 30% of the total number of cells express PD-L1. PD-L1 positive tumor or PD-L1 expression positive tumor can also be expressed herein as tumor expressing PD-L1. In other embodiments, the PD-L1 positive tumor or PD-L1 expression positive tumor means that at least about 0.1% to at least about 20% of the total number of cells express PD-L1. In certain embodiments, the PD-L1 positive tumor or PD-L1 expression positive tumor means that at least about 0.1% to at least about 10% of the total number of cells express PD-L1. In some embodiments, the PD-L1 positive or PD-L1 expression positive tumor means that at least about 1% of the total number of cells express PD-L1 on the cell surface. In other embodiments, the PD-L1 positive or PD-L1 expression positive tumor means that at least about 5% of the total number of cells express PD-L1 on the cell surface. In one particular embodiment, PD-L1 positive or PD-L1 expression positive tumor means that at least about 1%, or in the range of 1- 5% of the total number of cells express PD-L1 on the cell surface.

**[0048]** "Programmed Death-1 (PD-1)" refers to an immunoinhibitory receptor belonging to the CD28 family. PD-1 is expressed predominantly on previously activated T cells *in vivo*, and binds to two ligands, PD-L1 and PD-L2. The term "PD-1" as used herein includes human PD-1 (hPD-1), variants, isoforms, and species homologs of hPD-1, and analogs having at least one common epitope with hPD-1. The complete hPD-1 sequence can be found under GenBank Accession No. U64863.

**[0049]** "Programmed Death Ligand-1 (PD-L1)" is one of two cell surface glycoprotein ligands for PD-1 (the other being PD-L2) that down-regulate T cell activation and cytokine secretion upon binding to PD-1. The term "PD-L1" as used herein includes human PD-L1 (hPD-L1), variants, isoforms, and species homologs of hPD-L1, and analogs having at least one common epitope with hPD-L1. The complete hPD-L1 sequence can be found under GenBank Accession No. Q9NZQ7.

**[0050]** A "patient" as used herein includes any patient who is afflicted with a cancer (*e.g.*, melanoma). The terms "subject" and "patient" are used interchangeably herein.

**[0051]** A "therapeutically effective amount" or "therapeutically effective dosage" of a drug or therapeutic agent is any amount of the drug that, when used alone or in combination with another therapeutic agent, protects a subject against the onset of a disease or promotes disease regression evidenced by a decrease in severity of disease symptoms, an increase in frequency and duration of disease symptom-free periods, or a prevention of impairment or disability due to the disease affliction. The ability of a therapeutic agent to promote disease regression can be evaluated using a variety of methods known to the skilled practitioner, such as in human subjects during clinical trials, in animal model systems predictive of efficacy in humans, or by assaying the activity of the agent in *in vitro* assays.

**[0052]** "Treatment" or "therapy" of a subject refers to any type of intervention or process performed on, or the administration of an active agent to, the subject with the objective of reversing, alleviating, ameliorating, inhibiting, slowing down or preventing the onset, progression, development, severity or recurrence of a symptom, complication or condition, or biochemical indicia associated with a disease.

**[0053]** A "tumor-infiltrating inflammatory cell" is any type of cell that typically participates in an inflammatory response in a subject and which infiltrates tumor tissue. Such cells include tumor-infiltrating lymphocytes (TILs), macrophages, monocytes, eosinophils, histiocytes and dendritic cells.

**[0054]** The use of the alternative (*e.g.*, "or") should be understood to mean either one, both, or any combination thereof of the alternatives. As used herein, the indefinite articles "a" or "an" should be understood to refer to "one or more" of any recited or enumerated component.

**[0055]** The terms "about" or "comprising essentially of" refer to a value or composition that is within an acceptable error range for the particular value or composition as determined by one of ordinary skill in the art, which will depend in part on how the value or composition is measured or determined, *i.e.*, the limitations of the measurement system. For example, "about" or "comprising essentially of" can mean within 1 or more than 1 standard deviation per the practice in the art. Alternatively, "about" or "comprising essentially of" can mean a range of up to 10% or 20% (*i.e.,* ±10% or ±20%). For example, about 3mg can include any number between 2.7 mg and 3.3 mg (for 10%) or

between 2.4 mg and 3.6 mg (for 20%). Furthermore, particularly with respect to biological systems or processes, the terms can mean up to an order of magnitude or up to 5-fold of a value. When particular values or compositions are provided in the application and claims, unless otherwise stated, the meaning of "about" or "comprising essentially of" should be assumed to be within an acceptable error range for that particular value or composition.

**[0056]** The terms "about once a week," "once about every week," "once about every two weeks," or any other similar dosing interval terms as used herein means approximate number, and "about once a week" or "once about every week" can include every seven days ± two days, *i.e.,* every five days to every nine days. The dosing frequency of "once a week" thus can be every five days, every six days, every seven days, every eight days, or every nine days. "Once about every two weeks" can include every fourteen days ± three days, *i.e.*, every eleven days to every seventeen days. Similar approximations apply, for example, to once about every three weeks, once about every four weeks, once about every five weeks, once about every six weeks and once about every twelve weeks. In some embodiments, a dosing interval of once about every six weeks or once about every twelve weeks means that the first dose can be administered any day in the first week, and then the next dose can be administered any day in the sixth or twelfth week, respectively. In other embodiments, a dosing interval of once about every six weeks or once about every twelve weeks means that the first dose is administered on a particular day of the first week (e.g., Monday) and then the next dose is administered on the same day of the sixth or twelfth weeks (i.e., Monday), respectively.

**[0057]** As described herein, any concentration range, percentage range, ratio range or integer range is to be understood to include the value of any integer within the recited range and, when appropriate, fractions thereof (such as one-tenth and one-hundredth of an integer), unless otherwise indicated.

**[0058]** Various aspects of the invention are described in further detail in the following subsections.

*Methods of the Invention*

**[0059]** The present invention is directed to a method for treating a PD-L1-negative melanoma in a subject in need thereof. The present invention shows that for PD-L1 negative tumors, a combination therapy of an anti-PD-1 antibody and an anti-CTLA-4 antibody is more suitable than a monotherapy of either an anti-PD-1 antibody or an anti-CTLA-4 antibody.

**[0060]** Not bound to any theory, the present invention identifies that in patients having a PD-L1-negative tumor progression-free survival and overall response rate are higher following treatment with a combination therapy with an anti-PD-1 antibody and an anti-CTLA-4 antibody than following treatment with either an anti-PD-1 antibody or an anti-CTLA-4 antibody alone. Therefore, in order to increase the response of a patient having a PD-L1-negative tumor, the present invention provides identifying a patient suitable for the combination therapy of an anti-PD-1 antibody and an anti-CTLA-4 antibody.

**[0061]** In one embodiment, the invention includes a method of treating a PD-L1 negative melanoma comprising administering to a patient: (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4, wherein the patient is identified as having a PD-L1-negative melanoma tumor prior to the administration. In another embodiment, the invention includes a method for treating a melanoma in a patient in need thereof comprising: (i) identifying a patient having a PD-L1-negative melanoma tumor; and (ii) administering to the patient: (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4.

**[0062]** In certain embodiments, the invention includes a method for extending a progression-free survival period for over 9 months in a patient afflicted with a PD-L1 negative melanoma tumor comprising administering to the patient: (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4, wherein the patient is identified as having a PD-L1-negative melanoma tumor prior to the administration and wherein the patient

demonstrates progression-free survival for over 9 months. In other embodiments, the invention provides a method for extending a progression-free survival period for over 9 months in a patient afflicted with a melanoma tumor comprising: (i) identifying a patient having a PD-L1-negative melanoma tumor; and (ii) administering to the patient (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4, wherein the patient demonstrates progression-free survival for over 9 months. According to the invention, the progression-free survival of the patient can be extended, after the administration, over about 10 months, over about 11 months, over about 12 months, over about 13 months, about 14 months, about 15 months, about 16 months, about 17 months, about 18 months, about 2 years, about 3 years, about 4 years, about 5 years, about 6 years, about 7 years, about 8 years, about 9 years, or about 10 years. In a particular embodiment, the progression-free survival of the patient is extended for over 10 months.

**[0063]** In still other embodiments, the invention is directed to a method for reducing a tumor size at least by 10% in a patient afflicted with a PD-L1 negative melanoma tumor comprising administering to the patient: (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4, wherein the patient is identified as having a PD-L1-negative melanoma tumor prior to the administration and wherein the administration reduces the tumor size at least about 10%, about 20%, about 30%, about 40%, or about 50% compared to the tumor size prior to the administration. In yet other embodiments, the method comprises (i) identifying a patient having a PD-L1-negative melanoma tumor; and (ii) administering to the patient (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4, wherein the administration reduces the tumor size at least about 10%, about 20%, about 30%, about 40%, or about 50% compared to the tumor size prior to the administration. The tumor size, after the administration, can be reduced at least about 60%, 70%, 80%, 90% or 100%. The tumor can be completely eliminated from the patient's body after the administration.

**[0064]** The invention can also include a method of preventing a relapse and/or induce a remission to a patient having a PD-L1 negative melanoma tumor comprising administering to the patient: (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4, wherein the patient is identified as having a PD-L1-negative melanoma tumor prior to the administration. In some embodiments, the method of the invention comprises (i) identifying a patient having a PD-L1-negative melanoma tumor; and (ii) administering to the patient (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4.

**[0065]** In certain embodiments, the invention includes a method for increasing an objective response rate to be higher than 40% in a patient population, wherein each patient of the patient population is afflicted with a melanoma tumor, in a cancer treatment comprising administering to the patient: (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4, wherein each patient is identified as having a PD-L1-negative melanoma tumor prior to the administration and wherein the objective response rate is higher than 40%, 45%, 50%, 55%, 60%, 65%, 70%, or 75%. The methods can further comprise identifying each patient as having a PD-L1-negative melanoma tumor prior to the administration. In other embodiments, each patient in the methods can further be characterized by (i) extended progression-free survival for over 11 months, (ii) tumor size reduction at least about 10%, about 20%, about 30%, about 40%, or about 50% compared to the tumor size prior to the administration, or (iii) both. In some embodiments, the patient population can be at least 100 patients having a PD-L1-negative melanoma tumor. In some embodiments, the patient population can be at least 200, 300, 400, 500, 600, 700, 800, 900, or 1000 patients having a PD-L1-negative melanoma tumor.

**[0066]** In further embodiments, the invention provides a method for selecting a suitable cancer therapy course in a patient having a PD-L1 negative melanoma tumor comprising: (i) identifying a patient having a PD-L1-negative melanoma tumor; and (ii) instructing a

healthcare provider to administer to the patient: (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4. The method further comprises administering the antibodies to the patient.

**[0067]** The methods of the invention as a result of the combination therapy can treat the melanoma tumor, reduce the tumor size, prevent growth of the tumor, eliminate the tumor from the patient, prevent a relapse of a tumor, induce a remission in a patient, or any combination thereof. In certain embodiments, the combination therapy induces a complete response. In other embodiments, the combination therapy induces a partial response.

*Melanoma*

**[0068]** Melanoma (MEL) is a malignant tumor of melanocytes, the melanin-producing cells found predominantly in skin. Though less common than other skin cancers, it is the most dangerous of skin cancers if not diagnosed early and causes the majority (75%) of skin cancer deaths. The incidence of MEL is increasing worldwide in Caucasian populations, especially where peoples with low amounts of skin pigmentation receive excessive ultraviolet light exposure from the sun. In Europe, the incidence rate is < 10-20 per 100,000 population; in the USA 20-30 per 100,000; and in Australia, where the highest incidence is observed, 50-60 per 100,000 (Garbe *et al.*, *Eur. J. Cancer. 48(15)*:2375-90 (2012)). MEL accounts for about 5% of all new cases of cancer in the United States (U.S.), and the incidence continues to rise by almost 3% per year. This translates to an estimated 76,690 new cases in the U.S. in 2013 with 9,480 associated deaths (Siegel *et al.*, *CA Cancer J. Clin. 63(1)*:11-30 (2013)).

**[0069]** For in situ (stage 0) or early-stage MEL (Stages I-II), surgical excision is the primary treatment. In general, the prognosis is excellent for patients with localized disease and tumors 1.0 mm or less in thickness, with 5-year survival rates of more than 90% (NCCN GUIDELINES®, 2013- Melanoma). Where surgical excision is not feasible for in situ melanoma due to comorbidity or cosmetically sensitive tumor location, topical imiquimod (INN) and radiotherapy are emerging as treatments, especially for *lentigo maligna*. Chemotherapeutic agents for treating MEL include dacarbazine, temozolomide and imatinib for melanoma with a c-KIT mutation, high-dose interleukin-2, and

paclitaxel with or without carboplatin. However, these treatments have modest success, with response rates below 20% in first-line (1L) and second-line (2L) settings.

**[0070]** For patients with localized melanomas more than 1.0 mm in thickness, survival rates range from 50-90%. The likelihood of regional nodal involvement increases with increasing tumor thickness. With Stage III MEL (clinically positive nodes and/or in-transit disease), 5-year survival rates range from 20-70%. By far the most lethal is Stage IV MEL where long-term survival in patients with distant metastatic melanoma is less than 10% (NCCN GUIDELINES®, 2013 - melanoma).

**[0071]** The types of melanoma that can be treated with the present methods include, but are not limited to, lentigo maligna, lentigo maligna melanoma, superficial spreading melanoma, acral lentiginous melanoma, nucosal melanoma, nodular melanoma, polypoid melanoma, desmoplastic melanoma, amelanotic melanoma, soft-tissue melanoma, melanoma with small nevus-like cells, melanoma with features of a Spitz nevus, or uveal melanoma. The stages of melanoma that can be treated with the present methods include, but are not limited to, (i) Stage I / II (invasive melanoma): T1a characterized by less than 1.0 mm primary tumor thickness, without ulceration, and mitosis $< 1/mm^2$; T1b characterized by less than 1.0 mm primary tumor thickness, with ulceration or mitoses $\geq 1/mm^2$; T2a characterized by 1.01–2.0 mm primary tumor thickness, without ulceration; (ii) Stage II (high risk melanoma): T2b characterized by 1.01–2.0 mm primary tumor thickness, with ulceration; T3a characterized by 2.01–4.0 mm primary tumor thickness, without ulceration; T3b characterized by 2.01–4.0 mm primary tumor thickness, with ulceration; T4a characterized by greater than 4.0 mm primary tumor thickness, without ulceration; or T4b characterized by greater than 4.0 mm primary tumor thickness, with ulceration; (iii) Stage III (regional metastasis): N1 characterized by single positive lymph node; N2 characterized by two to three positive lymph nodes or regional skin/in-transit metastasis; or N3 characterized by four positive lymph nodes or one lymph node and regional skin/in-transit metastases; and (iv) Stage IV (distant metastasis): M1a characterized by distant skin metastasis, normal LDH; M1b characterized by Lung metastasis, normal LDH; or M1c characterized by other distant metastasis or any distant metastasis with elevated LDH. PD-L1-negative tumors that are treatable by the present

methods can lack PD-L1 expression on the surface of tumor cells and/or tumor infiltrating inflammatory cells.

*Measurement of PD-L1 expression*

**[0072]** In certain embodiments, identifying a patient suitable for a combination therapy for the present methods includes measuring or assessing a PD-L1 expression on the surface of the melanoma tumor cells or tumor infiltrating inflammatory cells. The phrases "tumors expressing PD-L1," "PD-L1 expressing tumor," "PD-L1 positive tumor," and "PD-L1 expression positive tumor" are used interchangeably herein. The meaning of the phrases is provided elsewhere herein. The methods of measuring or assessing the PD-L1 expression can be achieved by any methods applicable.

**[0073]** In order to assess the PD-L1 expression, in one embodiment, a test tissue sample is obtained from the patient who is in need of the therapy. In another embodiment, the assessment of PD-L1 expression can be achieved without obtaining a test tissue sample. In some embodiments, selecting a suitable patient includes (i) optionally providing a test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor cells and/or tumor-infiltrating inflammatory cells; and (ii) assessing the proportion of cells in the test tissue sample that express PD-L1 on the surface of the cells based on an assessment that the proportion of cells in the test tissue sample that express PD-L1 on the cell surface is lower than a predetermined threshold level. A test tissue sample can be considered to be PD-L1-negative if less than about 5% of the cells in the test tissue express PD-L1 on the cell surface. In other embodiments, a test tissue sample is considered to be PD-L1-negative if less than about 4%, less than about 3%, less than about 2%, less than about 1%, less than about 0.5%, less than about 0.1%, less than about 0.01% or 0% of the cells in the test tissue express PD-L1 on the cell surface. In one particular example, the test tissue sample can be considered to be PD-L1-negative if less than about 5% of the cells in the test tissue express PD-L1 on the cell surface.

**[0074]** In any of the methods comprising the measurement of PD-L1 expression in a test tissue sample, however, it should be understood that the step comprising the provision of a test tissue sample obtained from a patient is an optional step. That is, in certain embodiments the method includes this step, and in other embodiments, this step is not included in the method. It should also be understood that in certain embodiments the

"measuring" or "assessing" step to identify, or determine the number or proportion of, cells in the test tissue sample that express PD-L1 on the cell surface is performed by a transformative method of assaying for PD-L1 expression, for example by performing a reverse transcriptase-polymerase chain reaction (RT-PCR) assay or an IHC assay. In certain other embodiments, no transformative step is involved and PD-L1 expression is assessed by, for example, reviewing a report of test results from a laboratory. In certain embodiments, the steps of the methods up to, and including, assessing PD-L1 expression provides an intermediate result that may be provided to a physician or other healthcare provider for use in selecting a suitable candidate for the combination therapy of an anti-PD-1 antibody and an anti-CTLA-4 antibody. In certain embodiments, the steps that provide the intermediate result is performed by a medical practitioner or someone acting under the direction of a medical practitioner. In other embodiments, these steps are performed by an independent laboratory or by an independent person such as a laboratory technician.

**[0075]** In certain embodiments of any of the present methods, the proportion of cells that express PD-L1 is assessed by performing an assay to determine the presence of PD-L1 RNA. In further embodiments, the presence of PD-L1 RNA is determined by RT-PCR, *in situ* hybridization or RNase protection. In other embodiments, the proportion of cells that express PD-L1 is assessed by performing an assay to determine the presence of PD-L1 polypeptide. In further embodiments, the presence of PD-L1 polypeptide is determined by immunohistochemistry (IHC), enzyme-linked immunosorbent assay (ELISA), *in vivo* imaging, or flow cytometry. In some embodiments, PD-L1 expression is assayed by IHC. In other embodiments of all of these methods, cell surface expression of PD-L1 is assayed using, *e.g.*, IHC or *in vivo* imaging.

**[0076]** Imaging techniques have provided important tools in cancer research and treatment. Recent developments in molecular imaging systems, including positron emission tomography (PET), single-photon emission computed tomography (SPECT), fluorescence reflectance imaging (FRI), fluorescence-mediated tomography (FMT), bioluminescence imaging (BLI), laser-scanning confocal microscopy (LSCM) and multiphoton microscopy (MPM), will likely herald even greater use of these techniques in cancer research. Some of these molecular imaging systems allow clinicians to not only

see where a tumor is located in the body, but also to visualize the expression and activity of specific molecules, cells, and biological processes that influence tumor behavior and/or responsiveness to therapeutic drugs (Condeelis and Weissleder, *Cold Spring Harb. Perspect. Biol. 2(12)*:a003848 (2010)). Antibody specificity, coupled with the sensitivity and resolution of PET, makes immunoPET imaging particularly attractive for monitoring and assaying expression of antigens in tissue samples (McCabe and Wu, *Cancer Biother. Radiopharm. 25(3)*:253-61 (2010); Olafsen *et al.*, *Protein Eng. Des. Sel. 23(4)*:243-9 (2010)). In certain embodiments of any of the present methods, PD-L1 expression is assayed by immunoPET imaging. In certain embodiments of any of the present methods, the proportion of cells in a test tissue sample that express PD-L1 is assessed by performing an assay to determine the presence of PD-L1 polypeptide on the surface of cells in the test tissue sample. In certain embodiments, the test tissue sample is a FFPE tissue sample. In other embodiments, the presence of PD-L1 polypeptide is determined by IHC assay. In further embodiments, the IHC assay is performed using an automated process. In some embodiments, the IHC assay is performed using an anti-PD-L1 mAb to bind to the PD-L1 polypeptide, if present.

*Assaying Cell-Surface PD-L1 Expression by Automated IHC*

**[0077]** In one embodiment of the present methods, an automated IHC method is used to assay the expression of PD-L1 on the surface of cells in FFPE tissue specimens. This disclosure provides methods for detecting the presence of human PD-L1 antigen in a test tissue sample, or quantifying the level of human PD-L1 antigen or the proportion of cells in the sample that express the antigen, which methods comprise contacting the test sample, and a negative control sample, with a mAb that specifically binds to human PD-L1, under conditions that allow for formation of a complex between the antibody or portion thereof and human PD-L1. In certain embodiments, the test and control tissue samples are FFPE samples. The formation of a complex can then be detected, wherein a difference in complex formation between the test sample and the negative control sample is indicative of the presence of human PD-L1 antigen in the sample. Various methods are used to quantify PD-L1 expression.

**[0078]** In a particular embodiment, the automated IHC method comprises: (a) deparaffinizing and rehydrating mounted tissue sections in an autostainer; (b) retrieving

antigen using a decloaking chamber and pH 6 buffer, heated to 110°C for 10 min; (c) setting up reagents on an autostainer; and (d) running the autostainer to include steps of neutralizing endogenous peroxidase in the tissue specimen; blocking non-specific protein-binding sites on the slides; incubating the slides with primary Ab; incubating with a postprimary blocking agent; incubating with NovoLink Polymer; adding a chromogen substrate and developing; and counterstaining with hematoxylin.

**[0079]** For assessing PD-L1 expression in tumor tissue samples, a pathologist examines the number of membrane PD-L1$^+$ tumor cells in each field under a microscope and mentally estimates the percentage of cells that are positive, then averages them to come to the final percentage. The different staining intensities are defined as 0/negative, 1+/weak, 2+/moderate, and 3+/strong. Typically, percentage values are first assigned to the 0 and 3+ buckets, and then the intermediate 1+ and 2+ intensities are considered. For highly heterogeneous tissues, the specimen is divided into zones, and each zone is scored separately and then combined into a single set of percentage values. The percentages of negative and positive cells for the different staining intensities are determined from each area and a median value is given to each zone. A final percentage value is given to the tissue for each staining intensity category: negative, 1+, 2+, and 3+. The sum of all staining intensities needs to be 100%.

**[0080]** Staining is also assessed in tumor-infiltrating inflammatory cells such as macrophages and lymphocytes. In most cases macrophages serve as an internal positive control since staining is observed in a large proportion of macrophages. While not required to stain with 3+ intensity, an absence of staining of macrophages should be taken into account to rule out any technical failure. Macrophages and lymphocytes are assessed for plasma membrane staining and only recorded for all samples as being positive or negative for each cell category. Staining is also characterized according to an outside/inside tumor immune cell designation. "Inside" means the immune cell is within the tumor tissue and/or on the boundaries of the tumor region without being physically intercalated among the tumor cells. "Outside" means that there is no physical association with the tumor, the immune cells being found in the periphery associated with connective or any associated adjacent tissue.

**[0081]** In certain embodiments of these scoring methods, the samples are scored by two pathologists operating independently, and the scores are subsequently consolidated. In certain other embodiments, the identification of positive and negative cells is scored using appropriate software.

**[0082]** A histoscore is used as a more quantitative measure of the IHC data. The histoscore is calculated as follows:

Histoscore = [(% tumor x 1 (low intensity)) + (% tumor x 2 (medium intensity)) + (% tumor x 3 (high intensity)]

**[0083]** To determine the histoscore, the pathologist estimates the percentage of stained cells in each intensity category within a specimen. Because expression of most biomarkers is heterogeneous the histoscore is a truer representation of the overall expression. The final histoscore range is 0 (no expression) to 300 (maximum expression). A test sample can be designated as PD-L1-negative even though some level of PD-L1 expression is detected. For example, a designation of PD-L1-negative can be assigned to a specimen having a final histoscore of about 15 or less, of about 10 or less, about 9 or less, about 8 or less, about 7 or less, about 6 or less, about 5 or less, about 4 or less, about 3 or less, about 2 or less, about 1 or less, or of 0.

**[0084]** An alternative means of quantifying PD-L1 expression in a test tissue sample IHC is to determine the adjusted inflammation score (AIS) score defined as the density of inflammation multiplied by the percent PD-L1 expression by tumor-infiltrating inflammatory cells (Taube *et al.*, *Sci. Transl. Med. 4(127)*:127ra37 (2012)).

*Anti-PD-1 antibodies*

**[0085]** PD-1 is a key immune checkpoint receptor expressed by activated T and B cells and mediates immunosuppression. PD-1 is a member of the CD28 family of receptors, which includes CD28, CTLA-4, ICOS, PD-1, and BTLA. Two cell surface glycoprotein ligands for PD-1 have been identified, Programmed Death Ligand-1 (PD-L1) and Programmed Death Ligand-2 (PD-L2), that are expressed on antigen-presenting cells as well as many human cancers and have been shown to down regulate T cell activation and cytokine secretion upon binding to PD-1. Inhibition of the PD-1/PD-L1 interaction mediates potent antitumor activity in preclinical models.

**[0086]** HuMAbs that bind specifically to PD-1 with high affinity have been disclosed in U.S. Patent Nos. 8,008,449 and 8,779,105. Other anti-PD-1 mAbs have been described in, for example, U.S. Patent Nos. 6,808,710, 7,488,802, 8,168,757 and 8,354,509, and PCT Publication No. WO 2012/145493. Each of the anti-PD-1 HuMAbs disclosed in U.S. Patent No. 8,008,449 has been demonstrated to exhibit one or more of the following characteristics: (a) binds to human PD-1 with a $K_D$ of 1 x $10^{-7}$ M or less, as determined by surface plasmon resonance using a Biacore biosensor system; (b) does not substantially bind to human CD28, CTLA-4 or ICOS; (c) increases T-cell proliferation in a Mixed Lymphocyte Reaction (MLR) assay; (d) increases interferon-γ production in an MLR assay; (e) increases IL-2 secretion in an MLR assay; (f) binds to human PD-1 and cynomolgus monkey PD-1; (g) inhibits the binding of PD-L1 and/or PD-L2 to PD-1; (h) stimulates antigen-specific memory responses; (i) stimulates antibody responses; and (j) inhibits tumor cell growth *in vivo*. Anti-PD-1 antibodies useful for the present invention include mAbs that bind specifically to human PD-1 and exhibit at least one, at least two, at least three, at least four, or at least five, of the preceding characteristics.

**[0087]** In one embodiment, the anti-PD-1 antibody is nivolumab. Nivolumab (also known as "OPDIVO®"; formerly designated 5C4, BMS-936558, MDX-1106, or ONO-4538) is a fully human IgG4 (S228P) PD-1 immune checkpoint inhibitor antibody that selectively prevents interaction with PD-1 ligands (PD-L1 and PD-L2), thereby blocking the down-regulation of antitumor T-cell functions (U.S. Patent No. 8,008,449; Wang *et al.*, *Cancer Immunol Res*. 2(9):846-56 (2014)). In another embodiment, the anti-PD-1 antibody or fragment thereof cross-competes with nivolumab. In other embodiments, the anti-PD-1 antibody or fragment thereof binds to the same epitope as nivolumab. In certain embodiments, the anti-PD-1 antibody has the same CDRs as nivolumab.

**[0088]** In another embodiment, the anti-PD-1 antibody or fragment thereof cross-competes with pembrolizumab. In some embodiments, the anti-PD-1 antibody or fragment thereof binds to the same epitope as pembrolizumab. In certain embodiments, the anti-PD-1 antibody has the same CDRs as pembrolizumab. In another embodiment, the anti-PD-1 antibody is pembrolizumab. Pembrolizumab (also known as "KEYTRUDA®", lambrolizumab, and MK-3475) is a humanized monoclonal IgG4 antibody directed against human cell surface receptor PD-1 (programmed death-1 or

programmed cell death-1). Pembrolizumab is described, for example, in U.S. Patent Nos. 8,354,509 and 8,900,587; *see also* http://www.cancer.gov/drugdictionary?cdrid=695789 (last accessed: December 14, 2014). Pembrolizumab has been approved by the FDA for the treatment of relapsed or refractory melanoma.

**[0089]** In other embodiments, the anti-PD-1 antibody or fragment thereof cross-competes with MEDI0608. In still other embodiments, the anti-PD-1 antibody or fragment thereof binds to the same epitope as MEDI0608. In certain embodiments, the anti-PD-1 antibody has the same CDRs as MEDI0608. In other embodiments, the anti-PD-1 antibody is MEDI0608 (formerly AMP-514), which is a monoclonal antibody. MEDI0608 is described, for example, in US Pat. No. 8,609,089B2 or in http://www.cancer.gov/drugdictionary?cdrid=756047 (last accessed December 14, 2014).

**[0090]** In certain embodiments, the first antibody is an anti-PD-1 antagonist. One example of the anti-PD-1 antagonist is AMP-224, which is a B7-DC Fc fusion protein. AMP-224 is discussed in U.S. Publ. No. 2013/0017199 or in http://www.cancer.gov/publications/dictionaries/cancer-drug?cdrid=700595 (last accessed July 8, 2015).

**[0091]** In other embodiments, the anti-PD-1 antibody or fragment thereof cross-competes with BGB-A317. In some embodiments, the anti-PD-1 antibody or fragment thereof binds the same epitope as BGB-A317. In certain embodiments, the anti-PD-1 antibody has the same CDRs as BGB-A317. In certain embodiments, the anti-PD-1 antibody is BGB-A317, which is a humanized monoclonal antibody. BGB-A317 is described in U.S. Publ. No. 2015/0079109.

**[0092]** In some embodiments, the antibody is Pidilizumab (CT-011), which is an antibody previously reported to bind to PD-1 but which is believed to bind to a different target. Pidilizumab is described in US Pat. No. 8,686,119 B2 or WO 2013/014668 A1.

**[0093]** Anti-PD-1 antibodies useful for the disclosed compositions also include isolated antibodies that bind specifically to human PD-1 and cross-compete for binding to human PD-1 with nivolumab (*see, e.g.*, U.S. Patent Nos. 8,008,449 and 8,779,105; WO 2013/173223). The ability of antibodies to cross-compete for binding to an antigen indicates that these antibodies bind to the same epitope region of the antigen and sterically hinder the binding of other cross-competing antibodies to that particular epitope

region. These cross-competing antibodies are expected to have functional properties very similar to those of nivolumab by virtue of their binding to the same epitope region of PD-1. Cross-competing antibodies can be readily identified based on their ability to cross-compete with nivolumab in standard PD-1 binding assays such as Biacore analysis, ELISA assays or flow cytometry (*see, e.g.*, WO 2013/173223).

**[0094]** In certain embodiments, the antibodies that cross-compete for binding to human PD-1 with, or bind to the same epitope region of human PD-1 as, nivolumab are mAbs. For administration to human subjects, these cross-competing antibodies can be chimeric antibodies, or humanized or human antibodies. Such chimeric, humanized or human mAbs can be prepared and isolated by methods well known in the art.

**[0095]** Anti-PD-1 antibodies useful for the compositions of the disclosed invention also include antigen-binding portions of the above antibodies. It has been amply demonstrated that the antigen-binding function of an antibody can be performed by fragments of a full-length antibody. Examples of binding fragments encompassed within the term "antigen-binding portion" of an antibody include (i) a Fab fragment, a monovalent fragment consisting of the $V_L$, $V_H$, $C_L$ and $C_{H1}$ domains; (ii) a $F(ab')_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the $V_H$ and $C_{H1}$ domains; and (iv) a Fv fragment consisting of the $V_L$ and $V_H$ domains of a single arm of an antibody.

**[0096]** Anti-PD-1 antibodies suitable for use in the disclosed compositions are antibodies that bind to PD-1 with high specificity and affinity, block the binding of PD-L1 and or PD-L2, and inhibit the immunosuppressive effect of the PD-1 signaling pathway. In any of the compositions or methods disclosed herein, an anti-PD-1 "antibody" includes an antigen-binding portion or fragment that binds to the PD-1 receptor and exhibits the functional properties similar to those of whole antibodies in inhibiting ligand binding and upregulating the immune system. In certain embodiments, the anti-PD-1 antibody or antigen-binding portion thereof cross-competes with nivolumab for binding to human PD-1. In other embodiments, the anti-PD-1 antibody or antigen-binding portion thereof is a chimeric, humanized or human monoclonal antibody or a portion thereof. In certain embodiments, the antibody is a humanized antibody. In other embodiments, the antibody is a human antibody. Antibodies of an IgG1, IgG2, IgG3 or IgG4 isotype can be used.

**[0097]** In certain embodiments, the anti-PD-1 antibody or antigen-binding portion thereof comprises a heavy chain constant region which is of a human IgG1 or IgG4 isotype. In certain other embodiments, the sequence of the IgG4 heavy chain constant region of the anti-PD-1 antibody or antigen-binding portion thereof contains an S228P mutation which replaces a serine residue in the hinge region with the proline residue normally found at the corresponding position in IgG1 isotype antibodies. This mutation, which is present in nivolumab, prevents Fab arm exchange with endogenous IgG4 antibodies, while retaining the low affinity for activating Fc receptors associated with wild-type IgG4 antibodies (Wang *et al.*, *Cancer Immunol Res.* 2(9):846-56 (2014)). In yet other embodiments, the antibody comprises a light chain constant region which is a human kappa or lambda constant region. In other embodiments, the anti-PD-1 antibody or antigen-binding portion thereof is a mAb or an antigen-binding portion thereof. In certain embodiments of any of the therapeutic methods described herein comprising administration of an anti-PD-1 antibody, the anti-PD-1 antibody is nivolumab. In other embodiments, the anti-PD-1 antibody is pembrolizumab. In other embodiments, the anti-PD-1 antibody is chosen from the human antibodies 17D8, 2D3, 4H1, 4A11, 7D3 and 5F4 described in U.S. Patent No. 8,008,449. In still other embodiments, the anti-PD-1 antibody is MEDI0608 (formerly AMP-514), AMP-224, or BGB-A317.

**[0098]** Because anti-PD-1 and anti-PD-L1 target the same signaling pathway and have been shown in clinical trials to exhibit similar levels of efficacy in a variety of cancers, including RCC (*see* Brahmer *et al.* (2012) *N Engl J Med* 366:2455-65; Topalian *et al.* (2012a) *N Engl J Med* 366:2443-54; WO 2013/173223), an anti-PD-L1 antibody may be substituted for the anti-PD-1 Ab in any of the therapeutic methods disclosed herein. In certain embodiments, the anti-PD-L1 antibody is BMS-936559 (formerly 12A4 or MDX-1105) (*see, e.g.*, U.S. Patent No. 7,943,743; WO 2013/173223). In other embodiments, the anti-PD-L1 antibody is MPDL3280A (also known as RG7446) (*see, e.g.*, Herbst et al. (2013) *J Clin Oncol* 31(suppl):3000. Abstract; U.S. Patent No. 8,217,149) or MEDI4736 (Khleif (2013) In: Proceedings from the European Cancer Congress 2013; September 27-October 1, 2013; Amsterdam, The Netherlands. Abstract 802). In certain embodiments, the antibodies that cross-compete for binding to human PD-L1 with, or bind to the same epitope region of human PD-L1 as the above-references PD-L1 antibodies are mAbs. For

administration to human subjects, these cross-competing antibodies can be chimeric antibodies, or can be humanized or human antibodies. Such chimeric, humanized or human mAbs can be prepared and isolated by methods well known in the art.

*Anti-PD-L1 Antibodies*

**[0099]** In certain embodiments, the present application encompasses use of an anti-PD-L1 antibody in lieu of anti-PD-1 antibody. In one embodiment, the anti-PD-L1 antibody inhibits the binding of PD-L1 receptor, i.e., PD-1, with its ligand PD-L1.

**[0100]** Anti-PD-L1 antibodies useful for the invention include antibodies engineered starting from antibodies having one or more of the $V_H$ and/or $V_L$ sequences disclosed herein, which engineered antibodies can have altered properties from the starting antibodies. An anti-PD-L1 antibody can be engineered by a variety of modifications as described above for the engineering of modified anti-PD-1 antibodies of the invention.

**[0101]** Anti-PD-L1 antibodies of the invention also include isolated antibodies selected for their ability to bind to PD-L1 in formalin-fixed, paraffin-embedded (FFPE) tissue specimens. The use of FFPE samples is essential for the long-term follow-up analysis of the correlation between PD-L1 expression in tumors and disease prognosis or progression. The use of different antibodies to stain PD-L1 in frozen versus FFPE tissues, and the ability of certain antibodies to distinguish membranous and/or cytoplasmic forms of PD-Ll, may account for some of the disparate data reported in the literature correlating PD-Ll expression with disease prognosis (Hamanishi *et al.*, *Proc. Natl. Acad. Sci. USA 104(9)*:3360-3365 (2007); Gadiot *et al.*, *Cancer 117(10)*:2192-2201 (2011)). This disclosure provides several rabbit mAbs that bind with high affinity specifically to membranous human PD-Ll in FFPE tissue samples comprising tumor cells and tumor-infiltrating inflammatory cells.

**[0102]** In some embodiments, an anti-PD-L1 antibody useful for the present methods includes mAb 28-8 set forth in SEQ ID NOs. 1 and 2, respectively. The sequences of the heavy and light chain CDR domains of mAb 28-8, as delineated using the Kabat system, are set forth in SEQ ID NOs. 3-8. In other embodiments, an anti-PD-L1 antibody useful for the invention comprises mAbs 28-1, 28-12, 29-8 and 20-12 or an antigen-binding portion thereof, for example, including Fab, $F(ab')_2$ Fd, Fv, and scFv, di-scFv or bi-scFv, and scFv-Fc fragments, diabodies, triabodies, tetrabodies, and isolated CDRs.

*Anti-CTLA-4 antibodies*

**[0103]** Anti-CTLA-4 antibodies of the instant invention bind to human CTLA-4 so as to disrupt the interaction of CTLA-4 with a human B7 receptor. Because the interaction of CTLA-4 with B7 transduces a signal leading to inactivation of T-cells bearing the CTLA-4 receptor, disruption of the interaction effectively induces, enhances or prolongs the activation of such T cells, thereby inducing, enhancing or prolonging an immune response.

