(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
13 August 2015 (13.08.2015)

WIPO | PCT

(10) International Publication Number
WO 2015/119841 A1

(51) International Patent Classification:
A61P 37/00 (2006.01)

(21) International Application Number:
PCT/US2015/013642

(22) International Filing Date:
30 January 2015 (30.01.2015)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/936,234 5 February 2014 (05.02.2014) US

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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, 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, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, 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, SA, 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, ST, 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,

[Continued on next page]

(54) Title: ROLE OF IL-23 AND PD-1 IN AUTOREACTIVE IMMUNE RESPONSE

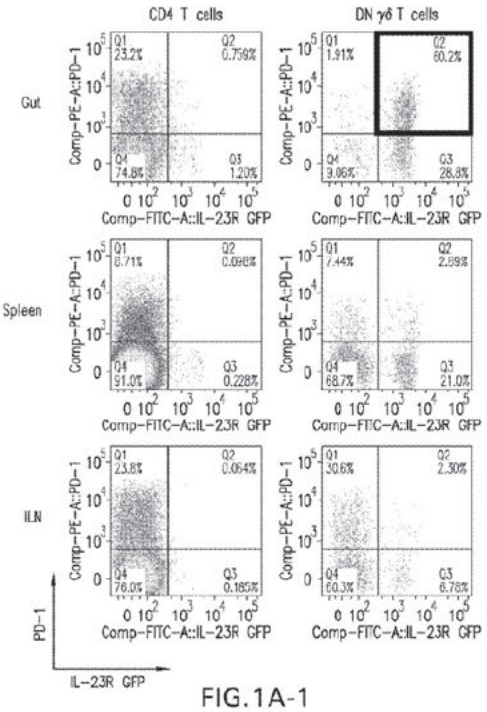


FIG.1A-1

(57) Abstract: The present invention provides methods of screening subjects for the presence of IL-23R+/PD-1$^+$ gamma-delta T-cells as a marker of autoreactive immune disorders, use of such identification in directing treatment, and related methods of treatment. The invention also provides use of such IL-23R+/PD-1$^+$ gamma-delta T-cells in screening for agents useful in treating autoreactive immune disorders.

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SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

Published:

— *with international search report (Art. 21(3))*

— *with sequence listing part of description (Rule 5.2(a))*

ROLE OF IL-23 AND PD-1 IN AUTOREACTIVE IMMUNE RESPONSE

FIELD OF THE INVENTION

The present invention relates generally to methods of treating and/or preventing diseases caused by autoreactive immune response mediated by cells of the innate immune system, and identifying patients likely to respond to specific methods of treatment.

BACKGROUND OF THE INVENTION

Autoimmune diseases result from misdirected immune responses against self-antigens, and can affect a variety of tissues. Interleukin 23 (IL-23) is a proinflammatory cytokine implicated in the pathology of various autoimmune diseases. Croxford *et al.* (2012) *Eur. J. Immunol.* 42:2263. Inhibitors of IL-23, such as the anti-IL-23p40 antibody ustekinumab, are approved for use in treating moderate to severe plaque psoriasis and active psoriatic arthritis. Other IL-23 antagonist antibodies, such as ustekinumab, briakinumab, tildrakizumab and guselkumab are in clinical trials for the treatment of various other disorders, such as ankylosing spondylitis and Crohn's disease, acute graft-versus-host disease, severe and chronic atopic dermatitis, sarcoidosis, rheumatoid arthritis, and palmoplantar pustulosis.

Although the response rates to anti-IL-23 therapy can be high for certain indications, such as psoriasis, the need exists for improved methods of treatment of IL-23 mediated autoreactive immune disorders for those patients that do not respond, or do not respond well, to treatment with IL-23 antagonists, and for treatment of disorders for which IL-23 antagonism alone fails to provide adequate relief.

SUMMARY OF THE INVENTION

In one aspect, the present invention provides methods of treating IL-23-mediated autoreactive immune disorders by selectively ablating IL-23R$^+$/PD-1$^+$ gamma delta (γδ) T cells, for example using one or more reagents, such as bi- or multi-specific binding molecules, *e.g.* antibodies or antigen binding fragments thereof, that bind to IL-23R and also bind to PD-1. In other embodiments, these methods of treatment are modified to target IL-1R$^+$/PD-1$^+$ gamma delta T cells, rather than IL-23R$^+$/PD-1$^+$ gamma delta T cells, and these cells are selectively ablated using one or more reagents, such as bi- or multi-specific binding molecules, *e.g.* antibodies or antigen binding fragments thereof, that bind to IL-1R and also bind to PD-1. In various embodiments, the binding molecule(s) is/are antibodies with

constant regions capable of eliciting ADCC or CDC, or antibody drug conjugates comprising a cytotoxic agent.

In another aspect, the present invention provides methods for efficiently treating and/or preventing IL-23-mediated autoreactive immune disorders by administration of agonists of the immunomodulatory receptor PD-1, comprising selectively administering the PD-1 agonist to patients demonstrated to have IL-23R$^+$/PD-1$^+$ gamma delta T cells, or IL-1R$^+$/PD-1$^+$ gamma delta T cells, in the affected tissue. The affected tissue will, of course, depend on the autoreactive immune disorder being treated.

In various embodiments involving treatment, the agonist of PD-1 comprises PD-L1, a PD-L1 fusion protein, such as an Ig fusion protein, or an agonist anti-PD-1 antibody or antigen binding fragment thereof. In further embodiments, the agonist of PD-1 comprises a small molecule agonist or peptide agonist.

In some embodiments involving treatment, the PD-1 agonist is administered in combination with an IL-23 antagonist. In various such embodiments, the IL-23 antagonist is an antagonist anti-IL-23 antibody that binds to the p40 or p19 subunit of IL-23, including but not limited to tildrakizumab, guselkumab, ustekinumab or briakinumab.In yet another aspect, the present invention provides methods of selecting subjects suffering from IL-23-mediated autoreactive immune disorders who are likely to respond to treatment with agonists of the immunomodulatory receptor PD-1 by determining the presence or level of IL-23R$^+$/PD-1$^+$ gamma delta T cells or IL-1R$^+$/PD-1$^+$ gamma delta T cells in affected tissue, *e.g.* as determined by biopsy.

In still another aspect, the present invention provides methods of screening for agents likely to be useful in treating IL-23-mediated an autoreactive immune disorder, such methods involving exposure of IL-23R$^+$/PD-1$^+$ gamma delta T cells to one or more candidate therapeutic agents and comparing the cytokine profile of the cells to the cytokine profile of control IL-23R$^+$/PD-1$^+$ gamma delta T cells that were not exposed to the one or more candidate therapeutic agent(s). In one embodiment, a candidate therapeutic agent is identified as a potential drug when it significantly reduces effector cytokine (*e.g.* IL-17A, IL-17F or IL-22) expression or secretion in activated IL-23R$^+$/PD-1$^+$ gamma delta T cells, where said activation is achieved by stimulation of the T cell receptor, treatment with IL-23 or IL-1, use in a mixed lymphocyte reaction, or in activated IL-23R$^+$/PD-1$^+$ gamma delta T cells obtained directly (*ex vivo*) from a subject, compared to control activated IL-23R$^+$/PD-1$^+$ gamma delta T cells not treated with drug.

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In another embodiment, activated IL-1R$^+$/PD-1$^+$ gamma delta T cells are used in place of activated IL-23R$^+$/PD-1$^+$ gamma delta T cells in the methods of the previous paragraph.

In various embodiments, the presence, absence, level or proportion of IL-23R$^+$/PD-1$^+$ or IL-1R$^+$/PD-1$^+$ gamma delta T cells is measured in a sample of affected tissue obtained from a subject. The "proportion" of IL-23R$^+$/PD-1$^+$ or IL-1R$^+$/PD-1$^+$ gamma delta T cells is the ratio of such cells to total γδ T cells in the sample, for example as described in Example 2. In various embodiments, an increase in the proportion of IL-23R$^+$/PD-1$^+$ or IL-1R$^+$/PD-1$^+$ gamma delta T cells of 1.5-, 2.0-, 3-, 5-, 10-fold (or more) the level in corresponding tissue from non-affected subject, or from corresponding non-affected tissue, indicates that the subject from which the sample was taken is a suitable candidate for treatment with an PD-1 agonist, such as an agonist PD-1 antibody.

In various embodiments of the methods of patient selection, treatment and drug screening, the IL-23-mediated autoreactive immune disorder is psoriasis, psoriatic arthritis, ankylosing spondylitis, uveitis,IBD or chronic obstructive pulmonary disease (COPD). In other embodiments, the IL-23-mediated disorder is rheumatoid arthritis, multiple sclerosis, or cancer or a chronic bacterial or fungal infection.

In one embodiment, the autoreactive immune disorder is psoriasis, and the tissue sample in which the proportion of IL-23R$^+$/PD-1$^+$ or IL-1R$^+$/PD-1$^+$ gamma delta T cells is measured is skin, obtained, *e.g.,* by punch biopsy. In another embodiment, the autoreactive immune disorder is inflammatory bowel disease, and the tissue sample in which the proportion of IL-23R$^+$/PD-1$^+$ or IL-1R$^+$/PD-1$^+$ gamma delta T cells is measured is intestine, obtained, *e.g.,* by resection. In yet another embodiment, the autoreactive immune disorder is an inflammatory disorder of the lung, such a chronic obstructive pulmonary disease (COPD), and the tissue sample in which the proportion of IL-23R$^+$/PD-1$^+$ or IL-1R$^+$/PD-1$^+$ gamma delta T cells is measured is lung, obtained, *e.g.,* by bronchoalveolar lavage (BAL) or biopsy.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1. shows

FIGs. 1A-1, 1A-2 and 1B show cell sorting data from various murine tissues illustrating the prevalence of PD-1$^+$ / IL-23R$^+$ double negative (CD4$^-$ / CD8$^-$) (DN) gamma-delta (γδ) T cells. Data are provided for gut, spleen, inguinal lymph node (ILN), hind joint, ear skin and visceral fat (FIGs. 1A-1 and 1A-2), and lung (FIG. 1B). *See* Example 2. PD-1$^+$ / IL-23R$^+$ double positive cells represent a significant proportion of γδ T cells in gut (60%), joint (40%), visceral fat (60%), and lung (26%). Cells were gated slightly differently for

FIG. 1B than for FIGs. 1A-1 and 1A-2, so the percentages of cells in each quadrant cannot be quantitatively compared between FIGs 1A-1 and 1A-2, and 1B.

FIG. 2 shows flow cytometry data from $CD4^+$ T cells and DN gamma-delta ($\gamma\delta$) T cells from a population of total stromal vascular cells isolated from murine adipose tissue. These cells were stimulated with IL-23 *in vitro*, and assayed for PD-1 and IL-17 expression via flow cytometry. *See* Example 3. $PD\text{-}1^+$ / $IL\text{-}17^+$ double positive cells represent a significant proportion of IL-23 treated DN gamma-delta ($\gamma\delta$) T cells (76%).

FIGS. 3A and 3B show IL-17A and IL-17F expression, respectively, in total stromal vascular cells isolated from murine adipose tissue, as a function of *in vitro* exposure to PD-L1-Fc, IL-23, or both. *See* Example 4. PD-L1-Fc treatment significantly reduces IL-23-induced IL-17A and IL-17F expression.

DETAILED DESCRIPTION

All references cited herein are incorporated by reference to the same extent as if each individual publication, patent application, or patent, was specifically and individually indicated to be incorporated by reference. Citation of the references herein is not intended as an admission that any of the foregoing is pertinent prior art, nor does it constitute any admission as to the contents or date of these publications or documents.

As used herein, including the appended claims, the singular forms of words such as "a," "an," and "the," include their corresponding plural references unless the context clearly dictates otherwise. Unless otherwise indicated, or clear from the context, references to proteins are intended to be references to the human form of the protein, rather than animal forms. Table 4 below provides a listing of sequence identifiers used in this application.

As used herein with reference to selection of subjects or patients based on the presence of absence of $IL\text{-}23R^+/PD\text{-}1^+$ gamma delta T cells, "if" is intended to mean "if and only if." For example, when a patient is treated with an IL-23 antagonist "if" he or she exhibits $IL\text{-}23R^+/PD\text{-}1^+$ gamma delta T cells, it is intended that the patient not be treated if he or she does not exhibit $IL\text{-}23R^+/PD\text{-}1^+$ gamma delta T cells. In some instances the complete phrase "if, and only if," is included to make this meaning clear, but otherwise is implicit when "if" is used in this context.

As used herein with reference to therapeutic treatment, the phrase "in combination with" means, unless otherwise indicated, that two or more therapeutic agents are administered as a mixture, simultaneously or substantially simultaneously, or sequentially to a subject.

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The agents are said to be administered "in combination with" each other if the drugs are administered sufficiently close in time that both agents can exert biological effects on the subject over at least some time interval. In contrast, two agents would not be considered to be administered "in combination with" each other if one drug was only administered at a time when a previously administered drug was no longer present at sufficient levels to exert biological activity. Subsets of "in combination with" include separate administration substantially simultaneously, administration of mixture of two or more agents, or administration of a single agent comprising two or more distinct biologically active moieties (*e.g.* a bispecific antibody).

