

DECLARATION OF ROBERT J. BLOCH

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I, Robert J. Bloch, make this declaration in opposition against European Patent No. 2161336 B1.

I. INTRODUCTION

1. I was retained by counsel for Opponent Merck & Co., Inc. (“Merck”) to design and perform Surface Plasmon Resonance (“SPR”) testing using a Biacore instrument to investigate the binding characteristics of certain antibodies to the human receptor programmed death-1 protein (“PD-1”).
2. The studies I performed were blinded as to the identity of the antibodies I was testing, other than to be provided a minimal number of characteristics of the antibody (e.g., the identity of the constant region of the antibody) necessary to design and perform the experiments.
3. I performed the testing explained below based on protocols that I developed.

4. I was provided European Patent No. 2161336 B1 (the “‘336 patent”) to review. I have considered the ‘336 patent in the course of providing my opinions about the binding properties of the antibodies I tested in my investigations.

II. QUALIFICATIONS AND BACKGROUND

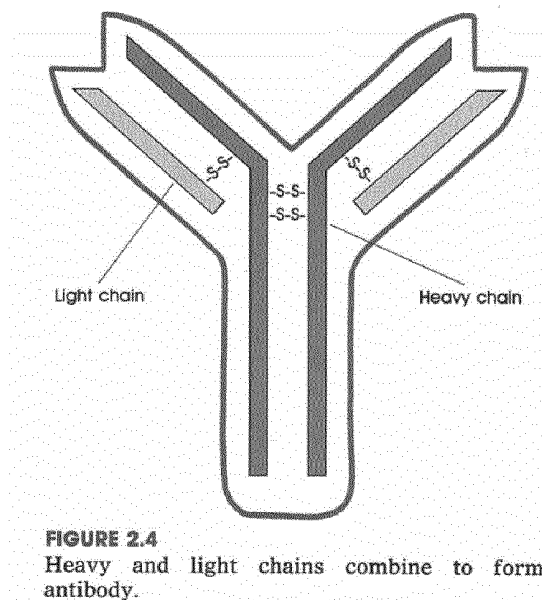
5. I hold a Ph.D. in biochemistry and molecular biology from Harvard University. My postdoctoral training was in biochemistry and cellular biology at the University of Basel, Switzerland, and in cellular neurobiology at the Salk Institute in La Jolla, CA. I have been working in the field of biochemistry and molecular biology for 40 years. I am currently a tenured professor of Physiology at the University of Maryland School of Medicine, Baltimore (UM). I have been at UM since 1980. My full CV is attached as **Annex A**.
6. As part of my responsibilities at the UM, I am the Director of the Biacore Core Facility, where I oversee the use of the Medical School’s Biacore 3000 instrument. In that capacity, I oversee all of the experiments run for UM scientists on that device. The Core Facility is staffed by a Ph.D. research scientist trained in the use of the Biacore instrument that I supervise and who assisted me in conducting the studies for this assignment. As Director of the Biacore Core Facility, I also oversee contract services on behalf of the UM for the use of the University’s Biacore equipment. For example, I routinely oversee contract experiments on the Biacore that contractors use to support publications and/or the filing of new drug or biological drug applications with the U.S. Food and Drug Administration. Such studies have included the kinetic and binding analysis of antibodies such as the ones conducted for this Opposition. I estimate that in the last ten years, I have carried out and/or supervised more than 500 Biacore studies for UM researchers and contractors.

III. ANTIBODIES

7. A great majority of the Biacore experiments that I have performed have investigated the binding properties of antibodies. I have extensive knowledge of both the structure and function of antibodies.

A. Antibody Structure

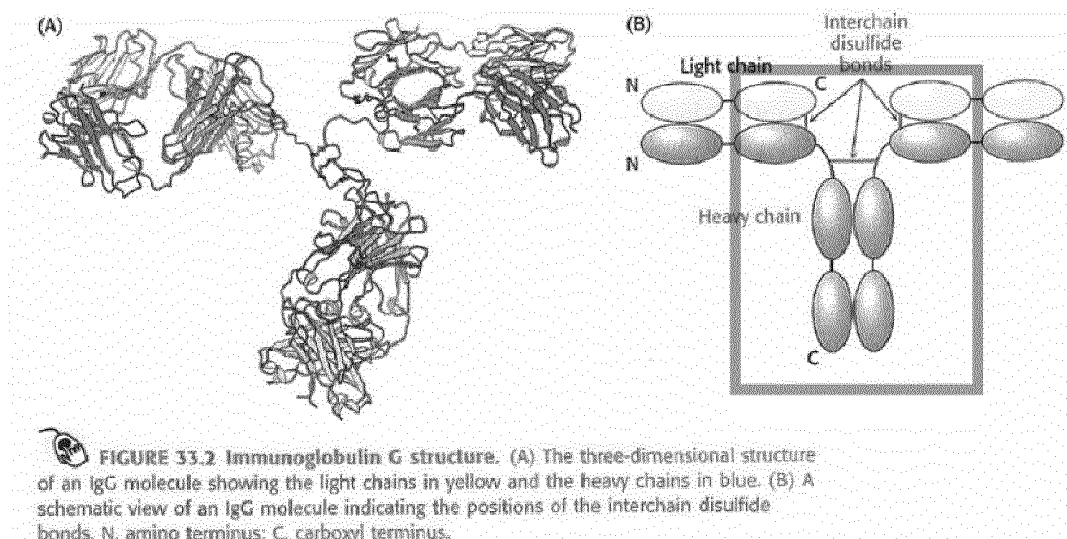
8. An antibody is a protein that can selectively bind to a substance. Antibodies are also referred to as immunoglobulins. The substance recognized by an antibody is referred to as the antigen.
9. An antibody is a “multimeric” protein – it consists of several polypeptide chains held together by covalent and non-covalent bonds. A typical tetrameric antibody structure contains four polypeptide chains – two identical “heavy” chains, and two identical “light” chains. In the tetrameric antibody structure, one heavy and one light chain pair up, and the two H-L pairs combine to form the tetrameric antibody complex. A depiction of the tetrameric structure of the antibody is provided in Ex. D1, Figure 2.4 from Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Press 9 (1988) (“Harlow 1988”):



10. The heavy chain of an antibody contains about twice as many amino acids as the light chain. See Ex. D2, Ed Harlow & David Lane, *Using Antibodies: A Laboratory Manual* 9-10 (1999) (“Harlow 1999”) (In the most prevalent antibody subclass, immunoglobulin G, there are approximately 440 residues in the heavy chain and about 220 amino acids in the light chain).
11. Each of the light and heavy antibody polypeptide chains contains a “variable” domain and a “constant domain.” The variable domain is generally responsible for the binding function of the antigen, while the constant region is generally responsible for interacting with immune system cells.
12. Antibodies are grouped into categories called isotypes based on the structure of the constant domain of the antibody. For example, there are five human isotypes of antibodies (i.e., IgG, IgA, IgD, IgE and IgM), each with a different constant region amino acid sequence. Ex. D3, Jeremy M. Berg et al., *Biochemistry* (5th ed. 2003), at p.294 (Figure 33.6: Classes of Immunoglobulin). In addition, certain antibody isotypes have

additional variations (e.g., the human IgG isotype contains IgG₁, IgG₂, IgG₃ and IgG₄ subtypes). These variations also are defined by unique constant region sequences that are species- and isotype-specific. Ex. D2, Harlow 1999, at p.8. Subject to minor allotypic variations, the constant domain of one isotype of an antibody within one animal species will have the same amino acid sequence as the constant domain in any other antibody of the same isotype from the same species, even if the two antibodies bind to different antigens.

13. A tetrameric IgG antibody is often depicted in two forms; namely: (a) as a three-dimensional ribbon model and (b) as a schematic figure. See Ex. D3, Berg at p.293 (Figure 33.2: Immunoglobulin G structure, reproduced below). In both depictions below, the yellow (lighter) segments denote the light chain of the antibody and the blue (darker) segments denote the heavy chain. The portion of the light and heavy chains within the gray box generally correspond to the constant domains of the antibody.