**[0104]** HuMAbs that bind specifically to CTLA-4 with high affinity have been disclosed in U.S. Patent Nos. 6,984,720 and 7,605,238. Other anti-PD-1 mAbs have been described in, for example, U.S. Patent Nos. 5,977,318, 6,051,227, 6,682,736, and 7,034,121. The anti-PD-1 HuMAbs disclosed in U.S. Patent No. Nos. 6,984,720 and 7,605,238 have been demonstrated to exhibit one or more of the following characteristics: (a) binds specifically to human CTLA-4 with a binding affinity reflected by an equilibrium association constant ($K_a$) of at least about $10^7$ $M^{-1}$, or about $10^9$ $M^{-1}$, or about $10^{10}$ $M^{-1}$ to $10^{11}$ $M^{-1}$ or higher, as determined by Biacore analysis; (b) a kinetic association constant ($k_a$) of at least about $10^3$, about $10^4$, or about $10^5$ $m^{-1}$ $s^{-1}$; (c) a kinetic disassociation constant ($k_d$) of at least about $10^3$, about $10^4$, or about $10^5$ $m^{-1}$ $s^{-1}$; and (d) inhibits the binding of CTLA-4 to B7-1 (CD80) and B7-2 (CD86). Anti-CTLA-4 antibodies useful for the present invention include mAbs that bind specifically to human CTLA-4 and exhibit at least one, at least two, or at least three of the preceding characteristics.

**[0105]** An exemplary clinical anti-CTLA-4 antibody is the human mAb 10D1 (now known as ipilimumab and marketed as YERVOY®) as disclosed in U.S. Patent No. 6,984,720. Ipilimumab is an anti-CTLA-4 antibody for use in the methods disclosed herein. Ipilimumab is a fully human, IgG1 monoclonal antibody that blocks the binding of CTLA-4 to its B7 ligands, thereby stimulating T cell activation and improving overall survival (OS) in patients with advanced melanoma.

**[0106]** Another anti-CTLA-4 antibody useful for the present methods is tremelimumab (also known as CP-675,206). Tremelimumab is human IgG2 monoclonal anti-CTLA-4 antibody. Tremelimumab is described in WO/2012/122444, U.S. Publ. No. 2012/263677, or WO Publ. No. 2007/113648 A2.

**[0107]** Anti-CTLA-4 antibodies useful for the disclosed composition also include isolated antibodies that bind specifically to human CTLA-4 and cross-compete for binding to human CTLA-4 with ipilimumab or tremelimumab or bind to the same epitope region of human CTLA-4 as ipilimumab or tremelimumab. In certain embodiments, the antibodies that cross-compete for binding to human CTLA-4 with, or bind to the same epitope region of human CTLA-4 as does ipilimumab or tremelimumab, are antibodies comprising a heavy chain of the human IgG1 isotype. For administration to human subjects, these cross-competing antibodies are chimeric antibodies, or humanized or human antibodies. Useful anti-CTLA-4 antibodies also include antigen-binding portions of the above antibodies such as Fab, $F(ab')_2$, Fd or Fv fragments.

*Dosages*

**[0108]** The combination of an anti-PD-1 antibody and an anti-CTLA-4 antibody can be administered to a suitable patient in therapeutically effective amounts. For example, each antibody can be administered at a dosage ranging from at least about 0.1 to at least about 20.0 mg/kg body weight. In certain embodiments, each of the anti-PD-1 and anti-CTLA-4 antibodies is individually administered at a dosage of at least about 0.1, at least about 0.3, at least about 0.5, at least about 1, at least about 3, at least about 5, at least about 10 or at least about 20 mg/kg, *e.g.*, at least about 1 to at least about 10 mg/kg, *e.g.*, at least about 1 to at least about 3 mg/kg, *e.g.*, at least about 3 mg/kg, *e.g.*, at least about 1 mg/kg. Each of the anti-PD-1 antibody and anti-CTLA-4 antibody can be administered at a dosing frequency of at least about once every week, at least about once every 2 weeks, at least about once every 3 weeks, or at least about once every 4 weeks, or at least about once a month, for up to 6 to up to 72 doses, or for as long as clinical benefit is observed, or until unmanageable toxicity or disease progression occurs. In some embodiments, the anti-PD-1 antibody is administered at a dosage of about 1 or about 3 mg/kg. In certain embodiments, the sequenced regimen comprises administering the anti-PD-1 antibody to the subject at a dosing frequency of once about every week, once about every 2 weeks, once about every 3 weeks, or once about every 4 weeks, or once a month for 6 to 72 doses, or for as long as clinical benefit is observed, or until unmanageable toxicity or disease progression occurs. In other embodiments, the anti-PD-1 is administered at a

dosage of about 1 mg/kg at a dosing frequency of once about every 3 weeks for up to 48 doses. In some embodiments, the anti-CTLA-4 antibody is administered at a dosage of about 1 or about 3 mg/kg. In certain embodiments, the sequenced regimen comprises administering the anti-CTLA-4 antibody to the subject at a dosing frequency of once about every week, at least about once every 2 weeks, at least about once every 3 weeks, or at least about once every 4 weeks, or about once a month for 6 to 72 doses, or for as long as clinical benefit is observed, or until unmanageable toxicity or disease progression occurs. In other embodiments, the anti-CTLA-4 antibody is administered at a dosage of about 3 mg/kg at a dosing frequency of once about every 3 weeks for up to 48 doses.

**[0109]** In other embodiments, the anti-PD-1 and anti-CTLA-4 antibodies are administered at the following dosages: (a) 0.1 mg/kg anti-PD-1 antibody and 3 mg/kg of anti-CTLA-4 antibody; (b) 0.3 mg/kg anti-PD-1 antibody and 3 mg/kg of anti- CTLA-4 antibody; (c) 1 mg/kg anti-PD-1 antibody and 3 mg/kg of anti-CTLA-4 antibody; (d) 3 mg/kg anti-PD-1 antibody and 3 mg/kg of anti-CTLA-4 antibody; (e) 5 mg/kg anti-PD-1 antibody and 3 mg/kg of anti-CTLA-4 antibody; (f) 10 mg/kg anti-PD-1 antibody and 3 mg/kg of anti-CTLA-4 antibody; (g) 0.1 mg/kg anti-PD-1 antibody and 1 mg/kg of anti-CTLA-4 antibody; (h) 0.3 mg/kg anti-PD-1 antibody and 1 mg/kg of anti-CTLA-4 antibody; (i) 1 mg/kg anti-PD-1 antibody and 1 mg/kg of anti-CTLA-4 antibody; (j) 3 mg/kg anti-PD-1 antibody and 1 mg/kg of anti-CTLA-4 antibody; (k) 5 mg/kg anti-PD-1 antibody and 1 mg/kg of anti-CTLA-4 antibody; or (1) 10 mg/kg anti-PD-1 antibody and 1 mg/kg of anti-CTLA-4 antibody. In a particular embodiment, the methods include administration of 1 mg/kg of an anti-PD-1 antibody and 3 mg/kg of an anti-CTLA-4 antibody.

**[0110]** In some embodiments, the dosage of each of the anti-PD-1 and anti-CTLA-4 antibodies is kept constant during the induction dosing schedule and the maintenance dosing schedule. In certain embodiments, a regimen comprises: (i) an induction dosing schedule comprising combined administration of the anti-PD-1 and anti-CTLA-4 antibodies at a dosing frequency of at least once about every 2 weeks, at least once about every 3 weeks, or at least once about every 4 weeks, or at least about once a month, for at least 2, 4, 6, 8 or 10 doses, followed by administration of the anti-PD-1 antibody alone at a dosing frequency of at least once every 2, 3 or 4 weeks, or at least once a month, for at

least 2, 4, 6, 8 or 12 doses; followed by (ii) a maintenance dosing schedule comprising combined administration of the anti-PD-1 and anti-CTLA-4 antibodies at a dosing frequency of at least about once every 8 weeks, once about every 12 weeks, or once about every 16 weeks, or at least once a quarter, for at least 4, 6, 8, 10, 12 or 16 doses, or for as long as clinical benefit is observed, or until unmanageable toxicity or disease progression occurs. In another embodiment, the dosing schedule comprises administering about 3 mg/kg of an anti-PD-1 antibody every 2 weeks, or about 1 mg/kg of an anti-PD-1 antibody every 3 weeks plus about 3 mg/kg of an anti-CTLA-4 antibody every 3 weeks for 4 doses, followed by about 3 mg/kg of an anti-PD-1 antibody every 2 weeks for cycle 3 and beyond, or 3 mg/kg of an anti-CTLA-4 antibody every 3 weeks for 4 doses. In one particular embodiment, the dosing schedule comprises administering about 1 mg/kg of an anti-PD-1 antibody followed by an anti-CTLA-4 antibody (*e.g.*, about 3 mg/kg anti-CTLA-4 antibody) on the same day every 3 weeks for 4 doses, followed by about 3 mg/kg of an anti-PD-1 antibody every 2 weeks.

**[0111]** In certain embodiments of this method, the maintenance dosing schedule comprises combined administration of up to 4, 6, 8, 10, 12 or 16 doses of the anti-PD-1 and anti-CTLA-4 antibodies. In other embodiments, the concurrent regimen comprises: (i) an induction dosing schedule comprising combined administration of the anti-PD-1 and anti- CTLA-4 antibodies at a dosing frequency of once about every 2 weeks, at least once about every 3 weeks, or at least once about every 4 weeks, or about once a month, for 2, 4, 6 or 8 doses, followed by administration of the anti-PD-1 antibody alone at a dosing frequency of once about every 2 weeks, at least once about every 3 weeks, or at least once about every 4 weeks, or about once a month, for 2, 4, 6, 8 or 12 doses; followed by (ii) a maintenance dosing schedule comprising combined administration of the anti-PD-1 and anti-CTLA-4 antibodies at a dosing frequency of once every once about every 8, once about every 12 or once about every 16 weeks, or once a quarter, for 4, 6, 8, 10, 12 or 16 doses, or for as long as clinical benefit is observed, or until unmanageable toxicity or disease progression occurs.

**[0112]** In a particular embodiment, the anti-PD-1 and anti-CTLA-4 antibodies are administered at dosages of about 1 mg/kg anti-PD-1 antibody every three weeks and about 3 mg/kg of anti-CTLA-4 antibody every three weeks. In another embodiment, the

patient is administered at a dose of about 1 mg/kg of an anti-PD-1 antibody, followed by an anti-CTLA-4 antibody on the same day, every 3 weeks for 4 doses, then 3 mg/kg of an anti-PD-1 antibody every 2 weeks.

**[0113]** In certain embodiments of the present methods, the anti-PD-1 and anti-CTLA-4 antibodies are formulated for parenteral administration, *e.g.*, intravenous administration. In certain other embodiments, when the anti-PD-1 and anti-CTLA-4 antibodies are administered in combination, they are administered within about 30 minutes of each other. Either antibody may be administered first, that is, in certain embodiments, the anti-PD-1 antibody is administered before the anti-CTLA-4 antibody, whereas in other embodiments, the anti-CTLA-4 antibody is administered before the anti-PD-1 antibody. Typically, each antibody is administered intravenously over a period of about 60 minutes. In further embodiments, the anti-PD-1 and anti-CTLA-4 antibodies are administered concurrently, either admixed as a single composition in a pharmaceutically acceptable formulation for concurrent administration, or concurrently as separate compositions with each antibody in a pharmaceutically acceptable formulation.

**[0114]** In some embodiments, the anti-PD-1 antibody and the anti-CTLA-4 antibody are formulated in a single composition. The ratio of the amount of the anti-PD-1 antibody and the amount of the anti-CTLA-4 antibody in the single composition can be 10:1 to 1:10. In other embodiments, the ratio of the amount of the anti-PD-1 antibody and the amount of the anti-CTLA-4 antibody in the single composition is 5:1, 4:1, 3:1, 2:1, 1:1, 1:2, 1:3, 1:4, or 1:5. In a particular embodiment, the ratio of the amount of the anti-PD-1 antibody and the amount of the anti-CTLA-4 antibody in the single composition is 1:3.

**[0115]** In some embodiments, the composition is administered at a flat dose regardless of the weight of the patient. For example, each of the anti-PD-1 antibody with the anti-CTLA-4 antibody may be administered at a flat dose of 20, 50, 75, 80, 160, 200, 240, 300, 360, 400, 480, 500, 750 or 1500 mg, without regard to the patient's weight. In some embodiments the composition is administered at a weight-based dose at any dose disclosed herein. In some embodiments, the amount of the anti-PD-1 antibody and the amount of the anti-CTLA-4 antibody administered to the patient at a single dose are identical.

**[0116]** In certain embodiments of the present methods, the therapeutically effective dosage of the anti-PD-1 antibody or antigen-binding portion thereof is a flat dosing (not a bodyweight based dosing) and comprises about 60 mg, about 80 mg, about 100 mg, about 120 mg, about 140 mg, about 160 mg, about 180 mg, about 200 mg, about 220 mg, about 240 mg, about 260 mg, about 280 mg, or about 300 mg. In other embodiments, the therapeutically effective dosage of the anti-PD-1 antibody or antigen-binding portion thereof comprises about 320 mg, 360mg, 400 mg, 420mg, 480 mg, 500mg, 540 mg, 550mg, 600mg, 620 mg, 650mg, 680mg, 700 mg, 720mg, 780 mg, 800mg, 840mg, or 900mg.. In some embodiments, the dose of the anti-PD-1 antibody in the composition is between about 60 mg and about 300 mg, between about 60 mg and about 100 mg, between about 100 mg and about 200 mg, or between about 200 mg and about 300 mg. In some embodiments, the amount of the anti-PD-1 antibody in the composition is at least about 80 mg, about 160 mg, or about 240mg. In some embodiments, the dose of the anti-PD-1 antibody in the composition is at least about 240 mg or at least about 80 mg.

**[0117]** In other embodiments, the dosages are given every week, every two weeks, every three weeks, every four weeks, every five weeks, every six weeks, every seven weeks, every eight weeks, every nine weeks, or every 10 weeks.

**[0118]** In some embodiments, the anti-PD-1 antibody or antigen-binding portion thereof is administered at a dose of about 240 mg. In embodiments, the anti-PD-1 antibody or antigen-binding portion thereof is administered at a dose of about 360 mg. In embodiments, the anti-PD-1 antibody or antigen-binding portion thereof is administered at a dose of about 480 mg. In one embodiment, 360mg of the anti-PD-1 antibody or antigen binding fragment is administered once every 3 weeks. In another embodiment, 480mg of the anti-PD-1 antibody or antigen binding fragment is administered once every 4 weeks.

**[0119]** In other embodiments, the therapeutically effective dosage of the anti-CTLA-4 antibody or antigen-binding portion thereof is a flat dosing (not a bodyweight based dosing) and comprises about 60 mg, about 80 mg, about 100 mg, about 120 mg, about 140 mg, about 160 mg, about 180 mg, about 200 mg, about 220 mg, about 240 mg, about 260 mg, about 280 mg, or about 300 mg. In other embodiments, the therapeutically effective dosage of the anti-CTLA-4 antibody or antigen-binding portion thereof

comprises about 320 mg, 360mg, 400 mg, 420mg, 480 mg, 500mg, 540 mg, 550mg, 600mg, 620 mg, 650mg, 680mg, 700 mg, 720mg, 780 mg, 800mg, 840mg, or 900mg. In some embodiments, the dose of the anti-CTLA-4 antibody in the composition is between about 60 mg and about 300 mg, between about 60 mg and about 100 mg, between about 100 mg and about 200 mg, or between about 200 mg and about 300 mg. In some embodiments, the amount of the anti-CTLA-4 antibody in the composition is at least about 80 mg, about 160 mg, or about 240 mg. In some embodiments, the dose of the anti-CTLA-4 antibody in the composition is at least about 240 mg or at least about 80 mg.

**[0120]** In some embodiments, a flat dose of an anti-PD-1 antibody is 80 mg, and a flat dose of an anti-CTLA-4 antibody is 240 mg.

**[0121]** In some embodiments, the anti-PD-1 antibody is administered at a subtherapeutic dose, *i.e.*, a dose of the therapeutic agent that is significantly lower than the usual or FDA-approved dose when administered as monotherapy for the treatment of the cancer. The quantity of the second antibody in the composition is calculated based on the desired ratio.

**[0122]** In some embodiments, the composition is administered by intravenous infusion about once per week, about once every 2 weeks, about once every 3 weeks, or about once a month. In certain embodiments, the composition is administered once every 3 weeks. In some embodiments, the infusion occurs over at least about 10 minutes, about 20 minutes, about 30 minutes, about 45 minutes, about 60 minutes, about 90 minutes, about 2 hours, about 3 hours, about 4 hours or about 5 hours.

**[0123]** Actual dosage levels of the active ingredients in the pharmaceutical compositions of the present invention can be flat or varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being unduly toxic to the patient. The selected dosage level will depend upon a variety of pharmacokinetic factors including the activity of the particular compositions of the present invention employed, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compositions employed, the age, sex, weight, condition, general health and prior medical history of the

patient being treated, and like factors well known in the medical arts. A composition of the present invention can be administered via one or more routes of administration using one or more of a variety of methods well known in the art. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results.

*Kits*

**[0124]** Also within the scope of the present invention are kits, including pharmaceutical kits, comprising a combination of an anti-PD-1 antibody and an anti-CTLA-4 antibody, for therapeutic uses, and diagnostic kits comprising an anti-PD-Ll antibody for assaying membranous PD-L1 expression as a biomarker for screening patients for the combination therapy or for predicting the efficacy of the combination therapy. Kits typically include a label indicating the intended use of the contents of the kit and instructions for use. The term "label" includes any writing, or recorded material supplied on or with the kit, or which otherwise accompanies the kit. In certain embodiments of a pharmaceutical kit, the anti-PD-1 antibody is co-packaged with an anti-CTLA-4 antibody in unit dosage form. In certain embodiments of a diagnostic kit, the anti-PD-Ll antibody is co-packaged with an anti-PD-1 antibody and an anti-CTLA-4 antibody for performing an assay to detect and/or quantify PD-L1 expression.

**[0125]** In certain embodiments, the pharmaceutical kit comprises the anti-human PD-1 HuMAb, nivolumab. In other embodiments, the pharmaceutical kit comprises the anti-human PD-L1 HuMAb, BMS-936559. In yet other embodiments, the pharmaceutical kit comprises the anti-human CTLA-4 HuMAb, ipilimumab. In certain embodiments, the diagnostic kit comprises the rabbit anti-human PD-L1 mAb, 28-8, comprising the $V_H$ and $V_L$ regions whose amino acid sequences are set forth in SEQ ID NOs. 1 and 2, respectively. In other embodiments, the diagnostic kit comprises the murine anti-human PD-L1 mAb, 5H1 (Dong *et al.*, *Nature Med. 8(8)*:793-800 (2002)).

**[0126]** In some embodiments, the invention provides a kit for treating a patient afflicted with a melanoma tumor, the kit comprising:

(a) a dosage ranging from at least about 0.1 to at least about 10 mg/kg body weight of an anti-PD-1 antibody or an antigen-binding portion thereof;

(b) a dosage ranging from at least about 0.1 to at least about 10 mg/kg body weight of an anti-CTLA-4 antibody or an antigen-binding portion thereof; and

(c) instructions for using the anti-PD-1 antibody or the antigen-binding portion thereof and the anti-CTLA-4 antibody or the antigen-binding portion thereof in the methods disclosed herein.

**[0127]** The present invention is further illustrated by the following examples which should not be construed as further limiting. The contents of all references cited throughout this application are expressly incorporated herein by reference.

## EXAMPLES

### EXAMPLE 1

**[0128]** A randomized, double-blind, multicenter, phase 3 trial was conducted to evaluate the safety and efficacy of nivolumab alone or nivolumab combined with ipilimumab in comparison with ipilimumab alone in previously untreated metastatic melanoma.

*Patients*

**[0129]** Eligible patients had histologically confirmed stage III (unresectable) or stage IV melanoma, and no prior systemic treatment for unresectable or metastatic melanoma. Other eligibility criteria included an age of at least 18 years, an Eastern Cooperative Oncology Group (ECOG) performance status score of 0 (indicating no symptoms) or 1 (indicating mild symptoms), measurable disease by computed tomography or magnetic resonance imaging per RECIST v1.1, availability of tissue collected from metastatic or unresectable tumors for the assessment of PD-L1 status, and known BRAF V600 mutation status (or consent to BRAF V600 mutation testing per local standards). Key exclusion criteria were presence of active brain metastases, ocular melanoma, or autoimmune disease, and any prior treatment with an anti-PD-1, anti-PD-L1, anti-PD-L2, or anti-CTLA-4 antibody. Patients who required systemic corticosteroid treatment or other immunosuppressive medications within 14 days of study drug administration were excluded.

*Study Design and Treatment*

**[0130]** In the double-blind, phase 3 study, enrolled patients were randomly assigned in a 1:1:1 ratio to receive 3 mg of nivolumab per kilogram of body weight every 2 weeks (plus ipilimumab-matched placebo), or 1 mg of nivolumab per kilogram every 3 weeks plus 3 mg of ipilimumab per kilogram every 3 weeks for 4 doses (plus nivolumab-matched placebo), followed by 3 mg of nivolumab per kilogram every 2 weeks for cycle 3 and beyond, or 3 mg of ipilimumab per kilogram every 3 weeks for 4 doses (plus nivolumab-matched placebo).

**[0131]** Both nivolumab and ipilimumab were administered by intravenous infusion. Randomization was stratified according to tumor PD-L1 status (positive vs. negative or indeterminate), BRAF mutation status (V600 mutation positive vs. wild-type), and American Joint Committee on Cancer metastasis stage (M0, M1a, or M1b vs. M1c). Treatment continued until RECIST v1.1-defined disease progression, unacceptable toxicity, or withdrawal of consent. Patients could be treated beyond progression provided they had a clinical benefit without clinical deterioration, and did not have substantial adverse effects, as assessed by the investigator.

**[0132]** Progression-free survival and overall survival were co-primary end points. Secondary end points included objective response rate, tumor PD-L1 expression as a predictive biomarker for progression-free and overall survival, and health-related quality of life. Exploratory end points include duration of objective response and safety/tolerability of study drug therapy.

*Assessments*

**[0133]** Patients were assessed for tumor response, according to RECIST v1.1 at 12 weeks after randomization and continuing every 6 weeks for 49 weeks, and then every 12 weeks until progression or treatment discontinuation, whichever occurred later. Progression-free survival was defined as the time between the date of randomization and the first date of documented progression or death, whichever occurred first. Patients treated beyond progression were considered to have progressive disease at the time of the initial progression event, as assessed by the investigator, regardless of subsequent tumor responses. Expression of PD-L1 on the surface of the tumor cells was assessed in a central laboratory by immunohistochemistry in formalin-fixed, paraffin-embedded tumor specimens using a rabbit monoclonal anti-human PD-L1 antibody (clone 28-8) and an

analytically validated automated assay developed by Dako (Carpinteria, CA). PD-L1 positivity was defined as at least 5% of tumor cells showing cell surface PD-L1 staining of any intensity in a section containing at least 100 tumor cells that could be evaluated. Indeterminate status was attributed to samples for which tumor cell–surface expression could not be discerned because of melanin content or strong cytoplasmic staining.

**[0134]** Any patient who received at least one dose of study drug in each of the 3 treatment groups was included in the assessment of safety. The severity of adverse events was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0.18. Safety assessments were made continuously during the treatment phase, and up to 100 days after the last dose of study drug.

*Statistical Analysis*

**[0135]** A study sample size of approximately 915 patients, randomized to the 3 treatment arms in a 1:1:1 ratio, was planned. For the comparison of progression-free survival, the number of events projected to be observed at a follow-up of at least 9 months provided approximately 83% power to detect an average hazard ratio of 0.71 with a type I error of 0.005 (two-sided). Progression-free survival was compared between nivolumab plus ipilimumab and ipilimumab alone, and between nivolumab alone and ipilimumab alone with the use of a two-sided log-rank test stratified according to PD-L1 status, BRAF mutation status, and metastasis stage (as described above). The study was not designed for a formal statistical comparison between the nivolumab alone and nivolumab plus ipilimumab groups. Hazard ratios and corresponding two-sided 99.5% confidence intervals (CIs) were estimated using a Cox proportional hazards model, with treatment group as a single covariate, stratified by the above factors. Progression-free survival curves, medians with 95% CIs, and progression-free survival rates at 6, 12, and 18 months with 95% CIs were estimated using Kaplan-Meier methodology. Overall survival will be analyzed when all patients have a minimum follow-up of 22 months.

**Results**

*Patients and Treatment*

**[0136]** From July 2013 through March 2014, a total of 1296 patients were enrolled at 137 centers in Australia, Europe, Israel, New Zealand, and North America. A total of 945 patients underwent randomization: 316 patients were assigned to the nivolumab group, 314 to the nivolumab plus ipilimumab group, and 315 to the ipilimumab group (~~Figure~~ Fig. 1). Baseline characteristics were balanced across the three groups. A total of 58.0% had stage M1c disease, 36.1% had an elevated lactate dehydrogenase level, 31.5% had a BRAF mutation, and 73.5% had negative PD-L1 status (Table 1).

**[0137]** All randomized patients had been followed for a minimum of 9 months at the time of database lock (February 17, 2015); 117 of 313 patients (37.4%) in the nivolumab group, 93 of 313 patients (29.7%) in the nivolumab plus ipilimumab group, and 50 of 311 patients (16.1%) in the ipilimumab group were continuing study treatment (Table 2). The most frequent reason for discontinuation was disease progression in the nivolumab and ipilimumab monotherapy groups (154 of 313 patients [49.2%] and 202 of 311 patients [65.0%], respectively), versus study drug toxicity in the nivolumab plus ipilimumab group (120 of 313 patients [38.3%]). The number of patients who had died was 85 (27.2%) in the nivolumab group, 86 (27.5%) in the nivolumab plus ipilimumab group, and 114 (36.7%) in the ipilimumab group.

**[0138]** The median number of doses in patients who received nivolumab alone or ipilimumab alone was 15 (range 1-38) and 4 (1-4), respectively. In the combination group, the median number of doses was 4 (range 1–39) for nivolumab and 4 (range 1–4) for ipilimumab; 147 of 313 patients (47%) received four or more doses of nivolumab monotherapy after combination treatment.

Table 1. Baseline Characteristics of the Patients.

| Characteristic | Nivolumab alone (N=316) | Nivolumab plus Ipilimumab (N=314) | Ipilimumab alone (N=315) | Total (N=945) |
|---|---|---|---|---|
| | | | | |
| Age — yr | | | | |
| Mean | 58.7 | 59.3 | 60.8 | 59.6 |
| Range | 25–90 | 18–88 | 18–89 | 18–90 |
| | | | | |
| Age groups — no. (%) | | | | |
| <65 yr | 198 (62.7) | 185 (58.9) | 182 (57.8) | 565 (59.8) |
| ≥ 65, < 75 yr | 79 (25.0) | 94 (29.9) | 89 (28.3) | 262 (27.7) |
| ≥ 75 yr | 39 (12.3) | 35 (11.1) | 44 (14.0) | 118 (12.5) |
| | | | | |
| Sex — no. (%) | | | | |
| Male | 202 (63.9) | 206 (65.6) | 202 (64.1) | 610 (64.6) |
| Female | 114 (36.1) | 108 (34.4) | 113 (35.9) | 335 (35.4) |
| | | | | |
| ECOG performance status — no. (%) | | | | |
| 0 | 238 (75.3) | 230 (73.2) | 224 (71.1) | 692 (73.2) |
| 1 | 77 (24.4) | 83 (26.4) | 91 (28.9) | 251 (26.6) |
| 2 | 1 (0.3) | 0 | 0 | 1 (0.1) |
| Not reported | 0 | 1 (0.3) | 0 | 1 (0.1) |
| | | | | |
| M stage — no. (%) | | | | |
| M1c | 184 (58.2) | 181 (57.6) | 183 (58.1) | 548 (58.0) |
| M0, M1a, or M1b | 132 (41.8) | 133 (42.4) | 132 (41.9) | 397 (42.0) |
| | | | | |
| Lactate dehydrogenase — no. (%)* | | | | |
| ≤ ULN | 196 ( 62.0) | 199 ( 63.4) | 194 ( 61.6) | 589 ( 62.3) |
| >ULN | 112 ( 35.4) | 114 ( 36.3) | 115 ( 36.5) | 341 ( 36.1) |
| ≤2x ULN | 271 ( 85.8) | 276 ( 87.9) | 279 ( 88.6) | 826 ( 87.4) |

| | | | | |
|---|---|---|---|---|
| >2x ULN | 37 ( 11.7) | 37 (11.8) | 30 (9.5) | 104 (11.0) |
| Unknown | 8 (2.5) | 1 (0.3) | 6 (1.9) | 15 (1.6) |
| | | | | |
| Brain metastases at baseline — no. (%) | | | | |
| Yes | 8 (2.5) | 11 (3.5) | 15 (4.8) | 34 (3.6) |
| No | 308 (37.5) | 303 (96.5) | 300 (95.2) | 911 (96.4) |
| | | | | |
| PD-L1 status — no. (%) | | | | |
| Positive | 80 (27.8) | 68 (24.5) | 75 (27.1) | 223(26.4) |
| Negative | 208 (72.2) | 210 (75.5) | 202 (72.9) | 620 (73.5) |
| | | | | |
| BRAF status — no. (%) | | | | |
| Mutation | 100 (31.6) | 101 (32.2) | 97 (30.8) | 298 (31.5) |
| No mutation | 216 (68.4) | 213 (67.8) | 218 (69.2) | 647 (68.5) |

* ULN denotes upper limit of normal.

*Efficacy*

**[0139]** The median progression-free survival was 6.5 months (95% confidence interval [CI], 4.3 to 9.5) in the nivolumab group, 11.5 months (95% CI, 8.9 to 16.5) in the nivolumab plus ipilimumab group, and 2.9 months (95% CI, 2.8 to 3.4) in the ipilimumab group (~~Figure~~ Fig. 2A). A significant improvement in progression-free survival was observed in the nivolumab plus ipilimumab group as compared with the ipilimumab group (hazard ratio, 0.42; 95% CI, 0.31 to 0.57; P<0.0001) (~~Figure~~ Fig. 2A). A significant improvement in progression-free survival was also observed in the nivolumab group as compared with the ipilimumab group (hazard ratio, 0.57; 95% CI, 0.43 to 0.76; P<0.00001) (~~Figure~~ Fig. 2A). The hazard ratio for the comparison between nivolumab plus ipilimumab and nivolumab groups was 0.74 (95% CI, 0.60 to 0.92).

**[0140]** Analyses of progression-free survival among prespecified patient subgroups showed a consistent improvement with nivolumab or nivolumab plus ipilimumab as compared with ipilimumab, including subgroups defined by PD-L1 status, BRAF mutation status, and metastasis stage (~~Figure~~ Figs. 3A and 3B). In the combination group, median PFS was 11.7 months (95% CI, 8.0 to not reached) among patients with a BRAF mutation and was 11.2 months (95% CI, 8.3 to not reached) in patients with wild-type BRAF (~~Figure~~ Fig. 3B). For patients with a positive PD-L1 tumor status, median progression-free survival in the nivolumab, nivolumab plus ipilimumab, and ipilimumab groups was 14.0 months (95% CI, 9.1 to not reached), 14.0 months (95% CI, 9.7 to not reached), and 3.9 months (95% CI, 2.8 to 4.2), respectively (~~Figure~~ Fig. 2B). For patients with a negative PD-L1 tumor status, median progression-free survival in the nivolumab, nivolumab plus ipilimumab, and ipilimumab groups was 5.3 months (95% CI, 2.8 to 7.1 months), 11.2 months (95% CI, 8.0 to not reached) and 2.8 months (95% CI, 2.8 to 3.1), respectively (Fig. 2C).

**[0141]** Investigator-assessed objective response rates were 43.7% (95% CI, 38.1 to 49.3%), 57.6% (95% CI, 52.0 to 63.2), and 19.0% (95% CI, 14.9 to 23.8) in the nivolumab, nivolumab plus ipilimumab, and ipilimumab groups, respectively (Table 3). The percentage of patients with a complete response was higher in the nivolumab plus ipilimumab group than in either the nivolumab or ipilimumab alone groups (11.5% vs.

8.9% and 2.2%) (Table 3). Time to objective response was similar in each group (Table 3), and the median duration of response was not reached in any of the groups.

**[0142]** Median reduction in the sum of the longest diameters of tumor target lesions was -34.5% (interquartile range: -75.4 to 15.4), -51.1% (-75.8 to -10.2), and 5.8% (-28.0 to 33.3) in the nivolumab, nivolumab plus ipilimumab, and ipilimumab groups, respectively (~~Figure~~ Figs. 4A-4C). Among patients with PD-L1-positive tumors, the objective response rates were 57.5% (95% CI, 45.9 to 68.5), 72.1% (95% CI, 59.9 to 82.3), and 21.3% (95% CI, 12.7 to 32.3) for the nivolumab, nivolumab plus ipilimumab, and ipilimumab groups, respectively; in patients with PD-L1-negative tumors, the objective response rates were 41.3% (95% CI, 34.6 to 48.4), 54.8% (95% CI, 47.8 to 61.6), and 17.8% (95% CI, 12.8 to 23.8) (Table 4).

*Adverse Events*

**[0143]** Treatment-related adverse events of any grade occurred in 82.1%, 95.5%, and 86.2% of patients in the nivolumab, nivolumab plus ipilimumab groups, and ipilimumab groups, respectively (Table 5). The most common adverse events in the nivolumab group were fatigue (in 34.2% of patients), rash (in 21.7%), and diarrhea (in 19.2%). In the nivolumab plus ipilimumab and ipilimumab groups, diarrhea (in 44.1% and 33.1% of patients, respectively), fatigue (in 35.1% and 28.0%), and pruritus (in 33.2% and 35.4%) were most common (Table 5). The incidence of treatment-related adverse events of grade 3 or 4 was also higher in the nivolumab plus ipilimumab group than in either the nivolumab or ipilimumab groups (55.0% vs. 16.3% and 27.3%), with diarrhea being the most common (2.2% , 9.3%, and 6.1% in the nivolumab, nivolumab plus ipilimumab, and ipilimumab groups, respectively) (Table 5). Treatment-related adverse events of any grade leading to discontinuation occurred in 7.7%, 36.4%, and 14.8% of patients in the nivolumab, nivolumab plus ipilimumab, and ipilimumab groups, respectively, with the most common being diarrhea (in 1.9%, 8.3%, and 4.5%, respectively) and colitis (in 0.6%, 8.3%, and 7.7%, respectively) (Table 5). One death due to study-drug toxicity was reported in the nivolumab group (neutropenia) and one in the ipilimumab group (cardiac arrest), although such adverse events have not been associated with these drugs in prior studies. No treatment-related deaths were reported in the combination group.

Table 2. Patient Disposition.*

| | Nivolumab alone (N=313) | Nivolumab plus Ipilimumab (N=313) | Ipilimumab alone (N=311) |
|---|---|---|---|
| | | | |
| **Patients in the treatment period — no. (%)** | | | |
| Continuing | 117 (37.4) | 93 (29.7) | 50 (16.1) |
| Not continuing | 196 (62.6) | 220 (70.3) | 261 (83.9) |
| | | | |
| **Reason for not continuing the treatment — no. (%)** | | | |
| Disease progression | 154 (49.2) | 69 (22.0) | 202 (65.0) |
| Study drug toxicity | 27 (8.6) | 120 (38.3) | 47 (15.1) |
| Adverse event unrelated to study drug | 5 (1.6) | 12 (3.8) | 4 (1.3) |
| Patient request to discontinue treatment | 5 (1.6) | 5 (1.6) | 4 (1.3) |
| Death | 5 (1.6) | 5 (1.6) | 4 (1.3) |
| Maximum clinical benefit | 2 (0.6) | 2 (0.6) | 0 |
| Poor/non-compliance | 1 (0.3) | 1 (0.3) | 1 (0.3) |
| Patient withdrew consent | 0 | 3 (1.0) | 0 |
| Lost to follow-up | 1 (0.3) | 0 | 0 |
| Patient no longer meets study criteria | 0 | 1 (0.3) | 0 |
| Other | 0 | 3 (1.0) | 2 (0.6) |
| | | | |
| **Patients in the study — no. (%)** | | | |
| Continuing | 223 (71.2) | 221 (70.6) | 189 (60.8) |
| Died | 85 (27.2) | 86 (27.5) | 114 (36.7) |
| Not continuing | 5 (1.6) | 6 (1.9) | 8 (2.6) |

****At the time of database lock on February 17, 2015*.

Table 3. Response to Treatment.

| Response | Nivolumab alone (N=316) | Nivolumab plus Ipilimumab (N=314) | Ipilimumab alone (N=315) |
|---|---|---|---|
| | | | |
| **Best overall response — no. (%)[†]** | | | |
| Complete response | 28 (8.9) | 36 (11.5) | 7 (2.2) |
| Partial response | 110 (34.8) | 145 (46.2) | 53 (16.8) |
| Stable disease | 34 (10.8) | 41 (13.1) | 69 (21.9) |
| Progressive disease | 119 (37.7) | 71 (22.6) | 154 (48.9) |
| Could not be determined | 25 (7.9) | 21 (6.7) | 32 (10.2) |
| | | | |
| **Objective response[‡]** | | | |
| No. of patients (% [95% CI]) | 138 (43.7 [38.1–49.3]) | 181 (57.6 [52.0–63.2]) | 60 (19.0 [14.9–23.8]) |
| Estimated odds ratio (95% CI)[§] | 3.40 (2.02–5.72) | 6.11 (3.59–10.38) | --- |
| Two-sided P value | <0.00001 | <0.00001 | --- |
| | | | |
| **Time to objective response — mo** | | | |
| No. of responders | 138 | 181 | 60 |
| Median | 2.78 | 2.76 | 2.79 |
| Range | 2.3–12.5 | 1.1–11.6 | 2.5–12.4 |

[†] ***Best overall response were assessed by the investigators with the use of RECIST v1.1.***

[‡] ***Data include patients with a complete response and those with a partial response.***

[§] ***Relative to ipilimumab alone.***

Table 4. Objective Response Rate by PD-L1 Status.

| **PD-L1 Positive** | | | |
|---|---|---|---|
| | **Nivolumab alone (N=80)** | **Nivolumab plus Ipilimumab (N=68)** | **Ipilimumab alone (N=75)** |
| | | | |
| **Objective response†** | | | |
| No. of patients (% [95% CI]) | 46 (57.5 [45.9–68.5]) | 49 (72.1 [59.9–82.3]) | 16 (21.3 [12.7–32.3]) |
| Estimated odds ratio (95% CI)§ | 5.03 (2.44–10.37) | 10.41 (4.63–23.40) | --- |
| | | | |
| **PD-L1 Negative** | | | |
| | **Nivolumab alone (N=208)** | **Nivolumab plus Ipilimumab (N=210)** | **Ipilimumab alone (N=202)** |
| **Objective response†** | | | |
| No. of patients (% [95% CI]) | 86 (41.3 [34.6–48.4]) | 115 (54.8 [47.8–61.6]) | 36 (17.8 [12.8–23.8]) |
| Estimated odds ratio (95% CI)§ | 3.25 (2.05–5.13) | 5.90 (3.71–9.38) | --- |

† ***Best overall response was assessed by the investigators with the use of RECIST v1.1.***

§ Relative to ipilimumab alone.