"PD-1," unless otherwise indicated, refers to "Programmed Death-1" or "Programmed Cell Death-1." The human gene is PDCD1, also known as PD1, CD279, and SLEB2. Human PD-1 is described in the NCBI Protein Reference Sequence Accession Nos. NM_005018.2 (mRNA) and NP_005009.2 (protein precursor, including 20 amino acid signal sequence), and at Gene ID No. 5133. "PD-1 agonist" refers to any agent that increases signaling through PD-1, *i.e.* induces the same or similar effects to the binding of PD-L1 to PD-1.

"PD-L1," unless otherwise indicated, refers to "Programmed Cell Death-1 Ligand-1." The human gene is CD274, also known as B7H1. Human PD-L1 is described at NCBI Reference Sequence Accession Nos. NM_014143.3 and NM_001267706.1 (mRNA for isoforms a and b precursors, respectively) and NP_054862.1 and NP_001254635.1 (protein for isoforms a and b precursors, respectively, including 18 amino acid signal sequences), and at Gene ID No. 29126. For the purposes of the present application, "PD-L1 agonist" refers to any agent that increases signaling through PD-1, *i.e.* induces the same or similar effects to the binding of PD-L1 to PD-1 in a target cell.

"PD-L2," unless otherwise indicated, refers to "Programmed Cell Death-1 Ligand-2." The human gene is PDCD1LG2, also known as CD273 and B7DC. Human PD-L2 is described at NCBI Reference Sequence Accession Nos. NM_025239.3 (mRNA) and NP_079515.2 (protein, including 19 amino acid signal sequence), and at Gene ID No. 80380. For the purposes of the present application, "PD-L2 agonist" refers to any agent that increases signaling through PD-1, *i.e.* induces the same or similar effects to the binding of PD-L2 to PD-1 in a target cell.

"IL-23p19," unless otherwise indicated, refers to the p19 subunit of human interleukin 23. Human IL-23p19, also known as IL-B30 and SGRF, is described at NCBI Reference

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Sequence Accession Nos. NM_016584.2 (mRNA) and NP_057668.1 (polypeptide), and at Gene ID No. 51561.

"IL-23R," unless otherwise indicated, refers to the human interleukin 23 receptor. Human IL-23R is described at NCBI Reference Sequence Accession Nos. NM_0144701.2 (mRNA) and NP_653302.2 (polypeptide), and at Gene ID No. 149233.

"IL-1R," unless otherwise indicated, refers to the human interleukin 1 receptor. The human gene is IL1R1, also known as IL1R, IL1RA, IL-1Rα, CD121A, D2S1473 and P80. Human IL-1R is described at NCBI Reference Sequence Accession Nos. NM_000877.2 (mRNA) and NP_000868.1 (polypeptide), and at Gene ID No. 3554.

"IL-23 antagonist" refers to any agent that inhibits signaling by IL-23. Because IL-23 signals via the IL-23 receptor complex, antagonists of the IL-23 receptor complex are also IL-23 antagonists. A "non-specific IL-23 antagonist" is an agent that blocks the activity of both IL-23 and IL-12. An "IL-23-specific antagonist" is an agent that selectively blocks the activity of IL-23 but does not block the activity of IL-12. Unless otherwise indicated, or clear from the context, "IL-23" and "IL-12" as used herein refer to human IL-23 and human IL-12. The antibodies disclosed herein bind to human IL-23."Anti-IL-23p19 antibody" includes, but is not limited to, the anti-human IL-23p19 antibodies disclosed in Int'l Pat. App. Pubs. WO 2007/027714, WO 2008/103432, and WO 2008/103473. In some embodiments the IL-23 antagonist is an anti-IL-23 antibody, or antigen binding fragment thereof, comprising the CDRs, variable domains, or heavy and light chains, of antibodies 13B8-a, 13B8-b, 13B8-c, ustekinumab, briakinumab, or guselkumab. Exemplary sequences are provided in the accompanying Sequence Listing.In various embodiments the anti-IL-23 antibodies or fragments thereof of the present invention are polyclonal, monoclonal, chimeric, humanized or fully human antibodies or fragments thereof. The present invention also contemplates that the antigen binding fragment is an antibody fragment selected from the group consisting of, e.g., Fab, Fab', Fab'-SH, Fv, scFv, $F(ab')_2$, and a diabody.

"Ablate," "ablating" and "ablation," as used herein, refer to the destruction of certain target cells, *e.g.* $IL\text{-}23R^+/PD\text{-}1^+$ γδ T cells. Such destruction can be accomplished by any suitable means, including but not limited to delivery of a cytoxotic agent, ADCC, CDC, or induction of apoptosis. Such destruction need not be complete, since mere reduction in the levels of autoreactive cells may be sufficient to provide therapeutic benefit. In preferred embodiments, ablation refers to selective destruction of target cells, rather than the broad, indiscriminant destruction associated with conventional chemotherapy.

"Proliferative activity" encompasses any activity that promotes, that is necessary for, or that is specifically associated with, *e.g.*, normal cell division, as well as cancer, tumors, dysplasia, cell transformation, metastasis, and angiogenesis.

"Administration" and "treatment," as it applies to an animal, human, experimental subject, cell, tissue, organ, or biological fluid, refers to contact of an exogenous pharmaceutical, therapeutic, diagnostic agent, or composition to the animal, human, subject, cell, tissue, organ, or biological fluid. "Administration" and "treatment" can refer, e.g., to therapeutic, pharmacokinetic, diagnostic, research, and experimental methods. Treatment of a cell encompasses contact of a reagent to the cell, as well as contact of a reagent to a fluid, where the fluid is in contact with the cell. "Administration" and "treatment" also means *in vitro* and *ex vivo* treatments, e.g., of a cell, by a reagent, diagnostic, binding composition, or by another cell. "Treatment," as it applies to a human, veterinary, or research subject, refers to therapeutic treatment, prophylactic or preventative measures, to research and diagnostic applications. "Treatment" as it applies to a human, veterinary, or research subject, or cell, tissue, or organ, encompasses contact of an agent with animal subject, a cell, tissue, physiological compartment, or physiological fluid. "Treatment of a cell" also encompasses situations where the agent contacts IL-23 receptor (IL-23R/IL-12Rβ1 heterodimer), e.g., in the fluid phase or colloidal phase, but also situations where the agonist or antagonist does not contact the cell or the receptor.

As used herein, the term "antibody" refers to any form of antibody that exhibits the desired biological activity. Thus, it is used in the broadest sense and specifically covers monoclonal antibodies (including full length monoclonal antibodies), polyclonal antibodies, multispecific antibodies (*e.g.*, bispecific antibodies), chimeric antibodies, humanized antibodies, fully human antibodies, etc. so long as they exhibit the desired biological activity.

As used herein, the terms "PD-1 binding fragment," "binding fragment thereof," "antigen binding fragment thereof" or "fragment" (when used with reference to an antibody) encompass a fragment or a derivative of an antibody that still substantially retains its biological activity of binding to and/or agonizing PD-1. Therefore, the term "antibody fragment" or "PD-1 binding fragment" refers to 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, e.g., sc-Fv; and multispecific antibodies formed from antibody fragments. Typically, a binding fragment or derivative retains at least 10% of its IL-23 inhibitory PD-1 binding and/or agonizing activity. Preferably, a binding fragment or derivative retains at least 25%,

50%, 60%, 70%, 80%, 90%, 95%, 99% or 100% (or more) of its IL-23 inhibitory PD-1 binding and/or agonizing activity, although any binding fragment with sufficient affinity to exert the desired biological effect will be useful. It is also intended that an IL-23a PD-1 binding fragment can include variants having conservative amino acid substitutions that do not substantially alter its biologic activity.

As used herein, the terms "IL-23 binding fragment," "binding fragment thereof," "antigen binding fragment thereof" or "fragment" (when used with reference to an antibody) encompass a fragment or a derivative of an antibody that still substantially retains its biological activity of inhibiting IL-23p19 activity. Therefore, the term "antibody fragment" or "IL-23 binding fragment" refers to 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, e.g., sc-Fv; and multispecific antibodies formed from antibody fragments. Typically, a binding fragment or derivative retains at least 10% of its IL-23 inhibitory activity. Preferably, a binding fragment or derivative retains at least 25%, 50%, 60%, 70%, 80%, 90%, 95%, 99% or 100% (or more) of its IL-23 inhibitory activity, although any binding fragment with sufficient affinity to exert the desired biological effect will be useful. It is also intended that an IL-23 binding fragment can include variants having conservative amino acid substitutions that do not substantially alter its biologic activity.

The term "monoclonal antibody," as used herein, refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic epitope. In contrast, conventional (polyclonal) antibody preparations typically include a multitude of antibodies directed against (or specific for) different epitopes. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler *et al.* (1975) *Nature* 256: 495, or may be made by recombinant DNA methods (*see, e.g.*, U.S. Pat. No. 4,816,567). The "monoclonal antibodies" may also be isolated from phage antibody libraries using the techniques described in Clackson *et al.* (1991) *Nature* 352: 624-628 and Marks *et al.* (1991) *J. Mol. Biol.* 222: 581-597, for example.

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Antibodies, and antigen binding fragments thereof, may be described herein as comprising a light and/or heavy chain variable domain, or as comprising a light and/or heavy chain. As used herein, and consistent with their plain meanings, such descriptions encompass embodiments in which the antibody, or antigen binding fragment thereof, includes more than one light and/or heavy chain variable domain and/or chain, such as two light and/or heavy chain variable domains and/or chains.

The monoclonal antibodies herein specifically include "chimeric" antibodies (immunoglobulins) in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity. U.S. Pat. No. 4,816,567; Morrison *et al.* (1984) *Proc. Natl. Acad. Sci. USA* 81: 6851-6855.

A "domain antibody" is an immunologically functional immunoglobulin fragment containing only the variable region of a heavy chain or the variable region of a light chain. In some instances, two or more V_H regions are covalently joined with a peptide linker to create a bivalent domain antibody. The two V_H regions of a bivalent domain antibody may target the same or different antigens.

A "bivalent antibody" comprises two antigen binding sites. In some instances, the two binding sites have the same antigen specificities. However, bivalent antibodies may be bispecific (see below).

As used herein, the term "single-chain Fv" or "scFv" antibody refers to antibody fragments comprising the V_H and V_L domains of antibody, wherein these domains are present in a single polypeptide chain. Generally, the Fv polypeptide further comprises a polypeptide linker between the V_H and V_L domains which enables the sFv to form the desired structure for antigen binding. For a review of sFv, see Pluckthun (1994) THE PHARMACOLOGY OF MONOCLONAL ANTIBODIES, vol. 113, Rosenburg and Moore eds. Springer-Verlag, New York, pp. 269-315.

The monoclonal antibodies herein also include camelized single domain antibodies. *See, e.g.,* Muyldermans *et al.* (2001) *Trends Biochem. Sci.* 26:230; Reichmann *et al.* (1999) *J. Immunol. Methods* 231:25; WO 94/04678; WO 94/25591; U.S. Pat. No. 6,005,079). In one embodiment, the present invention provides single domain antibodies comprising two V_H domains with modifications such that single domain antibodies are formed.

As used herein, the term "diabodies" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy chain variable domain (V_H) connected to a light chain variable domain (V_L) in the same polypeptide chain (V_H-V_L or V_L-V_H). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies are described more fully in, *e.g.*, EP 404,097; WO 93/11161; and Holliger *et al.* (1993) *Proc. Natl. Acad. Sci. USA* 90: 6444-6448. For a review of engineered antibody variants generally see Holliger and Hudson (2005) *Nat. Biotechnol.* 23:1126-1136.

As used herein, the term "humanized antibody" refers to forms of antibodies that contain sequences from non-human (*e.g.*, murine) antibodies as well as human antibodies. Such antibodies contain minimal sequence derived from non-human immunoglobulin. 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 loops correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions 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. The prefix "hum", "hu" or "h" is added to antibody clone designations when necessary to distinguish humanized antibodies (e.g. hum13B8) from parental rodent antibodies (e.g. mouse 13B8, or m13B8). The humanized forms of rodent antibodies will generally comprise the same CDR sequences of the parental rodent antibodies, although certain amino acid substitutions may be included to increase affinity, increase stability of the humanized antibody, or for other reasons.

The antibodies of the present invention also include antibodies with modified (or blocked) Fc regions to provide altered effector functions. See, e.g., U.S. Pat. No. 5,624,821; WO 2003/086310; WO 2005/120571; WO 2006/0057702; Presta (2006) *Adv. Drug Delivery Rev.* 58:640-656. Such modification can be used to enhance or suppress various reactions of the immune system, with possible beneficial effects in diagnosis and therapy. Alterations of the Fc region include amino acid changes (substitutions, deletions and insertions), glycosylation or deglycosylation, and adding multiple Fc. Changes to the Fc can also alter the half-life of antibodies in therapeutic antibodies, and a longer half-life would result in less frequent dosing, with the concomitant increased convenience and decreased use of material. *See* Presta (2005) *J. Allergy Clin. Immunol.*116:731 at 734-35.