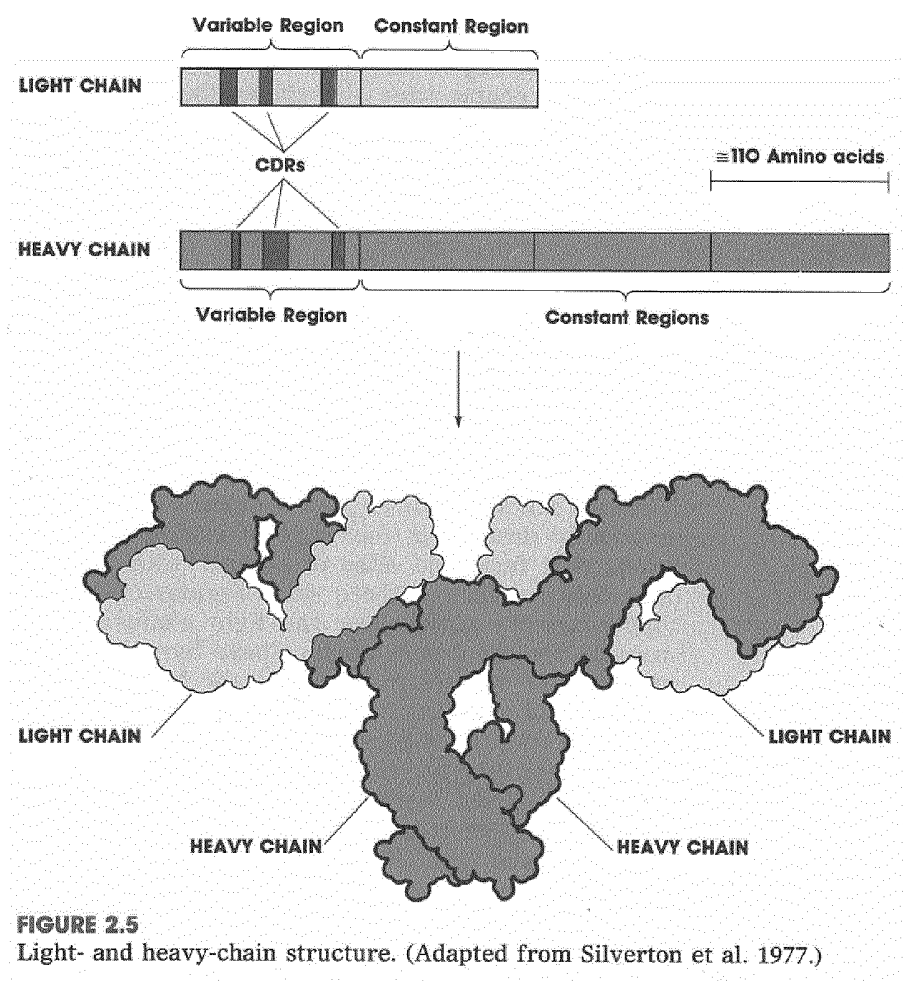


14. The constant domain of an antibody is the part of the antibody molecule that interacts with immune system cells and thereby mediates immune responses. Ex. D2, Harlow

1999, p.13. Different isotypes of antibodies mediate different types of immune responses, and at different levels. For example, in Antibody-Dependent Cell-mediated Cytotoxicity (ADCC), certain types of lymphocytes (e.g., natural killer or NK cells) kill target cells that have been marked by an antibody (i.e., by the antibody binding to the antigen on the target cell). The ADCC effector function is high for human IgG1 and IgG3, and but is relatively inactive for IgG2 and IgG4. See Ex. D4, Michael R. Clark, Antibody Engineering, Chemical Immunology 65, at p. 7. Another type of immune response – Complement-Dependent Cytotoxicity (CDC) – engages the complement pathway of the immune system to kill target cells marked by an antibody, effecting a complement cascade that activates the formation of a membrane attack complex on the surface of the target cell. The level of CDC effector function is high for human IgG1 and IgG3, low for IgG2, and null for IgG4. See Ex. D4, Clark at p.7.

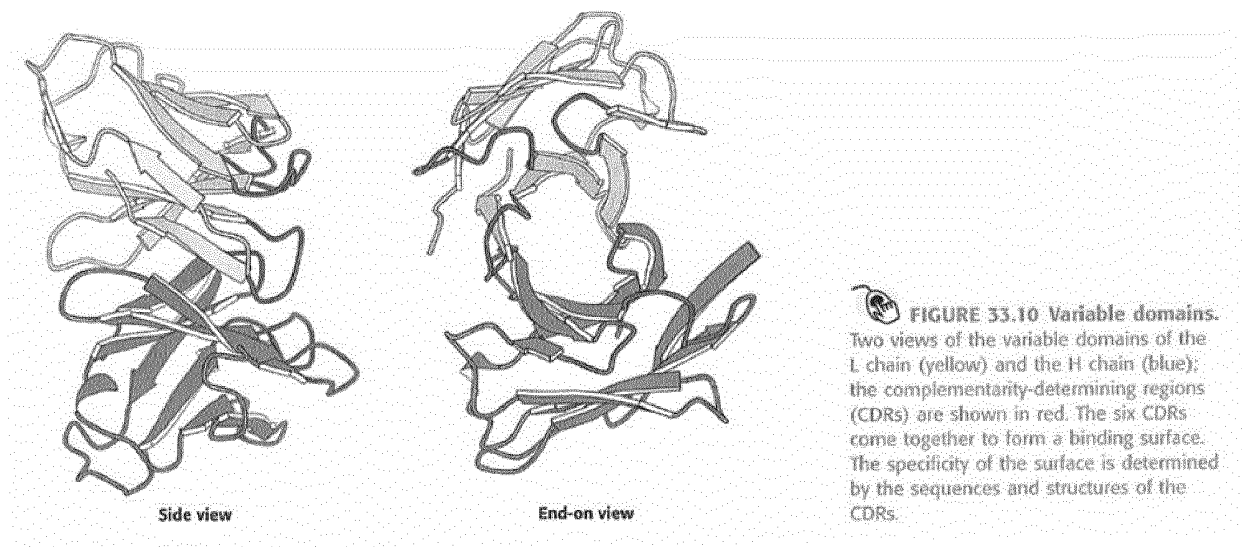
15. The variable domains of the light and heavy chains of the antibody contain the parts of the antibody molecule that physically interact with the antigen and enable the antibody to bind selectively to the antigen. See Ex. D3, Berg, at pp.926-27. The variable domains in the light and heavy chains of an antibody are called “variable” because they each contain amino acid sequences that vary from one antibody to the next, even if the antibodies have the same isotype and are from the same species. The unique amino acid sequences found in the heavy (V_H) and light (V_L) chains of the antibody give each antibody its unique antigen-binding capabilities. See Ex. D3, Berg, at pp.926-27.
16. The extensive variability in the amino acid sequences of the heavy and light chains of the antibody is localized to three sets of amino acid sequences termed the complementarity determining regions (CDR). The CDRs are also referred as “hypervariable” regions,

which is a name derived from the observation that the variability within these regions from one antibody to the next is extensive, as compared to the relatively unvarying amino acid sequences within the “framework regions” of the heavy and light chain variable domains of antibodies of a given isotype. See Ex. D3, Berg, at pp. 925-26. See also Ex. D1, Harlow 1988 at p.11 (Figure 2.5, reproduced below).



17. The three sets of CDRs in the antibody variable domain sequences play an important role in defining the unique three-dimensional shape of the antibody molecule that gives each antibody its unique antigen binding specificity. See Figure 33.10 from Ex. D3, Berg at p.927. Figure 33.10, reproduced below, illustrates how the six CDRs come together to

form a binding surface that interacts with a particular location on the antigen to which the antibody binds.



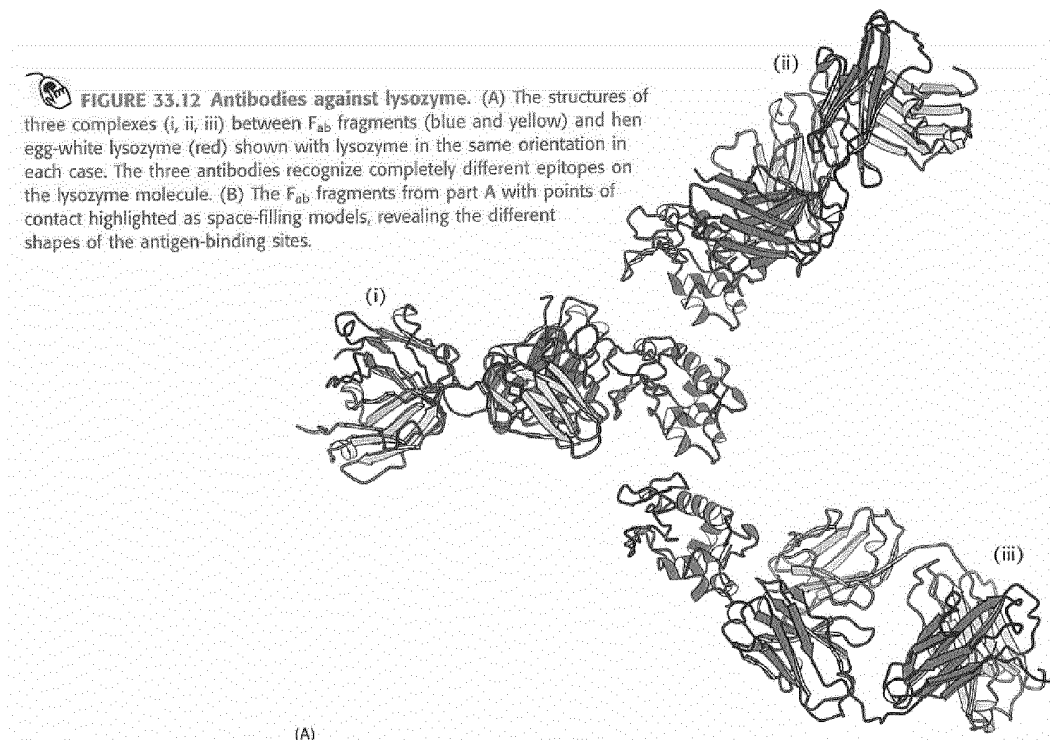
18. Antibodies exhibit tremendous variation in their antigen-binding properties, which is ultimately derived from variations in the CDR sequences. For example, it has been estimated that 5.8×10^6 possible distinct antigen-binding sites can be defined within the structure of an antibody's variable region. See Ex. D1, Harlow 1988 at 16. The localized nature of the specificity of antigen binding to the CDR sequences means that even minor changes to the CDR (or to the framework sequences that surround these sequences) can cause unpredictable and substantial effects on the binding properties of an antibody. See, e.g., Ex. D5, Urpo Lamminmäki et al., Modulating the Binding Properties of an Anti-17 β -estradiol Antibody by Systematic Mutation Combinations, *Protein Science* 12 (11): 2549 (2003); Ex. D6, Paul Carter et al., Humanization of an anti-p185^{HER2} antibody for human cancer therapy, *Proc. Natl. Acad. Sci. USA* 89(10):4285–9 (1992).