**[0144]** The most frequent grade 3 or 4 treatment-related select adverse events were diarrhea (2.2%, 9.3%, and 6.1% of patients in the nivolumab, nivolumab plus ipilimumab, and ipilimumab groups, respectively), colitis (in 0.6%, 7.7%, and 8.7%, respectively), increased alanine aminotransferase (in 1.3%, 8.3%, and 1.6%, respectively), and increased aspartate aminotransferase (in 1.0%, 6.1%, and 0.6%, respectively) (Table 6). With the use of immune modulatory agents, resolution rates for grade 3 or 4 select adverse events were generally similar across treatment groups, and were between 85-100% across organ categories in the nivolumab plus ipilimumab group. As observed in prior studies, most endocrine events in all treatment groups did not resolve (Table 7).

**Discussion**

**[0145]** In this randomized, double-blind, phase 3 study, both nivolumab alone and the combination of nivolumab and ipilimumab significantly increased progression-free survival and objective response rates, as compared with ipilimumab alone, in previously untreated advanced melanoma. These results were observed independently of PD-L1 tumor status, BRAF mutation status, or metastasis stage. Baseline characteristics of study participants were typical of patients with advanced melanoma, although the BRAF mutation rate (31.5%) was lower than the 40-50% generally reported for advanced disease. While not a primary end point of the study, the combination of nivolumab and ipilimumab resulted in numerically longer progression-free survival and a higher response rate as compared with nivolumab alone in the overall study population. While time to response was similar between groups, the first tumor assessment was done at week 12 and thus the possibility that responses may have occurred earlier with the combination remains unknown.

Table 5. Adverse Events (Safety Population).*

| Event | Nivolumab alone (N=313) | | Nivolumab plus Ipilimumab (N=313) | | Ipilimumab alone (N=311) | |
|---|---|---|---|---|---|---|
| | Total | Grade 3 or 4 | Total | Grade 3 or 4 | Total | Grade 3 or 4 |
| | *no. of patients with event (%)* | | | | | |
| Any adverse event | 311 (99.4) | 136 (43.5) | 312 (99.7) | 215 (68.7) | 308 (99.0) | 173 (55.6) |
| Treatment-related adverse event† | 257 (82.1) | 51 (16.3) | 299 (95.5) | 172 (55.0) | 268 (86.2) | 85 (27.3) |
| Diarrhea | 60 (19.2) | 7 (2.2) | 138 (44.1) | 29 (9.3) | 103 (33.1) | 19 (6.1) |
| Fatigue | 107 (34.2) | 4 (1.3) | 110 (35.1) | 13 (4.2) | 87 (28.0) | 3 (1.0) |
| Pruritus | 59 (18.8) | 0 | 104 (33.2) | 6 (1.9) | 110 (35.4) | 1 (0.3) |
| Rash | 81 (25.9) | 2 (0.6) | 126 (40.3) | 15 (4.8) | 102 (32.8) | 6 (1.9) |
| Nausea | 41 (13.1) | 0 | 81 (25.9) | 7 (2.2) | 50 (16.1) | 2 (0.6) |
| Pyrexia | 18 (5.8) | 0 | 58 (18.5) | 2 (0.6) | 21 (6.8) | 1 (0.3) |
| Decreased appetite | 34 (10.9) | 0 | 56 (17.9) | 4 (1.3) | 39 (12.5) | 1 (0.3) |
| Increase in alanine aminotransferase | 12 (3.8) | 4 (1.3) | 55 (17.6) | 26 (8.3) | 12 (3.9) | 5 (1.6) |
| Vomiting | 20 (6.4) | 1 (0.3) | 48 (15.3) | 8 (2.6) | 23 (7.4) | 1 (0.3) |
| Increase in aspartate aminotransferase | 12 (3.8) | 3 (1.0) | 48 (15.3) | 19 (6.1) | 11 (3.5) | 2 (0.6) |
| Hypothyroidism | 27 (8.6) | 0 | 47 (15.0) | 1 (0.3) | 13 (4.2) | 0 |
| Colitis | 4 (1.3) | 2 (0.6) | 37 (11.8) | 24 (7.7) | 36 (11.6) | 27 (8.7) |
| Arthralgia | 24 (7.7) | 0 | 33 (10.5) | 1 (0.3) | 19 (6.1) | 0 |
| Headache | 23 (7.3) | 0 | 32 (10.2) | 1 (0.3) | 24 (7.7) | 1 (0.3) |
| Dyspnea | 14 (4.5) | 1 (0.3) | 32 (10.2) | 2 (0.6) | 13 (4.2) | 0 |
| Treatment-related adverse event leading to discontinuation | 24 (7.7) | 16 (5.1) | 114 (36.4) | 92 (29.4) | 46 (14.8) | 41 (13.2) |

* The severity of adverse events was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0.
† The treatment-related adverse events listed here were reported in at least 10% of the patients in any of the three study groups.

Table 6. Treatment-related Select Adverse Events (Safety Population).*

| Event | Nivolumab alone (N=313) | | Nivolumab plus Ipilimumab (N=313) | | Ipilimumab alone (N=311) | |
|---|---|---|---|---|---|---|
| | Total | Grade 3 or 4 | Total | Grade 3 or 4 | Total | Grade 3 or 4 |
| | *no. of patients with event (%)* | | | | | |
| Any select adverse event | 194 (62.0) | 24 (7.7) | 275 (87.9) | 124 (39.6) | 229 (73.6) | 58 (18.6) |
| | | | | | | |
| Treatment-related select adverse event† | | | | | | |
| Skin | 131 (41.9) | 5 (1.6) | 185 (59.1) | 18 (5.8) | 168 (54.0) | 9 (2.9) |
| Pruritus | 59 (18.8) | 0 | 104 (33.2) | 6 (1.9) | 110 (35.4) | 1 (0.3) |
| Rash | 68 (21.7) | 1 (0.3) | 89 (28.4) | 9 (2.9) | 65 (20.9) | 5 (1.6) |
| Rash maculo-papular | 13 (4.2) | 1 (0.3) | 37 (11.8) | 6 (1.9) | 37 (11.9) | 1 (0.3) |
| Vitiligo | 23 (7.3) | 1 (0.3) | 21 (6.7) | 0 | 12 (3.9) | 0 |
| Gastrointestinal | 61 (19.5) | 7 (2.2) | 145 (46.3) | 46 (14.7) | 114 (36.7) | 36 (11.6) |
| Diarrhea | 60 (19.2) | 7 (2.2) | 138 (44.1) | 29 (9.3) | 103 (33.1) | 19 (6.1) |
| Colitis | 4 (1.3) | 2 (0.6) | 37 (11.8) | 24 (7.7) | 36 (11.6) | 27 (8.7) |
| Hepatic | 20 (6.4) | 8 (2.6) | 94 (30.0) | 59 (18.8) | 22 (7.1) | 5 (1.6) |
| Increase in alanine aminotransferase | 12 (3.8) | 4 (1.3) | 55 (17.6) | 26 (8.3) | 12 (3.9) | 5 (1.6) |
| Increase in aspartate aminotransferase | 12 (3.8) | 3 (1.0) | 48 (15.3) | 19 (6.1) | 11 (3.5) | 2 (0.6) |
| Endocrine | 45 (14.4) | 2 (0.6) | 94 (30.0) | 15 (4.8) | 34 (10.9) | 7 (2.3) |
| Hypothyroidism | 27 (8.6) | 0 | 47 (15.0) | 1 (0.3) | 13 (4.2) | 0 |
| Hyperthyroidism | 13 (4.2) | 0 | 31 (9.9) | 3 (1.0) | 3 (1.0) | 0 |
| Hypophysitis | 2 (0.6) | 1 (0.3) | 24 (7.7) | 5 (1.6) | 12 (3.9) | 6 (1.9) |
| Pulmonary | 5 (1.6) | 1 (0.3) | 22 (7.0) | 3 (1.0) | 6 (1.9) | 1 (0.3) |
| Pneumonitis | 4 (1.3) | 1 (0.3) | 20 (6.4) | 3 (1.0) | 5 (1.6) | 1 (0.3) |

* The severity of adverse events was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0.
† The treatment-related select adverse events listed here were reported in at least 5% of the patients in any of the three study groups.

Table 7. Management of Treatment-related Select Adverse Events With Immune Modulatory Medication (IMM).

| | Nivolumab | | | Nivolumab plus Ipilimumab | | | Ipilimumab | | |
|---|---|---|---|---|---|---|---|---|---|
| **Select Adverse Event Organ Category** | **Patients managed with IMM, n (%)** | **Patients with resolution of AE after treatment with IMM, n (%)** | **Median time to resolution, weeks (95% CI)** | **Patients managed with IMM, n (%)** | **Patients with resolution of AE after treatment with IMM, n (%)** | **Median time to resolution, weeks (95% CI)** | **Patients managed with IMM, n (%)** | **Patients with resolution of AE after treatment with IMM, n (%)** | **Median time to resolution, weeks (95% CI)** |
| **Skin**<br>Grade 3-4 | 33/131 (25.2)<br>3/5 (60.0) | 17 (54.8)<br>3 (75.0) | 34.9 (18.0, NE)<br>2.1 (0.9, NE) | 80/185 (43.2)<br>12/18 (66.7) | 55 (75.3)<br>12 (85.7) | 8.6 (7.0, 12.6)<br>3.4 (1.1, 12.4) | 58/168 (34.5)<br>5/9 (55.6) | 41 (74.5)<br>5 (83.3) | 12.4 (8.9, 19.1)<br>6.1 (4.4, NE) |
| **Gastrointestinal**<br>Grade 3-4 | 9/61 (14.8)<br>5/7 (71.4) | 5 (71.4)<br>3 (50.0) | 4.0 (0.8, NE)<br>NE (0.8, NE) | 71/145 (49.0)<br>41/46 (89.1) | 62 (93.9)<br>41 (97.6) | 4.5 (3.5, 5.7)<br>3.0 (1.9, 4.3) | 54/114 (47.4)<br>33/36 (91.7) | 44 (88.0)<br>31 (88.6) | 4.9 (2.9, 7.6)<br>4.7 (1.9, 6.1) |
| **Endocrine**<br>Grade 3-4 | 5/45 (11.1)<br>2/2 (100) | 2 (40.0)<br>0 | 24.3 (7.1, NE)<br>NE (NE, NE) | 36/94 (38.3)<br>10/15 (66.7) | 14 (41.2)<br>5 (45.5) | NE (10.4, NE)<br>NE (4.5, NE) | 15/34 (44.1)<br>7/7 (100.0) | 4 (28.6)<br>3 (42.9) | NE (0.71, NE)<br>NE (0.43, NE) |
| **Hepatic**<br>Grade 3-4 | 8/22 (36.4)<br>7/8 (87.5) | 6 (100.0)<br>6 (100.0) | 7.0 (2.0, 27.1)<br>7.0 (2.0, 27.1) | 46/95 (48.4)<br>37/60 (61.7) | 43 (95.6)<br>38 (100.0) | 5.9 (3.3, 6.6)<br>4.1 (3.0, 6.1) | 3/22 (13.6)<br>2/5 (40.0) | 3 (100.0)<br>2 (100.0) | 4.1 (4.0, 7.7)<br>5.9 (4.0, 7.7) |
| **Pulmonary**<br>Grade 3-4 | 4/5 (80.0)<br>1/1 (100) | 4 (100.0)<br>1 (100.0) | 3.3 (2.3, 9.1)<br>2.3 (NE, NE) | 17/22 (77.3)<br>2/3 (66.7) | 16 (94.1)<br>2 (100.0) | 6.1 (1.1, 8.3)<br>4.2 (1.1, 7.3) | 3/6 (50.0)<br>1/1 (100) | 2 (66.7)<br>1 (100.0) | 6.1 (6.0, 6.3)<br>4.7 (NE, NE) |
| **Renal**<br>Grade 3-4 | 1/3 (33.3)<br>0/1 | 1 (100.0)<br>0 | 0.3 (NE, NE)<br>- | 4/17 (23.5)<br>3/6 (50.0) | 3 (100.0)<br>3 (100.0) | 1.7 (0.4, 3.7)<br>1.7 (0.4, 3.6) | 3/8 (37.5)<br>1/1 (100) | 3 (100.0)<br>1 (100.0) | 4.6 (0.6, 16.1)<br>4.6 (NE, NE) |

**[0146]** The median progression-free survival reported for the combination of nivolumab and ipilimumab in this study (11.7 months in BRAF-mutant patients) is similar to that recently reported for the combination of BRAF and MEK inhibition in BRAF-mutant metastatic melanoma (9.9 months for vemurafenib and cobimetinib; 9.3 to 11.4 months for dabrafenib and trametinib). Resistance to such targeted therapies is almost inevitable when used as monotherapy and in many cases is very rapid. The confirmed rate of objective response for combined nivolumab and ipilimumab (57.6%) is numerically higher than observed with PD-1 blockade alone in advanced melanoma (nivolumab [40%] in treatment-naïve patients with wild-type BRAF or pembrolizumab [37%] in ipilimumab-naïve patients).

**[0147]** The results of subgroup analyses suggest that the greatest benefit for the combination of nivolumab and ipilimumab versus nivolumab alone may occur in the setting of negative PD-L1 tumor expression. In the PD-L1-positive group, both nivolumab alone and nivolumab plus ipilimumab resulted in a similar prolongation of progression-free survival as compared with ipilimumab alone, although objective response rates were numerically higher in the combination group versus either nivolumab or ipilimumab alone. Thus, the use of PD-L1 as a biomarker may allow clinicians to make more informed decisions about the risk-benefit of combination therapy versus monotherapy. Nonetheless, the observation of at least additive activity of the combination of ipilimumab and nivolumab in the setting of negative PD-L1 expression is of interest in melanoma as well as in other tumor types in which PD-1 checkpoint inhibitors are under evaluation.

**[0148]** The incidence of adverse events in this study was, in general, lowest in the nivolumab group and highest in the combination group. The overall incidence of grade 3 or 4 drug-related adverse events was higher in the combination group as compared with ipilimumab alone (39.6% versus 18.6%), as a result of a slightly higher incidence in most adverse events, particularly hepatic toxicity, where the rates of grade 3 or 4 ALT/AST elevations were 6-8% for the combination and approximately 1% for ipilimumab alone. One drug-related death was reported in each of the nivolumab and ipilimumab groups but none in the combination group. Overall, the safety profile of the combination of nivolumab and ipilimumab was consistent with previous experience with nivolumab or ipilimumab alone. No new safety signals were identified, and adverse events were manageable with established treatment guidelines as most

select adverse events resolved with immune modulatory agents. These data suggest that the combination of nivolumab and ipilimumab can be used safely in a broad range of clinical settings.

**[0149]** In summary, the report shows increased progression-free survival and objective response rates for nivolumab alone and the combination of nivolumab and ipilimumab, as compared with ipilimumab alone, in previously untreated advanced melanoma. Adverse events with the combination were managed with established algorithms, with no study drug-related deaths. The combination of nivolumab and ipilimumab may represent a means to improve outcomes with either agent as monotherapy, particularly for patients having PD-L1-negative tumors. Overall, nivolumab alone and the combination of nivolumab and ipilimumab are promising treatment options for previously untreated advanced melanoma.

## SEQUENCES

$V_H$ amino acid sequence of 28-8 (*Oryctolagus cuniculus*) (SEQ ID NO:1)

Met Glu Thr Gly Leu Arg Trp Leu Leu Leu Val Ala Val Leu Lys Gly Val Gln Cys Leu Ser Val Glu Glu Ser Gly Gly Arg Leu Val Thr Pro Gly Thr Pro Leu Thr Leu Thr Cys Thr Ala Ser Gly Phe Thr Ile Thr Asn Tyr His Met Phe Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile Gly Val Ile Thr Ser Ser Gly Ile Gly Ser Ser Ser Thr Thr Tyr Tyr Ala Thr Trp Ala Lys Gly Arg Phe Thr Ile Ser Lys Thr Ser Thr Thr Val Asn Leu Arg Ile Thr Ser Pro Thr Thr Glu Asp Thr Ala Thr Tyr Phe Cys Ala Arg Asp Tyr Phe Thr Asn Thr Tyr Tyr Ala Leu Asp Ile Trp Gly Pro Gly Thr Leu Val Thr Val Ser Ser

$V_L$ amino acid sequence of 28-8 (*Oryctolagus cuniculus*) (SEQ ID NO:2)

Met Asp Thr Arg Ala Pro Thr Gln Leu Leu Gly Leu Leu Leu Leu Trp Leu Pro Gly Ala Arg Cys Ala Leu Val Met Thr Gln Thr Pro Ser Ser Thr Ser Thr Ala Val Gly Gly Thr Val Thr Ile Lys Cys Gln Ala Ser Gln Ser Ile Ser Val Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro Lys Leu Leu Ile Tyr Ser Ala Ser Thr Leu Ala Ser Gly Val Pro Ser Arg Phe Lys Gly Ser Arg Ser Gly Thr Glu Tyr Thr Leu Thr Ile Ser Gly Val Gln Arg Glu Asp Ala Ala Thr Tyr Tyr Cys Leu Gly Ser Ala Gly Ser

Heavy Chain CDR1 sequence of 28-8 (*Oryctolagus cuniculus*) (SEQ ID NO:3)

Asn Tyr His Met Phe

Heavy Chain CDR2 sequence of 28-8 (*Oryctolagus cuniculus*) (SEQ ID NO:4)

Val Ile Thr Ser Ser Gly Ile Gly Ser Ser Ser Thr Thr Tyr Tyr Ala Thr Trp Ala Lys Gly

Heavy Chain CDR3 sequence of 28-8 (*Oryctolagus cuniculus*) (SEQ ID NO:5)

Asp Tyr Phe Thr Asn Thr Tyr Tyr Ala Leu Asp Ile

Light Chain CDR1 sequence of 28-8 (*Oryctolagus cuniculus*) (SEQ ID NO:6)

Gln Ala Ser Gln Ser Ile Ser Val Tyr Leu Ala

Light Chain CDR2 sequence of 28-8 (*Oryctolagus cuniculus*) (SEQ ID NO:7)

Ser Ala Ser Thr Leu Ala Ser

Light Chain CDR3 sequence of 28-8 (*Oryctolagus cuniculus*) (SEQ ID NO:8)

Leu Gly Ser Ala Gly Ser Asp Asp Ala Ala

# ABSTRACT

The invention provides a method of treating a melanoma comprising (i) identifying a patient having a PD-L1-negative melanoma and (ii) administering to the patient a combination of an anti-PD-1 antibody or an antigen-binding portion thereof and an anti-CTLA-4 antibody or an antigen-binding portion thereof. The methods of the invention can extend progression-free survival for over 8 months and/or reduces the tumor size at least about 10%, about 20%, about 30%, about 40%, or about 50% compared to the tumor size prior to the administration.

12549-US-NP

Atty. Dkt. No. 3338.0330001

PTO/AIA/01 (06-12)
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# DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

| Title of Invention | TREATMENT OF PD-L1-NEGATIVE MELANOMA USING AN ANTI-PD-1 ANTIBODY AND AN ANTI-CTLA-4 ANTIBODY |
|---|---|

As the below named inventor, I hereby declare that:

This declaration is directed to:

☐ The attached application, or

☒ United States application or PCT international application number 15/141,769

filed on April 28, 2016.

The above-identified application was made or authorized to be made by me.

I believe that I am the original inventor or an original joint inventor of a claimed invention in the application.

I hereby acknowledge that any willful false statement made in this declaration is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.

## WARNING:

Petitioner/applicant is cautioned to avoid submitting personal information in documents filed in a patent application that may contribute to identity theft. Personal information such as social security numbers, bank account numbers, or credit card numbers (other than a check or credit card authorization form PTO-2038 submitted for payment purposes) is never required by the USPTO to support a petition or an application. If this type of personal information is included in documents submitted to the USPTO, petitioners/applicants should consider redacting such personal information from the documents before submitting them to the USPTO. Petitioner/applicant is advised that the record of a patent application is available to the public after publication of the application (unless a non-publication request in compliance with 37 CFR 1.213(a) is made in the application) or issuance of a patent. Furthermore, the record from an abandoned application may also be available to the public if the application is referenced in a published application or an issued patent (see 37 CFR 1.14). Checks and credit card authorization forms PTO-2038 submitted for payment purposes are not retained in the application file and therefore are not publicly available.

LEGAL NAME OF INVENTOR

Inventor: Arvin YANG Date (~~Optional~~): August 3rd 2016

Signature: _____

Note: An application data sheet (PTO/SB/14 or equivalent), including naming the entire inventive entity, must accompany this form or must have been previously filed. Use an additional PTO/AIA/01 form for each additional inventor.

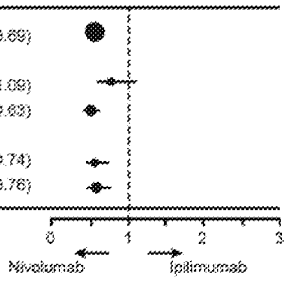
This collection of information is required by 35 U.S.C. 115 and 37 CFR 1.63. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 1 minute to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

*If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.*

# Fig. 3 – Subgroup Analyses of Progression-free Survival

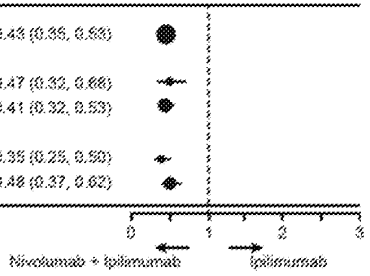
## Fig. 3A Nivolumab versus ipilimumab

| | N | Nivolumab: N of events (N of patients) | Nivolumab: Median PFS (95% CI) | Ipilimumab: N of events (N of patients) | Ipilimumab: Median PFS (95% CI) | Unstratified Hazard Ratio (95% CI) |
|---|---|---|---|---|---|---|
| **Overall** | 945 | 174 (316) | 6.87 (4.34, 9.46) | 234 (315) | 2.89 (2.79, 3.42) | 0.57 (0.47, 0.69) |
| BRAF Mutation Status | | | | | | |
| Mutant | 300 | 57 (98) | 5.62 (2.79, 9.46) | 66 (100) | 4.04 (2.76, 5.52) | 0.77 (0.54, 1.09) |
| Wild-type | 645 | 117 (218) | 7.89 (4.66, 12.68) | 168 (215) | 2.83 (2.76, 3.09) | 0.50 (0.39, 0.63) |
| M Stage at Study Entry | | | | | | |
| M0/M1a/M1b | 386 | 63 (131) | 9.30 (5.62, N.A.) | 92 (126) | 4.17 (2.96, 5.52) | 0.54 (0.39, 0.74) |
| M1c | 559 | 111 (185) | 5.39 (2.83, 8.87) | 142 (189) | 2.79 (2.73, 2.83) | 0.59 (0.46, 0.76) |




## Fig. 3B Nivolumab plus ipilimumab versus ipilimumab

| | N | Nivolumab + Ipilimumab: N of events (N of patients) | Nivolumab + Ipilimumab: Median PFS (95% CI) | Ipilimumab: N of events (N of patients) | Ipilimumab: Median PFS (95% CI) | Unstratified Hazard Ratio (95% CI) |
|---|---|---|---|---|---|---|
| **Overall** | 945 | 151 (314) | 11.50 (8.90, 16.72) | 234 (315) | 2.89 (2.79, 3.42) | 0.43 (0.35, 0.53) |
| BRAF Mutation Status | | | | | | |
| Mutant | 300 | 46 (102) | 11.73 (8.02, N.A.) | 66 (100) | 4.04 (2.79, 5.52) | 0.47 (0.33, 0.68) |
| Wild-type | 645 | 103 (212) | 11.24 (8.34, N.A.) | 168 (215) | 2.83 (2.76, 3.09) | 0.41 (0.32, 0.53) |
| M Stage at Study Entry | | | | | | |
| M0/M1a/M1b | 386 | 51 (129) | 15.54 (11.17, N.A.) | 92 (126) | 4.17 (2.96, 5.52) | 0.35 (0.25, 0.50) |
| M1c | 559 | 100 (185) | 8.51 (5.52, 12.35) | 142 (189) | 2.79 (2.73, 2.83) | 0.48 (0.37, 0.62) |




Inventor: Yang
Appl No: 15/141,769
Sheet 1 of 2
REPLACEMENT SHEET
5/6

Atty Dkt No 3338.03300001

**Fig. 4A Nivolumab**

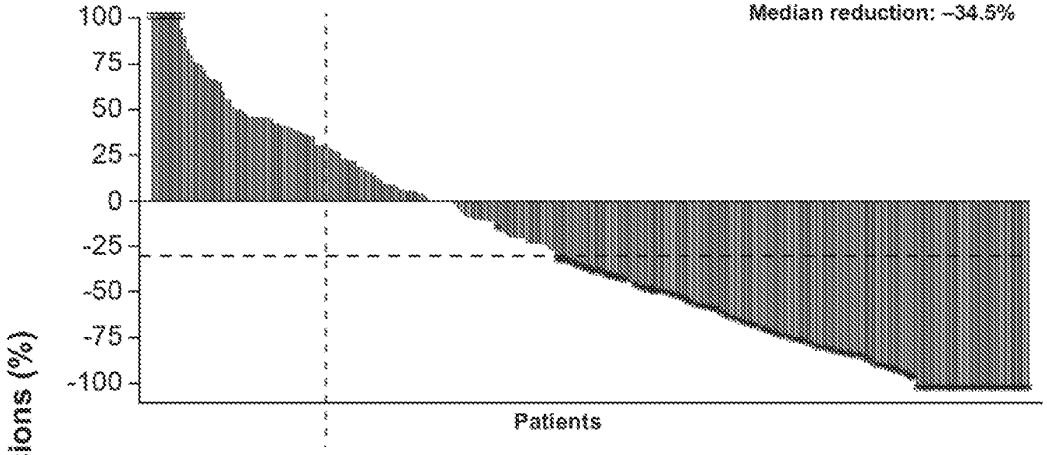



**Fig. 4B Nivolumab + Ipilimumab**

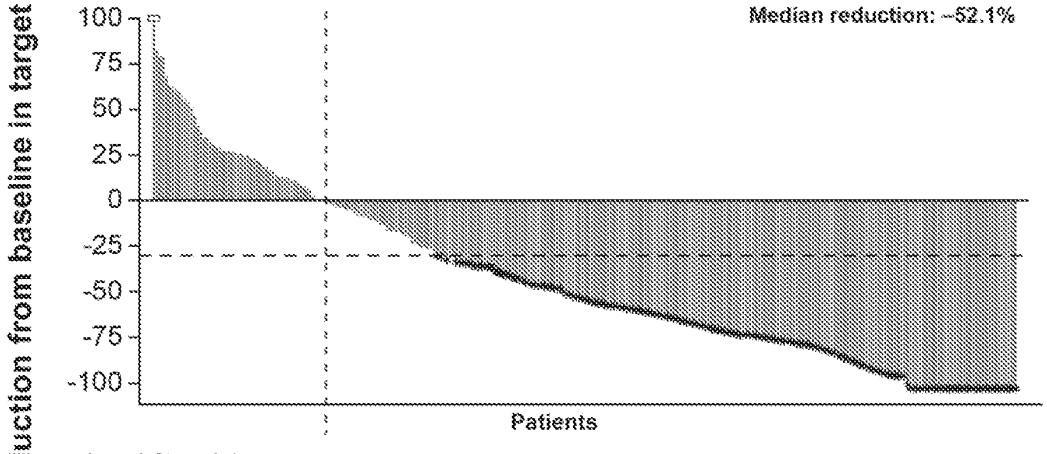
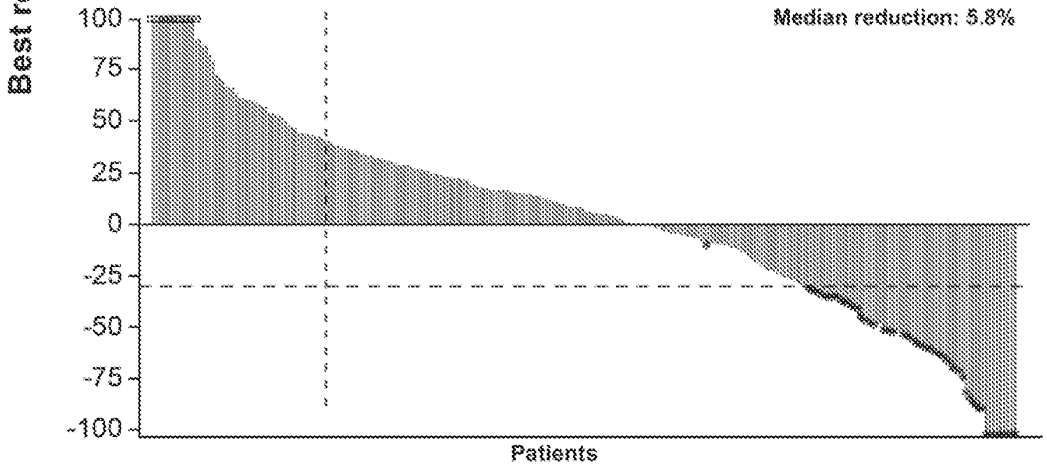



**Fig. 4C Ipilimumab**




Best reduction from baseline in target lesions (%)

Atty Dkt No 3338.0330001

# Electronic Patent Application Fee Transmittal

| | |
|---|---|
| **Application Number:** | 15141769 |
| **Filing Date:** | 28-Apr-2016 |
| **Title of Invention:** | Treatment of PD-L1-Negative Melanoma Using an Anti-PD-1 Antibody and an Anti-CTLA-4 Antibody |
| **First Named Inventor/Applicant Name:** | Arvin YANG |
| **Filer:** | Ji Eun Kim/Jeanette Yando |
| **Attorney Docket Number:** | 3338.0330001/ELE/C-K |

Filed as Large Entity

**Filing Fees for Utility under 35 USC 111(a)**

| Description | Fee Code | Quantity | Amount | Sub-Total in USD($) |
|---|---|---|---|---|
| **Basic Filing:** | | | | |
| **Pages:** | | | | |
| **Claims:** | | | | |
| **Miscellaneous-Filing:** | | | | |
| Late Filing Fee for Oath or Declaration | 1051 | 1 | 140 | 140 |
| **Petition:** | | | | |
| **Patent-Appeals-and-Interference:** | | | | |
| **Post-Allowance-and-Post-Issuance:** | | | | |

| Description | Fee Code | Quantity | Amount | Sub-Total in USD($) |
|---|---|---|---|---|
| **Extension-of-Time:** | | | | |
| Extension - 1 month with $0 paid | 1251 | 1 | 200 | 200 |
| **Miscellaneous:** | | | | |
| | | **Total in USD ($)** | | **340** |

| Electronic Acknowledgement Receipt | |
|---|---|
| **EFS ID:** | 26563020 |
| **Application Number:** | 15141769 |
| **International Application Number:** | |
| **Confirmation Number:** | 6926 |
| **Title of Invention:** | Treatment of PD-L1-Negative Melanoma Using an Anti-PD-1 Antibody and an Anti-CTLA-4 Antibody |
| **First Named Inventor/Applicant Name:** | Arvin YANG |
| **Customer Number:** | 115018 |
| **Filer:** | Ji Eun Kim/Jeanette Yando |
| **Filer Authorized By:** | Ji Eun Kim |
| **Attorney Docket Number:** | 3338.0330001/ELE/C-K |
| **Receipt Date:** | 05-AUG-2016 |
| **Filing Date:** | 28-APR-2016 |
| **Time Stamp:** | 17:13:27 |
| **Application Type:** | Utility under 35 USC 111(a) |

## Payment information:

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|---|---|
| Submitted with Payment | yes |
| Payment Type | CARD |
| Payment was successfully received in RAM | $340 |
| RAM confirmation Number | 080816INTEFSW17153200 |
| Deposit Account | 3007 |
| Authorized User | Jeanette Yando |
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## File Listing:

| Document Number | Document Description | File Name | File Size(Bytes)/ Message Digest | Multi Part /.zip | Pages (if appl.) |
|---|---|---|---|---|---|
| 1 | Transmittal Letter | 33380330001-CLUSPTO.pdf | 176945<br>f9cbd3c3f8a9ae723490c2016465cbfc07725f35 | no | 2 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 2 | Extension of Time | 33380330001-EOT.pdf | 82047<br>453972022058f679e0cb679d3325081f16561b53 | no | 1 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 3 | | 33380330001-PrelAmdt.pdf | 145383<br>8b5666a5013d87397121cebd3a1b965bc2394e8f | yes | 9 |

**Multipart Description/PDF files in .zip description**

| Document Description | Start | End |
|---|---|---|
| Preliminary Amendment | 1 | 1 |
| Specification | 2 | 2 |
| Claims | 3 | 6 |
| Drawings-only black and white line drawings | 7 | 7 |
| Applicant Arguments/Remarks Made in an Amendment | 8 | 9 |

| | | | | | |
|---|---|---|---|---|---|
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 4 | Specification | 33380330001-CleanSpec.pdf | 432991<br>2012d2827eb1bc6099e740a8975e153b3c698bbe | no | 57 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |

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|---|---|---|---|---|---|
| 5 | Specification | 33380330001-MarkedupSpec.pdf | 430473<br>19308bc6ce740a1efc88cbe63e1d0480903bd16f | no | 57 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 6 | Oath or Declaration filed | 33380330001-Dec.pdf | 291633<br>ab2ef363821781656c4df95a559b3dbf0cc3166f | no | 1 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| 7 | Drawings-only black and white line drawings | 33380330001ReplacementSheet.pdf | 352705<br>d632cf084517702dba7782bd4b411211ce54fd33 | no | 2 |
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| **Information:** | | | | | |
| 8 | Fee Worksheet (SB06) | fee-info.pdf | 32439<br>647cc312330e68253976eb416bd22da1006d63ae | no | 2 |
| **Warnings:** | | | | | |
| **Information:** | | | | | |
| | | **Total Files Size (in bytes):** | 1944616 | | |

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**New Applications Under 35 U.S.C. 111**
**If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.**

**National Stage of an International Application under 35 U.S.C. 371**
**If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.**

**New International Application Filed with the USPTO as a Receiving Office**
**If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.**

**JI-EUN KIM**
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Sterne Kessler Goldstein Fox
ATTORNEYS AT LAW

August 5, 2016

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

***Confirmation No. 6926***
***Mail Stop Missing Parts***

Re: U.S. Nonprovisional Patent Application
Appl. No. 15/141,769; Filed: April 28, 2016
For: **TREATMENT OF PD-L1-NEGATIVE MELANOMA USING AN ANTI-PD-1 ANTIBODY AND AN ANTI-CTLA-4 ANTIBODY**
Inventor: Arvin YANG
Our Ref: 3338.0330001/ELE/C-K/BMD

Commissioner:

In reply to the "Notice to File Corrected Application Papers," dated May 6, 2016, Applicant transmits the following documents for appropriate action by the U.S. Patent and Trademark Office:

1. Online Credit Card Payment Authorization for **$340.00** to cover:

   **$ 140.00** Surcharge for late filing fee or late Oath or Declaration;
   **$ 200.00** Extension of time fees;

2. Petition for Extension of Time Under 37 C.F.R. § 1.136(a);

3. Original Declaration, executed by the inventor;

4. Preliminary Amendment Under 37 C.F.R. § 1.115;

5. 2 sheets of replacement drawings: (Figures 3A-3B and 4A-4C);

6. Marked-up copy of Substitute Specification; and

7. Clean copy of Substitute Specification.

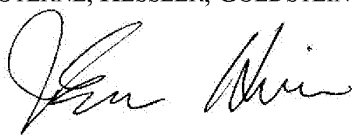
The above-listed documents are filed electronically.

Fee payment is provided through online credit card payment. The U.S. Patent and Trademark Office is hereby authorized to charge any fee deficiency, or credit any overpayment, to our Deposit Account No. 19-0036.

If extensions of time under 37 C.F.R. § 1.136 other than those otherwise provided for herewith are required to prevent abandonment of the present patent application, then such extensions of time are hereby petitioned, and any fees therefor are hereby authorized to be charged to our Deposit Account No. 19-0036.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.



Ji-Eun Kim
Attorney for Applicant
Registration No. 68,504

C-K/BMD/jly
Enclosure(s)

2847440_1

PTO/AIA/22 (03-13)
Approved for use through 7/31/2016. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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# PETITION FOR EXTENSION OF TIME UNDER 37 CFR 1.136(a)

Docket Number (Optional): 3338.0330001

| Application Number | Filed |
|---|---|
| 15/141,769 | April 28, 2016 |

For: TREATMENT OF PD-L1-NEGATIVE MELANOMA USING AN ANTI-PD-1 ANTIBODY AND AN ANTI-CTLA-4 ANTIBODY

| Art Unit | Examiner |
|---|---|
| 1618 | To Be Assigned |

This is a request under the provisions of 37 CFR 1.136(a) to extend the period for filing a reply in the above-identified application.