The term "fully human antibody" refers to an antibody that comprises human immunoglobulin protein sequences only. A fully human antibody may contain murine carbohydrate chains if produced in a mouse, in a mouse cell, or in a hybridoma derived from a mouse cell. Similarly, "mouse antibody" refers to an antibody which comprises mouse immunoglobulin sequences only. A fully human antibody may be generated in a human being, in a transgenic animal having human immunoglobulin germline sequences, by phage display or other molecular biological methods.

As used herein, the term "hypervariable region" refers to the amino acid residues of an antibody that are responsible for antigen-binding. The hypervariable region comprises amino acid residues from a "complementarity determining region" or "CDR" (*e.g.* residues 24-34 (CDRL1), 50-56 (CDRL2) and 89-97 (CDRL3) in the light chain variable domain and residues 31-35 (CDRH1), 50-65 (CDRH2) and 95-102 (CDRH3) in the heavy chain variable domain (Kabat *et al.* (1991) *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md.) 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 and Lesk (1987) *J. Mol. Biol.* 196: 901-917). As used herein, the term "framework" or "FR" residues refers to those variable domain residues other than the hypervariable region residues defined herein as CDR residues. The residue numbering above relates to the Kabat numbering system and does not necessarily correspond in detail to the sequence numbering in the accompanying Sequence Listing.

"Binding compound" refers to a molecule, small molecule, macromolecule, polypeptide, antibody or fragment or analogue thereof, or soluble receptor, capable of binding to a target. "Binding compound" also may refer to a complex of molecules, e.g., a non-covalent complex, to an ionized molecule, and to a covalently or non-covalently modified molecule, e.g., modified by phosphorylation, acylation, cross-linking, cyclization, or limited cleavage, which is capable of binding to a target. When used with reference to antibodies, the term "binding compound" refers to both antibodies and antigen binding fragments thereof. "Binding" refers to an association of the binding composition with a target where the association results in reduction in the normal Brownian motion of the binding composition, in cases where the binding composition can be dissolved or suspended in solution. "Binding composition" refers to a molecule, e.g. a binding compound, in combination with a stabilizer, excipient, salt, buffer, solvent, or additive, capable of binding to a target.

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"Conservatively modified variants" or "conservative substitution" refers to substitutions of amino acids that may be made, as known by those of skill in the art, with little or no impact on the biological activity of the resulting molecule, even in essential regions of the polypeptide. Such exemplary substitutions are preferably made in accordance with those set forth in Table 1 as follows:

Table 1
Exemplary Conservative Amino Acid Substitutions

Original residue	Conservative substitution
Ala (A)	Gly; Ser
Arg (R)	Lys, His
Asn (N)	Gln; His
Asp (D)	Glu; Asn
Cys (C)	Ser; Ala
Gln (Q)	Asn
Glu (E)	Asp; Gln
Gly (G)	Ala
His (H)	Asn; Gln
Ile (I)	Leu; Val
Leu (L)	Ile; Val
Lys (K)	Arg; His
Met (M)	Leu; Ile; Tyr
Phe (F)	Tyr; Met; Leu
Pro (P)	Ala
Ser (S)	Thr
Thr (T)	Ser
Trp (W)	Tyr; Phe
Tyr (Y)	Trp; Phe
Val (V)	Ile; Leu

In addition, those of skill in this art recognize that, in general, single amino acid substitutions in non-essential regions of a polypeptide do not substantially alter biological activity. *See, e.g.*, Watson *et al.* (1987) *Molecular Biology of the Gene*, The Benjamin/Cummings Pub. Co., p. 224 (4th Edition).

The phrase "consists essentially of," or variations such as "consist essentially of" or "consisting essentially of," as used throughout the specification and claims, indicate the inclusion of any recited elements or group of elements, and the optional inclusion of other

elements, of similar or different nature than the recited elements, that do not materially change the basic or novel properties of the specified dosage regimen, method, or composition. As a non-limiting example, a binding compound that consists essentially of a recited amino acid sequence may also include one or more amino acids, including substitutions of one or more amino acid residues, that do not materially affect the properties of the binding compound.

"Effective amount" encompasses an amount sufficient to ameliorate or prevent a symptom or sign of the medical condition. Effective amount also means an amount sufficient to allow or facilitate diagnosis. An effective amount for a particular patient or veterinary subject may vary depending on factors such as the condition being treated, the overall health of the patient, the method route and dose of administration and the severity of side affects. *See, e.g.*, U.S. Pat. No. 5,888,530. An effective amount can be the maximal dose or dosing protocol that avoids significant side effects or toxic effects. The effect will result in an improvement of a diagnostic measure or parameter by at least 5%, usually by at least 10%, more usually at least 20%, most usually at least 30%, preferably at least 40%, more preferably at least 50%, most preferably at least 60%, ideally at least 70%, more ideally at least 80%, and most ideally at least 90%, where 100% is defined as the diagnostic parameter shown by a normal subject. *See, e.g.*, Maynard *et al.* (1996) *A Handbook of SOPs for Good Clinical Practice*, Interpharm Press, Boca Raton, FL; Dent (2001) *Good Laboratory and Good Clinical Practice*, Urch Publ., London, UK.

"Specifically" or "selectively" binds, when referring to a ligand/receptor, antibody/antigen, or other binding pair, indicates a binding reaction which is determinative of the presence of the protein in a heterogeneous population of proteins and other biologics. Thus, under designated conditions, a specified ligand binds to a particular receptor and does not bind to a significant degree to other proteins present in the sample. As used herein, an antibody is said to bind specifically to a polypeptide *comprising* a given sequence (e.g. IL-23p19) if it binds to polypeptides comprising the sequence of IL-23p19 but does not bind to proteins lacking the sequence of IL-23p19. For example, an antibody that specifically binds to a polypeptide comprising IL-23p19 may bind to a FLAG®-tagged form of IL-23p19 but will not bind to other FLAG®-tagged proteins.

The antibody, or binding composition derived from the antigen-binding site of an antibody, of the contemplated method binds to its antigen with an affinity that is at least two fold greater, preferably at least ten times greater, more preferably at least 20-times greater, and most preferably at least 100-times greater than the affinity with unrelated antigens. In a

preferred embodiment the antibody will have an affinity that is greater than about 10^9 liters/mol, as determined, e.g., by Scatchard analysis. Munsen *et al.* (1980) *Analyt. Biochem.* 107:220-239.

I. The Role of PD-1 in IL-23 Mediated Response

The present invention is based, in part, on the discovery that murine adipose tissue resident IL-23R-expressing gamma delta T cells, which display IL-23-induced autoreactivity *in vitro*, also express PD-1. Gamma delta T cells are components of the innate immune system. Born *et al.* (2006) *Curr. Op. Immunol.* 18:31. Subsequent *in vitro* experiments, as disclosed herein, demonstrate that agonism of PD-1 is effective in reducing or eliminating IL-23-responsiveness in these cells, suggesting that agonism of PD-1 in these cells will be a effective method of treating diseases caused by IL-23 signaling in gamma delta T cells. *See* FIGS. 3A and 3B and Example 4.

Characterization of this unique pathogenic population of IL-23R$^+$ / PD-1$^+$ gamma delta T cells also enables selective ablation of this cell population, for example using a bi- or multi-specific binding agent, such as an antibody or fragment thereof, and that this ablation may be effective in treating IL-23 mediated immune disorders. Such targeting molecules will bind to PD-1 and either IL-23R or IL-1R. Multiple molecules can be used to ablate the cells, provided that the resulting ablation is reasonably limited to the desired cell population. Targeting molecules that selectively ablate the IL-23R$^+$ / PD-1$^+$ gamma delta T cells of the present invention include antibodies with constant regions capable of eliciting antigen-dependent cellular cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC), and also antibodies or antigen binding fragments thereof coupled to cytotoxic agents, and in antibody-drug conjugates. A PD-1 binding molecule for use in selectively ablating IL-23R$^+$ / PD-1$^+$ gamma delta T cells according to the present invention need not have agonist activity, and an IL-23R binding molecule need not have antagonist activity.

In light of the existence of this population of pathogenic IL-23R$^+$ / PD-1$^+$ gamma delta T cells, it is now possible to detect IL-23-mediated disease states, such as psoriasis and spondyloarthropathies, by screening for tissue-resident gamma delta T cells expressing IL-23R and PD-1. For example, in the case of psoriasis, the affected tissue would be skin, and samples may be obtained, *e.g.*, by punch biopsy. In the case of IBD, the affected tissue would be intestine, and samples may be obtained, *e.g.*, by resection. In the case of COPD, the affected tissue would be lung, and samples may be obtained, *e.g.*, by BAL or biopsy.

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Expression of IL-23R and PD-1 can be measured at the cellular, molecular (protein) or transcriptional levels, or any combination thereof.

In light of the existence of this population of pathogenic IL-23R$^+$ / PD-1$^+$ gamma delta T cells, it is also possible to screen for agents (*e.g.* drug candidates) capable of blocking IL-23-mediated autoreactive immune disorder. Populations of IL-23R$^+$ / PD-1$^+$ gamma delta T cells can be obtained (*e.g.* by cell sorting) and exposed to one or more drug candidates. Effector cytokine production is then measured, and compared to the levels of expression in the absence of the drug candidates. Analogous methods to screen for PD-1 agonists are disclosed at U.S. Pat. App. Pub. No. 2011/0008777 (to Univ. Montreal) and WO 2011/082400 (to Harvard), but for the purposes of the present invention the cells used in the assay are the IL-23R$^+$ / PD-1$^+$ gamma delta T cells of the invention, and the "read-out" of the assay is a reduction in pro-inflammatory effector cytokines of the Th-17 response, such as IL-17 and/or IL-22. Previous methods of screening for agents capable of blocking IL-23's activity on innate immune cells included isolation of total immune cells and assaying cytokine expression in cell supernatants as a function of exposure to IL-23. The method disclosed herein has the advantage of focusing on the specific pathogenic subset of immune cells involved in disease.

II. PD-1

Programmed Death 1 (PD-1), a member of the CD28 co-stimulatory gene family, is moderately expressed on naive T, B and NKT cells and up-regulated by T/B cell receptor signaling on lymphocytes, monocytes and myeloid cells. Sharpe *et al.* (2007) *Nat. Immunol.* 8:239. PD-1 has two known ligands with distinct expression profiles, PD-L1 (B7-H1) and PD-L2 (B7-DC). PD-L2 expression is relatively restricted and is found on activated dendritic cells, macrophages and monocytes and on vascular endothelial cells. *Id.*, and Greenwald *et al.* (2005) *Ann. Rev. Immunol.* 23:515; Okazaki & Honjo (2007) *Int. Immunol.* 19:813. In contrast, PD-L1 is expressed more broadly including on naive lymphocytes and its expression is induced on activated B and T cells, monocytes and dendritic cells. Furthermore, by mRNA, it is expressed by non-lymphoid tissues including vascular endothelial cells, epithelial cells and muscle cells.

PD-1 is recognized as an important player in immune regulation and the maintenance of peripheral tolerance. In the mouse, this was shown to require PD-L1 expression on peripheral tissues and ligation of PD-1 on potentially autoreactive T cells to negatively modulate T cell activation involving an ITIM sequence in the PD-1 cytoplasmic domain.

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Sharpe *et al.* (2007) *Nat. Immunol.* 8:239; Chemnitz *et al.* (2004) *J. Immunol.*:173:945. A polymorphism in the human PD-1 gene was also identified as a genetic modifier of the progression of the multiple sclerosis (MS). Kroner *et al.* (2005) *Ann. Neurol.* 58:50. It has also been reported that PD-1 inhibits inflammatory helper T-cell development through controlling innate immune response. Rui *et al.* (2013) *Proc. Nat'l Acad. Sci.* (USA) 110:16073.

III. Antagonism of PD-1

Although various embodiments of the present invention involve agonism of PD-1, rather than antagonism, experiments involving antagonism are helpful in understanding the biology of PD-1/PD-L1 signaling. In addition, PD-1 binding agents developed for use as PD-1 antagonists, although not preferred, may find use in bispecific targeting constructs of the present invention. Depending on the specific genetic background, $pdcd1^{-/-}$ (PD-1 deficient homozygous) mice spontaneously develop lupus-like phenomena or dilated cardiomyopathy. Nishimura *et al.* (1999) *Immunity* 11:141; Okazaki *et al.* (2003) *Nat. Med.* 9:1477. Furthermore, antibody-induced blockade of the PD-1 / PD-L1 pathway was demonstrated to accelerate the onset of autoimmune insulitis and diabetes in NOD mice. Ansari *et al.* (2003) *J Exp. Med.* 198:63.