B. Antibody-Antigen Binding

19. Depending on its size and other characteristics, a protein antigen can present hundreds or thousands of distinct locations where different antibodies can bind in a unique manner to the protein. Ex. D7, Marc H. V. van Regenmortel, The Recognition of Proteins and Peptides by Antibodies, *Journal of Immunoassay* 21(2-3): 85, 85 (2000), at p.89.
20. The specific location on the antigen molecule where each antibody binds is called the “epitope.” Each epitope is a unique, three-dimensional arrangement of atoms within the antigen molecule that forms non-covalent interactions with atoms within the antibody molecule. The set of these non-covalent interactions establish and stabilize the antibody-antigen binding complex. See Ex. D7, van Regenmortel, at p.87.
21. The corresponding three-dimensional arrangement of atoms in the antibody variable region that interacts with the “epitope” on the antigen is called the “paratope.” See, generally, Ex. D8, Ian A. Wilson and Robyn L. Stanfield, Antibody-Antigen Interactions, *Current Opinion in Structural Biology*, 3:113-118 (1993); Ex. D9, N. S. Greenspan, Epitopes, paratopes and other topes: do immunologists know what they are talking about?, *Bull. Inst. Pasteur*, 90:267-279 (1992). The amino acid sequences within the variable region establish and maintain the three-dimensional structure of the paratope of the antibody that enables the antibody to exhibit its unique antigen binding specificity consistently. See Ex. D7, van Regenmortel, at p.86; Ex. D8, Wilson, at pp.113-114.
22. Two different types of protein epitopes are known to exist. Conformational epitopes, also called discontinuous epitopes, are formed by non-continuous sequences of amino acid residues of a protein. See Ex. D10, Julius M. Cruse, *Atlas of Immunology* (2d Ed. 2004) at pp.112-113 (“Conformational epitopes are discontinuous determinants on a protein antigen formed from several separate regions in the primary sequence of a protein

brought together by protein folding. Antibodies that bind conformational epitopes bind only native folded proteins.”). By contrast, linear or continuous epitopes are defined by contiguous stretches of amino acids of the protein.

23. As I explained earlier, many different and unique epitopes can exist on a single protein antigen. This phenomenon is illustrated by Figure 33.12 from Ex. D3, Berg, at p.928, reproduced below. Items (i), (ii) and (iii) in Figure 33.12 show a representation of a single antigen, egg-white lysozyme (red), interacting with the F_{ab} portions of three different antibodies (blue and yellow). A “Fab” fragment of an antibody contains light and heavy chain variable region sequences, but omits constant region sequences. The lysozyme molecule in each figure has been maintained in the same orientation to show that the three antibodies are interacting with different locations (i.e., different epitopes) on the molecule.



24. Depending on the location of the epitopes and other factors, two or more antibodies may be able to bind to the same antigen at the same time. For example, Figure 1 from Ex. D7, van Regenmortel at p.88 (below) schematically shows two different antibodies binding at two different epitopes on a single antigen:

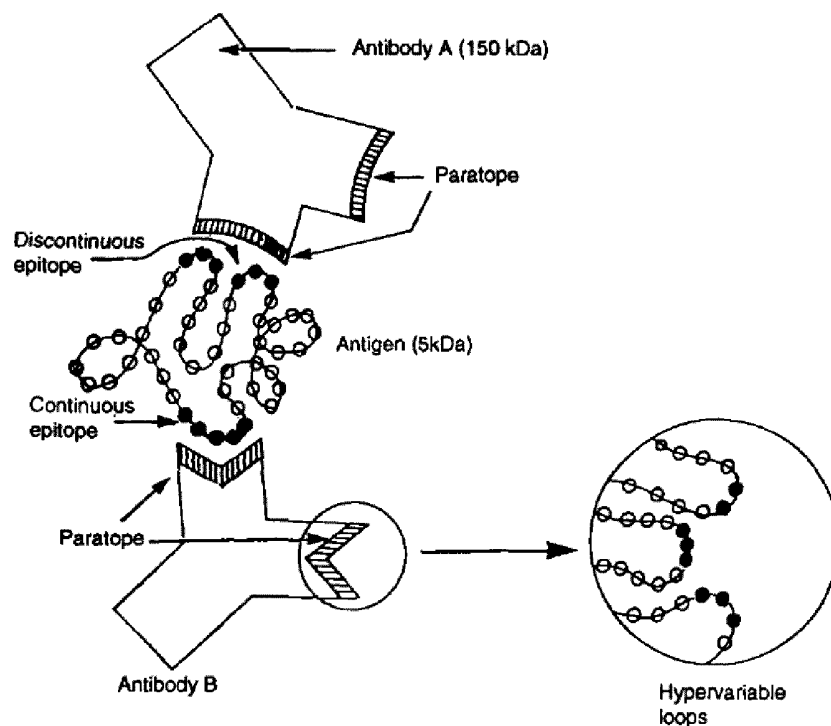


Figure 1 Interaction of two antibodies with a continuous and a discontinuous epitope of an antigen. Interacting amino acid residues are indicated in black.

25. In general terms, in order for a first and a second antibody to bind simultaneously to an antigen, (i) the antibodies must not physically interfere (cause “steric interference”) with each other’s binding to the antigen to such a degree that it prevents formation of a stable antibody-antigen binding complex; and (ii) the binding of the first antibody must not deform or alter the binding site (epitope) of the second antibody on the antigen (“allosteric interference”). See Ex. D1, Harlow 1988, at p.25.
26. The first of these parameters (“steric interference”) is influenced primarily by the three-dimensional space each antibody occupies when it is bound to the antigen. If a first

antibody occupies all or part of the three-dimensional space that a second antibody occupies when it binds to the antigen, there will be some amount of physical (steric) interference that can impede the ability of the second antibody to simultaneously bind to the antigen after the first antibody has bound to the antigen. Ex. D7, van Regenmortel, at p.98.

27. In some instances, steric interference may not entirely abolish the ability of a second antibody to bind to an antigen after a first antibody has bound. This is because several parts of an antibody molecule interact with the antigen molecule, and each of these interactions can contribute to varying degrees to forming and stabilizing the antibody-antigen complex. For example, the binding of a first antibody to the antigen may only interfere with the interactions between a part of a second antibody's paratope and the antigen, which may not be so severe as to prevent some amount of binding by the second antibody. In this case the binding of the second antibody might be reduced but not abolished.
28. The second parameter ("allosteric interference") is a consequence of the fact that proteins – including both antibodies and protein antigens – are large, complex structures and in solution do not maintain a static three-dimensional shape (conformation). Instead, the three-dimensional shape of a protein is dynamic, and will be influenced by the environment in which the protein is found and by interactions with other proteins, including antibodies. See Ex. D11, Rieko Ishima & Dennis A. Torchia, Protein Dynamics from NMR, *Nature Structural Biology* 7:740-743 (2000); Ex. D9, Greenspan, at p.273. In particular, the binding of a first antibody to a protein antigen can alter the three-dimensional shape of a portion of the antigen recognized by a second antibody, which

can interfere with the ability of that second antibody to bind simultaneously with the first antibody to that protein antigen. See Ex. D1, Harlow 1988, at p.25. This phenomenon can cause two antibodies to affect each other's binding properties to an antigen even if the epitopes for the two antibodies are physically remote and would not cause steric interference. Ex. D12, Application Note 1: Characterization of Monoclonal Antibody Epitope Specificity Using Biacore's SPR Technology, Biacore Manual *6 (February 2002) ("Application Note"); see also Ex. D13, Chung-Jung Tsai et al., Protein Allostery, Signal Transmission, and Dynamics: A Classification Scheme of Allosteric Mechanisms, *Mol. BioSyst.* 5: 207-216 (2009).

29. Like steric interference, sometimes allosteric interference may only partially hinder the ability of a second antibody to bind to the antigen after the first antibody has bound the antigen. This may be because the protein antigen has deformed in such a way as to only partially disrupt the binding site of the second antibody, such that the antibody's binding is reduced but not abolished..
30. Both factors – steric and allosteric interference – may affect the ability of two antibodies to interact simultaneously with an antigen. In addition, because antibodies themselves are large proteins, the physical structure (and therefore binding properties) of each of the antibodies may also be influenced by conformational changes associated with the binding of each antibody to the antigen. See, e.g., Ex. D8, Wilson, at p.117.