The requested extension and fee are as follows (check time period desired and enter the appropriate fee below):

| | | Fee | Small Entity Fee | Micro Entity Fee | |
|---|---|---|---|---|---|
| [X] | One month (37 CFR 1.17(a)(1)) | $200 | $100 | $50 | $ 200.00 |
| [ ] | Two months (37 CFR 1.17(a)(2)) | $600 | $300 | $150 | $ |
| [ ] | Three months (37 CFR 1.17(a)(3)) | $1,400 | $700 | $350 | $ |
| [ ] | Four months (37 CFR 1.17(a)(4)) | $2,200 | $1,100 | $550 | $ |
| [ ] | Five months (37 CFR 1.17(a)(5)) | $3,000 | $1,500 | $750 | $ |

[ ] Applicant asserts small entity status. See 37 CFR 1.27.

[ ] Applicant certifies micro entity status. See 37 CFR 1.29.
Form PTO/SB/15A or B or equivalent must either be enclosed or have been submitted previously.

[ ] A check in the amount of the fee is enclosed.

[ ] Payment by credit card. Form PTO-2038 is attached.

[ ] The Director has already been authorized to charge fees in this application to a Deposit Account.

[X] The Director is hereby authorized to charge any fees which may be required, or credit any overpayment, to Deposit Account Number 19-0036.

[X] Payment made via EFS-Web.

**WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.**

I am the

[ ] applicant.

[X] attorney or agent of record. Registration number 68,504.

[ ] attorney or agent acting under 37 CFR 1.34. Registration number _____.

| /Ji-Eun Kim/ | August 5, 2016 |
|---|---|
| Signature | Date |
| Ji-Eun Kim | (202) 371-2600 |
| Typed or printed name | Telephone Number |

**NOTE:** This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications. Submit multiple forms if more than one signature is required, see below*.

[X] * Total of 1 forms are submitted.

This collection of information is required by 37 CFR 1.136(a). The information is required to obtain or retain a benefit by the public, which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 6 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Mail Stop PCT, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

# SCORE Placeholder Sheet for IFW Content

Application Number: 15141769 Document Date: 08/05/2016

The presence of this form in the IFW record indicates that the following document type was received in electronic format on the date identified above. This content is stored in the SCORE database.

- Drawings – Other than Black and White Line Drawings

Since this was an electronic submission, there is no physical artifact folder, no artifact folder is recorded in PALM, and no paper documents or physical media exist. The TIFF images in the IFW record were created from the original documents that are stored in SCORE.

To access the documents in the SCORE database, refer to instructions below.

At the time of document entry (noted above):

- Examiners may access SCORE content via the eDAN interface.
- Other USPTO employees can bookmark the current SCORE URL (http://Score.uspto.gov/ScoreAccessWeb/).
- External customers may access SCORE content via the Public and Private PAIR interfaces.

Form Revision Date: September 30, 2013

PTO/SB/06 (09-11)
Approved for use through 1/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

| PATENT APPLICATION FEE DETERMINATION RECORD<br>Substitute for Form PTO-875 | Application or Docket Number<br>15/141,769 | Filing Date<br>04/28/2016 | ☐ To be Mailed |
|---|---|---|---|

**ENTITY**: ☒ LARGE ☐ SMALL ☐ MICRO

## APPLICATION AS FILED – PART I

| FOR | (Column 1) NUMBER FILED | (Column 2) NUMBER EXTRA | RATE ($) | FEE ($) |
|---|---|---|---|---|
| ☐ BASIC FEE (37 CFR 1.16(a), (b), or (c)) | N/A | N/A | N/A | |
| ☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m)) | N/A | N/A | N/A | |
| ☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q)) | N/A | N/A | N/A | |
| TOTAL CLAIMS (37 CFR 1.16(i)) | minus 20 = | * | X $ = | |
| INDEPENDENT CLAIMS (37 CFR 1.16(h)) | minus 3 = | * | X $ = | |
| ☐ APPLICATION SIZE FEE (37 CFR 1.16(s)) | If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s). | | | |
| ☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j)) | | | | |
| * If the difference in column 1 is less than zero, enter "0" in column 2. | | | TOTAL | |

## APPLICATION AS AMENDED – PART II

AMENDMENT **08/05/2016**

| | (Column 1) CLAIMS REMAINING AFTER AMENDMENT | | (Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR | (Column 3) PRESENT EXTRA | RATE ($) | ADDITIONAL FEE ($) |
|---|---|---|---|---|---|---|
| Total (37 CFR 1.16(i)) | * 20 | Minus | ** 20 | = 0 | X $80 = | 0 |
| Independent (37 CFR 1.16(h)) | * 3 | Minus | ***3 | = 0 | X $420 = | 0 |
| ☐ Application Size Fee (37 CFR 1.16(s)) | | | | | | |
| ☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j)) | | | | | | |
| | | | | | TOTAL ADD'L FEE | 0 |

AMENDMENT

| | (Column 1) CLAIMS REMAINING AFTER AMENDMENT | | (Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR | (Column 3) PRESENT EXTRA | RATE ($) | ADDITIONAL FEE ($) |
|---|---|---|---|---|---|---|
| Total (37 CFR 1.16(i)) | * | Minus | ** | = | X $ = | |
| Independent (37 CFR 1.16(h)) | * | Minus | *** | = | X $ = | |
| ☐ Application Size Fee (37 CFR 1.16(s)) | | | | | | |
| ☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j)) | | | | | | |
| | | | | | TOTAL ADD'L FEE | |

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

LIE
/PAMELA V. THERATT/

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**
*If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.*

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

| APPLICATION NUMBER | FILING or 371(c) DATE | GRP ART UNIT | FIL FEE REC'D | ATTY.DOCKET.NO | TOT CLAIMS | IND CLAIMS |
|---|---|---|---|---|---|---|
| 15/141,769 | 04/28/2016 | 1618 | 1600 | 3338.0330001/ELE/C-K | 47 | 6 |

**CONFIRMATION NO. 6926**

115018
Sterne, Kessler, Goldstein & Fox P.L.L.C.
1100 New York Avenue NW
Washington, DC 20005

**FILING RECEIPT**



Date Mailed: 05/06/2016

Receipt is acknowledged of this non-provisional patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please submit a written request for a Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections**

**Inventor(s)**
Arvin YANG, Princeton, NJ;

**Applicant(s)**
Bristol-Myers Squibb Company, Princeton, NJ

**Assignment For Published Patent Application**
Bristol-Myers Squibb Company, Princeton, NJ

**Power of Attorney:** The patent practitioners associated with Customer Number 115018

**Domestic Priority data as claimed by applicant**
This appln claims benefit of 62/153,973 04/28/2015

**Foreign Applications** for which priority is claimed (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see http://www.uspto.gov for more information.) - None.
*Foreign application information must be provided in an Application Data Sheet in order to constitute a claim to foreign priority. See 37 CFR 1.55 and 1.76.*

**Permission to Access Application via Priority Document Exchange:** Yes

**Permission to Access Search Results:** Yes

Applicant may provide or rescind an authorization for access using Form PTO/SB/39 or Form PTO/SB/69 as appropriate.

**If Required, Foreign Filing License Granted:** 05/06/2016

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 15/141,769**

**Projected Publication Date:** To Be Determined - pending completion of Corrected Papers

**Non-Publication Request:** No

**Early Publication Request:** No
**Title**

Treatment of PD-L1-Negative Melanoma Using an Anti-PD-1 Antibody and an Anti-CTLA-4 Antibody

**Preliminary Class**

424

**Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications:** No

## PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

Applicants also are advised that in the case of inventions made in the United States, the Director of the USPTO must issue a license before applicants can apply for a patent in a foreign country. The filing of a U.S. patent application serves as a request for a foreign filing license. The application's filing receipt contains further information and guidance as to the status of applicant's license for foreign filing.

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at http://www.uspto.gov/web/offices/pac/doc/general/index.html.

For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, http://www.stopfakes.gov. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4258).

## LICENSE FOR FOREIGN FILING UNDER

## Title 35, United States Code, Section 184

## Title 37, Code of Federal Regulations, 5.11 & 5.15

**GRANTED**

The applicant has been granted a license under 35 U.S.C. 184, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" followed by a date appears on this form. Such licenses are issued in all applications where the conditions for issuance of a license have been met, regardless of whether or not a license may be required as set forth in 37 CFR 5.15. The scope and limitations of this license are set forth in 37 CFR 5.15(a) unless an earlier license has been issued under 37 CFR 5.15(b). The license is subject to revocation upon written notification. The date indicated is the effective date of the license, unless an earlier license of similar scope has been granted under 37 CFR 5.13 or 5.14.

This license is to be retained by the licensee and may be used at any time on or after the effective date thereof unless it is revoked. This license is automatically transferred to any related applications(s) filed under 37 CFR 1.53(d). This license is not retroactive.

The grant of a license does not in any way lessen the responsibility of a licensee for the security of the subject matter as imposed by any Government contract or the provisions of existing laws relating to espionage and the national security or the export of technical data. Licensees should apprise themselves of current regulations especially with respect to certain countries, of other agencies, particularly the Office of Defense Trade Controls, Department of State (with respect to Arms, Munitions and Implements of War (22 CFR 121-128)); the Bureau of Industry and Security, Department of Commerce (15 CFR parts 730-774); the Office of Foreign AssetsControl, Department of Treasury (31 CFR Parts 500+) and the Department of Energy.

**NOT GRANTED**

No license under 35 U.S.C. 184 has been granted at this time, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" DOES NOT appear on this form. Applicant may still petition for a license under 37 CFR 5.12, if a license is desired before the expiration of 6 months from the filing date of the application. If 6 months has lapsed from the filing date of this application and the licensee has not received any indication of a secrecy order under 35 U.S.C. 181, the licensee may foreign file the application pursuant to 37 CFR 5.15(b).

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The United States represents the largest, most dynamic marketplace in the world and is an unparalleled location for business investment, innovation, and commercialization of new technologies. The U.S. offers tremendous resources and advantages for those who invest and manufacture goods here. Through SelectUSA, our nation works to promote and facilitate business investment. SelectUSA provides information assistance to the international investor community; serves as an ombudsman for existing and potential investors; advocates on behalf of U.S. cities, states, and regions competing for global investment; and counsels U.S. economic development organizations on investment attraction best practices. To learn more about why the United States is the best country in the world to develop technology, manufacture products, deliver services, and grow your business, visit http://www.SelectUSA.gov or call +1-202-482-6800.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

| APPLICATION NUMBER | FILING OR 371(C) DATE | FIRST NAMED APPLICANT | ATTY. DOCKET NO./TITLE |
|---|---|---|---|
| 15/141,769 | 04/28/2016 | Arvin YANG | 3338.0330001/ELE/C-K |

**CONFIRMATION NO. 6926**

115018
Sterne, Kessler, Goldstein & Fox P.L.L.C.
1100 New York Avenue NW
Washington, DC 20005

**FORMALITIES LETTER**

*OC000000082750138*

Date Mailed: 05/06/2016

## NOTICE TO FILE CORRECTED APPLICATION PAPERS

***Filing Date Granted***

An application number and filing date have been accorded to this application. The application is informal since it does not comply with the regulations for the reason(s) indicated below. Applicant is given TWO MONTHS from the date of this Notice within which to correct the informalities indicated below. Extensions of time may be obtained by filing a petition accompanied by the extension fee under the provisions of 37 CFR 1.136(a).

The required item(s) identified below must be timely submitted to avoid abandonment:

- Replacement drawings in compliance with 37 CFR 1.84 and 37 CFR 1.121(d) are required. The drawings submitted are not acceptable because:
  - The application contains drawings and the specification contains a brief description of the drawings. However, the specification does not contain a brief description of the several views of the drawings as required by 37 CFR 1.74 and 37 CFR 1.77(b)(7) and/or a drawing(s) has not been labeled in accordance with 37 CFR 1.84(u)(1). If each figure is not labeled "Fig." with a consecutive Arabic numeral (1, 2, etc.) or an Arabic numeral and capital letter in the English alphabet (A, B, etc.), then the drawing(s) must be relabeled in accordance with 37 CFR 1.84(u)(1). In addition, if the brief description of the several views of the drawings does not refer to the figure(s) as properly labeled, then the specification must be amended to correspond to the figure(s) as properly labeled and a substitute specification in compliance with 37 CFR 1.52, 1.121(b)(3), and 1.125, is required.

Applicant is cautioned that correction of the above items may cause the specification and drawings page count to exceed 100 pages. If the specification and drawings exceed 100 pages, applicant will need to submit the required application size fee.

- Additional claim fees of $ **4600** as an undiscounted entity, including any required multiple dependent claim fee, are required. Applicant must submit the additional claim fees or cancel the additional claims for which fees are due.
- Surcharge as set forth in 37 CFR 1.16(f) must be submitted.
  The surcharge is due for any one of:
  - late submission of the basic filing fee, search fee, or examination fee,
  - late submission of inventor's oath or declaration,
  - filing an application that does not contain at least one claim on filing, or
  - submission of an application filed by reference to a previously filed application.

**SUMMARY OF FEES DUE:**

The fee(s) required within **TWO MONTHS** from the date of this Notice to avoid abandonment is/are itemized below. No entity status discount is in effect. If applicant is qualified for small entity status, a written assertion of small entity status must be submitted to establish small entity status. (See 37 CFR 1.27). If applicant is qualified for micro entity status, an acceptable Certification of Micro Entity Status must be submitted to establish micro entity status. (See 37 CFR 1.29 and forms PTO/SB/15A and 15B.)

- $ **140** surcharge.
- $ **1260** for **3** independent claims over 3.
- $ **2560** for **32** total claims over 20.
- $ **780** for multiple dependent claim surcharge.
- $( **0**) previous unapplied payment amount.
- $ **4740** TOTAL FEE BALANCE DUE.

**Items Required To Avoid Processing Delays:**

Applicant is notified that the above-identified application contains the deficiencies noted below. No period for reply is set forth in this notice for correction of these deficiencies. However, if a deficiency relates to the inventor's oath or declaration, the applicant must file an oath or declaration in compliance with 37 CFR 1.63, or a substitute statement in compliance with 37 CFR 1.64, executed by or with respect to each actual inventor no later than the expiration of the time period set in the "Notice of Allowability" to avoid abandonment. See 37 CFR 1.53(f).

- A properly executed inventor's oath or declaration has not been received for the following inventor(s):
  Arvin YANG

Replies must be received in the USPTO within the set time period or must include a proper Certificate of Mailing or Transmission under 37 CFR 1.8 with a mailing or transmission date within the set time period. For more information and a suggested format, see Form PTO/SB/92 and MPEP 512.

Replies should be mailed to:

Mail Stop Missing Parts
Commissioner for Patents
P.O. Box 1450
Alexandria VA 22313-1450

Registered users of EFS-Web may alternatively submit their reply to this notice via EFS-Web, including a copy of this Notice and selecting the document description "Applicant response to Pre-Exam Formalities Notice". https://sportal.uspto.gov/authenticate/AuthenticateUserLocalEPF.html

For more information about EFS-Web please call the USPTO Electronic Business Center at 1-866-217-9197 or visit our website at http://www.uspto.gov/ebc.

If you are not using EFS-Web to submit your reply, you must include a copy of this notice.

Questions about the contents of this notice and the requirements it sets forth should be directed to the Office of Data Management, Application Assistance Unit, at **(571) 272-4000** or **(571) 272-4200** or **1-888-786-0101**.

/tpetros/

## PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number: 15/141,769

### APPLICATION AS FILED - PART I

| FOR | (Column 1) NUMBER FILED | (Column 2) NUMBER EXTRA | SMALL ENTITY RATE($) | SMALL ENTITY FEE($) | OR | OTHER THAN SMALL ENTITY RATE($) | OTHER THAN SMALL ENTITY FEE($) |
|---|---|---|---|---|---|---|---|
| BASIC FEE (37 CFR 1.16(a), (b), or (c)) | N/A | N/A | N/A | | | N/A | 280 |
| SEARCH FEE (37 CFR 1.16(k), (i), or (m)) | N/A | N/A | N/A | | | N/A | 600 |
| EXAMINATION FEE (37 CFR 1.16(o), (p), or (q)) | N/A | N/A | N/A | | | N/A | 720 |
| TOTAL CLAIMS (37 CFR 1.16(i)) | 52 minus 20 = | * 32 | | | OR | x 80 = | 2560 |
| INDEPENDENT CLAIMS (37 CFR 1.16(h)) | 6 minus 3 = | * 3 | | | | x 420 = | 1260 |
| APPLICATION SIZE FEE (37 CFR 1.16(s)) | If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s). | | | | | | 0.00 |
| MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j)) | | | | | | | 780 |
| * If the difference in column 1 is less than zero, enter "0" in column 2. | | | TOTAL | | | TOTAL | 6200 |

### APPLICATION AS AMENDED - PART II

| | | (Column 1) CLAIMS REMAINING AFTER AMENDMENT | | (Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR | (Column 3) PRESENT EXTRA | SMALL ENTITY RATE($) | SMALL ENTITY ADDITIONAL FEE($) | OR | OTHER THAN SMALL ENTITY RATE($) | OTHER THAN SMALL ENTITY ADDITIONAL FEE($) |
|---|---|---|---|---|---|---|---|---|---|---|
| AMENDMENT A | Total (37 CFR 1.16(i)) | * | Minus | ** | = | x = | | OR | x = | |
| | Independent (37 CFR 1.16(h)) | * | Minus | *** | = | x = | | OR | x = | |
| | Application Size Fee (37 CFR 1.16(s)) | | | | | | | | | |
| | FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j)) | | | | | | | OR | | |
| | | | | | | TOTAL ADD'L FEE | | OR | TOTAL ADD'L FEE | |

| | | (Column 1) CLAIMS REMAINING AFTER AMENDMENT | | (Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR | (Column 3) PRESENT EXTRA | RATE($) | ADDITIONAL FEE($) | | RATE($) | ADDITIONAL FEE($) |
|---|---|---|---|---|---|---|---|---|---|---|
| AMENDMENT B | Total (37 CFR 1.16(i)) | * | Minus | ** | = | x = | | OR | x = | |
| | Independent (37 CFR 1.16(h)) | * | Minus | *** | = | x = | | OR | x = | |
| | Application Size Fee (37 CFR 1.16(s)) | | | | | | | | | |
| | FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j)) | | | | | | | OR | | |
| | | | | | | TOTAL ADD'L FEE | | OR | TOTAL ADD'L FEE | |

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.

** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".

*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".

The "Highest Number Previously Paid For" (Total or Independent) is the highest found in the appropriate box in column 1.

# MULTIPLE DEPENDENT CLAIM FEE CALCULATION SHEET

Substitute for Form PTO-1360
(For use with Form PTO/SB/06)

| Application Number | Filing Date |
|---|---|
| 15141769 | |

Applicant(s) Arvin YANG

* May be used for additional claims or amendments

| CLAIMS | AS FILED | | AFTER FIRST AMENDMENT | | AFTER SECOND AMENDMENT | |
|---|---|---|---|---|---|---|
| | Indep | Depend | Indep | Depend | Indep | Depend |
| 1 | 1 | | | | | |
| 2 | 1 | | | | | |
| 3 | 1 | | | | | |
| 4 | | 1 | | | | |
| 5 | | 1 | | | | |
| 6 | 1 | | | | | |
| 7 | | 5 | | | | |
| 8 | | ( 1 ) | | | | |
| 9 | 1 | | | | | |
| 10 | | 1 | | | | |
| 11 | | 2 | | | | |
| 12 | | ( 1 ) | | | | |
| 13 | 1 | | | | | |
| 14 | | ( 1 ) | | | | |
| 15 | | ( 1 ) | | | | |
| 16 | | ( 1 ) | | | | |
| 17 | | ( 1 ) | | | | |
| 18 | | ( 1 ) | | | | |
| 19 | | ( 1 ) | | | | |
| 20 | | ( 1 ) | | | | |
| 21 | | ( 1 ) | | | | |
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| 25 | | ( 1 ) | | | | |
| 26 | | ( 1 ) | | | | |
| 27 | | ( 1 ) | | | | |
| 28 | | ( 1 ) | | | | |
| 29 | | ( 1 ) | | | | |
| 30 | | ( 1 ) | | | | |
| 31 | | ( 1 ) | | | | |
| 32 | | ( 1 ) | | | | |
| 33 | | ( 1 ) | | | | |
| 34 | | ( 1 ) | | | | |
| 35 | | ( 1 ) | | | | |
| 36 | | ( 1 ) | | | | |
| 37 | | ( 1 ) | | | | |
| 38 | | ( 1 ) | | | | |
| 39 | | ( 1 ) | | | | |
| 40 | | ( 1 ) | | | | |
| 41 | | ( 1 ) | | | | |
| 42 | | ( 1 ) | | | | |
| 43 | | ( 1 ) | | | | |
| 44 | | ( 1 ) | | | | |
| 45 | | ( 1 ) | | | | |
| 46 | | ( 1 ) | | | | |
| 47 | | ( 1 ) | | | | |
| 48 | | | | | | |
| 49 | | | | | | |
| 50 | | | | | | |
| Total Indep | 6 | | 0 | | 0 | |
| Total Depend | 46 | ↵ | 0 | ↵ | 0 | ↵ |
| Total Claims | 52 | | 0 | | 0 | |

| | * | | * | | * | |
|---|---|---|---|---|---|---|
| | Indep | Depend | Indep | Depend | Indep | Depend |
| 51 | | | | | | |
| 52 | | | | | | |
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| 54 | | | | | | |
| 55 | | | | | | |
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If you need help call the Patent Electronic Business Center at (866) 217-9197 (toll free).
Reviewer: Anjum, Durreshwar (CGI Federal)
Timestamp: [year=2016; month=5; day=5; hr=10; min=21; sec=25; ms=900; ]
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**Validated By CRFValidator v 1.0.5**

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Yang, Arvin

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# UTILITY PATENT APPLICATION TRANSMITTAL

*(Only for new nonprovisional applications under 37 CFR 1.53(b))*

| | |
|---|---|
| *Attorney Docket No.* | 3338.0330001/ELE/C-K |
| *First Named Inventor* | Arvin YANG |
| *Title* | See 1 in Addendum |
| *Express Mail Label No.* | |

## APPLICATION ELEMENTS

*See MPEP chapter 600 concerning utility patent application contents.*

1. [ ] **Fee Transmittal Form** (PTO/SB/17 or equivalent)
2. [ ] **Applicant asserts small entity status.** See 37 CFR 1.27
3. [ ] **Applicant certifies micro entity status.** See 37 CFR 1.29. Applicant must attach form PTO/SB/15A or B or equivalent.
4. [X] **Specification** [*Total Pages* 64] Both the claims and abstract must start on a new page. *(See MPEP § 608.01(a) for information on the preferred arrangement)*
5. [X] **Drawing(s)** (35 U.S.C. 113) [*Total Sheets* 6]
6. **Inventor's Oath or Declaration** [*Total Pages* ____] *(including substitute statements under 37 CFR 1.64 and assignments serving as an oath or declaration under 37 CFR 1.63(e))*
   a. [ ] Newly executed (original or copy)
   b. [ ] A copy from a prior application (37 CFR 1.63(d))
7. [X] **Application Data Sheet** ** See note below.* See 37 CFR 1.76 (PTO/AIA/14 or equivalent)
8. **CD-ROM or CD-R** in duplicate, large table, or Computer Program (*Appendix*)
   [ ] Landscape Table on CD
9. **Nucleotide and/or Amino Acid Sequence Submission** (*if applicable, items a. – c. are required*)
   a. [ ] Computer Readable Form (CRF)
   b. [ ] Specification Sequence Listing on:
      i. [ ] CD-ROM or CD-R (2 copies); or
      ii. [ ] Paper
   c. [ ] Statements verifying identity of above copies

## ADDRESS TO:

**Commissioner for Patents**
**P.O. Box 1450**
**Alexandria, VA 22313-1450**

## ACCOMPANYING APPLICATION PAPERS

10. [ ] **Assignment Papers** (cover sheet & document(s))
    Name of Assignee _____
11. [ ] **37 CFR 3.73(c) Statement** [X] **Power of Attorney** (*when there is an assignee*)
12. [ ] **English Translation Document** (*if applicable*)
13. [ ] **Information Disclosure Statement** (PTO/SB/08 or PTO-1449)
    [ ] Copies of citations attached
14. [ ] **Preliminary Amendment**
15. [ ] **Return Receipt Postcard** (*MPEP § 503*) (*Should be specifically itemized*)
16. [ ] **Certified Copy of Priority Document(s)** (*if foreign priority is claimed*)
17. [ ] **Nonpublication Request** Under 35 U.S.C. 122(b)(2)(B)(i). Applicant must attach form PTO/SB/35 or equivalent.
18. [X] **Other:** **Authorization under 37 CFR 1.136(a)(3)** ASCII text file of Sequence Listing

*Note: (1) Benefit claims under 37 CFR 1.78 and foreign priority claims under 1.55 **must** be included in an Application Data Sheet (ADS).
(2) For applications filed under 35 U.S.C. 111, the application must contain an ADS specifying the applicant if the applicant is an assignee, person to whom the inventor is under an obligation to assign, or person who otherwise shows sufficient proprietary interest in the matter. See 37 CFR 1.46(b).

## 19. CORRESPONDENCE ADDRESS

[X] The address associated with Customer Number: **115018** OR [ ] Correspondence address below

| | | | | | |
|---|---|---|---|---|---|
| Name | | | | | |
| Address | | | | | |
| City | | State | | Zip Code | |
| Country | | Telephone | | Email | |

| | | | |
|---|---|---|---|
| Signature | /Ji-Eun Kim/ | Date | April 28, 2016 |
| Name (Print/Type) | Ji-Eun Kim | Registration No. (Attorney/Agent) | 68,504 |

This collection of information is required by 37 CFR 1.53(b). The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

*If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.*

# Addendum

1. TREATMENT OF PD-L1-NEGATIVE MELANOMA USING AN ANTI-PD-1 ANTIBODY AND AN ANTI-CTLA-4 ANTIBODY

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

| | |
|---|---|
| Inventor: Arvin YANG | Confirmation No.: *To Be Assigned* |
| Applicant: Bristol-Myers Squibb Co. | Art Unit: *To Be Assigned* |
| Application No.: *To Be Assigned* | Examiner: *To Be Assigned* |
| Filing Date: *Herewith* | Atty. Docket: 3338.0330001 |

Title: **TREATMENT OF PD-L1 NEGATIVE MELANOMA USING AN ANTI-PD-1 ANTIBODY AND AN ANTI-CTLA-4 ANTIBODY**

## Authorization to Treat a Reply as Incorporating an Extension of Time Under 37 C.F.R. § 1.136(a)(3)

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

Commissioner:

The U.S. Patent and Trademark Office is hereby authorized to treat any concurrent or future reply that requires a petition for an extension of time under this paragraph for its timely submission, as incorporating a petition for extension of time for the appropriate length of time. The U.S. Patent and Trademark Office is hereby authorized to charge all required extension of time fees to our Deposit Account No. 19-0036, if such fees are not otherwise provided for in such reply.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.



Ji-Eun Kim
Attorney for Applicant
Registration No. 68,504

Date: April 28, 2016

1100 New York Avenue, N.W.
Washington, D.C. 20005-3934
(202) 371-2600

2802637_1

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| Application Data Sheet 37 CFR 1.76 | Attorney Docket Number | 3338.0330001/ELE/C-K |
|---|---|---|
| | Application Number | |
| Title of Invention | TREATMENT OF PD-L1-NEGATIVE MELANOMA USING AN ANTI-PD-1 ANTIBODY AND AN ANTI-CTLA-4 ANTIBODY | |

The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76.
This document may be completed electronically and submitted to the Office in electronic format using the Electronic Filing System (EFS) or the document may be printed and included in a paper filed application.

## Secrecy Order 37 CFR 5.2:

☐ Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)

## Inventor Information:

**Inventor 1** Remove

**Legal Name**

| Prefix | Given Name | Middle Name | Family Name | Suffix |
|---|---|---|---|---|
| | Arvin | | YANG | |

**Residence Information (Select One)** ◉ US Residency ○ Non US Residency ○ Active US Military Service

| City | | State/Province | | Country of Residence | |
|---|---|---|---|---|---|

**Mailing Address of Inventor:**

| | | | |
|---|---|---|---|
| **Address 1** | Rt. 206 & Province Line Road | | |
| **Address 2** | | | |
| **City** | Princeton | **State/Province** | NJ |
| **Postal Code** | 08543 | **Country** | US |

All Inventors Must Be Listed - Additional Inventor Information blocks may be generated within this form by selecting the **Add** button. Add

## Correspondence Information:

**Enter either Customer Number or complete the Correspondence Information section below.**
**For further information see 37 CFR 1.33(a).**

☐ **An Address is being provided for the correspondence Information of this application.**

| | | | |
|---|---|---|---|
| **Customer Number** | 115018 | | |
| **Email Address** | | Add Email | Remove Email |

## Application Information:

| | | | |
|---|---|---|---|
| **Title of the Invention** | TREATMENT OF PD-L1-NEGATIVE MELANOMA USING AN ANTI-PD-1 ANTIBODY AND AN ANTI-CTLA-4 ANTIBODY | | |
| **Attorney Docket Number** | 3338.0330001/ELE/C-K | **Small Entity Status Claimed** | ☐ |
| **Application Type** | Nonprovisional | | |
| **Subject Matter** | Utility | | |
| **Total Number of Drawing Sheets (if any)** | 6 | **Suggested Figure for Publication (if any)** | |

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| **Application Data Sheet 37 CFR 1.76** | Attorney Docket Number | 3338.0330001/ELE/C-K |
|---|---|---|
| | Application Number | |
| Title of Invention | TREATMENT OF PD-L1-NEGATIVE MELANOMA USING AN ANTI-PD-1 ANTIBODY AND AN ANTI-CTLA-4 ANTIBODY | |

## Filing By Reference:

Only complete this section when filing an application by reference under 35 U.S.C. 111(c) and 37 CFR 1.57(a). Do not complete this section if application papers including a specification and any drawings are being filed. Any domestic benefit or foreign priority information must be provided in the appropriate section(s) below (i.e., "Domestic Benefit/National Stage Information" and "Foreign Priority Information").

For the purposes of a filing date under 37 CFR 1.53(b), the description and any drawings of the present application are replaced by this reference to the previously filed application, subject to conditions and requirements of 37 CFR 1.57(a).

| Application number of the previously filed application | Filing date (YYYY-MM-DD) | Intellectual Property Authority or Country |
|---|---|---|
| | | |

## Publication Information:

☐ Request Early Publication (Fee required at time of Request 37 CFR 1.219)

☐ **Request Not to Publish.** I hereby request that the attached application not be published under 35 U.S.C. 122(b) and certify that the invention disclosed in the attached application **has not and will not** be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

## Representative Information:

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32).
Either enter Customer Number or complete the Representative Name section below. If both sections are completed the customer Number will be used for the Representative Information during processing.

| Please Select One: | ◉ Customer Number | ○ US Patent Practitioner | ○ Limited Recognition (37 CFR 11.9) |
|---|---|---|---|
| Customer Number | 115018 | | |

## Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, 365(c), or 386(c) or indicate National Stage entry from a PCT application. Providing benefit claim information in the Application Data Sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78.
When referring to the current application, please leave the "Application Number" field blank.

| Prior Application Status | Pending | | Remove |
|---|---|---|---|
| Application Number | Continuity Type | Prior Application Number | Filing or 371(c) Date (YYYY-MM-DD) |
| | Claims benefit of provisional | 62/153973 | 2015-04-28 |

Additional Domestic Benefit/National Stage Data may be generated within this form by selecting the **Add** button.

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| Application Data Sheet 37 CFR 1.76 | Attorney Docket Number | 3338.0330001/ELE/C-K |
|---|---|---|
| | Application Number | |
| Title of Invention | TREATMENT OF PD-L1-NEGATIVE MELANOMA USING AN ANTI-PD-1 ANTIBODY AND AN ANTI-CTLA-4 ANTIBODY | |

## Foreign Priority Information:

This section allows for the applicant to claim priority to a foreign application. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55. When priority is claimed to a foreign application that is eligible for retrieval under the priority document exchange program (PDX)[i] the information will be used by the Office to automatically attempt retrieval pursuant to 37 CFR 1.55(i)(1) and (2). Under the PDX program, applicant bears the ultimate responsibility for ensuring that a copy of the foreign application is received by the Office from the participating foreign intellectual property office, or a certified copy of the foreign priority application is filed, within the time period specified in 37 CFR 1.55(g)(1).

Remove

| Application Number | Country[i] | Filing Date (YYYY-MM-DD) | Access Code[i] (if applicable) |
|---|---|---|---|
| | | | |

Additional Foreign Priority Data may be generated within this form by selecting the **Add** button.

## Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

☐ This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013.
NOTE: By providing this statement under 37 CFR 1.55 or 1.78, this application, with a filing date on or after March 16, 2013, will be examined under the first inventor to file provisions of the AIA.

| Application Data Sheet 37 CFR 1.76 | Attorney Docket Number | 3338.0330001/ELE/C-K |
|---|---|---|
| | Application Number | |
| Title of Invention | TREATMENT OF PD-L1-NEGATIVE MELANOMA USING AN ANTI-PD-1 ANTIBODY AND AN ANTI-CTLA-4 ANTIBODY | |

## Authorization or Opt-Out of Authorization to Permit Access:

When this Application Data Sheet is properly signed and filed with the application, applicant has provided written authority to permit a participating foreign intellectual property (IP) office access to the instant application-as-filed (see paragraph A in subsection 1 below) and the European Patent Office (EPO) access to any search results from the instant application (see paragraph B in subsection 1 below).

Should applicant choose not to provide an authorization identified in subsection 1 below, applicant **must opt-out** of the authorization by checking the corresponding box A or B or both in subsection 2 below.

**NOTE**: This section of the Application Data Sheet is **ONLY** reviewed and processed with the **INITIAL** filing of an application. After the initial filing of an application, an Application Data Sheet cannot be used to provide or rescind authorization for access by a foreign IP office(s). Instead, Form PTO/SB/39 or PTO/SB/69 must be used as appropriate.

**1. Authorization to Permit Access by a Foreign Intellectual Property Office(s)**

**A. Priority Document Exchange (PDX)** - Unless box A in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the European Patent Office (EPO), the Japan Patent Office (JPO), the Korean Intellectual Property Office (KIPO), the State Intellectual Property Office of the People's Republic of China (SIPO), the World Intellectual Property Organization (WIPO), and any other foreign intellectual property office participating with the USPTO in a bilateral or multilateral priority document exchange agreement in which a foreign application claiming priority to the instant patent application is filed, access to: (1) the instant patent application-as-filed and its related bibliographic data, (2) any foreign or domestic application to which priority or benefit is claimed by the instant application and its related bibliographic data, and (3) the date of filing of this Authorization. See 37 CFR 1.14(h)(1).

**B. Search Results from U.S. Application to EPO** - Unless box B in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the EPO access to the bibliographic data and search results from the instant patent application when a European patent application claiming priority to the instant patent application is filed. See 37 CFR 1.14(h)(2).

The applicant is reminded that the EPO's Rule 141(1) EPC (European Patent Convention) requires applicants to submit a copy of search results from the instant application without delay in a European patent application that claims priority to the instant application.

**2. Opt-Out of Authorizations to Permit Access by a Foreign Intellectual Property Office(s)**

☐ A. Applicant **DOES NOT** authorize the USPTO to permit a participating foreign IP office access to the instant application-as-filed. If this box is checked, the USPTO will not be providing a participating foreign IP office with any documents and information identified in subsection 1A above.

☐ B. Applicant **DOES NOT** authorize the USPTO to transmit to the EPO any search results from the instant patent application. If this box is checked, the USPTO will not be providing the EPO with search results from the instant application.

**NOTE:** Once the application has published or is otherwise publicly available, the USPTO may provide access to the application in accordance with 37 CFR 1.14.

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| Application Data Sheet 37 CFR 1.76 | Attorney Docket Number | 3338.0330001/ELE/C-K |
|---|---|---|
| | Application Number | |
| Title of Invention | TREATMENT OF PD-L1-NEGATIVE MELANOMA USING AN ANTI-PD-1 ANTIBODY AND AN ANTI-CTLA-4 ANTIBODY | |

## Applicant Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

**Applicant 1**

If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section.

Clear

| ○ Assignee | ○ Legal Representative under 35 U.S.C. 117 | ○ Joint Inventor |
|---|---|---|
| ◉ Person to whom the inventor is obligated to assign. | ○ Person who shows sufficient proprietary interest | |

If applicant is the legal representative, indicate the authority to file the patent application, the inventor is:

Name of the Deceased or Legally Incapacitated Inventor:

If the Applicant is an Organization check here. ☒

| Organization Name | Bristol-Myers Squibb Company |
|---|---|

**Mailing Address Information For Applicant:**

| **Address 1** | Rt. 206 & Province Line Road | | |
|---|---|---|---|
| Address 2 | | | |
| **City** | Princeton | **State/Province** | NJ |
| **Country** US | | Postal Code | 08543 |
| Phone Number | | Fax Number | |
| Email Address | | | |

Additional Applicant Data may be generated within this form by selecting the Add button.

## Assignee Information including Non-Applicant Assignee Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

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| **Application Data Sheet 37 CFR 1.76** | Attorney Docket Number | 3338.0330001/ELE/C-K |
|---|---|---|
| | Application Number | |
| Title of Invention | TREATMENT OF PD-L1-NEGATIVE MELANOMA USING AN ANTI-PD-1 ANTIBODY AND AN ANTI-CTLA-4 ANTIBODY | |

**Assignee 1**

Complete this section if assignee information, including non-applicant assignee information, is desired to be included on the patent application publication. An assignee-applicant identified in the "Applicant Information" section will appear on the patent application publication as an applicant. For an assignee-applicant, complete this section only if identification as an assignee is also desired on the patent application publication.

If the Assignee or Non-Applicant Assignee is an Organization check here. ☒

| Organization Name | Bristol-Myers Squibb Company |
|---|---|

**Mailing Address Information For Assignee including Non-Applicant Assignee:**

| **Address 1** | Rt. 206 & Province Line Road | | |
|---|---|---|---|
| Address 2 | | | |
| **City** | Princeton | **State/Province** | NJ |
| **Country** | US | Postal Code | 08543 |
| Phone Number | | Fax Number | |
| Email Address | | | |

Additional Assignee or Non-Applicant Assignee Data may be generated within this form by selecting the Add button.