Human cancers arising in various tissues were found to over-express PD-L1 or PD-L2. In large sample sets of ovarian, renal, colorectal, pancreatic, liver cancers and melanoma it was shown that PD-L1 expression correlated with poor prognosis and reduced overall survival irrespective of subsequent treatment. Dong *et al.* (2002) *Nat Med.* 8:793; Yang *et al.* (2008) *Invest Ophthalmol Vis Sci.* 49:2518; Ghebeh *et al.* (2006) *Neoplasia* 8:190; Hamanishi *et al.* (2007) *Proc.Nat'l Acad. Sci.*:104:3360; Thompson *et al.* (2006) *Cancer* 5:206; Nomi *et al.* (2007) *Clin. Cancer Res.*13:2151; Ohigashi *et al.* (2005) *Clin. Cancer Res.* 11:2947; Inman *et al.* (2007) *Cancer* 109:1499; Shimauchi *et al.* (2007) *Int. J. Cancer* 121:2585; Gao *et al.* (2009) *Clin. Cancer Res.* 15:971; Nakanishi (2007) *Cancer Immunol Immunother.* 56:1173; Hino *et al.* (2010) *Cancer* 116:1757. Similarly, PD-1 expression on tumor infiltrating lymphocytes was found to mark dysfunctional T cells in breast cancer (Ghebeh *et al.* (2006) *Neoplasia* 8:190) and melanoma (Ahmadzadeh *et al.* (2009) *Blood* 114:1537) and to correlate with poor prognosis in renal cancer (Thompson *et al.* (2007) *Clin. Cancer Res.* 13:1757). Using primary patient samples, it was shown that blockade of PD-1 or PD-L1 *in vitro* results in enhancement of human tumor-specific T cell activation and cytokine production (Blank *et al.* (2006) *Int. J. Cancer* 119:317). Consequently, in several murine

syngeneic tumor models, blockade of either PD-1 or PD-L1 significantly inhibited tumor growth or induced complete regression.

A PD-1 blocking mAb (h409A11) was discovered and developed for use to treat human cancer patients and chronic virus-infected patients. *See* WO 2008/156712 (to NV Organon). *See also* U.S. Pat. App. Pub. Nos. 2011/0195068 (to Amplimmune) and 2009/0217401 (to Medarex).

Antigen-specific T cell dysfunction or tolerance is exemplified by the accumulated loss of the potential to produce interleukin 2 (IL-2), tumor necrosis factor (TNF)-α, perforin, interferon (IFN) γ (Riley & June (2006) *Trends Immunol.* 28:48) and inability to mount a proliferative response to T cell receptor triggering. Sharpe *et al.* (2007) *Nat. Immunol.* 8:239. The PD-1 pathway controls antigen-specific T cell tolerance and was found to be exploited in viral infection and tumor development to control and evade effective T cell immunity.

In chronic infection with LCMV (mouse), HIV, HBV or HCV (human), antigen-specific T cells were found to express aberrantly high levels of PD-1 correlating with their state of anergy or dysfunction. Barber *et al.* (2006) *Nature* 439:682. Blocking the PD-1 – PD-L1 interaction *in vivo* (LCMV) or *in vitro* (HIV, HCV, HBV) was shown to revive anti-viral T cell activity. Trautmann *et al.* (2006) *Nat. Med.* 12:1198; Petrovas *et al.* (2006) *J Exp. Med.* 203:2281; Day *et al.* (2006) *Nature* 443:350. PD-1 blockade in recently simian immunodeficiency virus (SIV) infected macaques resulted in strong reduction of viral load and increased survival. Velu *et al.* (2009) *Nature* 458:206. Similarly, reduction in viral load was confirmed in second study using long-term SIV-infected rhesus macaques. Finnefrock *et al.* (2009) *J. Immunol.* 182:980.

Overall, the PD-1/PD-Ll pathway is a well-validated target for the development of antibody therapeutics for cancer treatment. Anti-PD-1 antibodies are also useful for treating chronic viral infection. Memory $CD8^+$T cells generated after an acute viral infection are highly functional and constitute an important component of protective immunity. In contrast, chronic infections are often characterized by varying degrees of functional impairment (exhaustion) of virus-specific T-cell responses, and this defect is a principal reason for the inability of the host to eliminate the persisting pathogen. Although functional effector T cells are initially generated during the early stages of infection, they gradually lose function during the course of a chronic infection. Barber *et al.* showed that mice infected with a laboratory strain of LCMV developed chronic infection resulting in high levels of virus in the blood and other tissues. Barber *et al.* (2006) *Nature* 439:682. These mice initially developed a robust T cell response, but eventually succumbed to the infection upon T cell exhaustion. The authors

found that the decline in number and function of the effector T cells in chronically infected mice could be reversed by injecting an antibody that blocked the interaction between PD-1 and PD-Ll.

PD-1 has also been shown to be highly expressed on T cells from HIV infected individuals and that receptor expression correlates with impaired T cell function and disease progression. Day *et al.* (2006) *Nature* 443:350; Trautmann *et al.* (2006) *Nat. Med.* 12:1198. In both studies, blockade of the PD-1 pathway using antibodies against the ligand PD-Ll significantly increased the expansion of HIV-specific, IFN-gamma producing cells *in vitro*.

Other studies also implicate the importance of the PD-1 pathway in controlling viral infection. PD-1 knockout mice exhibit better control of adenovirus infection than wild- type mice. Iwai *et al.*, *Exp. Med.* 198:39-50 (2003). Also, adoptive transfer of HBV- specific T cells into HBV transgenic animals initiated hepatitis. Isogawa M. *et al.*, *Immunity* 23:53-63 (2005). The disease state of these animals oscillates as a consequence of antigen recognition in the liver and PD-1 upregulation by liver cells.

Exemplary therapeutic antagonist antibodies specific for human PD-1 are disclosed in commonly-assigned U.S. Patent Application Publication No. US2010/0266617, and in International Patent Publication No. WO2008/156712, the disclosures of which are hereby incorporated by reference in their entireties. Although such antagonist antibodies will not find use in embodiments of the present invention requiring use of PD-1 agonists, PD-1 binding domains from such antagonist antibodies, although less preferred than non-antagonist anti-PD-1 antibodies, may find use in the bispecific targeting constructs of the present invention.

IV. PD-1 Agonists

In some embodiments, the present invention involves agonism of PD-1 as a means of suppressing IL-23 mediated autoreactive immune disorders. As such, it may be performed with any suitable PD-1 agonist.

PD-1 is a receptor expressed on activated T and B lymphocytes. PD-1 agonists induce or increase one or more PD-1 associated activities. In some embodiments, a PD-1 agonist inhibits one or more of T cell intracellular signaling, proliferation, and cytokine production. Natural ligands for PD-1 include PD-L1 and PD-L2. These PD-1 ligands may be employed as PD-1 agonists in the methods described herein. Other types of PD-1 agonists include PD-1 agonistic antibodies, small molecules, and aptamers which comprise RNA or DNA molecules that can be substituted for antibodies. In some embodiments, a PD-1 agonist

is a soluble form of a PD-1 ligand (e.g., soluble PD-L1, soluble PD-L2). Soluble forms of PD-1 ligands typically include the extracellular domain of the ligand, or a portion thereof sufficient to bind to, and agonize, PD-1. In some embodiments, a soluble portion of PD-L1 includes amino acids 19-238 or amino acids 19-239, or a portion within including about 100, 150, 170, 180, 190 amino acid residues, of SEQ ID NO:8. A soluble PD-L2 can include an analogous portion. In some embodiments, a PD-1 agonist is a soluble PD-1 ligand fused to a heterologous polypeptide (e.g., a heterologous polypeptide that increases the circulating half-life of the ligand, such as an Fc region of an immunoglobulin). In some embodiments, a PD-1 ligand is fused to an Fc portion of a human IgG1, for example as available from R&D Systems (Minneapolis, Minn., USA). PD-1 ligand-Fc fusions are referred to herein "PD-L1-Ig" and "PD-L2-Ig". PD-L1-Ig and PD-L2-Ig are described, e.g., in Freeman et al.(2000) J. Exp. Med. 192(7): 1027; Latchman et al. (2001) Nat Immunol. 2(3):261; Watson et al. (2006) Invest Ophthalmol Vis Sci. :3417; and Youngnak et al. (2003) Biochem Biophys. Res Comm. 307:672. *See also* U.S. Pat. App. Pub. No. 2012/0269806 (to The General Hospital Corporation) for a discussion of PD-1 agonists for use in therapy.

PD-1 agonists include, but are not limited to, PD-1, PD-1 fusion proteins (such as PD-1 Ig), agonist antibodies that specifically bind to PD-1, or nucleic acids encoding any of these. Preferably, the PD-1 used as a PD-1 agonist comprises substantially the same or the same sequence as native PD-1 from the species being treated with the PD-1 agonist, *e.g.* human PD-1 is used for treating human beings. Preferably, the agonist anti-PD-1 antibodies used in humans are antibodies derived from humans; antibodies derived from phage libraries based on human germline antibody sequences; antibodies obtained from transgenic animals expressing at least some human immunoglobulin genes; humanized antibodies; or chimeric antibodies. As used herein, "deriving" and "obtaining" antibodies encompasses (but is not limited to) obtaining the polypeptide sequence of the antibody from the recited source, not necessarily the antibody *per se*. Exemplary agonist anti-human PD-1 antibodies are disclosed at U.S. Pat. No. 7,488,802 (to Medimmune); WO 2011/110621 (to UCB Pharma); and at U.S. Pat. App. Pub. No. 2011/0171220 (to Isis). Additional PD-1 agonists are disclosed at WO 2013/022091 (to Ono Pharmaceutical Co., Ltd). A bispecific reagent binding to CD3 and PD-1, mouse in the specific case, and acting as a PD-1 agonist is disclosed at U.S. Pat. App. Pub. No. 2004/0241745.

The invention also provides use of pharmaceutical compositions of PD-1 agonists in the therapeutic methods of the present invention. The PD-1 agonists of the present invention may be administered to mammals, including humans, either alone or, in combination with

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pharmaceutically acceptable carriers, excipients or diluents, in a pharmaceutical composition, according to standard pharmaceutical practice. The PD-1 agonists can be administered orally or parenterally, including the intravenous, intramuscular, intraperitoneal, subcutaneous, rectal and topical routes of administration.

V. IL-23 Antagonists

Interleukin-23 (IL-23) is a heterodimeric cytokine comprised of two subunits, p19 which is unique to IL-23, and p40, which is shared with IL-12. The p19 subunit is structurally related to IL-6, granulocyte-colony stimulating factor (G-CSF), and the p35 subunit of IL-12. IL-23 mediates signaling by binding to a heterodimeric receptor, comprised of IL-23R and IL-12β1, which is shared by the IL-12 receptor. A number of early studies demonstrated that the consequences of a genetic deficiency in p40 (p40 knockout mouse; p40KO mouse) were more severe than those found in a p35KO mouse. Some of these results were eventually explained by the discovery of IL-23, and the finding that the p40KO prevents expression of not only IL-12, but also of IL-23 (*see, e.g.*, Oppmann *et al.* (2000) *Immunity* 13:715-725; Wiekowski *et al.* (2001) *J. Immunol.* 166:7563-7570; Parham *et al.* (2002) *J. Immunol.* 168:5699-708; Frucht (2002) *Sci STKE* 2002, E1-E3; Elkins *et al.* (2002) *Infection Immunity* 70:1936-1948).

Recent studies, through the use of p40 KO mice, have shown that blockade of both IL-23 and IL-12 is an effective treatment for various inflammatory and autoreactive immune disorders. IL-23 is known to play a central role in psoriasis, and the IL-23/IL-12 antagonist antibody ustekinumab (anti-IL-12/23p40 mAb) has been approved in the U.S. and Europe for the treatment of psoriasis. However, the blockade of IL-12 through p40 appears to have various systemic consequences such as increased susceptibility to opportunistic microbial infections. Bowman *et al.* (2006) *Curr. Opin. Infect. Dis.* 19:245. Therapeutic antibodies may be used to block cytokine activity. The most significant limitation in using antibodies as a therapeutic agent *in vivo* is the immunogenicity of the antibodies. As most monoclonal antibodies are derived from rodents, repeated use in humans results in the generation of an immune response against the therapeutic antibody. Such an immune response results in a loss of therapeutic efficacy at a minimum and a potential fatal anaphylactic response at a maximum. Initial efforts to reduce the immunogenicity of rodent antibodies involved the production of chimeric antibodies, in which mouse variable regions were fused with human constant regions. Liu *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:3439-43. However, mice injected with hybrids of human variable regions and mouse constant regions develop a strong

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anti-antibody response directed against the human variable region, suggesting that the retention of the entire rodent Fv region in such chimeric antibodies may still result in unwanted immunogenicity in patients.In some embodiments the method of antagonizing IL-23 activity is a method that does not antagonize the activity of IL-12, *e.g.* by use of an IL-23-specific antagonist. Such methods of antagonizing IL-23 may involve blocking of the activity of the p19 subunit of IL-23, rather than the p40 subunit, since the p19 subunit is specific to IL-23 (p19 + p40) whereas the p40 subunit is shared with IL-12 (p35 + p40). Such methods of antagonizing IL-23 may also involve blocking of the activity of the IL-23R subunit of the IL-23 receptor complex (IL-23R + IL-12Rβ1), rather than the IL-12Rβ1 subunit that is shared with the IL-12 receptor (IL-12Rβ1 + IL-12Rβ2).In various embodiments the method of antagonism of IL-23 involves administration of an IL-23 antagonist. IL-23 antagonists include, but are not limited to, small molecule compounds, antisense nucleic acids, small interfering nucleic acids, aptamers, antibodies or antigen binding fragments thereof, and soluble forms of IL-23 receptor.In some embodiments the IL-23 antagonist is an IL-23-specific antagonist. Exemplary IL-23-specific antagonists include an antibody that binds specifically to IL-23p40 but not IL-12p40 (US 7,247,711 to Centocor) or an antibody that makes contacts with both the p19 and p40 subunits of IL-23 (WO 2011/056600 to Amgen, Inc.). Fibronectin-derived IL-23 antagonists are disclosed at WO 2011/103105 (developed at Adnexus Therapeutics Inc., now part of Bristol-Myers Squibb Co.).