IV. EXPERIMENTAL MEASUREMENTS OF ANTIBODY-ANTIGEN INTERACTIONS

31. Several experimental techniques are routinely used to investigate binding properties of antibodies. These techniques can, generally speaking, be divided into two categories. The first category of experiments can be used to identify the locations on the antigen molecule where the antibody molecule interacts (the "epitope") and forms the antibody-

antigen complex. The second category dynamically measures interactions between antibodies and the antigen in solution. For simplicity, I will refer the first type as “structural epitope mapping experiments” and the second type as “binding experiments.”

32. Examples of structural epitope mapping techniques include:

- (i) X-ray crystallography, which can reveal the spatial locations of atoms in the antigen and in the antibody molecules when the two molecules are in the antibody-antigen binding complex. Ex. D2, Harlow 1999, at p.400; Ex. D7, van Regenmortel, at p.90. Several groups have used X-ray crystallography to characterize the binding sites (epitopes) of different antibodies on a protein antigen. See, e.g., Ex. D14, Matthew C. Franklin et al., Insights into ErbB Signaling from the Structure of the ErbB2-Pertuzmab Complex, *Cancer Cell* 5:317-328 (April 2004); Ex. D15, Yvonne Chen et al., Selection and analysis of an optimized anti-VEGF antibody: crystal structure of an affinity-matured Fab in complex with antigen. *Journal of Molecular Biology*, 293(4):865–81 (1999); Ex. D16, David A. Dougan et al., Effects of substitutions in the binding surface of an antibody on antigen affinity, *Protein Eng.*, 11(1):65–74 (1998); Ex. D17, David R. Davies & Gerson H. Cohen, Interactions of protein antigens with antibodies, *Proc. Natl. Acad. Sci. USA* 93(1):7–12 (1996).
- (ii) Binding experiments using structural variants or fragments of a protein antigen can identify the parts of a protein antigen that are involved in binding to the antibody. If the antibody cannot bind or binds with reduced affinity to the modified antigen or a protein fragment, the site of the mutation or of the fragment is likely involved in antibody binding. Examples of structural variants typically

used in these types of experiments include proteins where one or more amino acids have been changed relative to the wild type sequence, or enzyme digests that have been fractionated and synthetic peptides matching sequences in the protein antigen. Ex. D2, Harlow 1999, at p.385-400.

- (iii) Nuclear Magnetic Resonance (“NMR”) spectroscopy can be used to identify the particular amino acid residues in a protein antigen that are interacting with amino acid residues in the antibody molecule when the antibody-antigen complex has been formed. See, e.g., Ex. D18, Wu et al., Structural Basis of a Flavivirus Recognized by Its Neutralizing Antibody, J. of Biological Chem. 278(46): 46007-46013 (2003).
33. In contrast to structural epitope mapping techniques, simultaneous binding experiments investigate interactions between two or more antibodies when they are present in solution with an antigen. Binding experiments by themselves cannot reveal where on the antigen each of the antibodies are binding because they are only measuring the impact each antibody might have on the binding of the other antibody to the antigen. Binding experiments are often used with other experiments that are capable of identifying the location on the antigen of the epitope for the different antibodies.
34. The ‘336 patent identifies a number of techniques or instruments that can measure binding properties of antibodies, including surface plasmon resonance (e.g., measure with a Biacore instrument), ELISA assays and flow cytometry. See ‘336 at ¶ 070.
35. Binding experiments can measure the interaction of antibodies with either isolated proteins (i.e., Biacore, ELISA) or with more complex structures, such as proteins present on a cell surface (i.e., flow cytometry / fluorescence activated cell sorting (FACS)). In

both types of binding experiments, the amount of antibody that binds to the antigen is measured, which can then be used to calculate certain functional properties of the antibody-antigen binding interaction, such as the affinity of the antibody.

36. Binding experiments can provide some insights into the relative location of two epitopes on an antigen. For example, experimental results showing that two antibodies can bind simultaneously to the antigen at levels similar to those seen when each antibody is binding to the antigen without the other antibody present suggest that the two antibodies are binding to sterically distinct epitopes on the antigen (i.e., the two antibodies are not occupying the same three-dimensional space when each binds to its respective epitope). See Ex. D19, BIAtechnology Handbook, Biacore (1998), at p.6-8; Ex. D2, Harlow 1999, at p.383.
37. On the other hand, an experimental result showing a partial or complete reduction in binding by a second antibody to the antigen after a first antibody has bound to the antigen by itself does not establish that the two antibodies are binding to the same epitope because the partial or complete elimination of binding could be due to steric or allosteric hindrance (or some combination of these factors) instead of the antibodies binding to the same epitope on the antigen. See Ex. D19, BIAtechnology Handbook, at p.6-8; Ex. D2, Harlow 1999, at p.400.
38. The ability of two antibodies to bind simultaneously to an antigen in an experimental setting may also depend on the order in which the antibodies are exposed to the antigen. For example, a first antibody may bind to an epitope that affects the conformation of the antigen and allosterically interfere with binding by the second antibody. The first antibody also may sterically obstruct access to a part of the epitope of the second

antibody that contributes to the antibody binding properties of the second antibody. In either situation, the converse may not be true (i.e., that the binding of the second antibody may not deform the epitope for the first antibody or may not interfere sterically with the binding of the first antibody to the antigen). See Ex. D19, BIAtechnology Handbook, at p.6-9.

39. As I noted above, binding experiments also can provide some insights into the location of an epitope on an antigen. For example, a Biacore instrument can be used to measure binding properties of antibodies to different forms of a protein (e.g., a wild type (native) sequence and a mutant having a substitution at a defined location of its amino acid sequence). If a first antibody binds to the wild-type protein but cannot bind to the mutated form of that protein, it is reasonable to infer that the mutated residue is in the epitope for that antibody. Conversely, if a second antibody binds equivalently to both the mutated and wild-type forms of the protein, one can reasonably infer that the residue is not located in the epitope for that antibody. Ex. D7, van Regenmortel, at p.97.

V. “CROSS-COMPETITION”

40. I have been asked to consider the terms “cross-compete” and “cross-competition” as they are used in the ‘336 patent and how they would be understood.
41. Claim 3 of the ‘336 patent states: “A monoclonal antibody, or antigen binding portion thereof, wherein the antibody cross-competes for binding to PD-1 with the antibody of claim 1 or 2.” I understand that the 5C4 antibody is one of the antibodies of claims 1 or 2.
42. Paragraph 070 of the ‘336 patent contains the following discussion of “cross-competition”:

In another embodiment, the invention provides antibodies that bind to the same epitope on human PD-1 as any of the PD-1 monoclonal antibodies of the invention (i.e., antibodies that have the ability to cross-compete for binding to PD-1 with any of the monoclonal antibodies of the invention). In preferred embodiments, the reference antibody for cross-competition studies can be the monoclonal antibody 5C4 (having V_H and V_L sequences as shown in SEQ ID NOs: 4 and 11, respectively). Such cross-competing antibodies can be identified based on their ability to cross-compete with 5C4 in standard PD-1 binding assays. For example, BIAcore analysis, ELISA assays or flow cytometry may be used to demonstrate cross-competition with the antibodies of the current invention. The ability of a test antibody to inhibit the binding of, for example, 5C4 to human PD-1 demonstrates that the test antibody can compete with 5C4 for binding to human PD-1 and thus binds to the same epitope on human PD-1 as 5C4. In a preferred embodiment, the antibody that binds to the same epitope on human PD-1 as 5C4 is a human monoclonal antibody. Such human monoclonal antibodies can be prepared and isolated as described in the Examples.

43. I find this passage to be unclear because it suggests there can be different meanings for the term “cross-compete.”
44. First, the ‘336 patent states that an antibody will “cross compete” with a reference antibody to PD-1 if the two antibodies bind to the same epitope. This is true – if two antibodies bind to the same epitope, they will compete for binding to the PD-1 protein because two antibodies cannot simultaneously occupy the same physical space, which would happen if the two antibodies are binding to the same epitope on the PD-1 protein.
45. However, the ‘336 patent also states that the “ability of a test antibody to inhibit the binding of, for example, 5C4 to human PD-1 demonstrates that the test antibody can

compete with 5C4 for binding to human PD-1 and *thus binds to the same epitope on human PD-1 as 5C4.*” (emphasis added). This statement is not scientifically accurate because inhibition in 5C4 binding to human PD-1, seen in a binding experiment where a test antibody is present may occur even if the test antibody is binding to a different epitope on the PD-1 relative to the 5C4 epitope.