## Signature:

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This Application Data Sheet **must** be signed by a patent practitioner if one or more of the applicants is a **juristic entity** (e.g., corporation or association). If the applicant is two or more joint inventors, this form must be signed by a patent practitioner, **all** joint inventors who are the applicant, or one or more joint inventor-applicants who have been given power of attorney (e.g., see USPTO Form PTO/AIA/81) on behalf of **all** joint inventor-applicants.

See 37 CFR 1.4(d) for the manner of making signatures and certifications.

| **Signature** | /Ji-Eun Kim/ | | | Date (YYYY-MM-DD) | 2016-04-28 |
|---|---|---|---|---|---|
| First Name | Ji-Eun | Last Name | Kim | Registration Number | 68504 |

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| **Application Data Sheet 37 CFR 1.76** | Attorney Docket Number | 3338.0330001/ELE/C-K |
|---|---|---|
| | Application Number | |
| Title of Invention | TREATMENT OF PD-L1-NEGATIVE MELANOMA USING AN ANTI-PD-1 ANTIBODY AND AN ANTI-CTLA-4 ANTIBODY | |

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| | |
|---|---|
| Application Number | To Be Assigned |
| Filing Date | Herewith |
| First Named Inventor | Arvin YANG |
| Title | TREATMENT OF PD-L1-NEGATIVE MELANOMA USING AN ANTI-PD-1 ANTIBODY AND AN ANTI-CTLA-4 ANTIBODY |
| Art Unit | To Be Assigned |
| Examiner Name | To Be Assigned |
| Attorney Docket Number | 3338.0330001/ELE/C-K |

**SIGNATURE of Applicant or Patent Practitioner**

| | | | |
|---|---|---|---|
| Signature | /Ji-Eun Kim/ | Date (Optional) | April 28, 2016 |
| Name | Ji-Eun Kim | Registration Number | 68,504 |
| Title (if Applicant is a juristic entity) | Attorney for Applicant | | |
| Applicant Name (if Applicant is a juristic entity) | Bristol-Myers Squibb Company | | |

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# POWER OF ATTORNEY BY APPLICANT

I hereby revoke all previous powers of attorney given in the application identified in either the attached transmittal letter or the boxes below.

| Application Number | Filing Date |
|---|---|
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OR

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| Firm or Individual Name | | | | | |
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| City | | State | | Zip | |
| Country | | | | | |
| Telephone | | Email | | | |

I am the Applicant (if the Applicant is a juristic entity, list the Applicant name in the box):

BRISTOL-MYERS SQUIBB COMPANY

[ ] Inventor or Joint Inventor (title not required below)

[ ] Legal Representative of a Deceased or Legally Incapacitated Inventor (title not required below)

[X] Assignee or Person to Whom the Inventor is Under an Obligation to Assign (provide signer's title if applicant is a juristic entity)

[ ] Person Who Otherwise Shows Sufficient Proprietary Interest (e.g., a petition under 37 CFR 1.46(b)(2) was granted in the application or is concurrently being filed with this document) (provide signer's title if applicant is a juristic entity)

**SIGNATURE of Applicant for Patent**

The undersigned (whose title is supplied below) is authorized to act on behalf of the applicant (e.g., where the applicant is a juristic entity).

| | | | |
|---|---|---|---|
| Signature | | Date (Optional) | July 31, 2015 |
| Name | Paul D. Golian | | |
| Title | Vice President & Assistant General Counsel, Bristol-Myers Squibb Company | | |

NOTE: Signature - This form must be signed by the applicant in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications. If more than one applicant, use multiple forms.

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# TREATMENT OF PD-L1-NEGATIVE MELANOMA USING AN ANTI-PD-1 ANTIBODY AND AN ANTI-CTLA-4 ANTIBODY

## CROSS-REFERENCE TO RELATED APPLICATIONS

**[0001]** The present application claims benefit to U.S. Provisional Application No. 62/153,973 filed April 28, 2015, which is incorporated herein by reference in its entirety.

## REFERENCE TO A SEQUENCE LISTING SUBMITTED ELECTRONICALLY VIA EFS-WEB

**[0002]** The instant application contains a Sequence Listing which has been submitted in ASCII format via EFS-Web, and is hereby incorporated by reference in its entirety. Said ASCII copy, created on April 28, 2016, is named 3338_0330001_SeqListing_ST25.txt and is 3,723 bytes in size.

**[0003]** Throughout this application, various publications are referenced in parentheses by author name and date, or by Patent No. or Patent Publication No. The disclosures of these publications are hereby incorporated in their entireties by reference into this application in order to more fully describe the state of the art as known to those skilled therein as of the date of the invention described and claimed herein. However, the citation of a reference herein should not be construed as an acknowledgement that such reference is prior art to the present invention.

## FIELD OF THE INVENTION

**[0004]** This invention relates to a method of treating PD-L1-negative melanoma comprising administering a combination of an anti-PD-1 antibody and an anti-CTLA-4 antibody.

## BACKGROUND OF THE INVENTION

**[0005]** Human cancers harbor numerous genetic and epigenetic alterations, generating neoantigens potentially recognizable by the immune system (Sjoblom *et al.* (2006)

*Science* 314:268-74). The adaptive immune system, comprised of T and B lymphocytes, has powerful anti-cancer potential, with a broad capacity and exquisite specificity to respond to diverse tumor antigens. Further, the immune system demonstrates considerable plasticity and a memory component. The successful harnessing of all these attributes of the adaptive immune system would make immunotherapy unique among all cancer treatment modalities.

**[0006]** Recently, several immune checkpoint pathway inhibitors have begun to provide new immunotherapeutic approaches for treating cancer, including the development of an antibody (Ab), ipilimumab (YERVOY®), that binds to and inhibits Cytotoxic T-Lymphocyte Antigen-4 (CTLA-4) for the treatment of patients with advanced melanoma and the development of antibodies such as nivolumab and pembrolizumab (formerly lambrolizumab; USAN Council Statement, (2013) Pembrolizumab: Statement on a nonproprietary name adopted by the USAN Council (ZZ-165), November 27, 2013) that bind specifically to the Programmed Death-1 (PD-1) receptor and block the inhibitory PD-1/PD-1 ligand pathway.

**[0007]** The promise of the emerging field of personalized medicine is that advances in pharmacogenomics will increasingly be used to tailor therapeutics to defined sub-populations, and ultimately, individual patients in order to enhance efficacy and minimize adverse effects. Recent successes include, for example, the development of imatinib mesylate (GLEEVEC®), a protein tyrosine kinase inhibitor that inhibits the bcr-abl tyrosine kinase, to treat Philadelphia chromosome-positive chronic myelogenous leukemia (CML); crizotinib (XALKORI®) to treat the 5% of patients with late-stage non-small cell lung cancers who express a mutant anaplastic lymphoma kinase (ALK) gene; and vemurafenib (ZELBORAF®), an inhibitor of mutated B-RAF protein (V600E-BRAF) which is expressed in around half of melanoma tumors. However, unlike the clinical development of small molecule agents that target discrete activating mutations found in select cancer populations, a particular challenge in cancer immunotherapy has been the identification of mechanism-based predictive biomarkers to enable patient selection and guide on-treatment management.

## SUMMARY OF THE INVENTION

**[0008]** The present disclosure provides a method for treating a melanoma comprising (i) identifying a patient having a PD-L1-negative melanoma tumor; and (ii) administering to the patient (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4. The present disclosure also provides a method for treating a melanoma comprising administering to a patient afflicted with a melanoma tumor (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4, wherein the patient is identified as having a PD-L1-negative melanoma tumor prior to the administration.

**[0009]** The present disclosure further provides a method for extending a progression-free survival period for over 8 months in a patient afflicted with a melanoma tumor comprising administering to the patient (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4, wherein the patient is identified as having a PD-L1-negative melanoma tumor prior to the administration and wherein the patient demonstrates progression-free survival for over 8 months. In certain embodiments, the progression-free survival of the patient is extended after the administration for over about 11 months, about 12 months, about 13 months, about 14 months, about 15 months, about 16 months, about 17 months, about 18 months, about 2 years, about 3 years, about 4 years, about 5 years, about 6 years, about 7 years, about 8 years, about 9 years, or about 10 years. In one particular embodiment, the progression-free survival of the patient is extended for over 11 months.

**[0010]** The present disclosure also provides a method for reducing a tumor size at least by 10% in a patient afflicted with a melanoma tumor comprising administering to the patient (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4, wherein the patient is identified as having a PD-L1-negative melanoma tumor prior to the administration and

wherein the administration reduces the tumor size at least about 10%, about 20%, about 30%, about 40%, or about 50% compared to the tumor size prior to the administration.

**[0011]** The present disclosure also provides a method for increasing an objective response rate to be higher than 40% in a patient population, each of whom is afflicted with a melanoma tumor, in a cancer treatment comprising administering to the patient (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4, wherein each patient is identified as having a PD-L1-negative melanoma tumor prior to the administration and wherein the objective response rate is higher than 40%, 45%, 50%, 55%, 60%, 65%, 70%, or 75%.

**[0012]** The present disclosure also provides a method for selecting a patient suitable for an anti-PD-1 antibody and anti-CTLA-4 antibody combination therapy comprising (i) identifying a patient having a PD-L1-negative melanoma tumor; and (ii) instructing a healthcare provider to administer to the patient (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4.

**[0013]** In certain embodiments, the methods disclosed herein further comprise identifying the patient as having a melanoma tumor that does not express PD-L1 prior to the administration. In some embodiments, the patient is characterized by (i) extended progression-free survival for over 8 months, (ii) tumor size reduction at least about 10%, about 20%, about 30%, about 40%, or about 50% compared to the tumor size prior to the administration, or (iii) both.

**[0014]** In some embodiments, the methods disclosed herein further comprise measuring a PD-L1 expression on the melanoma tumor. In certain embodiments, the measuring further comprises assessing the proportion of cells in the test tissue sample that express PD-L1 on the cell surface. In one particular embodiment, the presence of PD-L1 is determined using an automated IHC assay.

**[0015]** The present disclosure further provides a kit for treating a patient afflicted with a melanoma tumor, the kit comprising (a) a dosage ranging from 0.1 to 10 mg/kg body weight of an anti-PD-1 antibody or an antigen-binding portion thereof; (b) a dosage

ranging from 0.1 to 10 mg/kg body weight of an anti-CTLA-4 antibody or an antigen-binding portion thereof; and (c) instructions for using the anti-PD-1 antibody or the antigen-binding portion thereof and the anti-CTLA-4 antibody or the antigen-binding portion thereof in any of the methods disclosed herein.

## BRIEF DESCRIPTION OF THE FIGURES

**[0016]** Figure 1 shows a patient flow diagram of the randomized, double-blind, multicenter, phase 3 trial.

**[0017]** Figures 2A-C show progression-free survival data in the intention-to-treat population (Figure 2A), in patients with positive PD-L1 status (Figure 2B), and Negative PD-L1 Status (Figure 2C). Each graph shows the progression free survival for patients treated with nivolumab alone (solid line), ipilimumab alone (dashed line), or the combination of nivolumab and ipilimumab (dotted line) in months (Figures 2A-C). The number at risk in months for each of nivolumab, nivolumab plus ipilimumab, and ipilimumab is shown below each x-axis (Figures 2A-C). PD-L1 expression status is based on verified PD-L1 assay data (Figures 2B-C).

**[0018]** Figures 3A-B show subgroup analyses of progression-free survival among patients treated with nivolumab alone compared to ipilimumab alone (Figure 3A) and patients treated with nivolumab plus ipilimumab compared to ipilimumab alone (Figure 3B).

**[0019]** Figures 4A-C show the tumor burden change in target lesions in patients treated with nivolumab alone (Figure 4A), nivolumab plus ipilimumab (Figure 4B), and ipilimumab alone (Figure 4C). In each graph, the y-axis shows the best reduction from baseline in target lesions (%) and the x-axis represents each patient (Figures 4A-C).

## DETAILED DESCRIPTION OF THE INVENTION

**[0020]** The present invention relates to identifying an optimal strategy to treat a patient having a PD-L1-negative melanoma. The present invention shows that in a patient having a PD-L1-negative melanoma tumor, a combination therapy of an anti-PD-1 antibody and an anti-CTLA-4 antibody provides a better response (e.g., progression-free survival) than

a monotherapy of either an anti-PD-1 antibody or an anti-CTLA-4 antibody. Furthermore, the combination therapy when given to a population of patients can increase the objective response rate compared to the monotherapy.

*Definitions*

**[0021]** In order that the present disclosure may be more readily understood, certain terms are first defined. As used in this application, except as otherwise expressly provided herein, each of the following terms shall have the meaning set forth below. Additional definitions are set forth throughout the application.

**[0022]** The term "and/or" where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. Thus, the term "and/or" as used in a phrase such as "A and/or B" herein is intended to include "A and B," "A or B," "A" (alone), and "B" (alone). Likewise, the term "and/or" as used in a phrase such as "A, B, and/or C" is intended to encompass each of the following aspects: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).

**[0023]** It is understood that wherever aspects are described herein with the language "comprising," otherwise analogous aspects described in terms of "consisting of" and/or "consisting essentially of" are also provided.

**[0024]** Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure is related. For example, the Concise Dictionary of Biomedicine and Molecular Biology, Juo, Pei-Show, 2nd ed., 2002, CRC Press; The Dictionary of Cell and Molecular Biology, 3rd ed., 1999, Academic Press; and the Oxford Dictionary Of Biochemistry And Molecular Biology, Revised, 2000, Oxford University Press, provide one of skill with a general dictionary of many of the terms used in this disclosure.

**[0025]** Units, prefixes, and symbols are denoted in their Système International de Unites (SI) accepted form. Numeric ranges are inclusive of the numbers defining the range. The headings provided herein are not limitations of the various aspects of the disclosure, which can be had by reference to the specification as a whole. Accordingly, the terms

defined immediately below are more fully defined by reference to the specification in its entirety.

**[0026]** "Administering" refers to the physical introduction of a composition comprising a therapeutic agent to a subject, using any of the various methods and delivery systems known to those skilled in the art. Routes of administration for the formulations disclosed herein include intravenous, intramuscular, subcutaneous, intraperitoneal, spinal or other parenteral routes of administration, for example by injection or infusion. The phrase "parenteral administration" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intralymphatic, intralesional, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion, as well as *in vivo* electroporation. In some embodiments, the formulation is administered via a non-parenteral route, in some embodiments, orally. Other non-parenteral routes include a topical, epidermal or mucosal route of administration, for example, intranasally, vaginally, rectally, sublingually or topically. Administering can also be performed, for example, once, a plurality of times, and/or over one or more extended periods.

**[0027]** An "adverse event" (AE) as used herein is any unfavorable and generally unintended or undesirable sign (including an abnormal laboratory finding), symptom, or disease associated with the use of a medical treatment. For example, an adverse event may be associated with activation of the immune system or expansion of immune system cells (*e.g.*, T cells) in response to a treatment. A medical treatment may have one or more associated AEs and each AE may have the same or different level of severity. Reference to methods capable of "altering adverse events" means a treatment regime that decreases the incidence and/or severity of one or more AEs associated with the use of a different treatment regime.

**[0028]** An "antibody" (Ab) shall include, without limitation, a glycoprotein immunoglobulin which binds specifically to an antigen and comprises at least two heavy (H) chains and two light (L) chains interconnected by disulfide bonds, or an antigen-binding portion thereof. Each H chain comprises a heavy chain variable region

(abbreviated herein as $V_H$) and a heavy chain constant region. The heavy chain constant region comprises three constant domains, $C_{H1}$, $C_{H2}$ and $C_{H3}$. Each light chain comprises a light chain variable region (abbreviated herein as $V_L$) and a light chain constant region. The light chain constant region is comprises one constant domain, $C_L$. The $V_H$ and $V_L$ regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FR). Each $V_H$ and $V_L$ comprises three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The constant regions of the antibodies may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (*e.g.*, effector cells) and the first component (C1q) of the classical complement system.

**[0029]** An immunoglobulin may derive from any of the commonly known isotypes, including but not limited to IgA, secretory IgA, IgG and IgM. IgG subclasses are also well known to those in the art and include but are not limited to human IgG1, IgG2, IgG3 and IgG4. "Isotype" refers to the antibody class or subclass (*e.g.*, IgM or IgG1) that is encoded by the heavy chain constant region genes. The term "antibody" includes, by way of example, both naturally occurring and non-naturally occurring antibodies; monoclonal and polyclonal antibodies; chimeric and humanized antibodies; human or nonhuman antibodies; wholly synthetic antibodies; and single chain antibodies. A nonhuman antibody may be humanized by recombinant methods to reduce its immunogenicity in man. Where not expressly stated, and unless the context indicates otherwise, the term "antibody" also includes an antigen-binding fragment or an antigen-binding portion of any of the aforementioned immunoglobulins, and includes a monovalent and a divalent fragment or portion, and a single chain antibody.

**[0030]** The term "monoclonal antibody" ("mAb") refers to a non-naturally occurring preparation of antibody molecules of single molecular composition, *i.e.*, antibody molecules whose primary sequences are essentially identical, and which exhibits a single binding specificity and affinity for a particular epitope. A mAb is an example of an

isolated antibody. MAbs may be produced by hybridoma, recombinant, transgenic or other techniques known to those skilled in the art.

**[0031]** A "human" antibody (HuMAb) refers to an antibody having variable regions in which both the framework and CDR regions are derived from human germline immunoglobulin sequences. Furthermore, if the antibody contains a constant region, the constant region is also derived from human germline immunoglobulin sequences. The human antibodies of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (*e.g.*, mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*). However, the term "human antibody," as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences. The terms "human" antibodies and "fully human" antibodies and are used synonymously.

**[0032]** A "humanized antibody" refers to an antibody in which some, most or all of the amino acids outside the CDR domains of a non-human antibody are replaced with corresponding amino acids derived from human immunoglobulins. In one embodiment of a humanized form of an antibody, some, most or all of the amino acids outside the CDR domains have been replaced with amino acids from human immunoglobulins, whereas some, most or all amino acids within one or more CDR regions are unchanged. Small additions, deletions, insertions, substitutions or modifications of amino acids are permissible as long as they do not abrogate the ability of the antibody to bind to a particular antigen. A "humanized" antibody retains an antigenic specificity similar to that of the original antibody.

**[0033]** A "chimeric antibody" refers to an antibody in which the variable regions are derived from one species and the constant regions are derived from another species, such as an antibody in which the variable regions are derived from a mouse antibody and the constant regions are derived from a human antibody.

**[0034]** An "anti-antigen" antibody refers to an antibody that binds specifically to the antigen. For example, an anti-PD-1 antibody binds specifically to PD-1 and an anti-CTLA-4 antibody binds specifically to CTLA-4.

**[0035]** An "antigen-binding portion" of an antibody (also called an "antigen-binding fragment") refers to one or more fragments of an antibody that retain the ability to bind specifically to the antigen bound by the whole antibody.

**[0036]** A "cancer" refers a broad group of various diseases characterized by the uncontrolled growth of abnormal cells in the body. Unregulated cell division and growth results in the formation of malignant tumors that invade neighboring tissues and may also metastasize to distant parts of the body through the lymphatic system or bloodstream. A "cancer" or "cancer tissue" can include a tumor.

**[0037]** "Cytotoxic T-Lymphocyte Antigen-4" (CTLA-4) refers to an immunoinhibitory receptor belonging to the CD28 family. CTLA-4 is expressed exclusively on T cells *in vivo*, and binds to two ligands, CD80 and CD86 (also called B7-1 and B7-2, respectively). The term "CTLA-4" as used herein includes human CTLA-4 (hCTLA-4), variants, isoforms, and species homologs of hCTLA-4, and analogs having at least one common epitope with hCTLA-4. The complete hCTLA-4 sequence can be found under GenBank Accession No. AAB59385.

**[0038]** The term “progression-free survival,” which can be abbreviated as PFS, as used herein refers to the length of time during and after the treatment of a solid tumor (*i.e.*, melanoma) that a patient lives with the disease but it does not get worse.

**[0039]** "Dosing interval," as used herein, means the amount of time that elapses between multiple doses of a formulation disclosed herein being administered to a subject. Dosing interval can thus be indicated as ranges.

**[0040]** The term "dosing frequency" as used herein refers to the frequency of administering doses of a formulation disclosed herein in a given time. Dosing frequency can be indicated as the number of doses per a given time, *e.g.*, once a week or once in two weeks.

**[0041]** The use of the term "fixed dose" with regard to a composition of the invention means that two or more different antibodies in a single composition are present in the composition in particular (fixed) ratios with each other. In some embodiments, the fixed dose is based on the weight (*e.g.*, mg) of the antibodies. In certain embodiments, the fixed dose is based on the concentration (*e.g.*, mg/ml) of the antibodies. In some embodiments, the ratio is at least about 1:1, about 1:2, about 1:3, about 1:4, about 1:5, about 1:6, about

1:7, about 1:8, about 1:9, about 1:10, about 1:15, about 1:20, about 1:30, about 1:40, about 1:50, about 1:60, about 1:70, about 1:80, about 1:90, about 1:100, about 1:120, about 1:140, about 1:160, about 1:180, about 1:200, about 200:1, about 180:1, about 160:1, about 140:1, about 120:1, about 100:1, about 90:1, about 80:1, about 70:1, about 60:1, about 50:1, about 40:1, about 30:1, about 20:1, about 15:1, about 10:1, about 9:1, about 8:1, about 7:1, about 6:1, about 5:1, about 4:1, about 3:1, or about 2:1 mg first antibody to mg second antibody. For example, the 3:1 ratio of a first antibody and a second antibody can mean that a vial can contain about 240 mg of the first antibody and 80 mg of the second antibody or about 3 mg/ml of the first antibody and 1 mg/ml of the second antibody.

**[0042]** The use of the term "flat dose" with regard to the composition of the invention means a dose that is administered to a patient without regard for the weight or body surface area (BSA) of the patient. The flat dose is therefore not provided as a mg/kg dose, but rather as an absolute amount of the agent (*e.g.,* the anti-CTLA-4 antibody and/or anti-PD-1 antibody). For example, a 60 kg person and a 100 kg person would receive the same dose of the composition (*e.g.*, 240 mg of an anti-PD-1 antibody and 80 mg of an anti-CTLA-4 antibody in a single fixed dosing formulation vial containing both 240 mg of an anti-PD-1 antibody and 80 mg of an anti-CTLA-4 antibody (or two fixed dosing formulation vials containing 120 mg of an anti-PD-1 antibody and 40 mg of an anti-CTLA-4 antibody, etc.)).

**[0043]** The term "weight based dose" as referred to herein means that a dose that is administered to a patient is calculated based on the weight of the patient. For example, when a patient with 60 kg body weight requires 3 mg/kg of an anti-PD-1 antibody in combination with 1 mg/kg of an anti-CTLA-4 antibody, one can draw the appropriate amounts of the anti-PD-1 antibody (*i.e.*, 180 mg) and the anti-CTLA-4 antibody (*i.e.*, 60 mg) at once from a 3:1 ratio fixed dosing formulation of an anti-PD1 antibody and an anti-CTLA-4 antibody.

**[0044]** The term "anti-PD-1 antibody monotherapy" as used herein includes a therapy of an anti-PD-1 antibody without an anti-CTLA-4 antibody therapy. The anti-PD-1 antibody monotherapy comprises, consists essentially of, or consists of administering one or more doses of an anti-PD-1 antibody to a patient in need thereof, but does not include

administering an anti-CTLA-4 antibody. In one embodiment, the anti-PD-1 antibody monotherapy comprises administering one or more doses of an anti-PD-1 antibody to a patient in need thereof, but does not include administering an anti-CTLA-4 antibody. In another embodiment, the anti-PD-1 antibody monotherapy comprises administering one or more doses of an anti-PD-1 antibody to a patient in need thereof, but does not include administering an antibody specifically targeting a protein other than PD-1. In other embodiments, the anti-PD-1 antibody monotherapy comprises administering one or more doses of an anti-PD-1 antibody to a patient in need thereof, but does not include administering another anti-cancer agent.

**[0045]** An "immune response" refers to the action of a cell of the immune system (for example, T lymphocytes, B lymphocytes, natural killer (NK) cells, macrophages, eosinophils, mast cells, dendritic cells and neutrophils) and soluble macromolecules produced by any of these cells or the liver (including antibodies, cytokines, and complement) that results in selective targeting, binding to, damage to, destruction of, and/or elimination from a vertebrate's body of invading pathogens, cells or tissues infected with pathogens, cancerous or other abnormal cells, or, in cases of autoimmunity or pathological inflammation, normal human cells or tissues.

**[0046]** "PD-L1 negative" or "PD-L1 expression negative," relating to cell surface PD-L1 expression, refers to the lack of a detectable amount of cell surface PD-L1. For cell surface expression assayed by IHC, e.g., with the mAb 28-8, a PD-L1 negative tumor or PD-L1 expression negative tumor means that less than 0.01% of cells express a detectable level of PD-L1. In some embodiments, a PD-L1 negative tumor or PD-L1 expression negative tumor means that zero (0) cells express a detectable level of PD-L1. In some embodiments, a PD-L1 negative or a PD-L1 expression negative tumor is any tumor other than a PD-L1 positive or a PD-L1 expression positive tumor.

**[0047]** The term "PD-L1 positive" or "PD-L1 expression positive," relating to cell surface PD-L1 expression, refers to the proportion of cells in a test tissue sample comprising tumor cells and tumor- infiltrating inflammatory cells above which the sample is scored as expressing cell surface PD-L1. For cell surface expression assayed by immunohistochemistry (IHC), e.g., with the mAb 28- 8, the PD-L1 positive tumor or PD-L1 expression positive tumor means that at least about 0.01%, at least about 0.5%, at

least about 1%, at least about 2%, at least about 3%, at least about 4%, at least about 5%, at least about 6%, at least about 7%, at least about 8%, at least about 9%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, or at least about 30% of the total number of cells express PD-L1. PD-L1 positive tumor or PD-L1 expression positive tumor can also be expressed herein as tumor expressing PD-L1. In other embodiments, the PD-L1 positive tumor or PD-L1 expression positive tumor means that at least about 0.1% to at least about 20% of the total number of cells express PD-L1. In certain embodiments, the PD-L1 positive tumor or PD-L1 expression positive tumor means that at least about 0.1% to at least about 10% of the total number of cells express PD-L1. In some embodiments, the PD-L1 positive or PD-L1 expression positive tumor means that at least about 1% of the total number of cells express PD-L1 on the cell surface. In other embodiments, the PD-L1 positive or PD-L1 expression positive tumor means that at least about 5% of the total number of cells express PD-L1 on the cell surface. In one particular embodiment, PD-L1 positive or PD-L1 expression positive tumor means that at least about 1%, or in the range of 1- 5% of the total number of cells express PD-L1 on the cell surface.

**[0048]** "Programmed Death-1 (PD-1)" refers to an immunoinhibitory receptor belonging to the CD28 family. PD-1 is expressed predominantly on previously activated T cells *in vivo*, and binds to two ligands, PD-L1 and PD-L2. The term "PD-1" as used herein includes human PD-1 (hPD-1), variants, isoforms, and species homologs of hPD-1, and analogs having at least one common epitope with hPD-1. The complete hPD-1 sequence can be found under GenBank Accession No. U64863.

**[0049]** "Programmed Death Ligand-1 (PD-L1)" is one of two cell surface glycoprotein ligands for PD-1 (the other being PD-L2) that down-regulate T cell activation and cytokine secretion upon binding to PD-1. The term "PD-L1" as used herein includes human PD-L1 (hPD-L1), variants, isoforms, and species homologs of hPD-L1, and analogs having at least one common epitope with hPD-L1. The complete hPD-L1 sequence can be found under GenBank Accession No. Q9NZQ7.

**[0050]** A "patient" as used herein includes any patient who is afflicted with a cancer (*e.g.*, melanoma). The terms "subject" and "patient" are used interchangeably herein.

**[0051]** A "therapeutically effective amount" or "therapeutically effective dosage" of a drug or therapeutic agent is any amount of the drug that, when used alone or in combination with another therapeutic agent, protects a subject against the onset of a disease or promotes disease regression evidenced by a decrease in severity of disease symptoms, an increase in frequency and duration of disease symptom-free periods, or a prevention of impairment or disability due to the disease affliction. The ability of a therapeutic agent to promote disease regression can be evaluated using a variety of methods known to the skilled practitioner, such as in human subjects during clinical trials, in animal model systems predictive of efficacy in humans, or by assaying the activity of the agent in *in vitro* assays.

**[0052]** "Treatment" or "therapy" of a subject refers to any type of intervention or process performed on, or the administration of an active agent to, the subject with the objective of reversing, alleviating, ameliorating, inhibiting, slowing down or preventing the onset, progression, development, severity or recurrence of a symptom, complication or condition, or biochemical indicia associated with a disease.

**[0053]** A "tumor-infiltrating inflammatory cell" is any type of cell that typically participates in an inflammatory response in a subject and which infiltrates tumor tissue. Such cells include tumor-infiltrating lymphocytes (TILs), macrophages, monocytes, eosinophils, histiocytes and dendritic cells.

**[0054]** The use of the alternative (*e.g.*, "or") should be understood to mean either one, both, or any combination thereof of the alternatives. As used herein, the indefinite articles "a" or "an" should be understood to refer to "one or more" of any recited or enumerated component.

**[0055]** The terms "about" or "comprising essentially of" refer to a value or composition that is within an acceptable error range for the particular value or composition as determined by one of ordinary skill in the art, which will depend in part on how the value or composition is measured or determined, *i.e.*, the limitations of the measurement system. For example, "about" or "comprising essentially of" can mean within 1 or more than 1 standard deviation per the practice in the art. Alternatively, "about" or "comprising essentially of" can mean a range of up to 10% or 20% (*i.e.*, ±10% or ±20%). For example, about 3mg can include any number between 2.7 mg and 3.3 mg (for 10%) or

between 2.4 mg and 3.6 mg (for 20%). Furthermore, particularly with respect to biological systems or processes, the terms can mean up to an order of magnitude or up to 5-fold of a value. When particular values or compositions are provided in the application and claims, unless otherwise stated, the meaning of "about" or "comprising essentially of" should be assumed to be within an acceptable error range for that particular value or composition.

**[0056]** The terms "about once a week," "once about every week," "once about every two weeks," or any other similar dosing interval terms as used herein means approximate number, and "about once a week" or "once about every week" can include every seven days ± two days, *i.e.,* every five days to every nine days. The dosing frequency of "once a week" thus can be every five days, every six days, every seven days, every eight days, or every nine days. "Once about every two weeks" can include every fourteen days ± three days, *i.e.*, every eleven days to every seventeen days. Similar approximations apply, for example, to once about every three weeks, once about every four weeks, once about every five weeks, once about every six weeks and once about every twelve weeks. In some embodiments, a dosing interval of once about every six weeks or once about every twelve weeks means that the first dose can be administered any day in the first week, and then the next dose can be administered any day in the sixth or twelfth week, respectively. In other embodiments, a dosing interval of once about every six weeks or once about every twelve weeks means that the first dose is administered on a particular day of the first week (e.g., Monday) and then the next dose is administered on the same day of the sixth or twelfth weeks (i.e., Monday), respectively.

**[0057]** As described herein, any concentration range, percentage range, ratio range or integer range is to be understood to include the value of any integer within the recited range and, when appropriate, fractions thereof (such as one-tenth and one-hundredth of an integer), unless otherwise indicated.

**[0058]** Various aspects of the invention are described in further detail in the following subsections.

*Methods of the Invention*

**[0059]** The present invention is directed to a method for treating a PD-L1-negative melanoma in a subject in need thereof. The present invention shows that for PD-L1 negative tumors, a combination therapy of an anti-PD-1 antibody and an anti-CTLA-4 antibody is more suitable than a monotherapy of either an anti-PD-1 antibody or an anti-CTLA-4 antibody.

**[0060]** Not bound to any theory, the present invention identifies that in patients having a PD-L1-negative tumor progression-free survival and overall response rate are higher following treatment with a combination therapy with an anti-PD-1 antibody and an anti-CTLA-4 antibody than following treatment with either an anti-PD-1 antibody or an anti-CTLA-4 antibody alone. Therefore, in order to increase the response of a patient having a PD-L1-negative tumor, the present invention provides identifying a patient suitable for the combination therapy of an anti-PD-1 antibody and an anti-CTLA-4 antibody.

**[0061]** In one embodiment, the invention includes a method of treating a PD-L1 negative melanoma comprising administering to a patient: (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4, wherein the patient is identified as having a PD-L1-negative melanoma tumor prior to the administration. In another embodiment, the invention includes a method for treating a melanoma in a patient in need thereof comprising: (i) identifying a patient having a PD-L1-negative melanoma tumor; and (ii) administering to the patient: (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4.

**[0062]** In certain embodiments, the invention includes a method for extending a progression-free survival period for over 9 months in a patient afflicted with a PD-L1 negative melanoma tumor comprising administering to the patient: (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4, wherein the patient is identified as having a PD-L1-negative melanoma tumor prior to the administration and wherein the patient

demonstrates progression-free survival for over 9 months. In other embodiments, the invention provides a method for extending a progression-free survival period for over 9 months in a patient afflicted with a melanoma tumor comprising: (i) identifying a patient having a PD-L1-negative melanoma tumor; and (ii) administering to the patient (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4, wherein the patient demonstrates progression-free survival for over 9 months. According to the invention, the progression-free survival of the patient can be extended, after the administration, over about 10 months, over about 11 months, over about 12 months, over about 13 months, about 14 months, about 15 months, about 16 months, about 17 months, about 18 months, about 2 years, about 3 years, about 4 years, about 5 years, about 6 years, about 7 years, about 8 years, about 9 years, or about 10 years. In a particular embodiment, the progression-free survival of the patient is extended for over 10 months.

**[0063]** In still other embodiments, the invention is directed to a method for reducing a tumor size at least by 10% in a patient afflicted with a PD-L1 negative melanoma tumor comprising administering to the patient: (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4, wherein the patient is identified as having a PD-L1-negative melanoma tumor prior to the administration and wherein the administration reduces the tumor size at least about 10%, about 20%, about 30%, about 40%, or about 50% compared to the tumor size prior to the administration. In yet other embodiments, the method comprises (i) identifying a patient having a PD-L1-negative melanoma tumor; and (ii) administering to the patient (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4, wherein the administration reduces the tumor size at least about 10%, about 20%, about 30%, about 40%, or about 50% compared to the tumor size prior to the administration. The tumor size, after the administration, can be reduced at least about 60%, 70%, 80%, 90% or 100%. The tumor can be completely eliminated from the patient's body after the administration.

**[0064]** The invention can also include a method of preventing a relapse and/or induce a remission to a patient having a PD-L1 negative melanoma tumor comprising administering to the patient: (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4, wherein the patient is identified as having a PD-L1-negative melanoma tumor prior to the administration. In some embodiments, the method of the invention comprises (i) identifying a patient having a PD-L1-negative melanoma tumor; and (ii) administering to the patient (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4.

**[0065]** In certain embodiments, the invention includes a method for increasing an objective response rate to be higher than 40% in a patient population, wherein each patient of the patient population is afflicted with a melanoma tumor, in a cancer treatment comprising administering to the patient: (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4, wherein each patient is identified as having a PD-L1-negative melanoma tumor prior to the administration and wherein the objective response rate is higher than 40%, 45%, 50%, 55%, 60%, 65%, 70%, or 75%. The methods can further comprise identifying each patient as having a PD-L1-negative melanoma tumor prior to the administration. In other embodiments, each patient in the methods can further be characterized by (i) extended progression-free survival for over 11 months, (ii) tumor size reduction at least about 10%, about 20%, about 30%, about 40%, or about 50% compared to the tumor size prior to the administration, or (iii) both. In some embodiments, the patient population can be at least 100 patients having a PD-L1-negative melanoma tumor. In some embodiments, the patient population can be at least 200, 300, 400, 500, 600, 700, 800, 900, or 1000 patients having a PD-L1-negative melanoma tumor.

**[0066]** In further embodiments, the invention provides a method for selecting a suitable cancer therapy course in a patient having a PD-L1 negative melanoma tumor comprising: (i) identifying a patient having a PD-L1-negative melanoma tumor; and (ii) instructing a

healthcare provider to administer to the patient: (a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and (b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4. The method further comprises administering the antibodies to the patient.

**[0067]** The methods of the invention as a result of the combination therapy can treat the melanoma tumor, reduce the tumor size, prevent growth of the tumor, eliminate the tumor from the patient, prevent a relapse of a tumor, induce a remission in a patient, or any combination thereof. In certain embodiments, the combination therapy induces a complete response. In other embodiments, the combination therapy induces a partial response.

*Melanoma*

**[0068]** Melanoma (MEL) is a malignant tumor of melanocytes, the melanin-producing cells found predominantly in skin. Though less common than other skin cancers, it is the most dangerous of skin cancers if not diagnosed early and causes the majority (75%) of skin cancer deaths. The incidence of MEL is increasing worldwide in Caucasian populations, especially where peoples with low amounts of skin pigmentation receive excessive ultraviolet light exposure from the sun. In Europe, the incidence rate is < 10-20 per 100,000 population; in the USA 20-30 per 100,000; and in Australia, where the highest incidence is observed, 50-60 per 100,000 (Garbe *et al.*, *Eur. J. Cancer. 48(15)*:2375-90 (2012)). MEL accounts for about 5% of all new cases of cancer in the United States (U.S.), and the incidence continues to rise by almost 3% per year. This translates to an estimated 76,690 new cases in the U.S. in 2013 with 9,480 associated deaths (Siegel *et al.*, *CA Cancer J. Clin. 63(1)*:11-30 (2013)).

**[0069]** For in situ (stage 0) or early-stage MEL (Stages I-II), surgical excision is the primary treatment. In general, the prognosis is excellent for patients with localized disease and tumors 1.0 mm or less in thickness, with 5-year survival rates of more than 90% (NCCN GUIDELINES®, 2013- Melanoma). Where surgical excision is not feasible for in situ melanoma due to comorbidity or cosmetically sensitive tumor location, topical imiquimod (INN) and radiotherapy are emerging as treatments, especially for *lentigo maligna*. Chemotherapeutic agents for treating MEL include dacarbazine, temozolomide and imatinib for melanoma with a c-KIT mutation, high-dose interleukin-2, and

paclitaxel with or without carboplatin. However, these treatments have modest success, with response rates below 20% in first-line (1L) and second-line (2L) settings.