In other embodiments the IL-23-specific antagonist binds to p19. Exemplary IL-23-specific antagonists that bind to p19 include multimerized IL-23 receptors (US 2011/0052585 to Genzyme Corp.); protein constructs against IL-23p19 (WO 2010/142534 and WO 2009/068627 to Ablynx NV); an IL-23 aptamer (US 2006/0193821 to Archemix); and monoclonal antibody FM303 (Femta Pharmaceuticals). Further IL-23-specific antagonists that bind to p19 include antibodies or antigen-binding fragments thereof that specifically bind to the p19 subunit of IL-23, as disclosed at WO 2008/103432, US 2007/0048315 and WO 2008/103473 (to Schering Corp.); US 7,491,391, US 7,935,344 and EP 1971366 A2 (to Centocor Ortho Biotech, Inc.); US 7,872,102 (to Eli Lilly and Co.); WO 2007/147019, WO 2008/134659 and WO 2009/082624 (to Zymogenetics); US 2009/0311253 (to Abbott Bioresearch); and US 2009/0123479 and WO 2010/115786 (to Glaxo SmithKline), the disclosures of which are hereby incorporated by reference in their entireties.Anti-IL-23p19 antibodies that may be suitable for use in the methods of the present invention also include, but are not limited to, Merck's SCH 900222/MK-3222; Eli Lilly's LY2525623, and

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Centocor's CNTO 1959, all of which have entered human clinical trials. Specifically, the sequences of SEQ ID NOs: 48 and 52 (heavy chain variable domains), 57 (light chain variable domain), 28-37-40 (light chain CDRs 1-2-3, respectively) and 3-8-19 (light chain CDRs 1-2-3, respectively) of EP 1937721 B1 (to Eli Lilly and Company) are hereby incorporated by reference. In addition, the sequences of SEQ ID NOs: 106 (heavy chain variable domain), 116 (light chain variable domain), 50-56-73 (light chain CDRs 1-2-3, respectively) and 5-20-44 (light chain CDRs 1-2-3, respectively) of US 7,935,344 (to Centocor) are also hereby incorporated by reference.In some embodiments, the anti-IL-23p19 antibodies, or antigen binding fragments thereof, are based on antibody 13B8 of commonly assigned WO 2008/103432, the disclosure of which is hereby incorporated by reference in its entirety. The anti-human IL-23p19 antibody may comprise one, two, three, four, five or six of the CDR sequences, or the heavy and light chain variable domains, of the humanized antibodies disclosed in commonly assigned WO 2008/103432, for example antibodies hu13B8a, b or c. In another embodiment the anti-human IL-23p19 antibody competes with antibody hu13B8a, b or c for binding to human IL-23. In another embodiment the anti-human IL-23p19 antibody binds to the same epitope on human IL-23 as hu13B8a, b or c. Sequences for antibody 13B8 and variants thereof are provided in the accompanying Sequence Listing.A hybridoma expressing antibody 13B8 was deposited pursuant to the Budapest Treaty with American Type Culture Collection (ATCC - Manassas, Virginia, USA) on August 17, 2006 under Accession Number PTA-7803. All restrictions on the accessibility of this deposit will be irrevocably removed upon the granting of a U.S. patent based on the present application. In other embodiments, the anti-human IL-23p19 antibody is able to block binding of human IL-23p19 to the antibody produced by the hybridoma deposited with accession number PTA-7803 in a cross-blocking assay.

In yet further embodiments, the anti-human IL-23p19 antibody binds to the same epitope as the antibody produced by the hybridoma deposited with ATCC under accession number PTA-7803. In still further embodiments, the anti-human IL-23p19 antibody comprises the same CDR sequences as the antibody produced by the hybridoma deposited with ATCC with accession number PTA-7803.In some embodiments the IL-23 antagonist is a non-specific IL-23 antagonist Exemplary non-specific IL-23 antagonists include antibodies that bind to the p40 subunit of IL-23 and IL-12, such as ustekinumab (CNTO 1275) and briakinumab (ABT-874, J-695). Ustekinumab is marketed by Centocor for the treatment of psoriasis, and is described at US 6,902,734 and US 7,166,285 (to Centocor, Inc.), the disclosures of which are hereby incorporated by reference in their entireties. Specifically, the

sequences of SEQ ID NOs: 7 (heavy chain variable domain) and 8 (light chain variable domain), of US 6,902,734 are hereby incorporated by reference. SEQ ID NOs: 4-5-6 and 1-2-3 of US 6,902,734 are also incorporated by reference. Sequences for ustekinumab are also provided at SEQ ID NOs: 27 – 36 of the sequence listing of the present application. Briakinumab was developed by Abbott, and is described at US 6,914,128 and US 7,504,485, the disclosures of which are hereby incorporated by reference in their entireties. Specifically, the sequences of SEQ ID NOs: 31 (heavy chain variable domain), 32 (light chain variable domain) SEQ ID NOs; 30-28-26 (light chain CDRs 1-2-3, respectively) and 29-27-25 (heavy chain CDRs 1-2-3, respectively) of US 6,914,128 are hereby incorporated by reference. Sequences for briakinumab are also provided at SEQ ID NOs: 37 – 42 of the sequence listing of the present application.Further exemplary non-specific IL-23 antagonist antibodies that bind to the p40 subunit of IL-23 and IL-12 are disclosed at Clarke *et al.* (2010) *mAbs* 2:1-11 (Cephalon Australia, Pty., Ltd.). FM202 (Femta Pharmaceuticals) is also a monoclonal antibody that binds to the p40 subunit of both IL-12 and IL-23, as are the antibodies disclosed at WO 2010/017598 (Arana Therapeutics, Ltd.). Apilimod mesylate (STA-5326, Synta Pharmaceuticals Corp.), an oral non-specific IL-23 antagonist, may also be used in some embodiments of the present invention. Still further exemplary non-specific IL-23 antagonists include antibodies that bind to the IL-12Rβ1 subunit of both the IL-12 and IL-23 receptor complexes (WO 2010/112458 to Novartis AG).Other potential IL-23 antagonists for use in the methods of the present invention include the peptides disclosed in WO 2011/033493 (Peptinov SAS), the variant p19 polypeptides disclosed at WO 2011/011797 (Eleven Biotherapeutics, Inc. and Stanford University), and the oxidized lipid compounds disclosed at WO 2004/106486 and US 7,625,882 (Vascular Biogenics, Ltd.), such as VBL-201 (VBL Therapeutics).

VI. IL-23R Binding Domains

In other embodiments a bispecific targeting agent includes an arm that specifically binds to IL-23R, such as human IL-23R on the surface of the pathogenic IL-23R$^+$ / PD-1$^+$ gamma delta T cells disclosed for the first time herein. Exemplary IL-23R-specific antagonists that can serve as a source for IL-23R binding domains include anti-IL-23R antibodies (WO 2008/106134 and WO 2010/027767 to Schering Corp.); WO 2013/129454 (to Astellas Pharma); multimerized and multimerized polypeptides that binds to IL-23R (U.S. Pat. App. Pub. No. 2011/0086806 to Anaphore, Inc.); and IL-23 receptor antagonist peptides (WO 2009/007849 to Valorisation HSJ and Societe en Commandite), such as APG2305 (Allostera Pharma, Inc.).

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As stated above, binding regions from antibodies that bind to IL-23R may be used as components of bispecific reagents that specifically bind to both IL-23R and PD-1. Such bispecific reagents, such a bispecific antibodies or antigen binding fragments thereof, will find use in ablating pathogenic IL-23R$^+$ / PD-1$^+$ gamma delta T cells. Such bispecific reagents will further comprise sequences to elicit ADCC of CDC, or cytotoxic drugs, to kill target cells. The other binding arm of the bispecific reagents comprises a binding region from an antibody that specifically binds to PD-1. For treatment of humans, the bispecific reagent, e.g. a bispecific antibody of antigen binding fragment thereof, will bind specifically to human IL-23R and human PD-1. In embodiments in which a bispecific reagent is used to specifically ablate pathogenic IL-23R$^+$ / PD-1$^+$ gamma delta T cells, the antibody binding regions need only bind to their respective targets, and need not agonize of antagonize their respective targets, although agonistic or antagonistic properties would likely not interfere with ablation.

Bispecific antibodies are also useful in the present methods and compositions. As used herein, the term "bispecific antibody" refers to an antibody, typically a monoclonal antibody, having binding specificities for at least two different antigens, e.g., IL-23p19 and PD-1. In another embodiment, the epitopes are from two different antigens. Methods for making bispecific antibodies are known in the art. For example, bispecific antibodies can be produced recombinantly using the co-expression of two immunoglobulin heavy chain/light chain pairs. *See, e.g.,* Milstein *et al.* (1983) *Nature* 305: 537-39. Alternatively, bispecific antibodies can be prepared using chemical linkage. *See, e.g.,* Brennan *et al.* (1985) *Science* 229:81. Bispecific antibodies include bispecific antibody fragments. *See, e.g.,* Holliger *et al.* (1993) *Proc. Natl. Acad. Sci. U.S.A.* 90:6444-48, Gruber *et al.* (1994) *J. Immunol.* 152:5368. In yet other embodiments, different constant domains may be appended to the humanized V_L and V_H regions provided herein. For example, if a particular intended use of an antibody (or fragment) of the present invention were to call for altered effector functions, a heavy chain constant domain other than IgG1 may be used. Although IgG1 antibodies provide for long half-life and for effector functions, such as complement activation and antibody-dependent cellular cytotoxicity, such activities may not be desirable for all uses of the antibody. In such instances an IgG4 constant domain, for example, may be used. For example, IgG1 antibodies may be preferred in embodiments involving ablation of IL-23R$^+$/PD-1$^+$ gamma delta T cells, whereas IgG4 or other non-ADCC/non-CDC forms might be preferred in embodiments involving treatment with an agonist antibody to PD-1, with or without concurrent treatment with an antagonist antibody to IL-23.

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VII. Pharmaceutical Compositions of anti-IL-23 Antibodies, anti-PD-1 Antibodies and Bispecific anti-IL-23R/PD-1 Antibodies or anti-IL-1R/PD-1 Antibodies

To prepare pharmaceutical or sterile compositions of the antibodies of the present invention, the antibody (or antigen binding fragment thereof) is admixed with a pharmaceutically acceptable carrier or excipient. *See, e.g., Remington's Pharmaceutical Sciences* and *U.S. Pharmacopeia: National Formulary*, Mack Publishing Company, Easton, PA (1984).

Determination of the appropriate dose is made by the clinician, e.g., using parameters or factors known or suspected in the art to affect treatment or predicted to affect treatment. Generally, the dose begins with an amount somewhat less than the optimum dose and it is increased by small increments thereafter until the desired or optimum effect is achieved relative to any negative side effects. Important diagnostic measures include those of symptoms of, e.g., the inflammation or level of inflammatory cytokines produced. Preferably, a biologic that will be used is substantially derived from the same species as the animal targeted for treatment (e.g. a humanized antibody for treatment of human subjects), thereby minimizing any immune response to the reagent.

As used herein, the term "therapeutically effective amount" or "effective amount" refers to an amount of an antibody, or antigen binding fragment thereof, *e.g.* an antagonist IL-23p19-specific antibody or an agonist anti-PD-1 antibody, or antigen binding fragment thereof, that when administered alone or in combination with an additional therapeutic agent to a cell, tissue, or subject is effective to prevent or ameliorate an autoreactive immune disorder. A therapeutically effective dose further refers to that amount of the compound sufficient to result in amelioration of symptoms, *e.g.,* treatment, healing, prevention or amelioration of the relevant medical condition, or an increase in rate of treatment, healing, prevention or amelioration of such conditions. When applied to an individual active ingredient administered alone, a therapeutically effective dose refers to that ingredient alone. When applied to a combination, a therapeutically effective dose refers to combined amounts of the active ingredients that result in the therapeutic effect, whether administered in combination, serially or simultaneously. An effective amount of therapeutic will decrease the symptoms typically by at least 10%; usually by at least 20%; preferably at least about 30%; more preferably at least 40%, and most preferably by at least 50%.