46. As I have explained above, factors other than binding to the same epitope can cause inhibition of binding of one antibody when another antibody is present, including steric and allosteric interference caused when the test antibody or the 5C4 antibody binds to the PD-1 protein. Either of these factors – or both of them together – could cause the experimental result of inhibition of binding to be observed despite the fact that the two antibodies are binding to different epitopes on PD-1.
47. For example, inhibition caused by steric interference may be a factor given the relative sizes of the PD-1 extracellular domain and antibody proteins. The molecular weight of the extracellular domain (ECD) of the human PD-1 protein is only approximately 16.8 kDa. Ex. D20, ExPASy Bioinformatics Resource Portal calculation based on ECD of UniProt Sequence Q15116[21-170] (available at http://web.expasy.org/compute_pi/); Ex. D21, UniProt Sequence Q15116 (available at <http://www.uniprot.org/uniprot/Q15116>). By contrast, antibodies are much larger molecules (e.g., ~ 150 kDa). When the test antibody binds to its epitope on the ECD of PD-1, it may – due to its size – obscure access to the 5C4 epitope on the ECD of PD-1, even if the two epitopes are made up of entirely separate, spatially distant sets of atoms in the PD-1 molecule.
48. Allosteric effects may also be observed if the binding of PD-1 by the test antibody alters the conformation of the PD-1 protein in a way that deforms the three dimensional shape

of the 5C4 epitope, which could inhibit to some degree binding by 5C4 to the PD-1 protein.

49. The '336 patent indicates that "standard PD-1 binding assays" should be used to determine if an antibody "cross-competes" with 5C4 for binding to human PD-1, and explains that "cross competition" is shown if the test antibody inhibits the binding of 5C4 to human PD-1. The '336 patent, however, does not identify the experimental parameters to be used in these standard "cross-competition" assays. The '336 patent also does not contain any examples of cross-competition binding experiments. The examples of experiments in paragraphs 274 (BIAcore), 275 (flow cytometry) and 276 (ELISA) are not testing for simultaneous interactions between two different antibodies with human PD-1. Rather, they are only testing properties of a single antibody interacting with PD-1. The Biacore experiments described in paragraph 274 are measuring the affinities and binding kinetics of each the antibodies by itself to a human PD-1-Fc fusion protein. The flow cytometry experiments in paragraph 275 are testing the ability of each of the antibodies alone to bind to CHO cells that express recombinant human PD-1 at the cell surface. The ELISA experiments in paragraph 276 are testing whether individual antibodies are capable of binding to a number of other proteins (ICOS, CTLA-4 and CD28).
50. The '336 patent also does not indicate what degree of inhibition must be observed in a experimental binding assay for two antibodies to meet the "cross-compete" definition. For example, the '336 patent does not indicate whether "inhibition" must be complete (i.e., no detectable binding by the 5C4 or test antibody after the other antibody has been added) or partial (i.e., above some percentage of a reduction in the binding of the antibody relative to the amount of binding observed when the other antibody is not

present). I note that it is possible for even the same antibody in experimental binding assays to fail to achieve 100% inhibition of binding of itself to its antigen.

51. I also note that inhibition in experimental binding assays of 5C4 binding to PD-1 caused by a test antibody may not be observed to be reciprocal (i.e., 5C4 may not inhibit test antibody binding to PD-1 or may not inhibit it to the same degree). It is not uncommon to observe in a binding study different levels of binding inhibition depending on the order of loading the two antibodies being tested. Again, the '336 patent does not indicate whether the same amount of inhibition must be observed in each direction of a binding experiment in order for the antibodies to meet the definition of "cross-competition" in binding to PD-1.
52. In this declaration, I present results from my investigations using conventional binding studies performed using a Biacore instrument as to whether a number of test antibodies inhibit the binding of nivolumab (5C4) to human PD-1 proteins, and whether 5C4 inhibits the binding of those test antibodies to those human PD-1 proteins. I also present my opinions based on these investigations as to whether each of the test antibodies "cross-competes" for binding with 5C4 to human PD-1 within the meaning of the '336 patent.

VI. SURFACE PLASMON RESONANCE (BIACORE) BINDING STUDIES

A. Basic Principles of Surface Plasmon Resonance (Biacore) Studies

53. The Biacore system is widely used to measure antibody binding using a technique called Surface Plasmon Resonance (SPR). The Biacore instrument can measure the strength of the association between an antibody and antigen, and the rate at which the antibody binds to the antigen, and rate at which the antibody-antigen complex dissociates. See Ex. D19, BIAtechnology Handbook, at p.5-1.

54. Biacore instruments function by measuring changes in the index of refraction close to the surface of a sensor chip in real time and recording those changes as resonance units (RU). Figure 1-3 below illustrates how Biacore instruments measure the index of refraction.

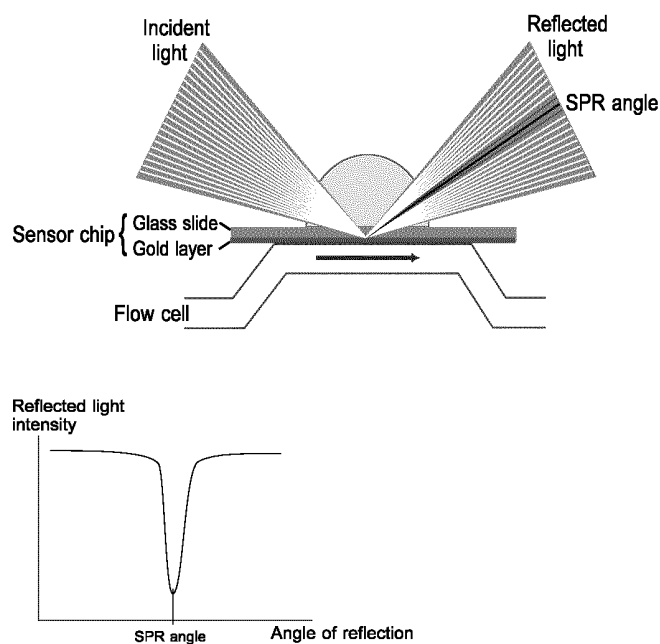


Figure 1-3. The SPR detection principle.

Ex. D22, Biacore Sensor Surface Handbook BR-1005-71 Edition AB, GE Healthcare (First published 2005), at p.9 (“Biacore Sensor Surface Handbook”).

55. The figure below illustrates how SPR can be used to detect changes to a surface:

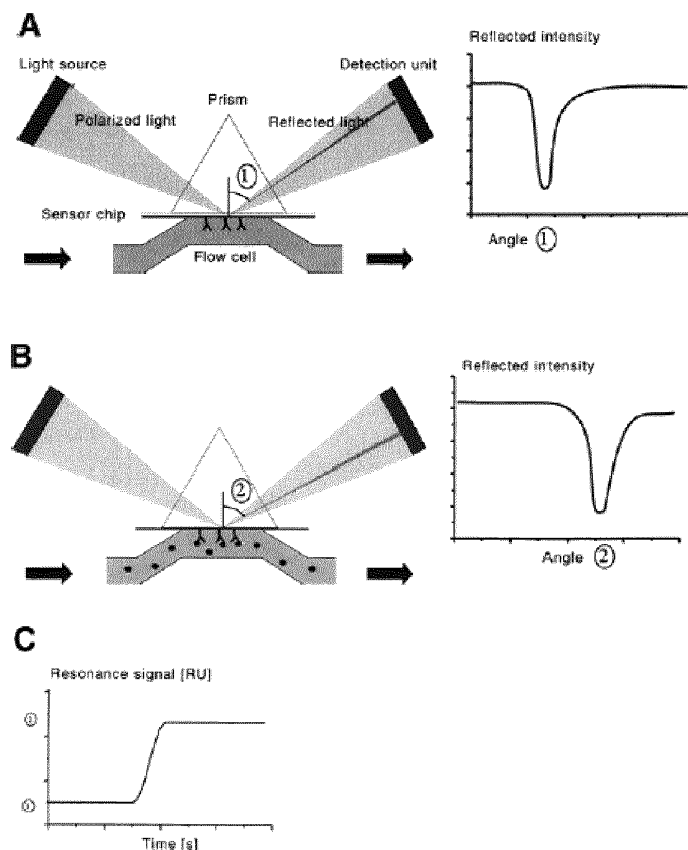


Fig. 1. The principle of SPR signal detection. Surface plasmon resonance detects changes in refractive index of the surface layer of a solution in contact with the sensor chip. The change in refractive index is caused by variation of the mass on the sensor chip surface owing to interactions of the biomolecules (A,B). A dip in the intensity of the reflected light occurs at a certain angle, which is referred to as the resonance signal (RU) (C). The shift in resonance signal is plotted against time and displayed in a sensorgram (from ref. 1).

Ex. D23, Berit Johne, Epitope Mapping by Surface Plasmon Resonance in the BIAcore, Molecular Biotechnology 9:65, 66 (1989).

56. In simple terms, in a Biacore instrument, light is reflected off a surface (the sensor chip) opposed to one containing the molecules being tested for interaction. At the resonance angle, a dip in intensity of the reflected light will be observed that correlates to the mass concentration at the surface containing the molecules. If the mass concentration increases (i.e., because another molecule has formed a complex with a first molecule

already bound to the surface), the angle of reflection will change. Ex. D24, John, at p.65.