**[0070]** For patients with localized melanomas more than 1.0 mm in thickness, survival rates range from 50-90%. The likelihood of regional nodal involvement increases with increasing tumor thickness. With Stage III MEL (clinically positive nodes and/or in-transit disease), 5-year survival rates range from 20-70%. By far the most lethal is Stage IV MEL where long-term survival in patients with distant metastatic melanoma is less than 10% (NCCN GUIDELINES®, 2013 - melanoma).

**[0071]** The types of melanoma that can be treated with the present methods include, but are not limited to, lentigo maligna, lentigo maligna melanoma, superficial spreading melanoma, acral lentiginous melanoma, nucosal melanoma, nodular melanoma, polypoid melanoma, desmoplastic melanoma, amelanotic melanoma, soft-tissue melanoma, melanoma with small nevus-like cells, melanoma with features of a Spitz nevus, or uveal melanoma. The stages of melanoma that can be treated with the present methods include, but are not limited to, (i) Stage I / II (invasive melanoma): T1a characterized by less than 1.0 mm primary tumor thickness, without ulceration, and mitosis $< 1/mm^2$; T1b characterized by less than 1.0 mm primary tumor thickness, with ulceration or mitoses $\geq 1/mm^2$; T2a characterized by 1.01–2.0 mm primary tumor thickness, without ulceration; (ii) Stage II (high risk melanoma): T2b characterized by 1.01–2.0 mm primary tumor thickness, with ulceration; T3a characterized by 2.01–4.0 mm primary tumor thickness, without ulceration; T3b characterized by 2.01–4.0 mm primary tumor thickness, with ulceration; T4a characterized by greater than 4.0 mm primary tumor thickness, without ulceration; or T4b characterized by greater than 4.0 mm primary tumor thickness, with ulceration; (iii) Stage III (regional metastasis): N1 characterized by single positive lymph node; N2 characterized by two to three positive lymph nodes or regional skin/in-transit metastasis; or N3 characterized by four positive lymph nodes or one lymph node and regional skin/in-transit metastases; and (iv) Stage IV (distant metastasis): M1a characterized by distant skin metastasis, normal LDH; M1b characterized by Lung metastasis, normal LDH; or M1c characterized by other distant metastasis or any distant metastasis with elevated LDH. PD-L1-negative tumors that are treatable by the present

methods can lack PD-L1 expression on the surface of tumor cells and/or tumor infiltrating inflammatory cells.

*Measurement of PD-L1 expression*

**[0072]** In certain embodiments, identifying a patient suitable for a combination therapy for the present methods includes measuring or assessing a PD-L1 expression on the surface of the melanoma tumor cells or tumor infiltrating inflammatory cells. The phrases "tumors expressing PD-L1," "PD-L1 expressing tumor," "PD-L1 positive tumor," and "PD-L1 expression positive tumor" are used interchangeably herein. The meaning of the phrases is provided elsewhere herein. The methods of measuring or assessing the PD-L1 expression can be achieved by any methods applicable.

**[0073]** In order to assess the PD-L1 expression, in one embodiment, a test tissue sample is obtained from the patient who is in need of the therapy. In another embodiment, the assessment of PD-L1 expression can be achieved without obtaining a test tissue sample. In some embodiments, selecting a suitable patient includes (i) optionally providing a test tissue sample obtained from a patient with cancer of the tissue, the test tissue sample comprising tumor cells and/or tumor-infiltrating inflammatory cells; and (ii) assessing the proportion of cells in the test tissue sample that express PD-L1 on the surface of the cells based on an assessment that the proportion of cells in the test tissue sample that express PD-L1 on the cell surface is lower than a predetermined threshold level. A test tissue sample can be considered to be PD-L1-negative if less than about 5% of the cells in the test tissue express PD-L1 on the cell surface. In other embodiments, a test tissue sample is considered to be PD-L1-negative if less than about 4%, less than about 3%, less than about 2%, less than about 1%, less than about 0.5%, less than about 0.1%, less than about 0.01% or 0% of the cells in the test tissue express PD-L1 on the cell surface. In one particular example, the test tissue sample can be considered to be PD-L1-negative if less than about 5% of the cells in the test tissue express PD-L1 on the cell surface.

**[0074]** In any of the methods comprising the measurement of PD-L1 expression in a test tissue sample, however, it should be understood that the step comprising the provision of a test tissue sample obtained from a patient is an optional step. That is, in certain embodiments the method includes this step, and in other embodiments, this step is not included in the method. It should also be understood that in certain embodiments the

"measuring" or "assessing" step to identify, or determine the number or proportion of, cells in the test tissue sample that express PD-L1 on the cell surface is performed by a transformative method of assaying for PD-L1 expression, for example by performing a reverse transcriptase-polymerase chain reaction (RT-PCR) assay or an IHC assay. In certain other embodiments, no transformative step is involved and PD-L1 expression is assessed by, for example, reviewing a report of test results from a laboratory. In certain embodiments, the steps of the methods up to, and including, assessing PD-L1 expression provides an intermediate result that may be provided to a physician or other healthcare provider for use in selecting a suitable candidate for the combination therapy of an anti-PD-1 antibody and an anti-CTLA-4 antibody. In certain embodiments, the steps that provide the intermediate result is performed by a medical practitioner or someone acting under the direction of a medical practitioner. In other embodiments, these steps are performed by an independent laboratory or by an independent person such as a laboratory technician.

**[0075]** In certain embodiments of any of the present methods, the proportion of cells that express PD-L1 is assessed by performing an assay to determine the presence of PD-L1 RNA. In further embodiments, the presence of PD-L1 RNA is determined by RT-PCR, *in situ* hybridization or RNase protection. In other embodiments, the proportion of cells that express PD-L1 is assessed by performing an assay to determine the presence of PD-L1 polypeptide. In further embodiments, the presence of PD-L1 polypeptide is determined by immunohistochemistry (IHC), enzyme-linked immunosorbent assay (ELISA), *in vivo* imaging, or flow cytometry. In some embodiments, PD-L1 expression is assayed by IHC. In other embodiments of all of these methods, cell surface expression of PD-L1 is assayed using, *e.g.*, IHC or *in vivo* imaging.

**[0076]** Imaging techniques have provided important tools in cancer research and treatment. Recent developments in molecular imaging systems, including positron emission tomography (PET), single-photon emission computed tomography (SPECT), fluorescence reflectance imaging (FRI), fluorescence-mediated tomography (FMT), bioluminescence imaging (BLI), laser-scanning confocal microscopy (LSCM) and multiphoton microscopy (MPM), will likely herald even greater use of these techniques in cancer research. Some of these molecular imaging systems allow clinicians to not only

see where a tumor is located in the body, but also to visualize the expression and activity of specific molecules, cells, and biological processes that influence tumor behavior and/or responsiveness to therapeutic drugs (Condeelis and Weissleder, *Cold Spring Harb. Perspect. Biol. 2(12)*:a003848 (2010)). Antibody specificity, coupled with the sensitivity and resolution of PET, makes immunoPET imaging particularly attractive for monitoring and assaying expression of antigens in tissue samples (McCabe and Wu, *Cancer Biother. Radiopharm. 25(3)*:253-61 (2010); Olafsen *et al.*, *Protein Eng. Des. Sel. 23(4)*:243-9 (2010)). In certain embodiments of any of the present methods, PD-L1 expression is assayed by immunoPET imaging. In certain embodiments of any of the present methods, the proportion of cells in a test tissue sample that express PD-L1 is assessed by performing an assay to determine the presence of PD-L1 polypeptide on the surface of cells in the test tissue sample. In certain embodiments, the test tissue sample is a FFPE tissue sample. In other embodiments, the presence of PD-L1 polypeptide is determined by IHC assay. In further embodiments, the IHC assay is performed using an automated process. In some embodiments, the IHC assay is performed using an anti-PD-L1 mAb to bind to the PD-L1 polypeptide, if present.

*Assaying Cell-Surface PD-L1 Expression by Automated IHC*

**[0077]** In one embodiment of the present methods, an automated IHC method is used to assay the expression of PD-L1 on the surface of cells in FFPE tissue specimens. This disclosure provides methods for detecting the presence of human PD-L1 antigen in a test tissue sample, or quantifying the level of human PD-L1 antigen or the proportion of cells in the sample that express the antigen, which methods comprise contacting the test sample, and a negative control sample, with a mAb that specifically binds to human PD-L1, under conditions that allow for formation of a complex between the antibody or portion thereof and human PD-L1. In certain embodiments, the test and control tissue samples are FFPE samples. The formation of a complex can then be detected, wherein a difference in complex formation between the test sample and the negative control sample is indicative of the presence of human PD-L1 antigen in the sample. Various methods are used to quantify PD-L1 expression.

**[0078]** In a particular embodiment, the automated IHC method comprises: (a) deparaffinizing and rehydrating mounted tissue sections in an autostainer; (b) retrieving

antigen using a decloaking chamber and pH 6 buffer, heated to 110°C for 10 min; (c) setting up reagents on an autostainer; and (d) running the autostainer to include steps of neutralizing endogenous peroxidase in the tissue specimen; blocking non-specific protein-binding sites on the slides; incubating the slides with primary Ab; incubating with a postprimary blocking agent; incubating with NovoLink Polymer; adding a chromogen substrate and developing; and counterstaining with hematoxylin.

**[0079]** For assessing PD-L1 expression in tumor tissue samples, a pathologist examines the number of membrane PD-L1$^+$ tumor cells in each field under a microscope and mentally estimates the percentage of cells that are positive, then averages them to come to the final percentage. The different staining intensities are defined as 0/negative, l+/weak, 2+/moderate, and 3+/strong. Typically, percentage values are first assigned to the 0 and 3+ buckets, and then the intermediate 1+ and 2+ intensities are considered. For highly heterogeneous tissues, the specimen is divided into zones, and each zone is scored separately and then combined into a single set of percentage values. The percentages of negative and positive cells for the different staining intensities are determined from each area and a median value is given to each zone. A final percentage value is given to the tissue for each staining intensity category: negative, 1+, 2+, and 3+. The sum of all staining intensities needs to be 100%.

**[0080]** Staining is also assessed in tumor-infiltrating inflammatory cells such as macrophages and lymphocytes. In most cases macrophages serve as an internal positive control since staining is observed in a large proportion of macrophages. While not required to stain with 3+ intensity, an absence of staining of macrophages should be taken into account to rule out any technical failure. Macrophages and lymphocytes are assessed for plasma membrane staining and only recorded for all samples as being positive or negative for each cell category. Staining is also characterized according to an outside/inside tumor immune cell designation. "Inside" means the immune cell is within the tumor tissue and/or on the boundaries of the tumor region without being physically intercalated among the tumor cells. "Outside" means that there is no physical association with the tumor, the immune cells being found in the periphery associated with connective or any associated adjacent tissue.

**[0081]** In certain embodiments of these scoring methods, the samples are scored by two pathologists operating independently, and the scores are subsequently consolidated. In certain other embodiments, the identification of positive and negative cells is scored using appropriate software.

**[0082]** A histoscore is used as a more quantitative measure of the IHC data. The histoscore is calculated as follows:

Histoscore = [(% tumor x 1 (low intensity)) + (% tumor x 2 (medium intensity)) + (% tumor x 3 (high intensity)]

**[0083]** To determine the histoscore, the pathologist estimates the percentage of stained cells in each intensity category within a specimen. Because expression of most biomarkers is heterogeneous the histoscore is a truer representation of the overall expression. The final histoscore range is 0 (no expression) to 300 (maximum expression). A test sample can be designated as PD-L1-negative even though some level of PD-L1 expression is detected. For example, a designation of PD-L1-negative can be assigned to a specimen having a final histoscore of about 15 or less, of about 10 or less, about 9 or less, about 8 or less, about 7 or less, about 6 or less, about 5 or less, about 4 or less, about 3 or less, about 2 or less, about 1 or less, or of 0.

**[0084]** An alternative means of quantifying PD-L1 expression in a test tissue sample IHC is to determine the adjusted inflammation score (AIS) score defined as the density of inflammation multiplied by the percent PD-L1 expression by tumor-infiltrating inflammatory cells (Taube *et al.*, *Sci. Transl. Med. 4(127)*:127ra37 (2012)).

*Anti-PD-1 antibodies*

**[0085]** PD-1 is a key immune checkpoint receptor expressed by activated T and B cells and mediates immunosuppression. PD-1 is a member of the CD28 family of receptors, which includes CD28, CTLA-4, ICOS, PD-1, and BTLA. Two cell surface glycoprotein ligands for PD-1 have been identified, Programmed Death Ligand-1 (PD-L1) and Programmed Death Ligand-2 (PD-L2), that are expressed on antigen-presenting cells as well as many human cancers and have been shown to down regulate T cell activation and cytokine secretion upon binding to PD-1. Inhibition of the PD-1/PD-L1 interaction mediates potent antitumor activity in preclinical models.

**[0086]** HuMAbs that bind specifically to PD-1 with high affinity have been disclosed in U.S. Patent Nos. 8,008,449 and 8,779,105. Other anti-PD-1 mAbs have been described in, for example, U.S. Patent Nos. 6,808,710, 7,488,802, 8,168,757 and 8,354,509, and PCT Publication No. WO 2012/145493. Each of the anti-PD-1 HuMAbs disclosed in U.S. Patent No. 8,008,449 has been demonstrated to exhibit one or more of the following characteristics: (a) binds to human PD-1 with a $K_D$ of 1 x $10^{-7}$ M or less, as determined by surface plasmon resonance using a Biacore biosensor system; (b) does not substantially bind to human CD28, CTLA-4 or ICOS; (c) increases T-cell proliferation in a Mixed Lymphocyte Reaction (MLR) assay; (d) increases interferon-γ production in an MLR assay; (e) increases IL-2 secretion in an MLR assay; (f) binds to human PD-1 and cynomolgus monkey PD-1; (g) inhibits the binding of PD-L1 and/or PD-L2 to PD-1; (h) stimulates antigen-specific memory responses; (i) stimulates antibody responses; and (j) inhibits tumor cell growth *in vivo*. Anti-PD-1 antibodies useful for the present invention include mAbs that bind specifically to human PD-1 and exhibit at least one, at least two, at least three, at least four, or at least five, of the preceding characteristics.

**[0087]** In one embodiment, the anti-PD-1 antibody is nivolumab. Nivolumab (also known as "OPDIVO®"; formerly designated 5C4, BMS-936558, MDX-1106, or ONO-4538) is a fully human IgG4 (S228P) PD-1 immune checkpoint inhibitor antibody that selectively prevents interaction with PD-1 ligands (PD-L1 and PD-L2), thereby blocking the down-regulation of antitumor T-cell functions (U.S. Patent No. 8,008,449; Wang *et al.*, *Cancer Immunol Res.* 2(9):846-56 (2014)). In another embodiment, the anti-PD-1 antibody or fragment thereof cross-competes with nivolumab. In other embodiments, the anti-PD-1 antibody or fragment thereof binds to the same epitope as nivolumab. In certain embodiments, the anti-PD-1 antibody has the same CDRs as nivolumab.

**[0088]** In another embodiment, the anti-PD-1 antibody or fragment thereof cross-competes with pembrolizumab. In some embodiments, the anti-PD-1 antibody or fragment thereof binds to the same epitope as pembrolizumab. In certain embodiments, the anti-PD-1 antibody has the same CDRs as pembrolizumab. In another embodiment, the anti-PD-1 antibody is pembrolizumab. Pembrolizumab (also known as "KEYTRUDA®", lambrolizumab, and MK-3475) is a humanized monoclonal IgG4 antibody directed against human cell surface receptor PD-1 (programmed death-1 or

programmed cell death-1). Pembrolizumab is described, for example, in U.S. Patent Nos. 8,354,509 and 8,900,587; *see also* http://www.cancer.gov/drugdictionary?cdrid=695789 (last accessed: December 14, 2014). Pembrolizumab has been approved by the FDA for the treatment of relapsed or refractory melanoma.

**[0089]** In other embodiments, the anti-PD-1 antibody or fragment thereof cross-competes with MEDI0608. In still other embodiments, the anti-PD-1 antibody or fragment thereof binds to the same epitope as MEDI0608. In certain embodiments, the anti-PD-1 antibody has the same CDRs as MEDI0608. In other embodiments, the anti-PD-1 antibody is MEDI0608 (formerly AMP-514), which is a monoclonal antibody. MEDI0608 is described, for example, in US Pat. No. 8,609,089B2 or in http://www.cancer.gov/drugdictionary?cdrid=756047 (last accessed December 14, 2014).

**[0090]** In certain embodiments, the first antibody is an anti-PD-1 antagonist. One example of the anti-PD-1 antagonist is AMP-224, which is a B7-DC Fc fusion protein. AMP-224 is discussed in U.S. Publ. No. 2013/0017199 or in http://www.cancer.gov/publications/dictionaries/cancer-drug?cdrid=700595 (last accessed July 8, 2015).

**[0091]** In other embodiments, the anti-PD-1 antibody or fragment thereof cross-competes with BGB-A317. In some embodiments, the anti-PD-1 antibody or fragment thereof binds the same epitope as BGB-A317. In certain embodiments, the anti-PD-1 antibody has the same CDRs as BGB-A317. In certain embodiments, the anti-PD-1 antibody is BGB-A317, which is a humanized monoclonal antibody. BGB-A317 is described in U.S. Publ. No. 2015/0079109.

**[0092]** In some embodiments, the antibody is Pidilizumab (CT-011), which is an antibody previously reported to bind to PD-1 but which is believed to bind to a different target. Pidilizumab is described in US Pat. No. 8,686,119 B2 or WO 2013/014668 A1.

**[0093]** Anti-PD-1 antibodies useful for the disclosed compositions also include isolated antibodies that bind specifically to human PD-1 and cross-compete for binding to human PD-1 with nivolumab (*see, e.g.*, U.S. Patent Nos. 8,008,449 and 8,779,105; WO 2013/173223). The ability of antibodies to cross-compete for binding to an antigen indicates that these antibodies bind to the same epitope region of the antigen and sterically hinder the binding of other cross-competing antibodies to that particular epitope

region. These cross-competing antibodies are expected to have functional properties very similar to those of nivolumab by virtue of their binding to the same epitope region of PD-1. Cross-competing antibodies can be readily identified based on their ability to cross-compete with nivolumab in standard PD-1 binding assays such as Biacore analysis, ELISA assays or flow cytometry (*see, e.g.*, WO 2013/173223).

**[0094]** In certain embodiments, the antibodies that cross-compete for binding to human PD-1 with, or bind to the same epitope region of human PD-1 as, nivolumab are mAbs. For administration to human subjects, these cross-competing antibodies can be chimeric antibodies, or humanized or human antibodies. Such chimeric, humanized or human mAbs can be prepared and isolated by methods well known in the art.

**[0095]** Anti-PD-1 antibodies useful for the compositions of the disclosed invention also include antigen-binding portions of the above antibodies. It has been amply demonstrated that the antigen-binding function of an antibody can be performed by fragments of a full-length antibody. Examples of binding fragments encompassed within the term "antigen-binding portion" of an antibody include (i) a Fab fragment, a monovalent fragment consisting of the $V_L$, $V_H$, $C_L$ and $C_{H1}$ domains; (ii) a F(ab')$_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the $V_H$ and $C_{H1}$ domains; and (iv) a Fv fragment consisting of the $V_L$ and $V_H$ domains of a single arm of an antibody.

**[0096]** Anti-PD-1 antibodies suitable for use in the disclosed compositions are antibodies that bind to PD-1 with high specificity and affinity, block the binding of PD-L1 and or PD-L2, and inhibit the immunosuppressive effect of the PD-1 signaling pathway. In any of the compositions or methods disclosed herein, an anti-PD-1 "antibody" includes an antigen-binding portion or fragment that binds to the PD-1 receptor and exhibits the functional properties similar to those of whole antibodies in inhibiting ligand binding and upregulating the immune system. In certain embodiments, the anti-PD-1 antibody or antigen-binding portion thereof cross-competes with nivolumab for binding to human PD-1. In other embodiments, the anti-PD-1 antibody or antigen-binding portion thereof is a chimeric, humanized or human monoclonal antibody or a portion thereof. In certain embodiments, the antibody is a humanized antibody. In other embodiments, the antibody is a human antibody. Antibodies of an IgG1, IgG2, IgG3 or IgG4 isotype can be used.

**[0097]** In certain embodiments, the anti-PD-1 antibody or antigen-binding portion thereof comprises a heavy chain constant region which is of a human IgG1 or IgG4 isotype. In certain other embodiments, the sequence of the IgG4 heavy chain constant region of the anti-PD-1 antibody or antigen-binding portion thereof contains an S228P mutation which replaces a serine residue in the hinge region with the proline residue normally found at the corresponding position in IgG1 isotype antibodies. This mutation, which is present in nivolumab, prevents Fab arm exchange with endogenous IgG4 antibodies, while retaining the low affinity for activating Fc receptors associated with wild-type IgG4 antibodies (Wang *et al.*, *Cancer Immunol Res.* 2(9):846-56 (2014)). In yet other embodiments, the antibody comprises a light chain constant region which is a human kappa or lambda constant region. In other embodiments, the anti-PD-1 antibody or antigen-binding portion thereof is a mAb or an antigen-binding portion thereof. In certain embodiments of any of the therapeutic methods described herein comprising administration of an anti-PD-1 antibody, the anti-PD-1 antibody is nivolumab. In other embodiments, the anti-PD-1 antibody is pembrolizumab. In other embodiments, the anti-PD-1 antibody is chosen from the human antibodies 17D8, 2D3, 4H1, 4A11, 7D3 and 5F4 described in U.S. Patent No. 8,008,449. In still other embodiments, the anti-PD-1 antibody is MEDI0608 (formerly AMP-514), AMP-224, or BGB-A317.

**[0098]** Because anti-PD-1 and anti-PD-L1 target the same signaling pathway and have been shown in clinical trials to exhibit similar levels of efficacy in a variety of cancers, including RCC (*see* Brahmer *et al.* (2012) *N Engl J Med* 366:2455-65; Topalian *et al.* (2012a) *N Engl J Med* 366:2443-54; WO 2013/173223), an anti-PD-L1 antibody may be substituted for the anti-PD-1 Ab in any of the therapeutic methods disclosed herein. In certain embodiments, the anti-PD-L1 antibody is BMS-936559 (formerly 12A4 or MDX-1105) (*see, e.g.*, U.S. Patent No. 7,943,743; WO 2013/173223). In other embodiments, the anti-PD-L1 antibody is MPDL3280A (also known as RG7446) (*see, e.g.*, Herbst et al. (2013) *J Clin Oncol* 31(suppl):3000. Abstract; U.S. Patent No. 8,217,149) or MEDI4736 (Khleif (2013) In: Proceedings from the European Cancer Congress 2013; September 27-October 1, 2013; Amsterdam, The Netherlands. Abstract 802). In certain embodiments, the antibodies that cross-compete for binding to human PD-L1 with, or bind to the same epitope region of human PD-L1 as the above-references PD-L1 antibodies are mAbs. For

administration to human subjects, these cross-competing antibodies can be chimeric antibodies, or can be humanized or human antibodies. Such chimeric, humanized or human mAbs can be prepared and isolated by methods well known in the art.

*Anti-PD-L1 Antibodies*

**[0099]** In certain embodiments, the present application encompasses use of an anti-PD-L1 antibody in lieu of anti-PD-1 antibody. In one embodiment, the anti-PD-L1 antibody inhibits the binding of PD-L1 receptor, i.e., PD-1, with its ligand PD-L1.

**[0100]** Anti-PD-L1 antibodies useful for the invention include antibodies engineered starting from antibodies having one or more of the $V_H$ and/or $V_L$ sequences disclosed herein, which engineered antibodies can have altered properties from the starting antibodies. An anti-PD-L1 antibody can be engineered by a variety of modifications as described above for the engineering of modified anti-PD-1 antibodies of the invention.

**[0101]** Anti-PD-L1 antibodies of the invention also include isolated antibodies selected for their ability to bind to PD-L1 in formalin-fixed, paraffin-embedded (FFPE) tissue specimens. The use of FFPE samples is essential for the long-term follow-up analysis of the correlation between PD-L1 expression in tumors and disease prognosis or progression. The use of different antibodies to stain PD-L1 in frozen versus FFPE tissues, and the ability of certain antibodies to distinguish membranous and/or cytoplasmic forms of PD-Ll, may account for some of the disparate data reported in the literature correlating PD-Ll expression with disease prognosis (Hamanishi *et al.*, *Proc. Natl. Acad. Sci. USA 104(9)*:3360-3365 (2007); Gadiot *et al.*, *Cancer 117(10)*:2192-2201 (2011)). This disclosure provides several rabbit mAbs that bind with high affinity specifically to membranous human PD-Ll in FFPE tissue samples comprising tumor cells and tumor-infiltrating inflammatory cells.

**[0102]** In some embodiments, an anti-PD-L1 antibody useful for the present methods includes mAb 28-8 set forth in SEQ ID NOs. 1 and 2, respectively. The sequences of the heavy and light chain CDR domains of mAb 28-8, as delineated using the Kabat system, are set forth in SEQ ID NOs. 3-8. In other embodiments, an anti-PD-L1 antibody useful for the invention comprises mAbs 28-1, 28-12, 29-8 and 20-12 or an antigen-binding portion thereof, for example, including Fab, $F(ab')_2$ Fd, Fv, and scFv, di-scFv or bi-scFv, and scFv-Fc fragments, diabodies, triabodies, tetrabodies, and isolated CDRs.

*Anti-CTLA-4 antibodies*

**[0103]** Anti-CTLA-4 antibodies of the instant invention bind to human CTLA-4 so as to disrupt the interaction of CTLA-4 with a human B7 receptor. Because the interaction of CTLA-4 with B7 transduces a signal leading to inactivation of T-cells bearing the CTLA-4 receptor, disruption of the interaction effectively induces, enhances or prolongs the activation of such T cells, thereby inducing, enhancing or prolonging an immune response.

**[0104]** HuMAbs that bind specifically to CTLA-4 with high affinity have been disclosed in U.S. Patent Nos. 6,984,720 and 7,605,238. Other anti-PD-1 mAbs have been described in, for example, U.S. Patent Nos. 5,977,318, 6,051,227, 6,682,736, and 7,034,121. The anti-PD-1 HuMAbs disclosed in U.S. Patent No. Nos. 6,984,720 and 7,605,238 have been demonstrated to exhibit one or more of the following characteristics: (a) binds specifically to human CTLA-4 with a binding affinity reflected by an equilibrium association constant ($K_a$) of at least about $10^7$ $M^{-1}$, or about $10^9$ $M^{-1}$, or about $10^{10}$ $M^{-1}$ to $10^{11}$ $M^{-1}$ or higher, as determined by Biacore analysis; (b) a kinetic association constant ($k_a$) of at least about $10^3$, about $10^4$, or about $10^5$ $m^{-1}$ $s^{-1}$; (c) a kinetic disassociation constant ($k_d$) of at least about $10^3$, about $10^4$, or about $10^5$ $m^{-1}$ $s^{-1}$; and (d) inhibits the binding of CTLA-4 to B7-1 (CD80) and B7-2 (CD86). Anti-CTLA-4 antibodies useful for the present invention include mAbs that bind specifically to human CTLA-4 and exhibit at least one, at least two, or at least three of the preceding characteristics.

**[0105]** An exemplary clinical anti-CTLA-4 antibody is the human mAb 10D1 (now known as ipilimumab and marketed as YERVOY®) as disclosed in U.S. Patent No. 6,984,720. Ipilimumab is an anti-CTLA-4 antibody for use in the methods disclosed herein. Ipilimumab is a fully human, IgG1 monoclonal antibody that blocks the binding of CTLA-4 to its B7 ligands, thereby stimulating T cell activation and improving overall survival (OS) in patients with advanced melanoma.

**[0106]** Another anti-CTLA-4 antibody useful for the present methods is tremelimumab (also known as CP-675,206). Tremelimumab is human IgG2 monoclonal anti-CTLA-4 antibody. Tremelimumab is described in WO/2012/122444, U.S. Publ. No. 2012/263677, or WO Publ. No. 2007/113648 A2.

**[0107]** Anti-CTLA-4 antibodies useful for the disclosed composition also include isolated antibodies that bind specifically to human CTLA-4 and cross-compete for binding to human CTLA-4 with ipilimumab or tremelimumab or bind to the same epitope region of human CTLA-4 as ipilimumab or tremelimumab. In certain embodiments, the antibodies that cross-compete for binding to human CTLA-4 with, or bind to the same epitope region of human CTLA-4 as does ipilimumab or tremelimumab, are antibodies comprising a heavy chain of the human IgG1 isotype. For administration to human subjects, these cross-competing antibodies are chimeric antibodies, or humanized or human antibodies. Useful anti-CTLA-4 antibodies also include antigen-binding portions of the above antibodies such as Fab, $F(ab')_2$, Fd or Fv fragments.

*Dosages*

**[0108]** The combination of an anti-PD-1 antibody and an anti-CTLA-4 antibody can be administered to a suitable patient in therapeutically effective amounts. For example, each antibody can be administered at a dosage ranging from at least about 0.1 to at least about 20.0 mg/kg body weight. In certain embodiments, each of the anti-PD-1 and anti-CTLA-4 antibodies is individually administered at a dosage of at least about 0.1, at least about 0.3, at least about 0.5, at least about 1, at least about 3, at least about 5, at least about 10 or at least about 20 mg/kg, *e.g.*, at least about 1 to at least about 10 mg/kg, *e.g.*, at least about 1 to at least about 3 mg/kg, *e.g.*, at least about 3 mg/kg, *e.g.*, at least about 1 mg/kg. Each of the anti-PD-1 antibody and anti-CTLA-4 antibody can be administered at a dosing frequency of at least about once every week, at least about once every 2 weeks, at least about once every 3 weeks, or at least about once every 4 weeks, or at least about once a month, for up to 6 to up to 72 doses, or for as long as clinical benefit is observed, or until unmanageable toxicity or disease progression occurs. In some embodiments, the anti-PD-1 antibody is administered at a dosage of about 1 or about 3 mg/kg. In certain embodiments, the sequenced regimen comprises administering the anti-PD-1 antibody to the subject at a dosing frequency of once about every week, once about every 2 weeks, once about every 3 weeks, or once about every 4 weeks, or once a month for 6 to 72 doses, or for as long as clinical benefit is observed, or until unmanageable toxicity or disease progression occurs. In other embodiments, the anti-PD-1 is administered at a

dosage of about 1 mg/kg at a dosing frequency of once about every 3 weeks for up to 48 doses. In some embodiments, the anti-CTLA-4 antibody is administered at a dosage of about 1 or about 3 mg/kg. In certain embodiments, the sequenced regimen comprises administering the anti-CTLA-4 antibody to the subject at a dosing frequency of once about every week, at least about once every 2 weeks, at least about once every 3 weeks, or at least about once every 4 weeks, or about once a month for 6 to 72 doses, or for as long as clinical benefit is observed, or until unmanageable toxicity or disease progression occurs. In other embodiments, the anti-CTLA-4 antibody is administered at a dosage of about 3 mg/kg at a dosing frequency of once about every 3 weeks for up to 48 doses.

**[0109]** In other embodiments, the anti-PD-1 and anti-CTLA-4 antibodies are administered at the following dosages: (a) 0.1 mg/kg anti-PD-1 antibody and 3 mg/kg of anti-CTLA-4 antibody; (b) 0.3 mg/kg anti-PD-1 antibody and 3 mg/kg of anti- CTLA-4 antibody; (c) 1 mg/kg anti-PD-1 antibody and 3 mg/kg of anti-CTLA-4 antibody; (d) 3 mg/kg anti-PD-1 antibody and 3 mg/kg of anti-CTLA-4 antibody; (e) 5 mg/kg anti-PD-1 antibody and 3 mg/kg of anti-CTLA-4 antibody; (f) 10 mg/kg anti-PD-1 antibody and 3 mg/kg of anti-CTLA-4 antibody; (g) 0.1 mg/kg anti-PD-1 antibody and 1 mg/kg of anti-CTLA-4 antibody; (h) 0.3 mg/kg anti-PD-1 antibody and 1 mg/kg of anti-CTLA-4 antibody; (i) 1 mg/kg anti-PD-1 antibody and 1 mg/kg of anti-CTLA-4 antibody; (j) 3 mg/kg anti-PD-1 antibody and 1 mg/kg of anti-CTLA-4 antibody; (k) 5 mg/kg anti-PD-1 antibody and 1 mg/kg of anti-CTLA-4 antibody; or (1) 10 mg/kg anti-PD-1 antibody and 1 mg/kg of anti-CTLA-4 antibody. In a particular embodiment, the methods include administration of 1 mg/kg of an anti-PD-1 antibody and 3 mg/kg of an anti-CTLA-4 antibody.

**[0110]** In some embodiments, the dosage of each of the anti-PD-1 and anti-CTLA-4 antibodies is kept constant during the induction dosing schedule and the maintenance dosing schedule. In certain embodiments, a regimen comprises: (i) an induction dosing schedule comprising combined administration of the anti-PD-1 and anti-CTLA-4 antibodies at a dosing frequency of at least once about every 2 weeks, at least once about every 3 weeks, or at least once about every 4 weeks, or at least about once a month, for at least 2, 4, 6, 8 or 10 doses, followed by administration of the anti-PD-1 antibody alone at a dosing frequency of at least once every 2, 3 or 4 weeks, or at least once a month, for at

least 2, 4, 6, 8 or 12 doses; followed by (ii) a maintenance dosing schedule comprising combined administration of the anti-PD-1 and anti-CTLA-4 antibodies at a dosing frequency of at least about once every 8 weeks, once about every 12 weeks, or once about every 16 weeks, or at least once a quarter, for at least 4, 6, 8, 10, 12 or 16 doses, or for as long as clinical benefit is observed, or until unmanageable toxicity or disease progression occurs. In another embodiment, the dosing schedule comprises administering about 3 mg/kg of an anti-PD-1 antibody every 2 weeks, or about 1 mg/kg of an anti-PD-1 antibody every 3 weeks plus about 3 mg/kg of an anti-CTLA-4 antibody every 3 weeks for 4 doses, followed by about 3 mg/kg of an anti-PD-1 antibody every 2 weeks for cycle 3 and beyond, or 3 mg/kg of an anti-CTLA-4 antibody every 3 weeks for 4 doses. In one particular embodiment, the dosing schedule comprises administering about 1 mg/kg of an anti-PD-1 antibody followed by an anti-CTLA-4 antibody (*e.g.*, about 3 mg/kg anti-CTLA-4 antibody) on the same day every 3 weeks for 4 doses, followed by about 3 mg/kg of an anti-PD-1 antibody every 2 weeks.

**[0111]** In certain embodiments of this method, the maintenance dosing schedule comprises combined administration of up to 4, 6, 8, 10, 12 or 16 doses of the anti-PD-1 and anti-CTLA-4 antibodies. In other embodiments, the concurrent regimen comprises: (i) an induction dosing schedule comprising combined administration of the anti-PD-1 and anti- CTLA-4 antibodies at a dosing frequency of once about every 2 weeks, at least once about every 3 weeks, or at least once about every 4 weeks, or about once a month, for 2, 4, 6 or 8 doses, followed by administration of the anti-PD-1 antibody alone at a dosing frequency of once about every 2 weeks, at least once about every 3 weeks, or at least once about every 4 weeks, or about once a month, for 2, 4, 6, 8 or 12 doses; followed by (ii) a maintenance dosing schedule comprising combined administration of the anti-PD-1 and anti-CTLA-4 antibodies at a dosing frequency of once every once about every 8, once about every 12 or once about every 16 weeks, or once a quarter, for 4, 6, 8, 10, 12 or 16 doses, or for as long as clinical benefit is observed, or until unmanageable toxicity or disease progression occurs.

**[0112]** In a particular embodiment, the anti-PD-1 and anti-CTLA-4 antibodies are administered at dosages of about 1 mg/kg anti-PD-1 antibody every three weeks and about 3 mg/kg of anti-CTLA-4 antibody every three weeks. In another embodiment, the

patient is administered at a dose of about 1 mg/kg of an anti-PD-1 antibody, followed by an anti-CTLA-4 antibody on the same day, every 3 weeks for 4 doses, then 3 mg/kg of an anti-PD-1 antibody every 2 weeks.

**[0113]** In certain embodiments of the present methods, the anti-PD-1 and anti-CTLA-4 antibodies are formulated for parenteral administration, *e.g.*, intravenous administration. In certain other embodiments, when the anti-PD-1 and anti-CTLA-4 antibodies are administered in combination, they are administered within about 30 minutes of each other. Either antibody may be administered first, that is, in certain embodiments, the anti-PD-1 antibody is administered before the anti-CTLA-4 antibody, whereas in other embodiments, the anti-CTLA-4 antibody is administered before the anti-PD-1 antibody. Typically, each antibody is administered intravenously over a period of about 60 minutes. In further embodiments, the anti-PD-1 and anti-CTLA-4 antibodies are administered concurrently, either admixed as a single composition in a pharmaceutically acceptable formulation for concurrent administration, or concurrently as separate compositions with each antibody in a pharmaceutically acceptable formulation.

**[0114]** In some embodiments, the anti-PD-1 antibody and the anti-CTLA-4 antibody are formulated in a single composition. The ratio of the amount of the anti-PD-1 antibody and the amount of the anti-CTLA-4 antibody in the single composition can be 10:1 to 1:10. In other embodiments, the ratio of the amount of the anti-PD-1 antibody and the amount of the anti-CTLA-4 antibody in the single composition is 5:1, 4:1, 3:1, 2:1, 1:1, 1:2, 1:3, 1:4, or 1:5. In a particular embodiment, the ratio of the amount of the anti-PD-1 antibody and the amount of the anti-CTLA-4 antibody in the single composition is 1:3.

**[0115]** In some embodiments, the composition is administered at a flat dose regardless of the weight of the patient. For example, each of the anti-PD-1 antibody with the anti-CTLA-4 antibody may be administered at a flat dose of 20, 50, 75, 80, 160, 200, 240, 300, 360, 400, 480, 500, 750 or 1500 mg, without regard to the patient's weight. In some embodiments the composition is administered at a weight-based dose at any dose disclosed herein. In some embodiments, the amount of the anti-PD-1 antibody and the amount of the anti-CTLA-4 antibody administered to the patient at a single dose are identical.