The broad scope of this invention is best understood with reference to the following examples, which are not intended to limit the inventions to the specific embodiments. The

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specific embodiments described herein are offered by way of example only, and the invention is to be limited by the terms of the appended claims, along with the full scope of equivalents to which such claims are entitled.

EXAMPLES

Example 1

General Methods

Standard methods in molecular biology are described. Maniatis *et al.* (1982) *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY; Sambrook and Russell (2001) *Molecular Cloning, 3rd ed.*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY; Wu (1993) *Recombinant DNA*, Vol. 217, Academic Press, San Diego, CA. Standard methods also appear in Ausbel *et al.* (2001) *Current Protocols in Molecular Biology, Vols.1-4*, John Wiley and Sons, Inc. New York, NY, which describes cloning in bacterial cells and DNA mutagenesis (Vol. 1), cloning in mammalian cells and yeast (Vol. 2), glycoconjugates and protein expression (Vol. 3), and bioinformatics (Vol. 4).

Methods for protein purification including immunoprecipitation, chromatography, electrophoresis, centrifugation, and crystallization are described. Coligan *et al.* (2000) *Current Protocols in Protein Science, Vol. 1*, John Wiley and Sons, Inc., New York. Chemical analysis, chemical modification, post-translational modification, production of fusion proteins, glycosylation of proteins are described. *See, e.g.*, Coligan *et al.* (2000) *Current Protocols in Protein Science, Vol. 2*, John Wiley and Sons, Inc., New York; Ausubel *et al.* (2001) *Current Protocols in Molecular Biology, Vol. 3*, John Wiley and Sons, Inc., NY, NY, pp. 16.0.5-16.22.17; Sigma-Aldrich, Co. (2001) *Products for Life Science Research*, St. Louis, MO; pp. 45-89; Amersham Pharmacia Biotech (2001) *BioDirectory*, Piscataway, N.J., pp. 384-391. Production, purification, and fragmentation of polyclonal and monoclonal antibodies are described. Coligan *et al.* (2001) *Current Protocols in Immunology, Vol. 1*, John Wiley and Sons, Inc., New York; Harlow and Lane (1999) *Using Antibodies*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY; Harlow and Lane, *supra*. Standard techniques for characterizing ligand/receptor interactions are available. *See, e.g.*, Coligan *et al.* (2001) *Current Protocols in Immunology, Vol. 4*, John Wiley, Inc., New York.

Methods for flow cytometry, including fluorescence activated cell sorting detection systems (FACS®), are available. *See, e.g.*, Owens *et al.* (1994) *Flow Cytometry Principles for Clinical Laboratory Practice*, John Wiley and Sons, Hoboken, NJ; Givan (2001) *Flow Cytometry, 2nd ed.*; Wiley-Liss, Hoboken, NJ; Shapiro (2003) *Practical Flow Cytometry*, John Wiley and Sons, Hoboken, NJ. Fluorescent reagents suitable for modifying nucleic acids, including nucleic acid primers and probes, polypeptides, and antibodies, for use, e.g., as diagnostic reagents, are available. Molecular Probes (2003) *Catalogue*, Molecular Probes, Inc., Eugene, OR; Sigma-Aldrich (2003) *Catalogue*, St. Louis, MO.

Standard methods of histology of the immune system are described. *See, e.g.*, Muller-Harmelink (ed.) (1986) *Human Thymus: Histopathology and Pathology*, Springer Verlag, New York, NY; Hiatt, *et al.* (2000) *Color Atlas of Histology*, Lippincott, Williams, and Wilkins, Phila, PA; Louis, *et al.* (2002) *Basic Histology: Text and Atlas*, McGraw-Hill, New York, NY.

Software packages and databases for determining, e.g., antigenic fragments, leader sequences, protein folding, functional domains, glycosylation sites, and sequence alignments, are available. *See, e.g.*, GenBank, Vector NTI® Suite (Informax, Inc, Bethesda, MD); GCG Wisconsin Package (Accelrys, Inc., San Diego, CA); DeCypher® (TimeLogic Corp., Crystal Bay, Nevada); Menne *et al.* (2000) *Bioinformatics* 16: 741-742; Menne *et al.* (2000) *Bioinformatics Applications Note* 16:741-742; Wren *et al.* (2002) *Comput. Methods Programs Biomed.* 68:177-181; von Heijne (1983) *Eur. J. Biochem.* 133:17-21; von Heijne (1986) *Nucleic Acids Res.* 14:4683-4690.

Example 2

Presence of PD-1$^+$ and IL-23R$^+$ T Cells in Various Mouse Tissues

Single cell suspensions were isolated from murine gut, spleen, inguinal lymph node (ILN), hind joint, ear skin, visceral fat and lung. The suspensions were stained for immune cell markers. Various immune cell subsets were then assessed for IL-23R reporter (GFP) and PD-1 expression via flow cytometry. *See* FIGs. 1A-1, 1A-2 and 1B. Quadrant 2 (Q2) represents double positive PD-1$^+$ / IL-23R$^+$ T cells, and percentages of total sorted cells are provided for each quadrant

Briefly, total immune cells were isolated from tissue obtained from IL-23R-GFP reporter mice. The cells were stained with LIVE/DEAD® cell viability stain (Invitrogen, San Diego, Calif., USA) and with fluorescently labeled antibodies against CD3e, TCRb, TCRd (all three from BD Biosciences, San Jose, Calif., USA), CD4 (Invitrogen), and CD8a and PD-

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1 (both from eBioscience, San Diego, Calif., USA). Live, $CD3^+$ lymphocytes were gated and assessed for TCRb vs TCRd expression. $TCRb^+$ cells were further assessed for CD4 and CD8 expression. Gamma delta T cells were defined as $TCRb^-/TCRd^+$. All cells were assessed for PD-1 and IL-23R GFP expression. Only CD4 and gamma delta T cells are shown. CD4 staining is representative of similar staining found in other tissue T cell subsets.

The proportions of $IL\text{-}23R^+/PD\text{-}1^+$ gamma delta T cells, as a fraction of total γδ T cells in the samples, are provided as % values in quadrant 2 (the upper right quadrant) of each of the "DN γδ T cells" panels of FIGs. 1A-1, 1A-2 and 1B. This quadrant is outlined on bold in gut, joint and visceral fat data, which exhibit particularly elevated proportions of $IL\text{-}23R^+/PD\text{-}1^+$ gamma delta T cells. The % value is calculated by dividing the number of cells in quadrant 2 ($IL\text{-}23R^+/PD\text{-}1^+$) by the total number of cells sorted (all four quadrants). Cells were gated slightly differently for FIG. 1B than for FIGs. 1A-1 and 1A-2, so the percentages of cells in each quadrant cannot be quantitatively compared between FIGs 1A-1 and 1A-2, and 1B.

Example 3

PD-1 and IL-17A Expression in IL-23-Treated T Cells from Mouse Adipose Tissue

Total stromal vascular cells were isolated from murine adipose tissue and were treated with IL-23, *in vitro*, for 48 hours. Cells were then stimulated with PMA, ionomycin, and Brefeldin A for three hours and assayed for immune cell markers, IL-17A expression, and PD-1 expression (as described in greater detail below). *See* FIG. 2. Quadrant 2 (Q2) represents double positive $PD\text{-}1^+$ / $IL\text{-}17A^+$ T cells, and percentages of total sorted cells are provided for each quadrant.

Briefly, cells were stained with LIVE/DEAD® cell viability stain (Invitrogen, San Diego, Calif., USA) and with fluorescently labeled antibodies against CD3e, TCRb, TCRd and PD-1 (all four from BD Biosciences, San Jose, Calif., USA), CD4 (Invitrogen), and CD8a (eBioscience, San Diego, Calif., USA). Live, $CD3^+$ lymphocytes were gated and assessed for TCRb vs TCRd expression. $TCRb^+$ cells were further assessed for CD4 and CD8 expression. Gamma delta T cells were defined as $TCRb^-/TCRd^+$. All cells were assessed for PD-1 and IL-17 expression. Only CD4 and gamma delta T cells are shown. CD4 staining is representative of similar staining found in other tissue T cell subsets.

The proportion of $IL\text{-}17A^+/PD\text{-}1^+$ gamma delta T cells, as a fraction of total γδ T cells in the sample, is provided as a % value (75.7%) in the quadrant 2 of the "DN γδ T cells" panel of FIG. 2. The % value is calculated by dividing the number of cells in quadrant 2 ($IL\text{-}17A^+/PD\text{-}1^+$) by the total number of cells sorted (all four quadrants).

Example 4

Suppression of IL-17A and IL-22 Expression by PD-L1 Ig

Total stromal vascular cells were isolated from murine adipose tissue and were treated with IL-23, PD-L1-Fc, or both, *in vitro*, for 48 hours. PD-L1-Fc was obtained from R&D Systems (Minneapolis, Minn., USA). A control experiment was included ("No Rx") in which cells were not exposed to either of IL-23 or PD-L1-Fc. Supernatants were then assayed for IL-17A and IL-17F. *See* FIGS. 3A and 3B.

Table 4 provides a brief description of the sequences in the sequence listing.

Table 4

Sequence Identifiers

SEQ ID NO.	Description
1	IL-23R Nucleic Acid
2	IL-23R
3	IL-1R Nucleic Acid
4	IL-1R
5	PD-1 Nucleic Acid
6	PD-1
7	PD-L1α Nucleic Acid
8	PD-L1α
9	PD-L1β Nucleic Acid
10	PD-L1β
11	IL-23p19 Nucleic Acid
12	IL-23p19
13	13B8 CDRH1
14	13B8-a CDRH2
15	13B8-b CDRH2
16	13B8-c CDRH2
17	13B8 CDRH3
18	13B8 CDRL1
19	13B8 CDRL2
20	13B8 CDRL3

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SEQ ID NO.	Description
21	13B8-a HC
22	13B8-b HC
23	13B8-c HC
24	13B8 LC
25	13B8-b HC DNA
26	13B8 LC DNA
27	ustekinumab CDRH1
28	ustekinumab CDRH2
29	ustekinumab CDRH3
30	ustekinumab CDRL1
31	ustekinumab CDRL2
32	ustekinumab CDRL3
33	ustekinumab V_H
34	ustekinumab V_L
35	ustekinumab HC
36	ustekinumab LC
37	briakinumab CDRH1
38	briakinumab CDRH2
39	briakinumab CDRH3
40	briakinumab CDRL1
41	briakinumab CDRL2
42	briakinumab CDRL3
43	briakinumab V_H
44	briakinumab V_L
45	briakinumab HC
46	briakinumab LC
47	guselkumab CDRH1
48	guselkumab CDRH2
49	guselkumab CDRH3
50	guselkumab CDRL1
51	guselkumab CDRL2
52	guselkumab CDRL3

SEQ ID NO.	Description
53	guselkumab VH
54	guselkumab VL
55	guselkumab HC
56	guselkumab LC

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CLAIMS

WHAT IS CLAIMED IS:

1. A method of treating an IL-23-mediated autoreactive immune disorder in a subject by selectively ablating IL-23R$^+$/PD-1$^+$ gamma delta T cells.

2. The method of Claim 1 wherein the ablation is achieved by administering to the subject a multi-specific antibody, or antigen binding fragment thereof, that specifically binds to:

a) PD-1, and;

b) at least one of IL-23R and IL-1Rβ1,

wherein the antibody or antigen binding fragment thereof further comprises a constant region sequence capable of eliciting an ADCC response or a CDC response.

3. The method of Claim 1 wherein the ablation is achieved by administering to the subject a multi-specific antibody, or antigen binding fragment thereof, that specifically binds to:

a) PD-1, and;

b) at least one of IL-23R and IL-1Rβ1,

wherein the antibody or antigen binding fragment thereof further comprises a cytotoxic agent.

4. A method of treating an IL-23-mediated autoreactive immune disorder in a subject, comprising administering to the subject a pharmaceutical composition comprising an agonist of PD-1 if and only if the subject exhibits at least a 2-fold higher proportion of IL-23R$^+$/PD-1$^+$ gamma delta T cells in the affected tissue as compared to the proportion in a corresponding tissue in non-affected subjects.

5. A method of treating an IL-23-mediated autoreactive immune disorder in a subject, comprising:

a) determining the proportion of IL-23R$^+$/PD-1$^+$ gamma delta T cells, as a fraction of total γδ T cells, in a sample of affected tissue obtained from the subject; and

b) administering to the subject a pharmaceutical composition comprising an agonist of PD-1 if and only if the subject exhibits at least a 2-fold higher proportion of IL-

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$23R^+/PD\text{-}1^+$ gamma delta T cells in the affected tissue as compared to the proportion in a corresponding tissue in non-affected subjects.