57. The measured resonance angle is plotted against time to generate a sensorgram. A schematic illustration of a sensorgram provided by the manufacturer of the Biacore instrument is provided below. See Ex. D22, Biacore Sensor Surface Handbook, at p.8 (Figure 1-2). The sensorgram shows a baseline value in buffer, after which a solution with an analyte that binds to the surface is injected. This value is defined in “Resonance Units,” or RU, in which 1 RU is equivalent to the change that occurs when 1 picogram of analyte associates with the surface. The increase in the RU measured over time, as in the figure below, indicates that the analyte has bound to the surface. The binding can be followed in real time until the report point where the interaction is at steady state equilibrium. See also Ex. D19, BIAtechnology Handbook, at pp.4-3 to 4-5. The sensorgram also shows what occurs when the analyte is removed from the solution and dissociates from the surface. The dissociation, too, can be followed in real time until the system reaches its original baseline RU, or, as illustrated here, the system is regenerated in preparation for a second study.

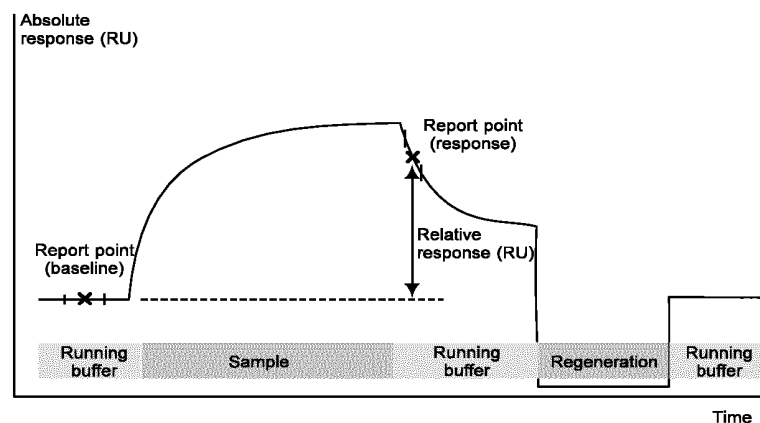
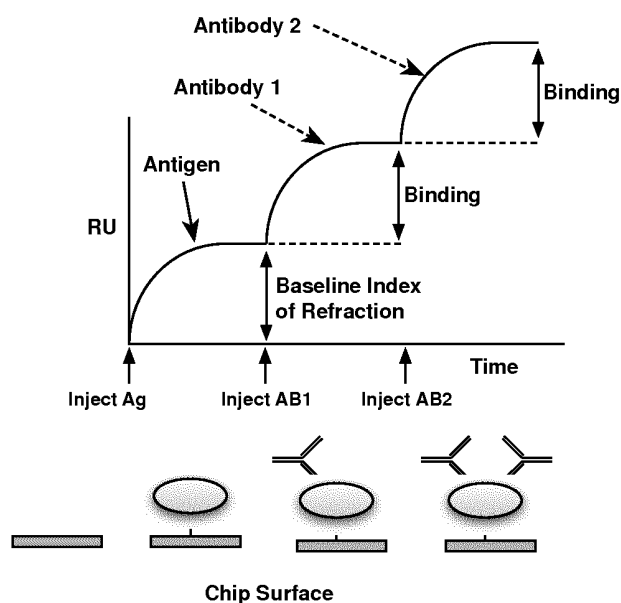


Figure 1-2. Schematic illustration of a sensorgram. The bars below the sensorgram curve indicate the solutions that pass over the sensor surface.

B. Direct and Indirect Capture Binding Studies

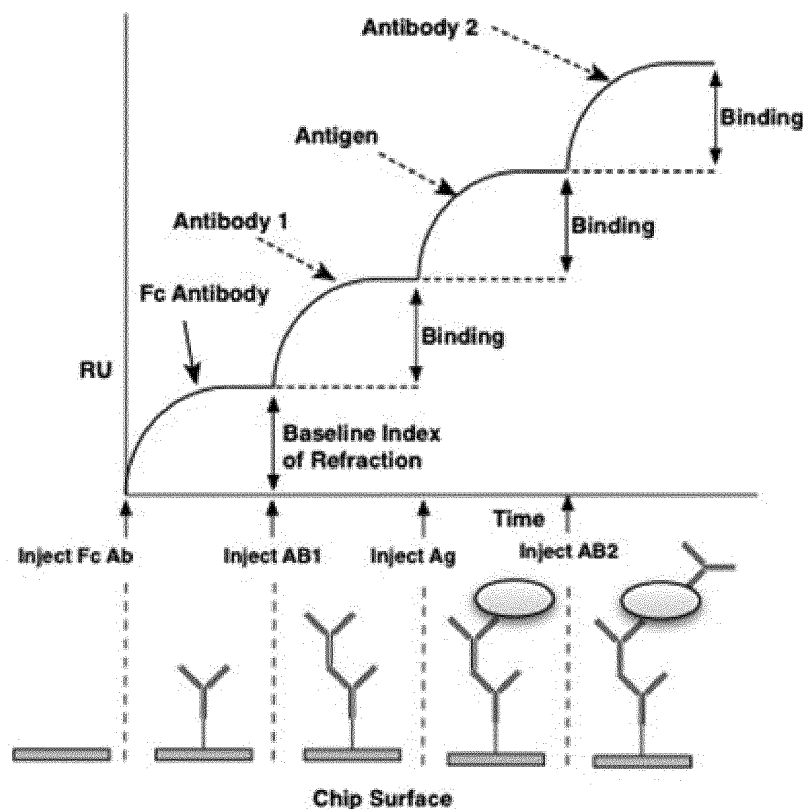
58. Two general experimental designs are typically used to conduct binding studies with one or more antibodies and an antigen on a Biacore instrument.
59. In a “direct capture” experiment, the antigen is first bound to the surface of the sensor chip through a covalent linkage. The RU measured for this binding event provides a baseline value that is used to quantify the amount of other analytes (antibodies) that interact with the antigen.
60. To test the binding properties of a first antibody, a solution containing that first antibody is flowed across the sensor chip. If the first antibody binds to the antigen, the Biacore instrument will record a change in RU. Repeated application of the first antibody at near saturating concentrations will lead to smaller changes in RU as saturation of its sites on the antigen on the surface is approached.
61. At this point, while the first antibody is bound to the antigen, a second antibody can be added to the reaction environment to determine if it can simultaneously bind to the antigen. This is done by injecting a solution containing the second antibody into the Biacore instrument. The second antibody will flow across the sensor chip containing the antigen bound to saturation with the first antibody. If the second antibody also binds to the antigen, the Biacore instrument will record an additional change in RU. See, e.g., Ex. D23, Johne, at pp.67-68.
62. A hypothetical direct binding experiment with two antibodies that bind to different binding sites on an antigen is illustrated in the figure below.



63. In an “indirect capture” experiment, an anti-Fc antibody specific to the constant region of the first test antibody is first bound to the surface of the sensor chip. Again, this will provide a baseline index of refraction. Next, a solution containing the first test antibody is injected into the Biacore instrument, which causes the antibodies to flow over the chip surface to which are bound the anti-Fc antibodies. This will cause the first antibody to be captured by its constant region, leaving its variable domains available to engage antigen. After this equilibrium is reached, a solution containing the antigen is flowed across the sensor chip. If the first captured antibody binds to the antigen, the Biacore instrument will record a change in RU.
64. Again, a second antibody can be added to the reaction environment to determine if it can simultaneously bind to the antigen at the same time as the first antibody. I note that if the second antibody is of the same isotype and species as the first antibody, it will first be necessary to inject a blocking antibody (i.e., an antibody of the same species/isotype as the first antibody but which does not bind to the antigen). This is typically done to block

any remaining open anti-F_c antibody binding sites on the chip, which would record a change in resonance if those binding sites were to capture the second test antibody. Then, a solution containing the second antibody is flowed across the sensor chip on which is the antigen, which is bound to the first test antibody. If the second test antibody binds to the antigen, the Biacore instrument will record an additional change in RU. See, e.g., Ex. D23, Johne, at p.67 (Fig. 2).

65. The indirect capture design is depicted in the figure below:



66. Both types of experiments will reveal if there is binding between the antibody and the antigen. If binding occurs, the kinetics of that binding can be determined, including the affinity of each antibody for the antigen and the “on” (k_{on}) and “off” (k_{off}) rates for the association and dissociation reactions. See Ex. D23, Johne, at pp.67-68.