[0116] In certain embodiments of the present methods, the therapeutically effective dosage of the anti-PD-1 antibody or antigen-binding portion thereof is a flat dosing (not a bodyweight based dosing) and comprises about 60 mg, about 80 mg, about 100 mg, about 120 mg, about 140 mg, about 160 mg, about 180 mg, about 200 mg, about 220 mg, about 240 mg, about 260 mg, about 280 mg, or about 300 mg. In other embodiments, the therapeutically effective dosage of the anti-PD-1 antibody or antigen-binding portion thereof comprises about 320 mg, 360mg, 400 mg, 420mg, 480 mg, 500mg, 540 mg, 550mg, 600mg, 620 mg, 650mg, 680mg, 700 mg, 720mg, 780 mg, 800mg, 840mg, or 900mg.. In some embodiments, the dose of the anti-PD-1 antibody in the composition is between about 60 mg and about 300 mg, between about 60 mg and about 100 mg, between about 100 mg and about 200 mg, or between about 200 mg and about 300 mg. In some embodiments, the amount of the anti-PD-1 antibody in the composition is at least about 80 mg, about 160 mg, or about 240mg. In some embodiments, the dose of the anti-PD-1 antibody in the composition is at least about 240 mg or at least about 80 mg.

[0117] In other embodiments, the dosages are given every week, every two weeks, every three weeks, every four weeks, every five weeks, every six weeks, every seven weeks, every eight weeks, every nine weeks, or every 10 weeks.

[0118] In some embodiments, the anti-PD-1 antibody or antigen-binding portion thereof is administered at a dose of about 240 mg. In embodiments, the anti-PD-1 antibody or antigen-binding portion thereof is administered at a dose of about 360 mg. In embodiments, the anti-PD-1 antibody or antigen-binding portion thereof is administered at a dose of about 480 mg. In one embodiment, 360mg of the anti-PD-1 antibody or antigen binding fragment is administered once every 3 weeks. In another embodiment, 480mg of the anti-PD-1 antibody or antigen binding fragment is administered once every 4 weeks.

[0119] In other embodiments, the therapeutically effective dosage of the anti-CTLA-4 antibody or antigen-binding portion thereof is a flat dosing (not a bodyweight based dosing) and comprises about 60 mg, about 80 mg, about 100 mg, about 120 mg, about 140 mg, about 160 mg, about 180 mg, about 200 mg, about 220 mg, about 240 mg, about 260 mg, about 280 mg, or about 300 mg. In other embodiments, the therapeutically effective dosage of the anti-CTLA-4 antibody or antigen-binding portion thereof

comprises about 320 mg, 360mg, 400 mg, 420mg, 480 mg, 500mg, 540 mg, 550mg, 600mg, 620 mg, 650mg, 680mg, 700 mg, 720mg, 780 mg, 800mg, 840mg, or 900mg. In some embodiments, the dose of the anti-CTLA-4 antibody in the composition is between about 60 mg and about 300 mg, between about 60 mg and about 100 mg, between about 100 mg and about 200 mg, or between about 200 mg and about 300 mg. In some embodiments, the amount of the anti-CTLA-4 antibody in the composition is at least about 80 mg, about 160 mg, or about 240 mg. In some embodiments, the dose of the anti-CTLA-4 antibody in the composition is at least about 240 mg or at least about 80 mg.

**[0120]** In some embodiments, a flat dose of an anti-PD-1 antibody is 80 mg, and a flat dose of an anti-CTLA-4 antibody is 240 mg.

**[0121]** In some embodiments, the anti-PD-1 antibody is administered at a subtherapeutic dose, *i.e.*, a dose of the therapeutic agent that is significantly lower than the usual or FDA-approved dose when administered as monotherapy for the treatment of the cancer. The quantity of the second antibody in the composition is calculated based on the desired ratio.

**[0122]** In some embodiments, the composition is administered by intravenous infusion about once per week, about once every 2 weeks, about once every 3 weeks, or about once a month. In certain embodiments, the composition is administered once every 3 weeks. In some embodiments, the infusion occurs over at least about 10 minutes, about 20 minutes, about 30 minutes, about 45 minutes, about 60 minutes, about 90 minutes, about 2 hours, about 3 hours, about 4 hours or about 5 hours.

**[0123]** Actual dosage levels of the active ingredients in the pharmaceutical compositions of the present invention can be flat or varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being unduly toxic to the patient. The selected dosage level will depend upon a variety of pharmacokinetic factors including the activity of the particular compositions of the present invention employed, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compositions employed, the age, sex, weight, condition, general health and prior medical history of the

patient being treated, and like factors well known in the medical arts. A composition of the present invention can be administered via one or more routes of administration using one or more of a variety of methods well known in the art. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results.

*Kits*

**[0124]** Also within the scope of the present invention are kits, including pharmaceutical kits, comprising a combination of an anti-PD-1 antibody and an anti-CTLA-4 antibody, for therapeutic uses, and diagnostic kits comprising an anti-PD-Ll antibody for assaying membranous PD-L1 expression as a biomarker for screening patients for the combination therapy or for predicting the efficacy of the combination therapy. Kits typically include a label indicating the intended use of the contents of the kit and instructions for use. The term "label" includes any writing, or recorded material supplied on or with the kit, or which otherwise accompanies the kit. In certain embodiments of a pharmaceutical kit, the anti-PD-1 antibody is co-packaged with an anti-CTLA-4 antibody in unit dosage form. In certain embodiments of a diagnostic kit, the anti-PD-Ll antibody is co-packaged with an anti-PD-1 antibody and an anti-CTLA-4 antibody for performing an assay to detect and/or quantify PD-L1 expression.

**[0125]** In certain embodiments, the pharmaceutical kit comprises the anti-human PD-1 HuMAb, nivolumab. In other embodiments, the pharmaceutical kit comprises the anti-human PD-L1 HuMAb, BMS-936559. In yet other embodiments, the pharmaceutical kit comprises the anti-human CTLA-4 HuMAb, ipilimumab. In certain embodiments, the diagnostic kit comprises the rabbit anti-human PD-L1 mAb, 28-8, comprising the $V_H$ and $V_L$ regions whose amino acid sequences are set forth in SEQ ID NOs. 1 and 2, respectively. In other embodiments, the diagnostic kit comprises the murine anti-human PD-L1 mAb, 5H1 (Dong *et al.*, *Nature Med. 8(8)*:793-800 (2002)).

**[0126]** In some embodiments, the invention provides a kit for treating a patient afflicted with a melanoma tumor, the kit comprising:

(a) a dosage ranging from at least about 0.1 to at least about 10 mg/kg body weight of an anti-PD-1 antibody or an antigen-binding portion thereof;

(b) a dosage ranging from at least about 0.1 to at least about 10 mg/kg body weight of an anti-CTLA-4 antibody or an antigen-binding portion thereof; and

(c) instructions for using the anti-PD-1 antibody or the antigen-binding portion thereof and the anti-CTLA-4 antibody or the antigen-binding portion thereof in the methods disclosed herein.

**[0127]** The present invention is further illustrated by the following examples which should not be construed as further limiting. The contents of all references cited throughout this application are expressly incorporated herein by reference.

## EXAMPLES

### EXAMPLE 1

**[0128]** A randomized, double-blind, multicenter, phase 3 trial was conducted to evaluate the safety and efficacy of nivolumab alone or nivolumab combined with ipilimumab in comparison with ipilimumab alone in previously untreated metastatic melanoma.

*Patients*

**[0129]** Eligible patients had histologically confirmed stage III (unresectable) or stage IV melanoma, and no prior systemic treatment for unresectable or metastatic melanoma. Other eligibility criteria included an age of at least 18 years, an Eastern Cooperative Oncology Group (ECOG) performance status score of 0 (indicating no symptoms) or 1 (indicating mild symptoms), measurable disease by computed tomography or magnetic resonance imaging per RECIST v1.1, availability of tissue collected from metastatic or unresectable tumors for the assessment of PD-L1 status, and known BRAF V600 mutation status (or consent to BRAF V600 mutation testing per local standards). Key exclusion criteria were presence of active brain metastases, ocular melanoma, or autoimmune disease, and any prior treatment with an anti-PD-1, anti-PD-L1, anti-PD-L2, or anti-CTLA-4 antibody. Patients who required systemic corticosteroid treatment or other immunosuppressive medications within 14 days of study drug administration were excluded.

*Study Design and Treatment*

**[0130]** In the double-blind, phase 3 study, enrolled patients were randomly assigned in a 1:1:1 ratio to receive 3 mg of nivolumab per kilogram of body weight every 2 weeks (plus ipilimumab-matched placebo), or 1 mg of nivolumab per kilogram every 3 weeks plus 3 mg of ipilimumab per kilogram every 3 weeks for 4 doses (plus nivolumab-matched placebo), followed by 3 mg of nivolumab per kilogram every 2 weeks for cycle 3 and beyond, or 3 mg of ipilimumab per kilogram every 3 weeks for 4 doses (plus nivolumab-matched placebo).

**[0131]** Both nivolumab and ipilimumab were administered by intravenous infusion. Randomization was stratified according to tumor PD-L1 status (positive vs. negative or indeterminate), BRAF mutation status (V600 mutation positive vs. wild-type), and American Joint Committee on Cancer metastasis stage (M0, M1a, or M1b vs. M1c). Treatment continued until RECIST v1.1-defined disease progression, unacceptable toxicity, or withdrawal of consent. Patients could be treated beyond progression provided they had a clinical benefit without clinical deterioration, and did not have substantial adverse effects, as assessed by the investigator.

**[0132]** Progression-free survival and overall survival were co-primary end points. Secondary end points included objective response rate, tumor PD-L1 expression as a predictive biomarker for progression-free and overall survival, and health-related quality of life. Exploratory end points include duration of objective response and safety/tolerability of study drug therapy.

*Assessments*

**[0133]** Patients were assessed for tumor response, according to RECIST v1.1 at 12 weeks after randomization and continuing every 6 weeks for 49 weeks, and then every 12 weeks until progression or treatment discontinuation, whichever occurred later. Progression-free survival was defined as the time between the date of randomization and the first date of documented progression or death, whichever occurred first. Patients treated beyond progression were considered to have progressive disease at the time of the initial progression event, as assessed by the investigator, regardless of subsequent tumor responses. Expression of PD-L1 on the surface of the tumor cells was assessed in a central laboratory by immunohistochemistry in formalin-fixed, paraffin-embedded tumor specimens using a rabbit monoclonal anti-human PD-L1 antibody (clone 28-8) and an

analytically validated automated assay developed by Dako (Carpinteria, CA). PD-L1 positivity was defined as at least 5% of tumor cells showing cell surface PD-L1 staining of any intensity in a section containing at least 100 tumor cells that could be evaluated. Indeterminate status was attributed to samples for which tumor cell–surface expression could not be discerned because of melanin content or strong cytoplasmic staining.

**[0134]** Any patient who received at least one dose of study drug in each of the 3 treatment groups was included in the assessment of safety. The severity of adverse events was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0.18. Safety assessments were made continuously during the treatment phase, and up to 100 days after the last dose of study drug.

*Statistical Analysis*

**[0135]** A study sample size of approximately 915 patients, randomized to the 3 treatment arms in a 1:1:1 ratio, was planned. For the comparison of progression-free survival, the number of events projected to be observed at a follow-up of at least 9 months provided approximately 83% power to detect an average hazard ratio of 0.71 with a type I error of 0.005 (two-sided). Progression-free survival was compared between nivolumab plus ipilimumab and ipilimumab alone, and between nivolumab alone and ipilimumab alone with the use of a two-sided log-rank test stratified according to PD-L1 status, BRAF mutation status, and metastasis stage (as described above). The study was not designed for a formal statistical comparison between the nivolumab alone and nivolumab plus ipilimumab groups. Hazard ratios and corresponding two-sided 99.5% confidence intervals (CIs) were estimated using a Cox proportional hazards model, with treatment group as a single covariate, stratified by the above factors. Progression-free survival curves, medians with 95% CIs, and progression-free survival rates at 6, 12, and 18 months with 95% CIs were estimated using Kaplan-Meier methodology. Overall survival will be analyzed when all patients have a minimum follow-up of 22 months.

**Results**

*Patients and Treatment*

**[0136]** From July 2013 through March 2014, a total of 1296 patients were enrolled at 137 centers in Australia, Europe, Israel, New Zealand, and North America. A total of 945 patients underwent randomization: 316 patients were assigned to the nivolumab group, 314 to the nivolumab plus ipilimumab group, and 315 to the ipilimumab group (Figure 1). Baseline characteristics were balanced across the three groups. A total of 58.0% had stage M1c disease, 36.1% had an elevated lactate dehydrogenase level, 31.5% had a BRAF mutation, and 73.5% had negative PD-L1 status (Table 1).

**[0137]** All randomized patients had been followed for a minimum of 9 months at the time of database lock (February 17, 2015); 117 of 313 patients (37.4%) in the nivolumab group, 93 of 313 patients (29.7%) in the nivolumab plus ipilimumab group, and 50 of 311 patients (16.1%) in the ipilimumab group were continuing study treatment (Table 2). The most frequent reason for discontinuation was disease progression in the nivolumab and ipilimumab monotherapy groups (154 of 313 patients [49.2%] and 202 of 311 patients [65.0%], respectively), versus study drug toxicity in the nivolumab plus ipilimumab group (120 of 313 patients [38.3%]). The number of patients who had died was 85 (27.2%) in the nivolumab group, 86 (27.5%) in the nivolumab plus ipilimumab group, and 114 (36.7%) in the ipilimumab group.

**[0138]** The median number of doses in patients who received nivolumab alone or ipilimumab alone was 15 (range 1-38) and 4 (1-4), respectively. In the combination group, the median number of doses was 4 (range 1–39) for nivolumab and 4 (range 1–4) for ipilimumab; 147 of 313 patients (47%) received four or more doses of nivolumab monotherapy after combination treatment.

Table 1. Baseline Characteristics of the Patients.

| Characteristic | Nivolumab alone (N=316) | Nivolumab plus Ipilimumab (N=314) | Ipilimumab alone (N=315) | Total (N=945) |
|---|---|---|---|---|
| | | | | |
| Age — yr | | | | |
| Mean | 58.7 | 59.3 | 60.8 | 59.6 |
| Range | 25–90 | 18–88 | 18–89 | 18–90 |
| | | | | |
| Age groups — no. (%) | | | | |
| <65 yr | 198 (62.7) | 185 (58.9) | 182 (57.8) | 565 (59.8) |
| ≥ 65, < 75 yr | 79 (25.0) | 94 (29.9) | 89 (28.3) | 262 (27.7) |
| ≥ 75 yr | 39 (12.3) | 35 (11.1) | 44 (14.0) | 118 (12.5) |
| | | | | |
| Sex — no. (%) | | | | |
| Male | 202 (63.9) | 206 (65.6) | 202 (64.1) | 610 (64.6) |
| Female | 114 (36.1) | 108 (34.4) | 113 (35.9) | 335 (35.4) |
| | | | | |
| ECOG performance status — no. (%) | | | | |
| 0 | 238 (75.3) | 230 (73.2) | 224 (71.1) | 692 (73.2) |
| 1 | 77 (24.4) | 83 (26.4) | 91 (28.9) | 251 (26.6) |
| 2 | 1 (0.3) | 0 | 0 | 1 (0.1) |
| Not reported | 0 | 1 (0.3) | 0 | 1 (0.1) |
| | | | | |
| M stage — no. (%) | | | | |
| M1c | 184 (58.2) | 181 (57.6) | 183 (58.1) | 548 (58.0) |
| M0, M1a, or M1b | 132 (41.8) | 133 (42.4) | 132 (41.9) | 397 (42.0) |
| | | | | |
| Lactate dehydrogenase — no. (%)* | | | | |
| ≤ ULN | 196 ( 62.0) | 199 ( 63.4) | 194 ( 61.6) | 589 ( 62.3) |
| >ULN | 112 ( 35.4) | 114 ( 36.3) | 115 ( 36.5) | 341 ( 36.1) |
| ≤2x ULN | 271 ( 85.8) | 276 ( 87.9) | 279 ( 88.6) | 826 ( 87.4) |

| | | | | |
|---|---|---|---|---|
| >2x ULN | 37 ( 11.7) | 37 (11.8) | 30 (9.5) | 104 (11.0) |
| Unknown | 8 (2.5) | 1 (0.3) | 6 (1.9) | 15 (1.6) |
| | | | | |
| Brain metastases at baseline — no. (%) | | | | |
| Yes | 8 (2.5) | 11 (3.5) | 15 (4.8) | 34 (3.6) |
| No | 308 (37.5) | 303 (96.5) | 300 (95.2) | 911 (96.4) |
| | | | | |
| PD-L1 status — no. (%) | | | | |
| Positive | 80 (27.8) | 68 (24.5) | 75 (27.1) | 223(26.4) |
| Negative | 208 (72.2) | 210 (75.5) | 202 (72.9) | 620 (73.5) |
| | | | | |
| BRAF status — no. (%) | | | | |
| Mutation | 100 (31.6) | 101 (32.2) | 97 (30.8) | 298 (31.5) |
| No mutation | 216 (68.4) | 213 (67.8) | 218 (69.2) | 647 (68.5) |

* ULN denotes upper limit of normal.

*Efficacy*

**[0139]** The median progression-free survival was 6.5 months (95% confidence interval [CI], 4.3 to 9.5) in the nivolumab group, 11.5 months (95% CI, 8.9 to 16.5) in the nivolumab plus ipilimumab group, and 2.9 months (95% CI, 2.8 to 3.4) in the ipilimumab group (Figure 2A). A significant improvement in progression-free survival was observed in the nivolumab plus ipilimumab group as compared with the ipilimumab group (hazard ratio, 0.42; 95% CI, 0.31 to 0.57; P<0.0001) (Figure 2A). A significant improvement in progression-free survival was also observed in the nivolumab group as compared with the ipilimumab group (hazard ratio, 0.57; 95% CI, 0.43 to 0.76; P<0.00001) (Figure 2A). The hazard ratio for the comparison between nivolumab plus ipilimumab and nivolumab groups was 0.74 (95% CI, 0.60 to 0.92).

**[0140]** Analyses of progression-free survival among prespecified patient subgroups showed a consistent improvement with nivolumab or nivolumab plus ipilimumab as compared with ipilimumab, including subgroups defined by PD-L1 status, BRAF mutation status, and metastasis stage (Figure 3). In the combination group, median PFS was 11.7 months (95% CI, 8.0 to not reached) among patients with a BRAF mutation and was 11.2 months (95% CI, 8.3 to not reached) in patients with wild-type BRAF (Figure 3B). For patients with a positive PD-L1 tumor status, median progression-free survival in the nivolumab, nivolumab plus ipilimumab, and ipilimumab groups was 14.0 months (95% CI, 9.1 to not reached), 14.0 months (95% CI, 9.7 to not reached), and 3.9 months (95% CI, 2.8 to 4.2), respectively (Figure 2B). For patients with a negative PD-L1 tumor status, median progression-free survival in the nivolumab, nivolumab plus ipilimumab, and ipilimumab groups was 5.3 months (95% CI, 2.8 to 7.1 months), 11.2 months (95% CI, 8.0 to not reached) and 2.8 months (95% CI, 2.8 to 3.1), respectively (Fig. 2C).

**[0141]** Investigator-assessed objective response rates were 43.7% (95% CI, 38.1 to 49.3%), 57.6% (95% CI, 52.0 to 63.2), and 19.0% (95% CI, 14.9 to 23.8) in the nivolumab, nivolumab plus ipilimumab, and ipilimumab groups, respectively (Table 3). The percentage of patients with a complete response was higher in the nivolumab plus ipilimumab group than in either the nivolumab or ipilimumab alone groups (11.5% vs.

8.9% and 2.2%) (Table 3). Time to objective response was similar in each group (Table 3), and the median duration of response was not reached in any of the groups.

**[0142]** Median reduction in the sum of the longest diameters of tumor target lesions was -34.5% (interquartile range: -75.4 to 15.4), -51.1% (-75.8 to -10.2), and 5.8% (-28.0 to 33.3) in the nivolumab, nivolumab plus ipilimumab, and ipilimumab groups, respectively (Figure 4). Among patients with PD-L1-positive tumors, the objective response rates were 57.5% (95% CI, 45.9 to 68.5), 72.1% (95% CI, 59.9 to 82.3), and 21.3% (95% CI, 12.7 to 32.3) for the nivolumab, nivolumab plus ipilimumab, and ipilimumab groups, respectively; in patients with PD-L1-negative tumors, the objective response rates were 41.3% (95% CI, 34.6 to 48.4), 54.8% (95% CI, 47.8 to 61.6), and 17.8% (95% CI, 12.8 to 23.8) (Table 4).

*Adverse Events*

**[0143]** Treatment-related adverse events of any grade occurred in 82.1%, 95.5%, and 86.2% of patients in the nivolumab, nivolumab plus ipilimumab groups, and ipilimumab groups, respectively (Table 5). The most common adverse events in the nivolumab group were fatigue (in 34.2% of patients), rash (in 21.7%), and diarrhea (in 19.2%). In the nivolumab plus ipilimumab and ipilimumab groups, diarrhea (in 44.1% and 33.1% of patients, respectively), fatigue (in 35.1% and 28.0%), and pruritus (in 33.2% and 35.4%) were most common (Table 5). The incidence of treatment-related adverse events of grade 3 or 4 was also higher in the nivolumab plus ipilimumab group than in either the nivolumab or ipilimumab groups (55.0% vs. 16.3% and 27.3%), with diarrhea being the most common (2.2% , 9.3%, and 6.1% in the nivolumab, nivolumab plus ipilimumab, and ipilimumab groups, respectively) (Table 5). Treatment-related adverse events of any grade leading to discontinuation occurred in 7.7%, 36.4%, and 14.8% of patients in the nivolumab, nivolumab plus ipilimumab, and ipilimumab groups, respectively, with the most common being diarrhea (in 1.9%, 8.3%, and 4.5%, respectively) and colitis (in 0.6%, 8.3%, and 7.7%, respectively) (Table 5). One death due to study-drug toxicity was reported in the nivolumab group (neutropenia) and one in the ipilimumab group (cardiac arrest), although such adverse events have not been associated with these drugs in prior studies. No treatment-related deaths were reported in the combination group.

Table 2. Patient Disposition.*

| | Nivolumab alone (N=313) | Nivolumab plus Ipilimumab (N=313) | Ipilimumab alone (N=311) |
|---|---|---|---|
| | | | |
| **Patients in the treatment period — no. (%)** | | | |
| Continuing | 117 (37.4) | 93 (29.7) | 50 (16.1) |
| Not continuing | 196 (62.6) | 220 (70.3) | 261 (83.9) |
| | | | |
| **Reason for not continuing the treatment — no. (%)** | | | |
| Disease progression | 154 (49.2) | 69 (22.0) | 202 (65.0) |
| Study drug toxicity | 27 (8.6) | 120 (38.3) | 47 (15.1) |
| Adverse event unrelated to study drug | 5 (1.6) | 12 (3.8) | 4 (1.3) |
| Patient request to discontinue treatment | 5 (1.6) | 5 (1.6) | 4 (1.3) |
| Death | 5 (1.6) | 5 (1.6) | 4 (1.3) |
| Maximum clinical benefit | 2 (0.6) | 2 (0.6) | 0 |
| Poor/non-compliance | 1 (0.3) | 1 (0.3) | 1 (0.3) |
| Patient withdrew consent | 0 | 3 (1.0) | 0 |
| Lost to follow-up | 1 (0.3) | 0 | 0 |
| Patient no longer meets study criteria | 0 | 1 (0.3) | 0 |
| Other | 0 | 3 (1.0) | 2 (0.6) |
| | | | |
| **Patients in the study — no. (%)** | | | |
| Continuing | 223 (71.2) | 221 (70.6) | 189 (60.8) |
| Died | 85 (27.2) | 86 (27.5) | 114 (36.7) |
| Not continuing | 5 (1.6) | 6 (1.9) | 8 (2.6) |

***At the time of database lock on February 17, 2015.***

Table 3. Response to Treatment.

| Response | Nivolumab alone (N=316) | Nivolumab plus Ipilimumab (N=314) | Ipilimumab alone (N=315) |
|---|---|---|---|
| | | | |
| **Best overall response — no. (%)[†]** | | | |
| Complete response | 28 (8.9) | 36 (11.5) | 7 (2.2) |
| Partial response | 110 (34.8) | 145 (46.2) | 53 (16.8) |
| Stable disease | 34 (10.8) | 41 (13.1) | 69 (21.9) |
| Progressive disease | 119 (37.7) | 71 (22.6) | 154 (48.9) |
| Could not be determined | 25 (7.9) | 21 (6.7) | 32 (10.2) |
| | | | |
| **Objective response[‡]** | | | |
| No. of patients (% [95% CI]) | 138 (43.7 [38.1–49.3]) | 181 (57.6 [52.0–63.2]) | 60 (19.0 [14.9–23.8]) |
| Estimated odds ratio (95% CI)[§] | 3.40 (2.02–5.72) | 6.11 (3.59–10.38) | --- |
| Two-sided P value | <0.00001 | <0.00001 | --- |
| | | | |
| **Time to objective response — mo** | | | |
| No. of responders | 138 | 181 | 60 |
| Median | 2.78 | 2.76 | 2.79 |
| Range | 2.3–12.5 | 1.1–11.6 | 2.5–12.4 |

*[†] Best overall response were assessed by the investigators with the use of RECIST v1.1.*

*[‡] Data include patients with a complete response and those with a partial response.*

*[§] Relative to ipilimumab alone.*

Table 4. Objective Response Rate by PD-L1 Status.

| **PD-L1 Positive** | | | |
|---|---|---|---|
| | **Nivolumab alone (N=80)** | **Nivolumab plus Ipilimumab (N=68)** | **Ipilimumab alone (N=75)** |
| | | | |
| **Objective response**[†] | | | |
| No. of patients (% [95% CI]) | 46 (57.5 [45.9–68.5]) | 49 (72.1 [59.9–82.3]) | 16 (21.3 [12.7–32.3]) |
| Estimated odds ratio (95% CI)[§] | 5.03 (2.44–10.37) | 10.41 (4.63–23.40) | --- |
| | | | |
| **PD-L1 Negative** | | | |
| | **Nivolumab alone (N=208)** | **Nivolumab plus Ipilimumab (N=210)** | **Ipilimumab alone (N=202)** |
| **Objective response**[†] | | | |
| No. of patients (% [95% CI]) | 86 (41.3 [34.6–48.4]) | 115 (54.8 [47.8–61.6]) | 36 (17.8 [12.8–23.8]) |
| Estimated odds ratio (95% CI)[§] | 3.25 (2.05–5.13) | 5.90 (3.71–9.38) | --- |

[†] ***Best overall response was assessed by the investigators with the use of RECIST v1.1.***

[§] Relative to ipilimumab alone.

[0144] The most frequent grade 3 or 4 treatment-related select adverse events were diarrhea (2.2%, 9.3%, and 6.1% of patients in the nivolumab, nivolumab plus ipilimumab, and ipilimumab groups, respectively), colitis (in 0.6%, 7.7%, and 8.7%, respectively), increased alanine aminotransferase (in 1.3%, 8.3%, and 1.6%, respectively), and increased aspartate aminotransferase (in 1.0%, 6.1%, and 0.6%, respectively) (Table 6). With the use of immune modulatory agents, resolution rates for grade 3 or 4 select adverse events were generally similar across treatment groups, and were between 85-100% across organ categories in the nivolumab plus ipilimumab group. As observed in prior studies, most endocrine events in all treatment groups did not resolve (Table 7).

**Discussion**

[0145] In this randomized, double-blind, phase 3 study, both nivolumab alone and the combination of nivolumab and ipilimumab significantly increased progression-free survival and objective response rates, as compared with ipilimumab alone, in previously untreated advanced melanoma. These results were observed independently of PD-L1 tumor status, BRAF mutation status, or metastasis stage. Baseline characteristics of study participants were typical of patients with advanced melanoma, although the BRAF mutation rate (31.5%) was lower than the 40-50% generally reported for advanced disease. While not a primary end point of the study, the combination of nivolumab and ipilimumab resulted in numerically longer progression-free survival and a higher response rate as compared with nivolumab alone in the overall study population. While time to response was similar between groups, the first tumor assessment was done at week 12 and thus the possibility that responses may have occurred earlier with the combination remains unknown.

Table 5. Adverse Events (Safety Population).*

| Event | Nivolumab alone (N=313) | | Nivolumab plus Ipilimumab (N=313) | | Ipilimumab alone (N=311) | |
|---|---|---|---|---|---|---|
| | Total | Grade 3 or 4 | Total | Grade 3 or 4 | Total | Grade 3 or 4 |
| | *no. of patients with event (%)* | | | | | |
| Any adverse event | 311 (99.4) | 136 (43.5) | 312 (99.7) | 215 (68.7) | 308 (99.0) | 173 (55.6) |
| Treatment-related adverse event† | 257 (82.1) | 51 (16.3) | 299 (95.5) | 172 (55.0) | 268 (86.2) | 85 (27.3) |
| Diarrhea | 60 (19.2) | 7 (2.2) | 138 (44.1) | 29 (9.3) | 103 (33.1) | 19 (6.1) |
| Fatigue | 107 (34.2) | 4 (1.3) | 110 (35.1) | 13 (4.2) | 87 (28.0) | 3 (1.0) |
| Pruritus | 59 (18.8) | 0 | 104 (33.2) | 6 (1.9) | 110 (35.4) | 1 (0.3) |
| Rash | 81 (25.9) | 2 (0.6) | 126 (40.3) | 15 (4.8) | 102 (32.8) | 6 (1.9) |
| Nausea | 41 (13.1) | 0 | 81 (25.9) | 7 (2.2) | 50 (16.1) | 2 (0.6) |
| Pyrexia | 18 (5.8) | 0 | 58 (18.5) | 2 (0.6) | 21 (6.8) | 1 (0.3) |
| Decreased appetite | 34 (10.9) | 0 | 56 (17.9) | 4 (1.3) | 39 (12.5) | 1 (0.3) |
| Increase in alanine aminotransferase | 12 (3.8) | 4 (1.3) | 55 (17.6) | 26 (8.3) | 12 (3.9) | 5 (1.6) |
| Vomiting | 20 (6.4) | 1 (0.3) | 48 (15.3) | 8 (2.6) | 23 (7.4) | 1 (0.3) |
| Increase in aspartate aminotransferase | 12 (3.8) | 3 (1.0) | 48 (15.3) | 19 (6.1) | 11 (3.5) | 2 (0.6) |
| Hypothyroidism | 27 (8.6) | 0 | 47 (15.0) | 1 (0.3) | 13 (4.2) | 0 |
| Colitis | 4 (1.3) | 2 (0.6) | 37 (11.8) | 24 (7.7) | 36 (11.6) | 27 (8.7) |
| Arthralgia | 24 (7.7) | 0 | 33 (10.5) | 1 (0.3) | 19 (6.1) | 0 |
| Headache | 23 (7.3) | 0 | 32 (10.2) | 1 (0.3) | 24 (7.7) | 1 (0.3) |
| Dyspnea | 14 (4.5) | 1 (0.3) | 32 (10.2) | 2 (0.6) | 13 (4.2) | 0 |
| Treatment-related adverse event leading to discontinuation | 24 (7.7) | 16 (5.1) | 114 (36.4) | 92 (29.4) | 46 (14.8) | 41 (13.2) |

* The severity of adverse events was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0.

† The treatment-related adverse events listed here were reported in at least 10% of the patients in any of the three study groups.

Table 6. Treatment-related Select Adverse Events (Safety Population).*

| Event | Nivolumab alone (N=313) | | Nivolumab plus Ipilimumab (N=313) | | Ipilimumab alone (N=311) | |
|---|---|---|---|---|---|---|
| | Total | Grade 3 or 4 | Total | Grade 3 or 4 | Total | Grade 3 or 4 |
| | *no. of patients with event (%)* | | | | | |
| Any select adverse event | 194 (62.0) | 24 (7.7) | 275 (87.9) | 124 (39.6) | 229 (73.6) | 58 (18.6) |
| | | | | | | |
| Treatment-related select adverse event† | | | | | | |
| Skin | 131 (41.9) | 5 (1.6) | 185 (59.1) | 18 (5.8) | 168 (54.0) | 9 (2.9) |
| Pruritus | 59 (18.8) | 0 | 104 (33.2) | 6 (1.9) | 110 (35.4) | 1 (0.3) |
| Rash | 68 (21.7) | 1 (0.3) | 89 (28.4) | 9 (2.9) | 65 (20.9) | 5 (1.6) |
| Rash maculo-papular | 13 (4.2) | 1 (0.3) | 37 (11.8) | 6 (1.9) | 37 (11.9) | 1 (0.3) |
| Vitiligo | 23 (7.3) | 1 (0.3) | 21 (6.7) | 0 | 12 (3.9) | 0 |
| Gastrointestinal | 61 (19.5) | 7 (2.2) | 145 (46.3) | 46 (14.7) | 114 (36.7) | 36 (11.6) |
| Diarrhea | 60 (19.2) | 7 (2.2) | 138 (44.1) | 29 (9.3) | 103 (33.1) | 19 (6.1) |
| Colitis | 4 (1.3) | 2 (0.6) | 37 (11.8) | 24 (7.7) | 36 (11.6) | 27 (8.7) |
| Hepatic | 20 (6.4) | 8 (2.6) | 94 (30.0) | 59 (18.8) | 22 (7.1) | 5 (1.6) |
| Increase in alanine aminotransferase | 12 (3.8) | 4 (1.3) | 55 (17.6) | 26 (8.3) | 12 (3.9) | 5 (1.6) |
| Increase in aspartate aminotransferase | 12 (3.8) | 3 (1.0) | 48 (15.3) | 19 (6.1) | 11 (3.5) | 2 (0.6) |
| Endocrine | 45 (14.4) | 2 (0.6) | 94 (30.0) | 15 (4.8) | 34 (10.9) | 7 (2.3) |
| Hypothyroidism | 27 (8.6) | 0 | 47 (15.0) | 1 (0.3) | 13 (4.2) | 0 |
| Hyperthyroidism | 13 (4.2) | 0 | 31 (9.9) | 3 (1.0) | 3 (1.0) | 0 |
| Hypophysitis | 2 (0.6) | 1 (0.3) | 24 (7.7) | 5 (1.6) | 12 (3.9) | 6 (1.9) |
| Pulmonary | 5 (1.6) | 1 (0.3) | 22 (7.0) | 3 (1.0) | 6 (1.9) | 1 (0.3) |
| Pneumonitis | 4 (1.3) | 1 (0.3) | 20 (6.4) | 3 (1.0) | 5 (1.6) | 1 (0.3) |

* The severity of adverse events was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0.
† The treatment-related select adverse events listed here were reported in at least 5% of the patients in any of the three study groups.

Table 7. Management of Treatment-related Select Adverse Events With Immune Modulatory Medication (IMM).

| | Nivolumab | | | Nivolumab plus Ipilimumab | | | Ipilimumab | | |
|---|---|---|---|---|---|---|---|---|---|
| Select Adverse Event Organ Category | Patients managed with IMM, n (%) | Patients with resolution of AE after treatment with IMM, n (%) | Median time to resolution, weeks (95% CI) | Patients managed with IMM, n (%) | Patients with resolution of AE after treatment with IMM, n (%) | Median time to resolution, weeks (95% CI) | Patients managed with IMM, n (%) | Patients with resolution of AE after treatment with IMM, n (%) | Median time to resolution, weeks (95% CI) |
| **Skin** | 33/131 (25.2) | 17 (54.8) | 34.9 (18.0, NE) | 80/185 (43.2) | 55 (75.3) | 8.6 (7.0, 12.6) | 58/168 (34.5) | 41 (74.5) | 12.4 (8.9, 19.1) |
| Grade 3-4 | 3/5 (60.0) | 3 (75.0) | 2.1 (0.9, NE) | 12/18 (66.7) | 12 (85.7) | 3.4 (1.1, 12.4) | 5/9 (55.6) | 5 (83.3) | 6.1 (4.4, NE) |
| **Gastrointestinal** | 9/61 (14.8) | 5 (71.4) | 4.0 (0.8, NE) | 71/145 (49.0) | 62 (93.9) | 4.5 (3.5, 5.7) | 54/114 (47.4) | 44 (88.0) | 4.9 (2.9, 7.6) |
| Grade 3-4 | 5/7 (71.4) | 3 (50.0) | NE (0.8, NE) | 41/46 (89.1) | 41 (97.6) | 3.0 (1.9, 4.3) | 33/36 (91.7) | 31 (88.6) | 4.7 (1.9, 6.1) |
| **Endocrine** | 5/45 (11.1) | 2 (40.0) | 24.3 (7.1, NE) | 36/94 (38.3) | 14 (41.2) | NE (10.4, NE) | 15/34 (44.1) | 4 (28.6) | NE (0.71, NE) |
| Grade 3-4 | 2/2 (100) | 0 | NE (NE, NE) | 10/15 (66.7) | 5 (45.5) | NE (4.5, NE) | 7/7 (100.0) | 3 (42.9) | NE (0.43, NE) |
| **Hepatic** | 8/22 (36.4) | 6 (100.0) | 7.0 (2.0, 27.1) | 46/95 (48.4) | 43 (95.6) | 5.9 (3.3, 6.6) | 3/22 (13.6) | 3 (100.0) | 4.1 (4.0, 7.7) |
| Grade 3-4 | 7/8 (87.5) | 6 (100.0) | 7.0 (2.0, 27.1) | 37/60 (61.7) | 38 (100.0) | 4.1 (3.0, 6.1) | 2/5 (40.0) | 2 (100.0) | 5.9 (4.0, 7.7) |
| **Pulmonary** | 4/5 (80.0) | 4 (100.0) | 3.3 (2.3, 9.1) | 17/22 (77.3) | 16 (94.1) | 6.1 (1.1, 8.3) | 3/6 (50.0) | 2 (66.7) | 6.1 (6.0, 6.3) |
| Grade 3-4 | 1/1 (100) | 1 (100.0) | 2.3 (NE, NE) | 2/3 (66.7) | 2 (100.0) | 4.2 (1.1, 7.3) | 1/1 (100) | 1 (100.0) | 4.7 (NE, NE) |
| **Renal** | 1/3 (33.3) | 1 (100.0) | 0.3 (NE, NE) | 4/17 (23.5) | 3 (100.0) | 1.7 (0.4, 3.7) | 3/8 (37.5) | 3 (100.0) | 4.6 (0.6, 16.1) |
| Grade 3-4 | 0/1 | 0 | - | 3/6 (50.0) | 3 (100.0) | 1.7 (0.4, 3.6) | 1/1 (100) | 1 (100.0) | 4.6 (NE, NE) |

**[0146]** The median progression-free survival reported for the combination of nivolumab and ipilimumab in this study (11.7 months in BRAF-mutant patients) is similar to that recently reported for the combination of BRAF and MEK inhibition in BRAF-mutant metastatic melanoma (9.9 months for vemurafenib and cobimetinib; 9.3 to 11.4 months for dabrafenib and trametinib). Resistance to such targeted therapies is almost inevitable when used as monotherapy and in many cases is very rapid. The confirmed rate of objective response for combined nivolumab and ipilimumab (57.6%) is numerically higher than observed with PD-1 blockade alone in advanced melanoma (nivolumab [40%] in treatment-naïve patients with wild-type BRAF or pembrolizumab [37%] in ipilimumab-naïve patients).