6. The method of Claim 5 wherein the PD-1 agonist is administered in combination with an IL-23 antagonist.

7. The method of Claim 6 wherein the IL-23 antagonist is an anti-IL-23 antibody or antigen binding fragment thereof that binds specifically to the p40 subunit of IL-23.

8. The method of Claim 6 wherein the IL-23 antagonist is an anti-IL-23 antibody or antigen binding fragment thereof that binds specifically to the p19 subunit of IL-23..

9. A method of identifying subjects that are likely to be good responders to treatment of an IL-23-mediated autoreactive immune disorder with a PD-1 agonist, comprising:

a) determining the proportion of $IL\text{-}23R^+/PD\text{-}1^+$ gamma delta T cells, as a fraction of total γδ T cells, in a sample of affected tissue obtained from the subject;

b) selecting the subject for treatment with a PD-1 agonist if, and only if, the subject exhibits at least a 2-fold higher proportion of $IL\text{-}23R^+/PD\text{-}1^+$ gamma delta T cells in the affected tissue as compared to the proportion in a corresponding tissue in non-affected subjects.

10. The method of identifying agents likely to be useful in the treatment of IL-23-mediated autoreactive immune disorder, comprising:

a) treating $IL\text{-}23R^+/PD\text{-}1^+$ gamma delta T cells with IL-23 *in vitro* in the presence of, and in the absence of, one or more candidate therapeutic agents;

b) measuring the levels of the effector cytokines IL-17A and IL-22 produced by the $IL\text{-}23R^+/PD\text{-}1^+$ gamma delta T cells; and

c) selecting one or more of the candidate therapeutic agents to advance as a potential drug based on its ability to reduce expression of IL-17A, IL-22, or both, compared to their levels of expression in control experiments performed in the absence of any candidate therapeutic agents.

11. The method of Claim 10 further comprising evaluating the one or more selected candidate therapeutic agents in an animal model or other pre-clinical test, wherein most or all

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of the candidate therapeutic agents that were not selected are not evaluated in the animal model or pre-clinical test.

12. The method of Claim 10 wherein the candidate therapeutic agents are anti-PD-1 antibodies, or antigen binding fragments thereof.

13. A method of any of Claim 1, 4, 5, 9 and 10 wherein the IL-23-mediated autoreactive immune disorder is selected from the group consisting of psoriasis, psoriatic arthritis, ankylosing spondylitis, inflammatory bowel disease,uveitis and COPD.

14. The method of Claim 9 wherein the autoreactive immune disorder is psoriasis.

15. The method of Claim 14 wherein the tissue is skin, and the sample is obtained by punch biopsy.

16. The method of Claim 9 wherein the autoreactive immune disorder is inflammatory bowel disease.

17. The method of Claim 16 wherein the tissue is intestine, and the sample is obtained by resection.

18. The method of Claim 9 wherein the autoreactive immune disorder is COPD.

19. The method of Claim 18 wherein the tissue is lung, and the sample is obtained by BAL or biopsy.

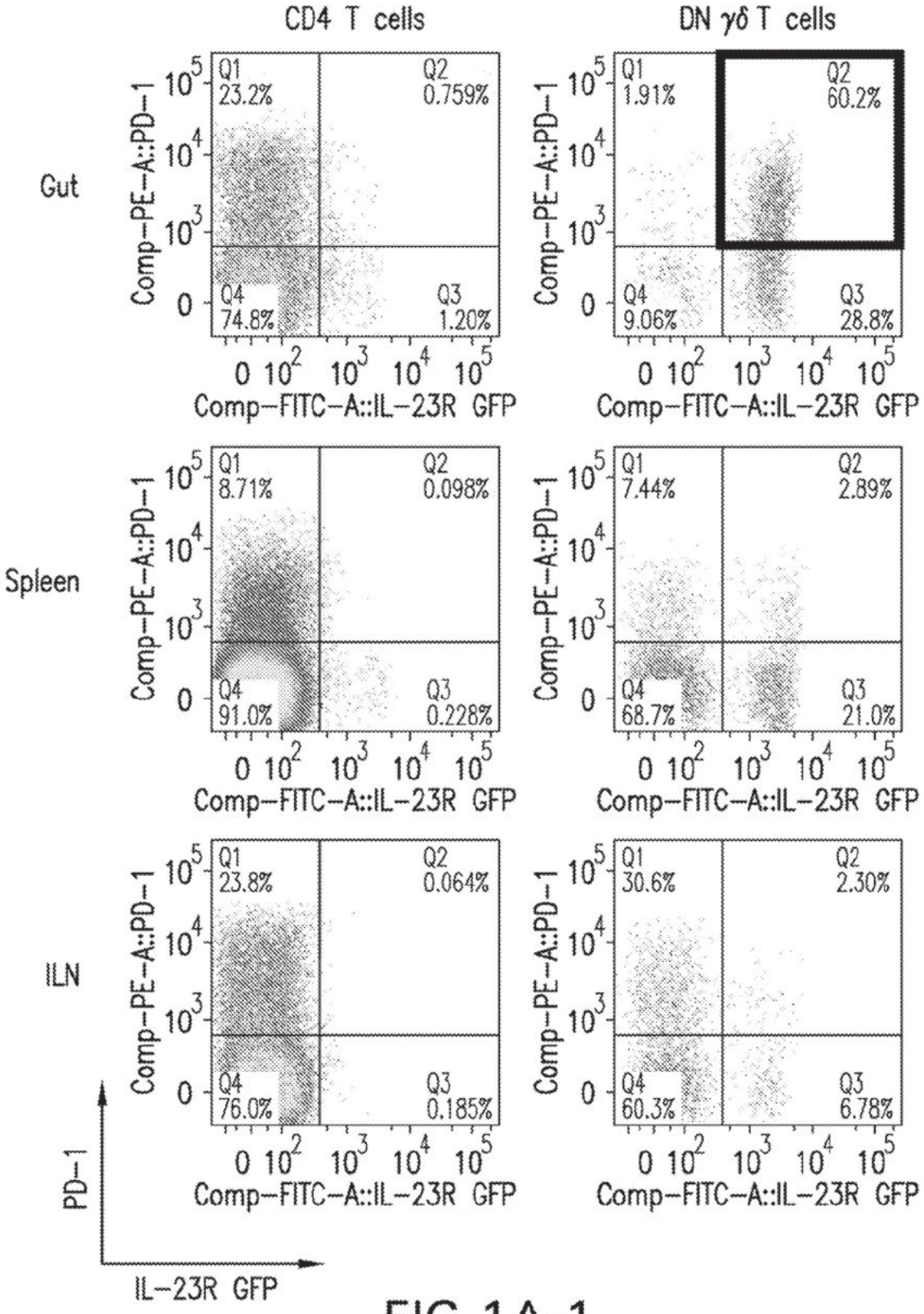

CD4 T cells
DN γδ T cells
Gut
Spleen
ILN
Q1 23.2%
Q2 0.759%
Q4 74.8%
Q3 1.20%
Q1 1.91%
Q2 60.2%
Q4 9.06%
Q3 28.8%
Q1 8.71%
Q2 0.098%
Q4 91.0%
Q3 0.228%
Q1 7.44%
Q2 2.89%
Q4 68.7%
Q3 21.0%
Q1 23.8%
Q2 0.064%
Q4 76.0%
Q3 0.185%
Q1 30.6%
Q2 2.30%
Q4 60.3%
Q3 6.78%
Comp-PE-A::PD-1
Comp-FITC-A::IL-23R GFP
PD-1
IL-23R GFP

FIG.1A-1

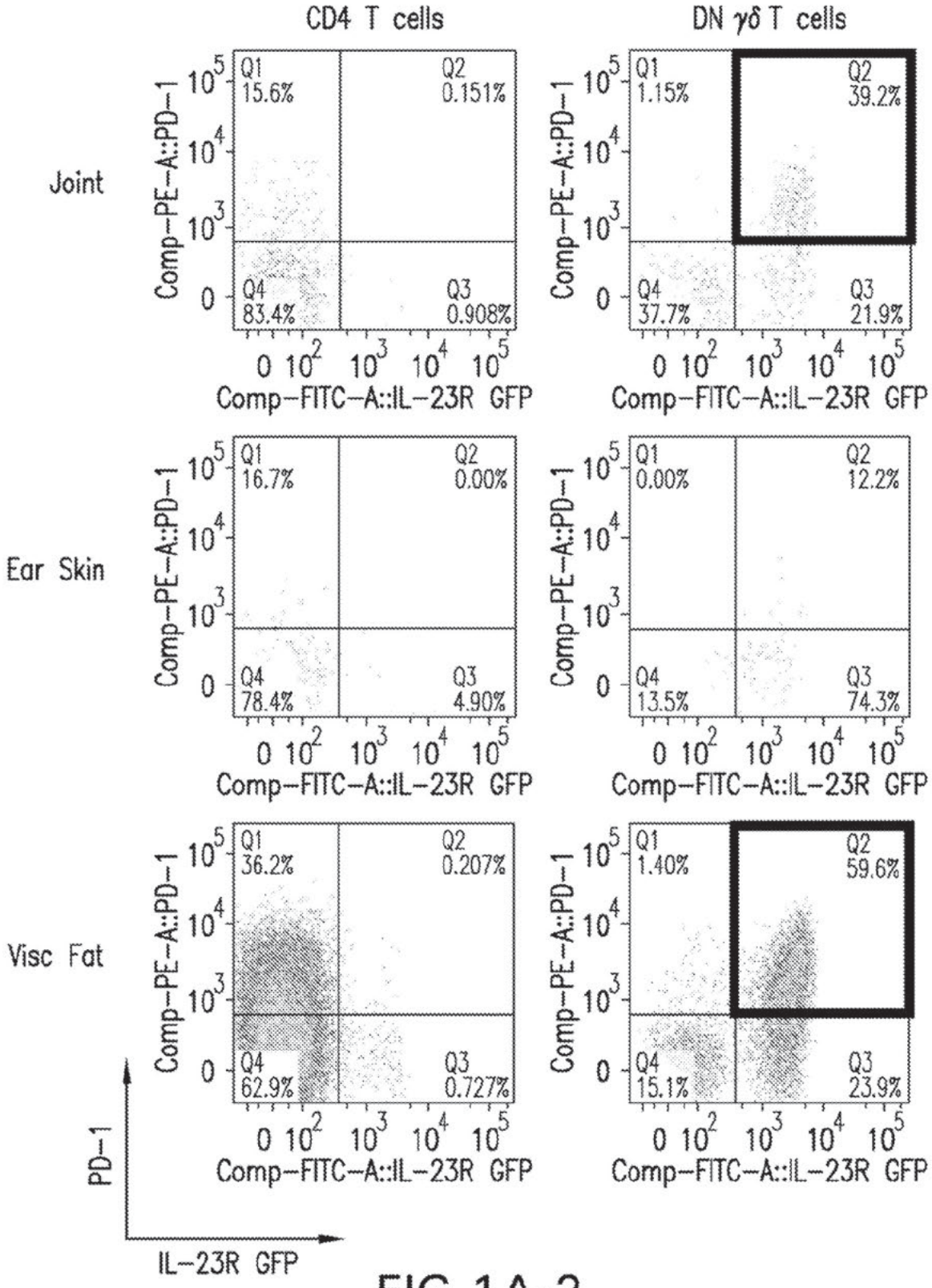

CD4 T cells
DN γδ T cells
Joint
Ear Skin
Visc Fat
Comp-PE-A::PD-1
Comp-FITC-A::IL-23R GFP
Q1 15.6%
Q2 0.151%
Q4 83.4%
Q3 0.908%
Q1 1.15%
Q2 39.2%
Q4 37.7%
Q3 21.9%
Q1 16.7%
Q2 0.00%
Q4 78.4%
Q3 4.90%
Q1 0.00%
Q2 12.2%
Q4 13.5%
Q3 74.3%
Q1 36.2%
Q2 0.207%
Q4 62.9%
Q3 0.727%
Q1 1.40%
Q2 59.6%
Q4 15.1%
Q3 23.9%
PD-1
IL-23R GFP