67. The indirect capture design can reduce the contribution of avidity to the measured binding affinity of an antibody-antigen interaction. See, e.g., Ex. D24, Laure Jason-Moller et al., *Current Protocols in Protein Science* 19.13.1-19.13.14 (2006), at p.19.13.8. An avidity effect can be observed if multiple antigens are in close proximity to the two arms of the antibody, and can influence the experimentally measured binding affinity for the antibody (e.g., it can make it appear that the antibody is binding the antigen more tightly). See also Ex. D2, Harlow 1999, at pp.34 and 386.
68. In studies of antibody binding with Biacore technology, changes in RU are measured over time from the point of injection of a sample containing a test or reference antibody, or the antigen. Additional injections of the same analyte (e.g., the reference antibody) are often made to ensure that all available binding sites on the chip are occupied before introducing the second antibody into the machine. See, e.g., Ex. D12, Application Note, at p.*6 (“in this type of experiment [i.e., a competitive binding experiment between two antibodies binding to a single antigen], saturation of the surface binding sites at each stage is essential. Each MAb [i]s therefore injected over a longer time period than for the pair-wise binding tests, until a plateau [i]s reached in the SPR signal.”).
69. A model sensorgram created by multiple “saturating” injections of an analyte is illustrated in Ex. D22, Biacore Sensor Surface Handbook, in Figure 7-1, reproduced below:

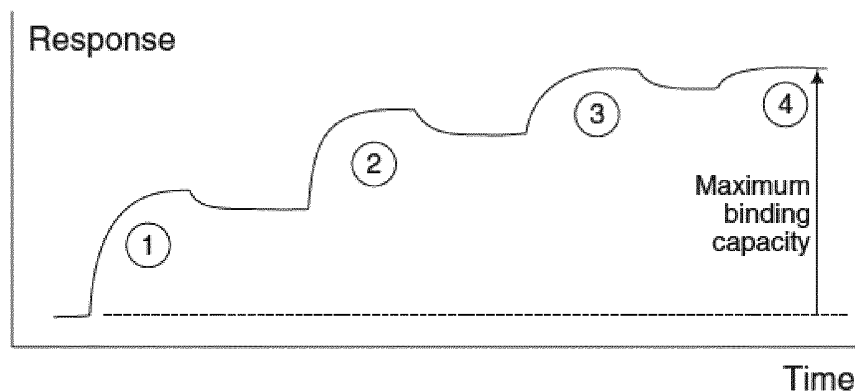


Figure 7-1. Repeated injections of analyte without regeneration can be used to estimate the maximum analyte binding capacity of the surface.

Ex. D22, Biacore Sensor Surface Handbook, at p.79.

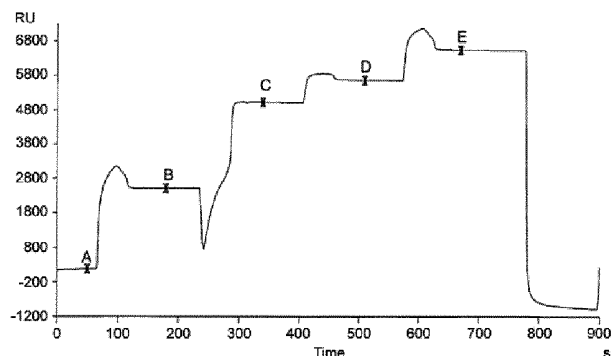
70. Saturating the binding sites on the chip surface in multiple analyte binding experiments is important because it helps to ensure that an increase in measured RU is due to interactions between the analyte and the ligand. For example, in a direct capture experiment, multiple injections of the first antibody are typically made to occupy all accessible binding sites on the chip (i.e., all of the antigen molecules coupled to the chip), which “saturates” the chip. Then, when the second antibody is injected, it will either bind to the antigen simultaneously with the first antibody or not bind at all. If the chip surface is saturated, and the RU measured when the second antibody is injected increases to a level above the RU value reached at saturation for the first antibody, that experimental result supports the conclusion that the first and second antibodies are binding simultaneously to the antigen. If the RU measured when the second antibody is injected is at or below the RU value at saturation for the first antibody, that increase in RU may be due to binding of the second antibody to free antigen molecules on the surface (i.e.,

antigen molecules coupled to the chip from which the first antibody dissociated in the intervening time interval, needed for washing and introduction of the second antibody).

71. In the Biacore instrument, a buffer solution continuously flows over the chip surface and removes any unbound analytes from the reaction environment. Because, antibodies form complexes with antigens that exist in an equilibrium, at any point in time, some amount of the antibody bound to an antigen on the Biacore chip surface will dissociate from the antigen and will be washed out of the reaction environment, which will cause the RU value at equilibrium to gradually decrease over time. Thus, in a typical sensorgram measuring interactions of an antibody with an antigen over time, the RU values will show a plateau indicating that a binding equilibrium has been established, and will then gradually decrease once the buffer solution containing the antibody is replaced with buffer without antibody. See Ex. D19, BIA technology Handbook, at p.5-6 (Figure 5-1).
72. In the “direct capture” assay, the magnitude of a change in RU for the first binding interaction (reference antibody to antigen) is compared to the magnitude of the change in RU (if any) associated with the second binding interaction (test antibody to antigen). If the RU value measured after equilibrium has been established with the second binding interaction exceeds the RU value seen at the point of saturation for the first binding interaction, it is reasonable to conclude that the two antibodies are simultaneously binding to the antigen. See, e.g., Ex. D23, Johne, at pp.67-68 (Figures 2 & 3). In other words, if the magnitudes of the increases in RU measured at equilibrium for the first and the second antibodies are equivalent in scale, this suggests that the reference and test antibodies are binding to the antigen simultaneously without interfering with one another. One can infer from such data that the reference antibody binds at a different and

- independent epitope relative to the epitope bound by the test antibody. See Ex. D19, BIAtechnology Handbook, at p.6-8.
73. If, on the other hand, there is in a “direct capture” assay less than an equivalent increase in the magnitude of RU measured when the second antibody is added, or no change at all, this suggests the first antibody and second antibody are interfering with each other’s ability to bind to their respective epitopes in some manner. See Ex. D19, BIAtechnology Handbook, at p.6-8.
74. Care must be taken in evaluating increases in RU values seen with the second binding interaction in “direct capture” assays. For example, if the increase in RU observed at equilibrium after injection of a second antibody does not exceed the RU values observed after injection of a saturating amount of the first antibody, the increase may be simply be due to the second antibody occupying open binding sites available on the chip surface (i.e., sites that were never occupied by the first antibody or which were occupied by the first antibody but have become available through dissociation subsequent to the time saturation was achieved). When evaluating direct binding results, it is important to compare any RU increases at equilibrium for the second antibody relative to the RU seen at saturation with the first antibody. See Ex. D19, BIAtechnology Handbook, at p.6-9.
75. Biacore instruments can also be used to determine if different antibodies can simultaneously bind to an antigen by “indirect capture.” Figure 2 from Ex. D12, Application Note of the Biacore 3000 Manual, at p.4 (reproduced below) showing a sensorgram for an example of “indirect capture” Biacore experiment.

Figure 2
Example of a sensorgram
obtained from epitope
specificity determination for
two MAbs directed against
independent epitopes. The
SPR response gives the
amount of surface-bound
component at each stage as
follows: (A) baseline signal,
(B)-(A) first MAb, (C)-(B)
blocking antibody, (D)-(C) p24,
(E)-(D) second MAb.



76. In Figure 2, the first antibody is injected into the system at point (A); it has reached equilibrium and is bound to the surface at point (B). Next, a blocking antibody is injected to block any remaining binding sites, (B)-(C). After all of the remaining binding sites are occupied, the protein antigen p24 is injected, which binds to the first antibody, as is shown by the increase in RU from point (C) to point (D). Once the antigen has reached equilibrium with the captured first antibody, a second antibody is injected at point (D). The injection of the second antibody results in an increase in RU from (D) to (E) which, in this case, is greater than the saturation RU value seen after addition of the antigen at point (D). Because the antigen has already bound to the first antibody when the second antibody is injected (point D), and because the RU values increase further when the second antibody is injected (point E), one can infer that the two antibodies bind at different epitopes on the antigen.
77. The steady values for RU seen in the flat lines at B, C and D in Figure 2 of D12 are not always observed in experimental settings. Experiments in which the first analyte must first be washed away with buffer will often show a decrease in RU during the washout phase, due to dissociation of the first analyte of interest (e.g., the antigen). In the experiment depicted in Figure 2 of D12, washout is needed in order to remove the antigen

from the system before introducing the second antibody. Depending on the affinity of the first antibody and other experimental conditions, the decrease in RU seen during the washout and before the introduction of the second antibody can be significant, resulting in the presence of less antigen captured on the chip surface at the time the second antibody is injected. Nevertheless, if simultaneous binding can occur, even small amounts of antigen retained on the chip surface are sufficient to generate a significant increase in RU due to binding of the second antibody. Thus, for simultaneous binding to be detected in “indirect” experiments, the overall RU signal seen upon introduction of the second antibody must increase significantly over the RU value immediately before its addition, but it need not exceed the previous levels.

VII. ANTIBODY BINDING KINETICS

78. The affinity of an antibody is a measure of how tightly and stably the antibody binds to the antigen. Affinity measures the interactions between one antigen-binding site and one antigen, and results from the nature of the non-covalent interactions that occur between the antigen-binding site and the antigen. Ex. D2, Harlow 1999, at p.28.
79. The affinity of an antibody is expressed as a measure of the equilibrium in solution of the complex of antibody and antigen molecules on the one hand, and the two individual molecules (the antibody and the antigen) on the other. In other words, how many of the antibody and antigen molecules form a complex rather than staying apart in solution. This is often referred to as the K_D or dissociation constant. Ex. D2, Harlow 1999, at p.28.
80. The affinity of an antibody is calculated by comparing measurements of how quickly the antibody-antigen complex forms and dissociates. One measurement evaluates the rate of association or binding of the antibody to the antigen (designated k_{on} , k_a or k_{assoc}). A second measurement evaluates the rate of separation or dissociation of the antibody-

antigen complex (designated k_{off} , k_d or k_{dissoc}). The dissociation constant for the antibody can then be represented as $K_D = k_{\text{off}}/k_{\text{on}}$, k_d/k_a , or $k_{\text{dissoc}}/k_{\text{assoc}}$. See '336 patent at ¶ [0039].