**[0147]** The results of subgroup analyses suggest that the greatest benefit for the combination of nivolumab and ipilimumab versus nivolumab alone may occur in the setting of negative PD-L1 tumor expression. In the PD-L1-positive group, both nivolumab alone and nivolumab plus ipilimumab resulted in a similar prolongation of progression-free survival as compared with ipilimumab alone, although objective response rates were numerically higher in the combination group versus either nivolumab or ipilimumab alone. Thus, the use of PD-L1 as a biomarker may allow clinicians to make more informed decisions about the risk-benefit of combination therapy versus monotherapy. Nonetheless, the observation of at least additive activity of the combination of ipilimumab and nivolumab in the setting of negative PD-L1 expression is of interest in melanoma as well as in other tumor types in which PD-1 checkpoint inhibitors are under evaluation.

**[0148]** The incidence of adverse events in this study was, in general, lowest in the nivolumab group and highest in the combination group. The overall incidence of grade 3 or 4 drug-related adverse events was higher in the combination group as compared with ipilimumab alone (39.6% versus 18.6%), as a result of a slightly higher incidence in most adverse events, particularly hepatic toxicity, where the rates of grade 3 or 4 ALT/AST elevations were 6-8% for the combination and approximately 1% for ipilimumab alone. One drug-related death was reported in each of the nivolumab and ipilimumab groups but none in the combination group. Overall, the safety profile of the combination of nivolumab and ipilimumab was consistent with previous experience with nivolumab or ipilimumab alone. No new safety signals were identified, and adverse events were manageable with established treatment guidelines as most

select adverse events resolved with immune modulatory agents. These data suggest that the combination of nivolumab and ipilimumab can be used safely in a broad range of clinical settings.

**[0149]** In summary, the report shows increased progression-free survival and objective response rates for nivolumab alone and the combination of nivolumab and ipilimumab, as compared with ipilimumab alone, in previously untreated advanced melanoma. Adverse events with the combination were managed with established algorithms, with no study drug-related deaths. The combination of nivolumab and ipilimumab may represent a means to improve outcomes with either agent as monotherapy, particularly for patients having PD-L1-negative tumors. Overall, nivolumab alone and the combination of nivolumab and ipilimumab are promising treatment options for previously untreated advanced melanoma.

## SEQUENCES

$V_H$ amino acid sequence of 28-8 (*Oryctolagus cuniculus*) (SEQ ID NO:1)

Met Glu Thr Gly Leu Arg Trp Leu Leu Leu Val Ala Val Leu Lys Gly Val Gln Cys Leu Ser Val Glu Glu Ser Gly Gly Arg Leu Val Thr Pro Gly Thr Pro Leu Thr Leu Thr Cys Thr Ala Ser Gly Phe Thr Ile Thr Asn Tyr His Met Phe Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile Gly Val Ile Thr Ser Ser Gly Ile Gly Ser Ser Ser Thr Thr Tyr Tyr Ala Thr Trp Ala Lys Gly Arg Phe Thr Ile Ser Lys Thr Ser Thr Thr Val Asn Leu Arg Ile Thr Ser Pro Thr Thr Glu Asp Thr Ala Thr Tyr Phe Cys Ala Arg Asp Tyr Phe Thr Asn Thr Tyr Tyr Ala Leu Asp Ile Trp Gly Pro Gly Thr Leu Val Thr Val Ser Ser

$V_L$ amino acid sequence of 28-8 (*Oryctolagus cuniculus*) (SEQ ID NO:2)

Met Asp Thr Arg Ala Pro Thr Gln Leu Leu Gly Leu Leu Leu Leu Trp Leu Pro Gly Ala Arg Cys Ala Leu Val Met Thr Gln Thr Pro Ser Ser Thr Ser Thr Ala Val Gly Gly Thr Val Thr Ile Lys Cys Gln Ala Ser Gln Ser Ile Ser Val Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro Lys Leu Leu Ile Tyr Ser Ala Ser Thr Leu Ala Ser Gly Val Pro Ser Arg Phe Lys Gly Ser Arg Ser Gly Thr Glu Tyr Thr Leu Thr Ile Ser Gly Val Gln Arg Glu Asp Ala Ala Thr Tyr Tyr Cys Leu Gly Ser Ala Gly Ser

Heavy Chain CDR1 sequence of 28-8 (*Oryctolagus cuniculus*) (SEQ ID NO:3)

Asn Tyr His Met Phe

Heavy Chain CDR2 sequence of 28-8 (*Oryctolagus cuniculus*) (SEQ ID NO:4)

Val Ile Thr Ser Ser Gly Ile Gly Ser Ser Ser Thr Thr Tyr Tyr Ala Thr Trp Ala Lys Gly

Heavy Chain CDR3 sequence of 28-8 (*Oryctolagus cuniculus*) (SEQ ID NO:5)

Asp Tyr Phe Thr Asn Thr Tyr Tyr Ala Leu Asp Ile

Light Chain CDR1 sequence of 28-8 (*Oryctolagus cuniculus*) (SEQ ID NO:6)

Gln Ala Ser Gln Ser Ile Ser Val Tyr Leu Ala

Light Chain CDR2 sequence of 28-8 (*Oryctolagus cuniculus*) (SEQ ID NO:7)

Ser Ala Ser Thr Leu Ala Ser

Light Chain CDR3 sequence of 28-8 (*Oryctolagus cuniculus*) (SEQ ID NO:8)

Leu Gly Ser Ala Gly Ser Asp Asp Ala Ala

What is claimed is:

1. A method for treating a melanoma comprising:

(i) identifying a patient having a PD-L1-negative melanoma tumor; and

(ii) administering to the patient:

(a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and

(b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4.

2. A method for treating a melanoma comprising administering to a patient afflicted with a melanoma tumor:

(a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and

(b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4,

wherein the patient is identified as having a PD-L1-negative melanoma tumor prior to the administration.

3. A method for extending a progression-free survival period for over 8 months in a patient afflicted with a melanoma tumor comprising administering to the patient:

(a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and

(b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4,

wherein the patient is identified as having a PD-L1-negative melanoma tumor prior to the administration and wherein the patient demonstrates progression-free survival for over 8 months.

4. The method of claim 3, wherein the progression-free survival of the patient is extended after the administration for over about 11 months, about 12 months, about 13 months, about 14

months, about 15 months, about 16 months, about 17 months, about 18 months, about 2 years, about 3 years, about 4 years, about 5 years, about 6 years, about 7 years, about 8 years, about 9 years, or about 10 years.

5. The method of claim 4, wherein the progression-free survival of the patient is extended for over 11 months.

6. A method for reducing a tumor size at least by 10% in a patient afflicted with a melanoma tumor comprising administering to the patient:

(a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and

(b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4,

wherein the patient is identified as having a PD-L1-negative melanoma tumor prior to the administration and wherein the administration reduces the tumor size at least about 10%, about 20%, about 30%, about 40%, or about 50% compared to the tumor size prior to the administration.

7. The method of any one of claims 2 to 6, further comprising identifying the patient as having a melanoma tumor that does not express PD-L1 prior to the administration.

8. The method of any one of claims 1 to 7, wherein the patient is characterized by (i) extended progression-free survival for over 8 months, (ii) tumor size reduction at least about 10%, about 20%, about 30%, about 40%, or about 50% compared to the tumor size prior to the administration, or (iii) both.

9. A method for increasing an objective response rate to be higher than 40% in a patient population, each of whom is afflicted with a melanoma tumor, in a cancer treatment comprising administering to the patient:

(a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and

(b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4,

wherein each patient is identified as having a PD-L1-negative melanoma tumor prior to the administration and wherein the objective response rate is higher than 40%, 45%, 50%, 55%, 60%, 65%, 70%, or 75%.

10. The method of claim 9, further comprising identifying each patient of the patient population as having a PD-L1-negative melanoma tumor prior to the administration.

11. The method of claim 9 or 10, wherein each patient of the patient population is further characterized by (i) extended progression-free survival for over 8 months, (ii) tumor size reduction at least about 10%, about 20%, about 30%, about 40%, or about 50% compared to the tumor size prior to the administration, or (iii) both.

12. The method of any one of claims 9 to 11, wherein the patient population comprises at least about 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1000 patients having a PD-L1-negative melanoma tumor.

13. A method for selecting a patient suitable for an anti-PD-1 antibody and anti-CTLA-4 antibody combination therapy comprising:

(i) identifying a patient having a PD-L1-negative melanoma tumor; and

(ii) instructing a healthcare provider to administer to the patient:

(a) an anti-PD-1 antibody or an antigen-binding portion thereof that binds specifically to a human PD-1; and

(b) an anti-CTLA-4 antibody or an antigen-binding portion thereof that binds specifically to a human CTLA-4.

14. The method of any one of claims 1, 7, 10, and 13, wherein the identifying comprises measuring a PD-L1 expression on the melanoma tumor.

15. The method of any one of claims 1 to 14, wherein the administration treats the melanoma tumor.

16. The method of claim 14 or 15, wherein the measuring comprises providing a test tissue sample obtained from the patient, the test tissue sample comprising tumor cells and/or tumor-infiltrating inflammatory cells.

17. The method of claim 16, wherein the measuring further comprises assessing the proportion of cells in the test tissue sample that express PD-L1 on the cell surface.

18. The method of claim 17, wherein the test tissue sample is a formalin-fixed paraffin-embedded (FFPE) tissue sample.

19. The method of claim 18, wherein the presence of PD-L1 is determined using an automated IHC assay.

20. The method of claim 19, wherein the IHC assay is performed using an anti-PD-L1 monoclonal antibody that specifically binds to the PD-L1 and wherein the anti-PD-L1 monoclonal antibody is selected from the group consisting of 28-8, 28-1, 28-12, 29-8, 5H1, and any combination thereof.

21. The method of any one of claims 1 to 20, wherein the PD-L1-negative melanoma tumor is characterized by having less than about 5%, about 4%, about 3%, about 2%, about 1%, or about 0% of tumor cells showing binding to the anti-PD-L1 antibody or an antigen-binding portion thereof.

22. The method of any one of claims 1 to 21, wherein the anti-PD-1 antibody or antigen-binding portion thereof cross-competes with nivolumab for binding to human PD-1.

23. The method of any one of claims 1 to 22, wherein the anti-PD-1 antibody or antigen-binding portion thereof is a chimeric, humanized or human monoclonal antibody or a portion thereof.

24. The method of any one of claims 1 to 23, wherein the anti-PD-1 antibody or antigen-binding portion thereof comprises a heavy chain constant region which is of a human IgG1 or IgG4 isotype.

25. The method of any one of claims 1 to 24, wherein the anti-PD-1 antibody is nivolumab.

26. The method of any one of claims 1 to 24, wherein the anti-PD-1 antibody is pembrolizumab.

27. The method of any one of claims 1 to 26, wherein the anti-PD-1 antibody or antigen-binding portion thereof is administered at a dose ranging from 0.1 to 10.0 mg/kg body weight once every 2, 3 or 4 weeks.

28. The method of claim 27, wherein the anti-PD-1 antibody or antigen-binding portion thereof is administered at a dose of 1 or 3 mg/kg body weight once every 3 weeks.

29. The method of any one of claims 1 to 28, wherein the anti-CTLA-4 antibody or antigen-binding portion thereof cross-competes with ipilimumab for binding to human CTLA-4.

30. The method of any one of claims 1 to 29, wherein the anti-CTLA-4 antibody or antigen-binding portion thereof is a chimeric, humanized or human monoclonal antibody or a portion thereof.

31. The method of any one of claims 1 to 30, wherein the anti-CTLA-4 antibody or antigen-binding portion thereof comprises a heavy chain constant region which is of a human IgG1 isotype.

32. The method of any one of claims 1 to 31, wherein the anti-CTLA-4 antibody is ipilimumab.

33. The method of any one of claims 1 to 31, wherein the anti-CTLA-4 antibody is tremelimumab.

34. The method of any one of claims 1 to 33, wherein the anti-CTLA-4 antibody or antigen-binding portion thereof is administered at a dose ranging from 0.1 to 10.0 mg/kg body weight once every 2, 3 or 4 weeks.

35. The method of claim 34, wherein the anti-CTLA-4 antibody or antigen-binding portion thereof is administered at a dose of 1 or 3 mg/kg body weight once every 3 weeks.

36. The method of claim 35, wherein the anti-PD-1 antibody or an antigen-binding portion thereof is administered at a dose of 1 mg/kg body weight once every 3 weeks and the anti-

CTLA-4 antibody or an antigen-binding portion thereof is administered at a dose of 3 mg/kg body weight once every 3 weeks.

37. The method of any of claims 1 to 36, wherein the anti-PD-1 antibody or an antigen-binding portion thereof is administered concurrently with the anti-CTLA-4 antibody or an antigen-binding portion thereof.

38. The method of any of claims 1 to 36, wherein the anti-PD-1 antibody or an antigen-binding portion thereof is administered prior to or after the anti-CTLA-4 antibody or an antigen-binding portion thereof.

39. The method of any of claims 1 to 36, wherein the anti-PD-1 antibody or an antigen-binding portion thereof and the anti-CTLA-4 antibody or an antigen-binding portion thereof are in a single pharmaceutical composition.

40. The method of claim 39, wherein the pharmaceutical composition comprises the anti-PD-1 antibody and the anti-CTLA-4 antibody at a ratio of 50:1 to 1:50.

41. The method of claim 39, wherein the pharmaceutical composition comprises the anti-PD-1 antibody and the anti-CTLA-4 antibody at a ratio of 10:1 to 1:10.

42. The method of claim 39, wherein the pharmaceutical composition comprises the anti-PD-1 antibody and the anti-CTLA-4 antibody at a ratio of 3:1 to 1:3.

43. The method of claim 39, wherein the pharmaceutical composition comprises the anti-PD-1 antibody and the anti-CTLA-4 antibody at a ratio of 1:3.

44. The method of claim 39, wherein the pharmaceutical composition comprises 80 mg of the anti-PD-1 antibody and 240 mg of the anti-CTLA-4 antibody.

45. The method of claim any of claims 1 to 38, wherein the anti-PD-1 and anti-CTLA-4 antibodies are administered within 30 minutes of each other.

46. The method of any one of claims 1 to 45, further comprising administering an anti-cancer agent.

47. A kit for treating a patient afflicted with a melanoma tumor, the kit comprising:

(a) a dosage ranging from 0.1 to 10 mg/kg body weight of an anti-PD-1 antibody or an antigen-binding portion thereof;

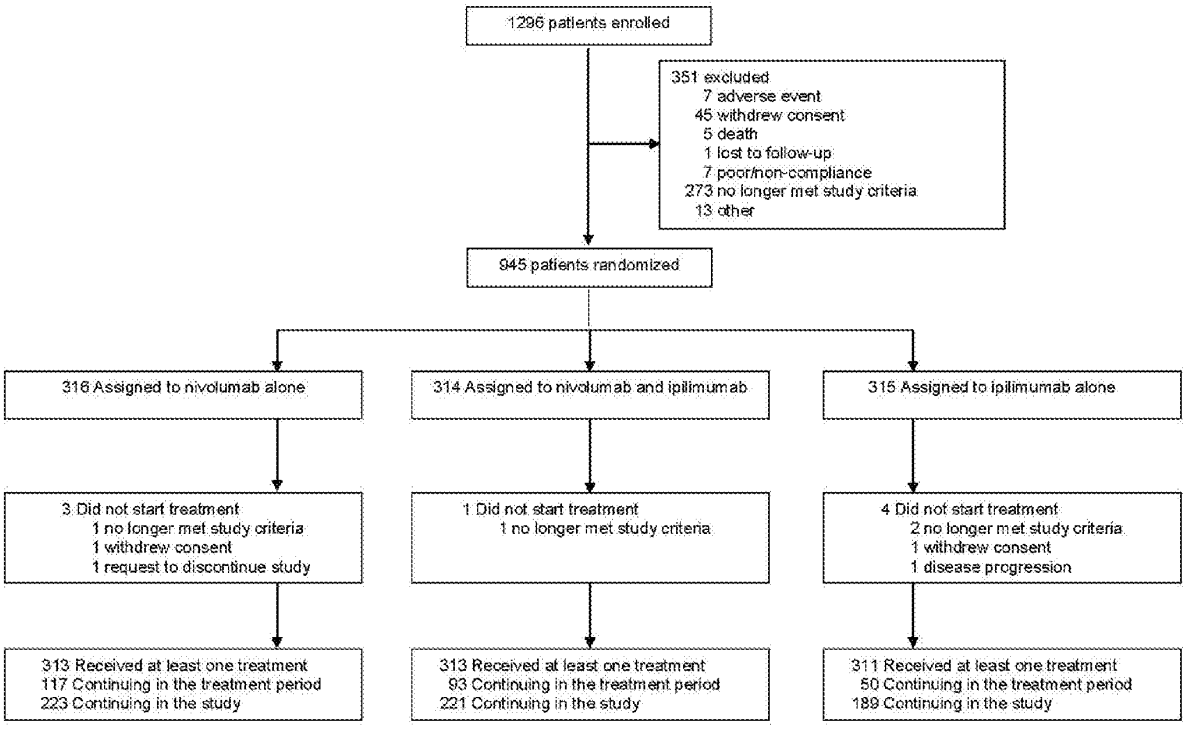
(b) a dosage ranging from 0.1 to 10 mg/kg body weight of an anti-CTLA-4 antibody or an antigen-binding portion thereof; and

(c) instructions for using the anti-PD-1 antibody or the antigen-binding portion thereof and the anti-CTLA-4 antibody or the antigen-binding portion thereof in the method of any one of claims 1 to 46.

# ABSTRACT

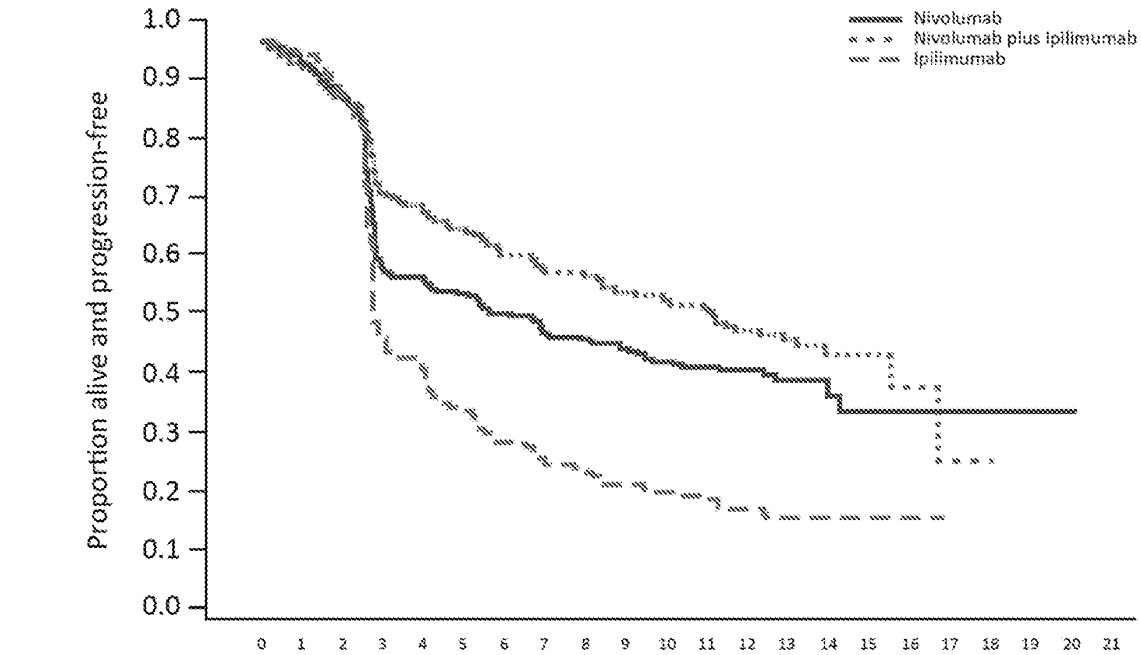
The invention provides a method of treating a melanoma comprising (i) identifying a patient having a PD-L1-negative melanoma and (ii) administering to the patient a combination of an anti-PD-1 antibody or an antigen-binding portion thereof and an anti-CTLA-4 antibody or an antigen-binding portion thereof. The methods of the invention can extend progression-free survival for over 8 months and/or reduces the tumor size at least about 10%, about 20%, about 30%, about 40%, or about 50% compared to the tumor size prior to the administration.

**Fig. 1 – Patient Flow Diagram: Enrollment, Randomization, and Follow-up of Patients in the Study**




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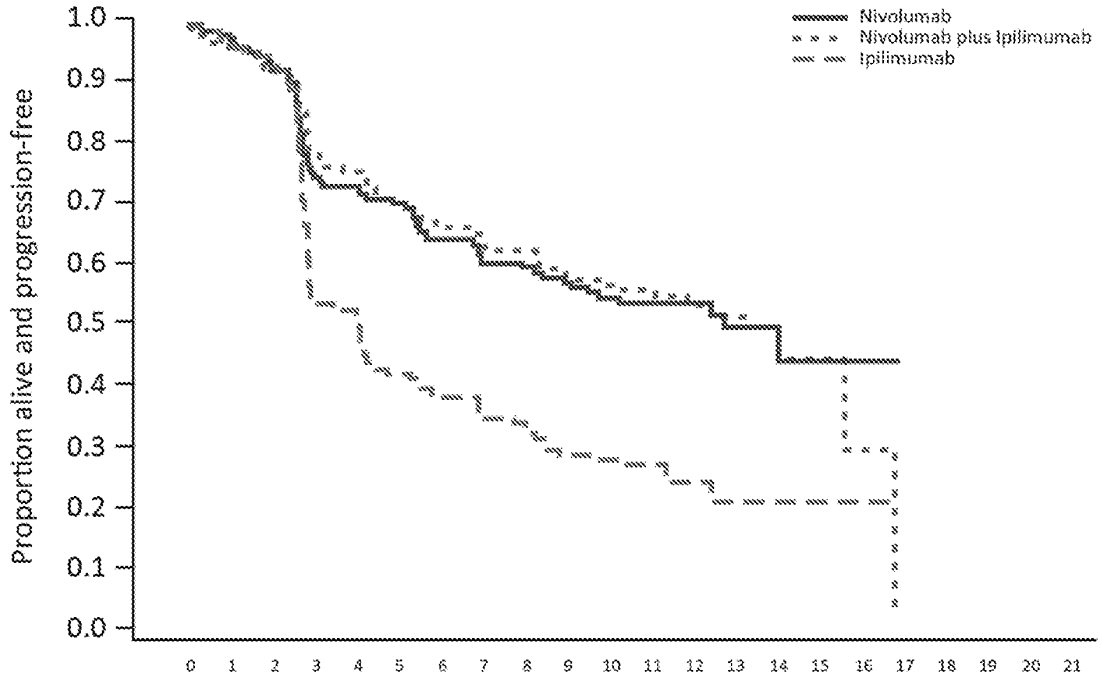
## Fig. 2A – Progression-free Survival (Intention-to-Treat Population)




| No. at Risk | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Nivolumab | 316 | 292 | 271 | 177 | 170 | 160 | 147 | 136 | 132 | 124 | 106 | 86 | 50 | 38 | 14 | 9 | 6 | 2 | 1 | 1 | 1 | 0 |
| Nivolumab plus Ipilimumab | 314 | 293 | 275 | 219 | 208 | 191 | 173 | 164 | 163 | 151 | 137 | 116 | 65 | 54 | 18 | 11 | 7 | 2 | 1 | 0 | 0 | 0 |
| Ipilimumab | 315 | 285 | 265 | 137 | 118 | 95 | 77 | 68 | 63 | 54 | 47 | 42 | 24 | 17 | 7 | 4 | 3 | 0 | 0 | 0 | 0 | 0 |

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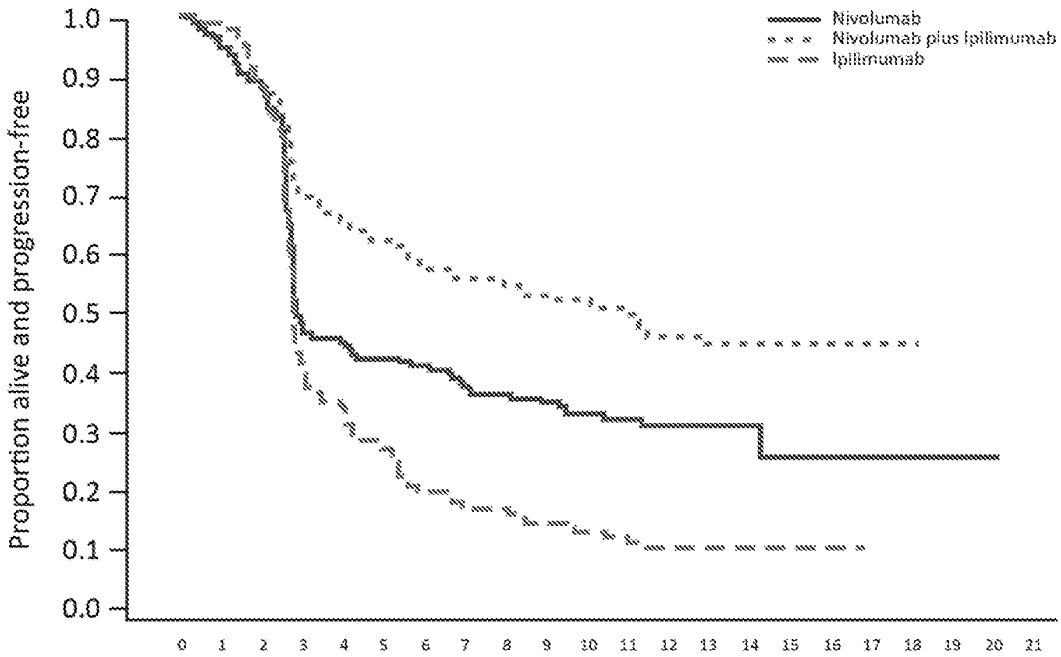
**Fig. 2B – Progression-free Survival in Patients with Positive PD-L1 Status**




| No. at Risk | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Nivolumab | 143 | 134 | 126 | 101 | 98 | 92 | 83 | 77 | 76 | 70 | 63 | 52 | 28 | 22 | 8 | 6 | 3 | 0 | 0 | 0 | 0 | 0 |
| Nivolumab plus Ipilimumab | 144 | 137 | 131 | 107 | 103 | 93 | 85 | 80 | 80 | 73 | 67 | 53 | 32 | 27 | 7 | 4 | 2 | 0 | 0 | 0 | 0 | 0 |
| Ipilimumab | 144 | 131 | 125 | 73 | 66 | 53 | 46 | 42 | 38 | 33 | 29 | 26 | 15 | 11 | 5 | 3 | 2 | 0 | 0 | 0 | 0 | 0 |

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## Fig. 2C – Progression-free Survival in Patients with Negative PD-L1 Status



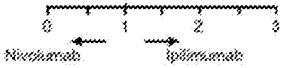

| No. at risk | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Nivolumab | 165 | 150 | 137 | 72 | 68 | 64 | 60 | 55 | 52 | 50 | 40 | 32 | 20 | 15 | 6 | 3 | 3 | 2 | 1 | 1 | 1 | 0 |
| Nivolumab plus ipilimumab | 154 | 142 | 131 | 101 | 95 | 88 | 79 | 76 | 75 | 70 | 64 | 57 | 31 | 25 | 11 | 7 | 5 | 2 | 1 | 0 | 0 | 0 |
| Ipilimumab | 162 | 146 | 132 | 60 | 48 | 38 | 27 | 23 | 23 | 19 | 16 | 14 | 8 | 5 | 2 | 1 | 1 | 0 | 0 | 0 | 0 | 0 |

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## Fig. 3 – Subgroup Analyses of Progression-free Survival

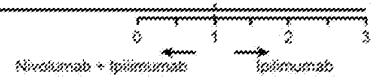
### (A) Nivolumab versus ipilimumab

| | N | Nivolumab N of events (N of patients) | Nivolumab Median PFS (95% CI) | Ipilimumab N of events (N of patients) | Ipilimumab Median PFS (95% CI) | Unstratified Hazard Ratio (95% CI) |
|---|---|---|---|---|---|---|
| **Overall** | 945 | 174 (316) | 6.87 (4.34, 9.46) | 234 (315) | 2.89 (2.79, 3.42) | 0.57 (0.47, 0.69) |
| BRAF Mutation Status | | | | | | |
| *Mutant* | 300 | 57 (98) | 5.62 (2.79, 9.46) | 66 (100) | 4.04 (2.79, 5.52) | 0.77 (0.54, 1.09) |
| *Wild-type* | 645 | 117 (218) | 7.89 (3.86, 12.68) | 168 (215) | 3.83 (2.76, 3.09) | 0.50 (0.39, 0.63) |
| M Stage at Study Entry | | | | | | |
| *M0/M1a/M1b* | 386 | 63 (131) | 9.30 (5.62, N.A.) | 92 (126) | 4.17 (2.96, 5.52) | 0.54 (0.39, 0.74) |
| *M1c* | 559 | 111 (185) | 5.39 (2.83, 8.87) | 142 (189) | 2.79 (2.73, 2.83) | 0.59 (0.46, 0.76) |




### (B) Nivolumab plus ipilimumab versus ipilimumab

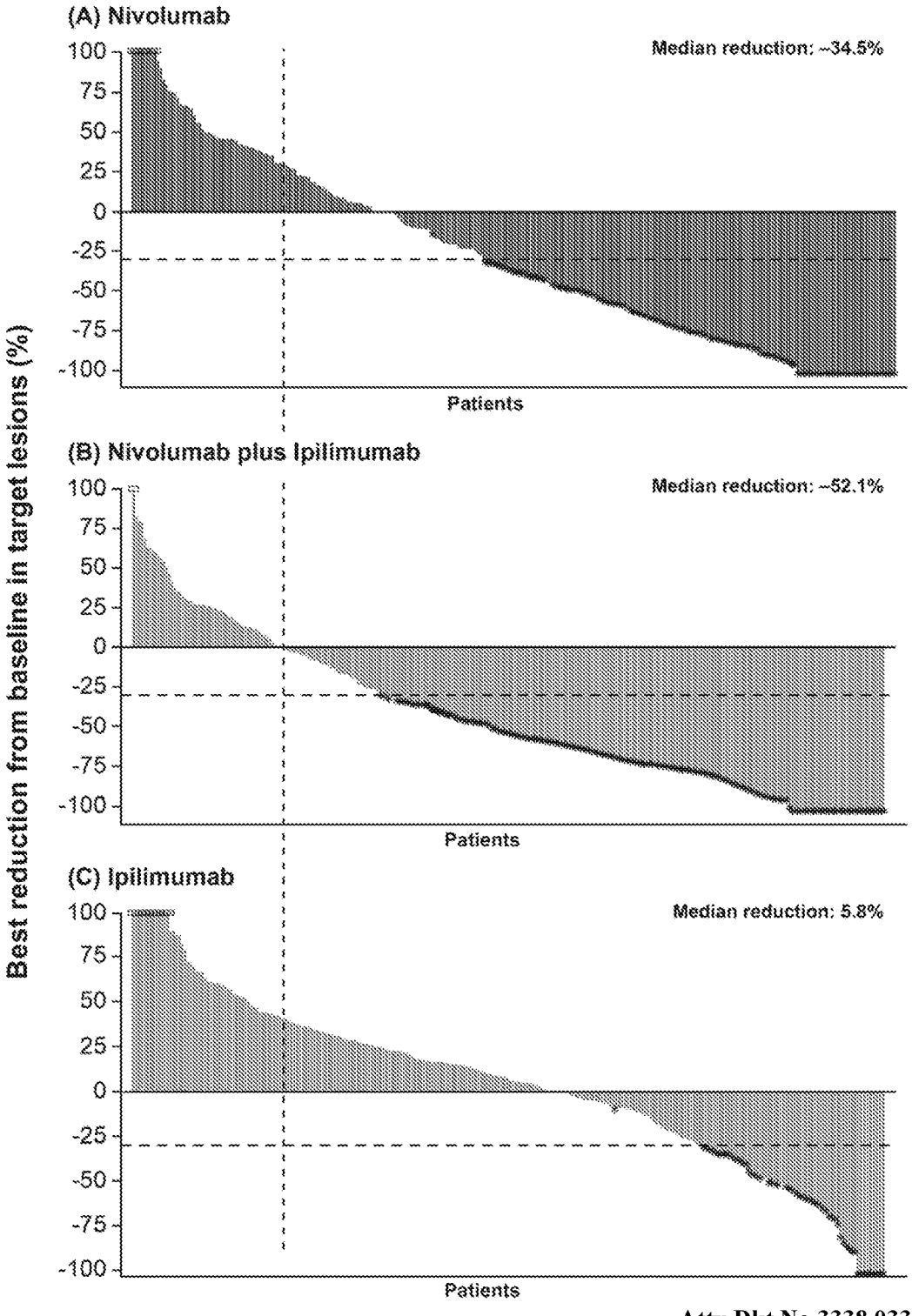
| | N | Nivolumab + Ipilimumab N of events (N of patients) | Nivolumab + Ipilimumab Median PFS (95% CI) | Ipilimumab N of events (N of patients) | Ipilimumab Median PFS (95% CI) | Unstratified Hazard Ratio (95% CI) |
|---|---|---|---|---|---|---|
| **Overall** | 945 | 151 (314) | 11.50 (8.90, 16.72) | 234 (315) | 2.89 (2.79, 3.42) | 0.43 (0.35, 0.53) |
| BRAF Mutation Status | | | | | | |
| *Mutant* | 300 | 48 (102) | 11.73 (8.02, N.A.) | 66 (100) | 4.04 (2.79, 5.52) | 0.47 (0.32, 0.68) |
| *Wild-type* | 645 | 103 (212) | 11.24 (8.34, N.A.) | 168 (215) | 3.83 (2.76, 3.09) | 0.41 (0.32, 0.53) |
| M Stage at Study Entry | | | | | | |
| *M0/M1a/M1b* | 386 | 51 (129) | 15.54 (11.17, N.A.) | 92 (126) | 4.17 (2.96, 5.52) | 0.35 (0.25, 0.50) |
| *M1c* | 559 | 100 (185) | 8.51 (5.52, 12.35) | 142 (189) | 2.79 (2.73, 2.83) | 0.48 (0.37, 0.62) |






Atty Dkt No 3338.0330001

## Fig. 4 – Tumor Burden Change in Target Lesions




Atty Dkt No 3338.0330001

# Electronic Patent Application Fee Transmittal

| | |
|---|---|
| **Application Number:** | |
| **Filing Date:** | |
| **Title of Invention:** | Treatment of PD-L1-Negative Melanoma Using an Anti-PD-1 Antibody and an Anti-CTLA-4 Antibody |
| **First Named Inventor/Applicant Name:** | Arvin YANG |
| **Filer:** | Ji Eun Kim/Kim Perry |
| **Attorney Docket Number:** | 3338.0330001/ELE/C-K |

Filed as Large Entity

**Filing Fees for Utility under 35 USC 111(a)**

| Description | Fee Code | Quantity | Amount | Sub-Total in USD($) |
|---|---|---|---|---|
| **Basic Filing:** | | | | |
| Utility application filing | 1011 | 1 | 280 | 280 |
| Utility Search Fee | 1111 | 1 | 600 | 600 |
| Utility Examination Fee | 1311 | 1 | 720 | 720 |
| **Pages:** | | | | |
| **Claims:** | | | | |
| **Miscellaneous-Filing:** | | | | |
| **Petition:** | | | | |
| **Patent-Appeals-and-Interference:** | | | | |

| Description | Fee Code | Quantity | Amount | Sub-Total in USD($) |
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| **Post-Allowance-and-Post-Issuance:** | | | | |
| **Extension-of-Time:** | | | | |
| **Miscellaneous:** | | | | |
| | | **Total in USD ($)** | | **1600** |

| Electronic Acknowledgement Receipt | |
|---|---|
| **EFS ID:** | 25631032 |
| **Application Number:** | 15141769 |
| **International Application Number:** | |
| **Confirmation Number:** | 6926 |
| **Title of Invention:** | Treatment of PD-L1-Negative Melanoma Using an Anti-PD-1 Antibody and an Anti-CTLA-4 Antibody |
| **First Named Inventor/Applicant Name:** | Arvin YANG |
| **Customer Number:** | 115018 |
| **Filer:** | Ji Eun Kim/Kim Perry |
| **Filer Authorized By:** | Ji Eun Kim |
| **Attorney Docket Number:** | 3338.0330001/ELE/C-K |
| **Receipt Date:** | 28-APR-2016 |
| **Filing Date:** | |
| **Time Stamp:** | 21:08:56 |
| **Application Type:** | Utility under 35 USC 111(a) |

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| Application Data Sheet | 4 | 10 |
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| 3 | Fee Worksheet (SB06) | fee-info.pdf | 35054<br>4bab73d7d64312c56d75629b98b3f1d3653e845b | no | 2 |

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