FIG.1A-2

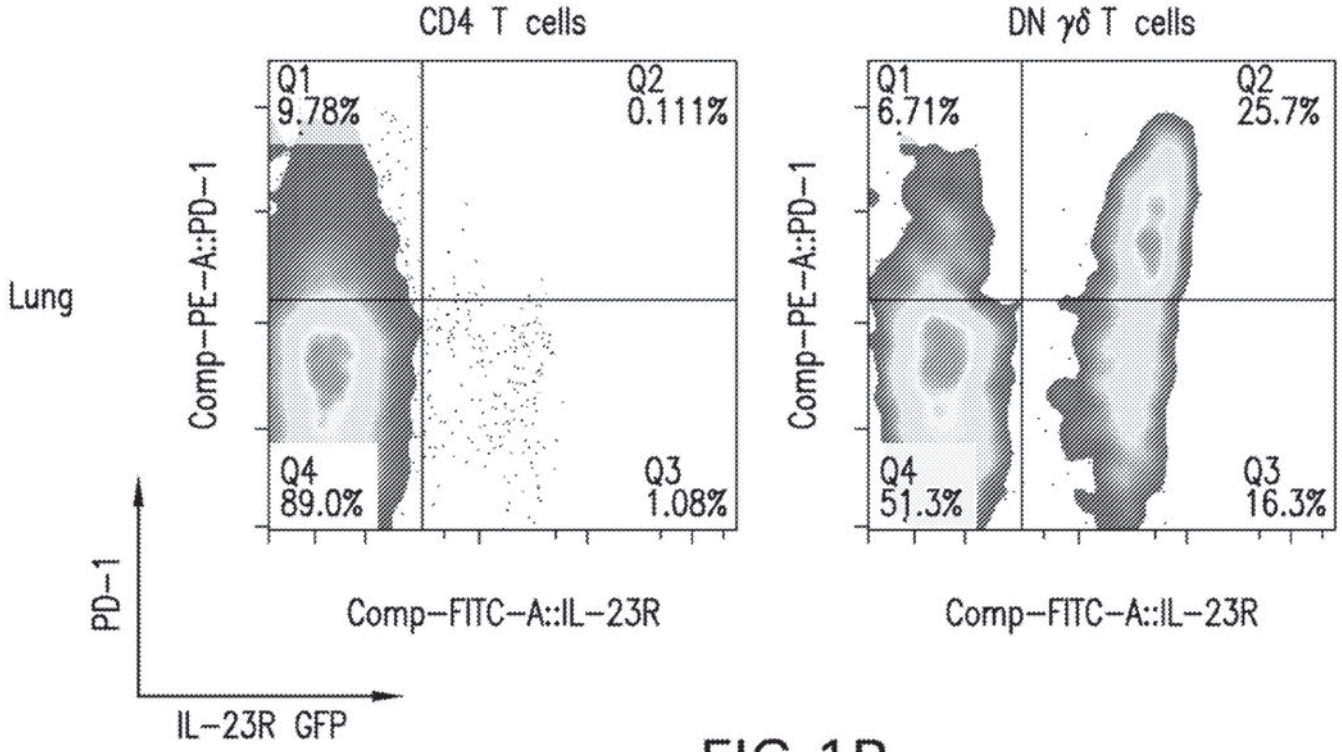

CD4 T cells
DN γδ T cells
Lung
Q1
9.78%
Q2
0.111%
Q4
89.0%
Q3
1.08%
Q1
6.71%
Q2
25.7%
Q4
51.3%
Q3
16.3%
Comp-PE-A::PD-1
Comp-FITC-A::IL-23R
PD-1
IL-23R GFP

FIG. 1B

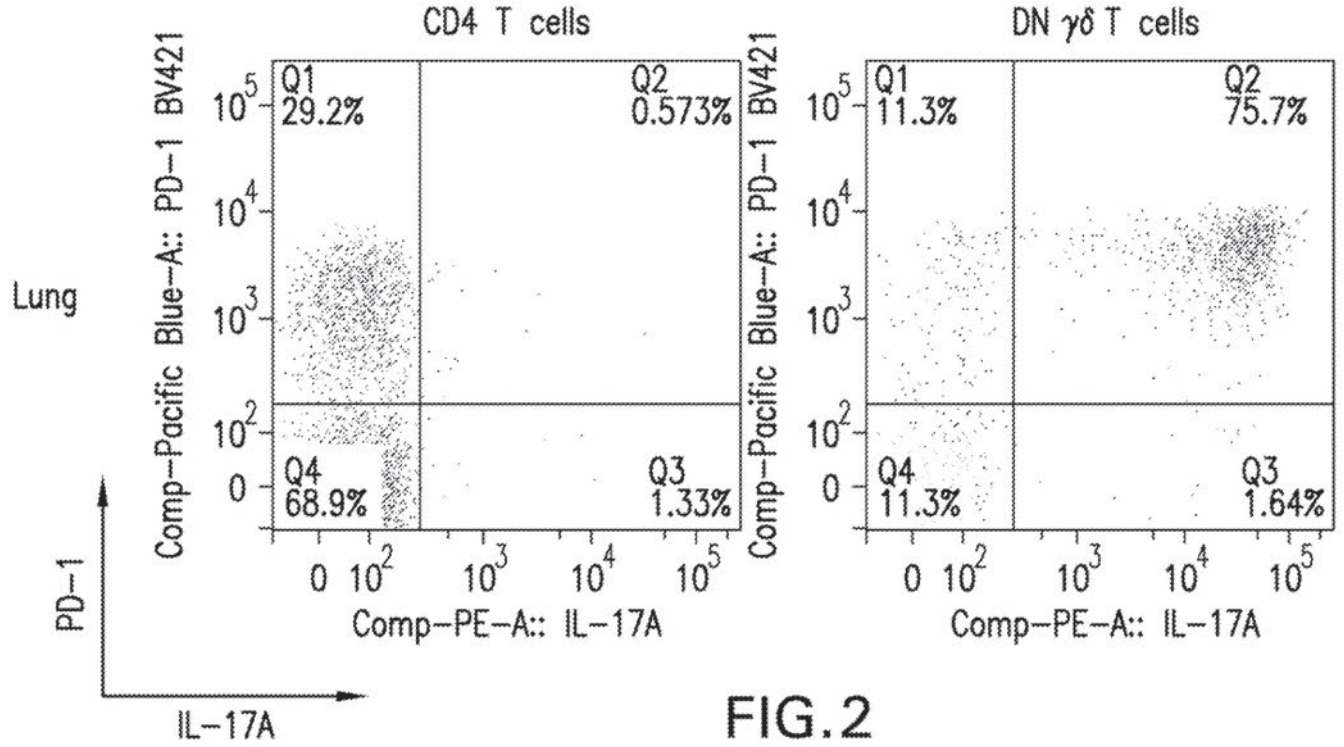

Lung
CD4 T cells
DN γδ T cells
Comp-Pacific Blue-A:: PD-1 BV421
Comp-PE-A:: IL-17A
Q1
29.2%
Q2
0.573%
Q4
68.9%
Q3
1.33%
Q1
11.3%
Q2
75.7%
Q4
11.3%
Q3
1.64%
PD-1
IL-17A

FIG.2

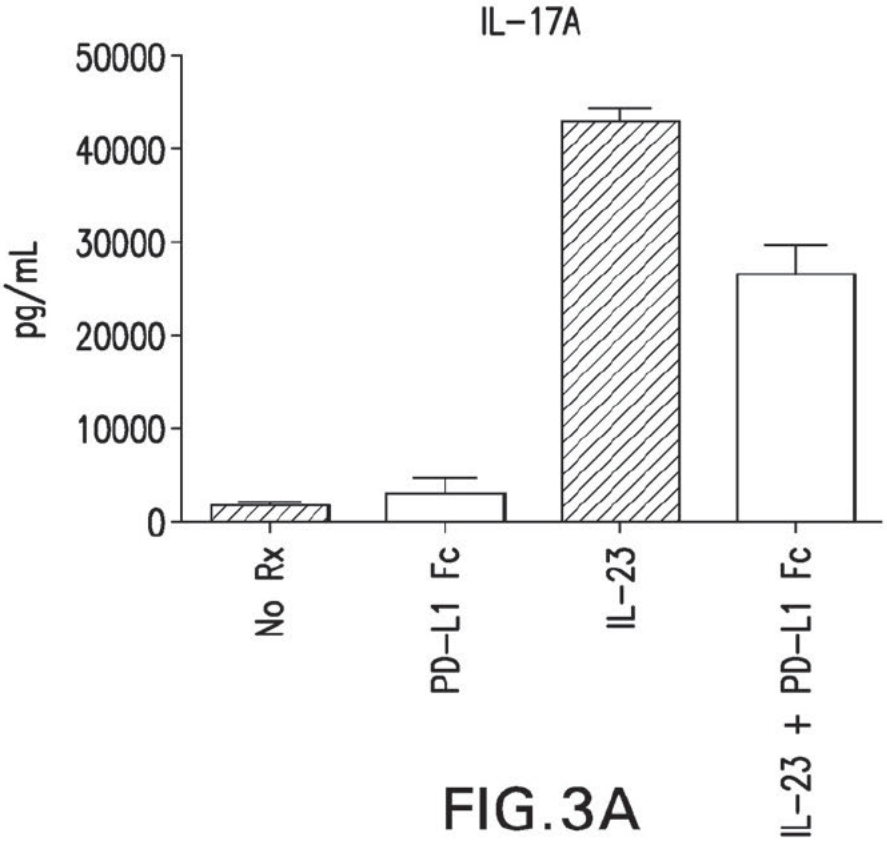

IL-17A
50000
40000
30000
20000
10000
0
pg/mL
No Rx
PD-L1 Fc
IL-23
IL-23 + PD-L1 Fc

FIG.3A

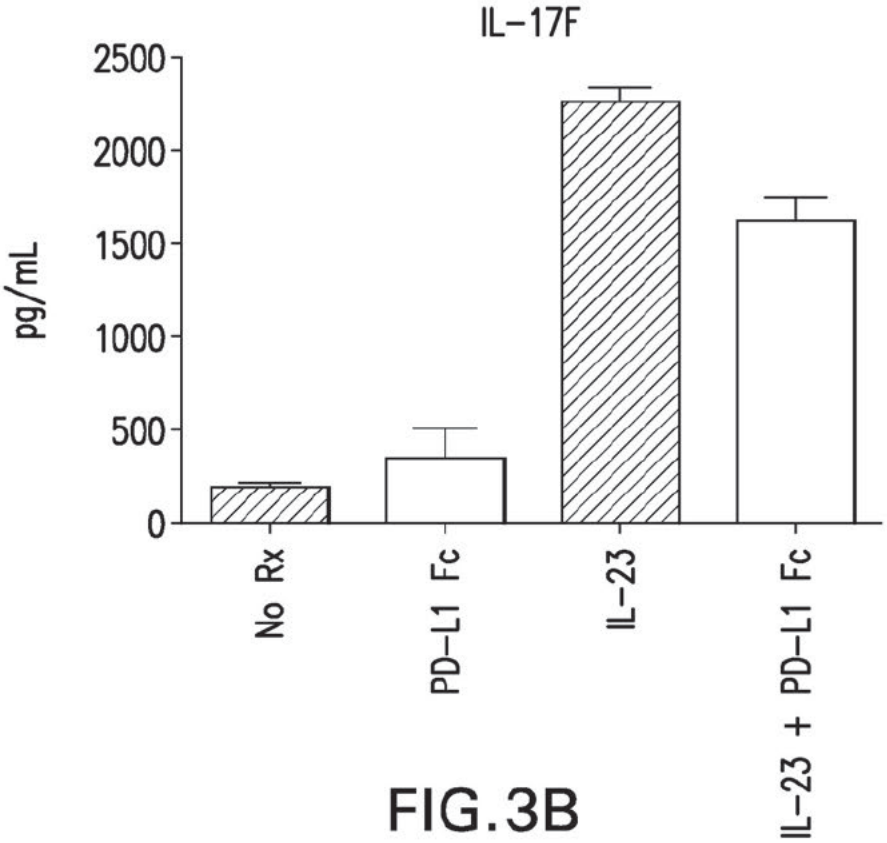

IL-17F
2500
2000
1500
1000
500
0
pg/mL
No Rx
PD-L1 Fc
IL-23
IL-23 + PD-L1 Fc

FIG.3B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2015/013642

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61P 37/00 (2015.01)
CPC - C07K 16/28 (2015.01)
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(8) - A61K 39/00, 39/395; A61P 37/00; C07K 16/00, 16/28 (2015.01)
USPC - 435/325, 375; 530/380, 386, 387.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
CPC - C07K 16/00, 16/18, 16/28, 2317/70, 2317/75 (2015.01)
(keyword delimited)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Orbit, Google Patents, PubMed, Google

Search terms used: treat psoriasis uveitis ibd copd selectively ablating gamma-delta γδ t cells il-23+ pd-1+

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,223,426 A (SKIBBENS et al) 29 June 1993 (29.06.1993) entire document	1-3, 13
Y	US 2013/0156790 A1 (INSTITUT GUSTAVE ROUSSY) 20 June 2013 (20.06.2013) entire document	1-3, 13
Y	US 8,193,321 B2 (BOSTROM et al) 05 June 2012 (05.06.2012) entire document	2, 3
A	PETERMANN et al. "γδ T Cells Enhance Autoimmunity by Restraining Regulatory T Cell Responses via an Interleukin-23-Dependent Mechanism," Immunity, 24 September 2010 (24.09.2010), Vol. 33, Iss. 3, Pgs. 351–363. entire document	1-19
A	US 2012/0213781 A1 (HILBERT) 23 August 2012 (23.08.2012) entire document	1-19

☐ 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
31 March 2015	06 MAY 2015

Name and mailing address of the ISA/US	Authorized officer:
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

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