81. An antibody must bind with a minimum affinity to exhibit specific binding to an antigen, otherwise the binding cannot be differentiated from non-specific interactions that occur between proteins. A non-specific interaction is one that results from essentially random interactions between parts of the protein antigen and the protein antibody via non-covalent forces of attraction. In the context of Biacore testing, non-specific binding gives response levels that continue to increase with increasing antibody or antigen concentration, instead of reaching a saturation plateau. See Ex. D25, Matthew Fivash et al., *BIACore for Macromolecular Interaction*, *Current Opinion in Biotechnology* 9(1): 97, 101 n.28 (1998).

VIII. EXPERIMENTAL DATA

82. I personally oversaw the performance of binding studies described below at the Biosensor Core Facility at the University of Maryland School of Medicine in Baltimore, Maryland (USA). I was assisted in my studies by Dr. Yinghua Zhang who operated the Biacore instrument, prepared the chips and solutions, and performed injections of the analytes used in the experiments.
83. I performed “yes-no” binding studies using procedures outlined in the Biacore instrument documentation to obtain information regarding the reaction conditions for the kinetic studies detailed below in Section 7. Results from these studies are reported in **Table 1**, below. Reactions for association, specific binding, and dissociation were observed and analyzed for the antibodies/antigens used in the studies. Based on the RU values determined from these preliminary “yes-no” binding studies, the range of concentrations

to use for studies of the binding kinetics discussed below was calculated for each pair of reagents.

A. Binding Kinetics

84. The following reagents and equipment were used to evaluate binding kinetics:
- i. Biacore 3000 (Biosensor Core Facility, University of Maryland School of Medicine, Baltimore)
 - ii. CM5 chips (Biacore/GE Healthcare, Piscataway, NJ)
 - iii. Amine coupling Kit [N-hydroxysuccinimide (NHS) and 1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC)] (Biacore/GE Healthcare, Piscataway, NJ)
 - iv. HBS-EP (10 mM Hepes, pH 7.4, 150 mM NaCl, 3 mM EDTA, 0.05% P-20) buffer (Biacore/GE Healthcare, Piscataway, NJ)
 - v. Regeneration buffer (10 mM Glycine pH 1.5) (Biacore/GE Healthcare, Piscataway, NJ)
 - vi. Recombinant murine PD1, extracellular domain (ECD), his-tagged (Merck, lot #92AFY) (“mouse PD-1”)
 - vii. Recombinant human PD1, ECD, his-tagged (MednaBio Human PD1-10xHis, Product No. E1951MG1, lot 1127-868873) (“wild type PD-1”)
 - viii. Recombinant human PD1, ECD, his-tagged (Merck, lot #26AFY) (“C62S PD-1”)
 - ix. Recombinant mutant human PD1, ECD, his-tagged (Merck, lot #61AFH) (“A101L PD-1”)
 - x. Recombinant human CTLA4, ECD, his-tagged (Merck, lot #29AFY) (“CTLA-4”)
 - xi. Recombinant human ICOS ECD his-tagged, Fc protein (Creative Biomart item # ICOS-1628H) (“ICOS”)
 - xii. Recombinant human CD28 ECD, his-tagged (Creative Biomart item #CD28-3910H) (“CD28”)
 - xiii. Human kappa light chain (Bence Jones protein) (Bethyl Laboratories item #P80-126)
 - xiv. Test antibodies: nivolumab (Merck lot #86AFL); J105 mouse anti-human PD1 (eBioscience item #14-2799); J105 mouse anti-human PD-1 (MBL item #D133-

- 3); J110 mouse anti-human PD1 (MBL item #D132-3); J116 mouse anti-human PD1 (eBioscience item #14-9989)
- xv. Positive control antibodies: Mouse anti-human CTLA4 (Affymetrix eBioscience item #16-1529-82); Mouse anti-human CD28 (Affymetrix eBioscience item #16-0288-81); Mouse anti-human ICOS (Affymetrix eBioscience item #16-9948-82); Mouse anti-human kappa light chain (Sigma item #K4377); Goat Anti-Mouse IgG, Fc specific, AffiniPure (Jackson ImmunoResearch item #115-005-071); Goat Anti-Human IgG, Fc-Specific, AffiniPure (Jackson ImmunoResearch item #109-005-098)
 - xvi. Negative control antibodies: Human IgG (Jackson ImmunoResearch item #009-000-002); Mouse IgG (Jackson ImmunoResearch item #015-000-002)
85. The materials were either obtained from commercial suppliers or were shipped from Merck, as indicated in paragraph 84.
86. The '336 patent identifies the antibody "5C4" as a reference antibody to be used in binding experiments. I was provided a patent application owned by Bristol Myers Squibb that indicates the 5C4 antibody is also called nivolumab and BMS-936558. Ex. D26 WO2013/173223 A1 at 20:2-3. ("U.S. Pat. No. 8,008,449 exemplifies seven anti-PD-1 HuMAbs: 17D8, 2D3, 4H1, 5C4 (also referred to herein as nivolumab or BMS-936558), 4A11, 7D3 and 5F4."). I have been instructed by Merck's counsel to assume that the 5C4 antibody in the '336 patent has the same amino acid sequence as the nivolumab antibody that I evaluated in my experimental studies.
87. One of the antibodies that was tested is the J105 antibody. The J105 antibody is sold commercially by different providers. Aliquots of the J105 antibody used in these experiments were purchased from two different commercial vendors; namely, MBL International, based in Woburn, MA and eBioscience Inc., located in San Diego, CA.
88. The purchase orders for aliquots of J105 antibody (item D133-3) from MBL International are attached as Exhibit D29. The purchase orders indicate that the aliquots of D133-3

antibody were from lots 020 and 021. These purchases were made at my direction and were received on October 2, 2013 and March 13, 2014.

89. I reviewed two declarations from officials from MBL Biological Laboratories Co., Ltd. in Japan. Exhibits D27 (Declaration of Masako Ikeda) and D28 (Declaration of Hiroyuki Yamada). Exhibit D28 at ¶ 11 indicates that MBL International is the U.S. distributor of the D133-3 antibody produced by MBL Biological Laboratories Co., Ltd. in Japan.
90. Exhibit D28 indicates that MBL has produced and is selling aliquots from lots 020 and 021 of the D133-3 antibody, which match the lot numbers of the aliquots of antibodies my lab purchased from MBL International in the United States. Ex. D29, D133-3 Purchase Receipts.
91. The MBL datasheet for the D133-3 antibody is attached as Exhibit D30. The datasheet indicates that the D133-3 antibody is produced by hybridoma clone J105 and refers to publications by Ex. D31, Iwai et al., Immunol. Lett. 83, 215-220 (2002) and Ex. D32, Kanai et al., J. Immunol. 171, 4156-4163 (2003). Those two publications indicate the J105 antibody was produced by a hybridoma clone designated J105 that was generated by immunizing BALB/c mice with the extracellular domain of human PD-1 protein fused to the human $\gamma 1$ heavy chain (hPD-1-Ig), and selected for its ability to produce an antibody that bound specifically to the human PD-1 protein. The D133-3 datasheet also indicates that the J105 hybridoma clone was used to produce the D133-3 antibody sold by MBL.
92. Exhibit D27 describes the quality control experiments that were performed by MBL on the D133-3 antibody from various lots of the antibody. Exhibit D27 indicates that samples of the D133-3 antibody from lots 010, 020 and 021 were each tested for their ability to bind to human PD-1 protein expressed on the surface of X63 cells using flow

cytometry techniques. The flow cytometry plots in Exhibit D27 and in the quality control documents referenced in that Exhibit show that the D133-3 antibody from each of lots 010, 020 and 021 was able to bind to the X63 cells expressing human PD-1 protein. Binding is shown by a shift in the fluorescence values when the D133-3 antibody is added to the cells relative to the values when an unrelated control antibody is added to the these human PD-1 expressing X63 cells. The quality control documents also show that the D133-3 antibody did not cause a fluorescence shift when added to untransfected X63 cells, which indicates that no binding occurred by the antibodies to these untransfected cells. The control antibody also did not cause a fluorescence shift when added to either the untransfected or transfected X63 cells.

93. Exhibit D27 shows that MBL produced each of lots 010, 020 and 021 of the D133-3 antibody using the same hybridoma clone J105. Because the same hybridoma clone was used to produce the D133-3 antibody in these three lots, the D133-3 antibody in each lot will have the same amino acid sequence – this is the necessary consequence of the antibody being produced by the same hybridoma clone. Exhibit D27 and the quality control documents also show that the D133-3 antibody produced in each lot of antibody for each lot specifically bound to human PD-1 on transfected X63 cells, which indicates the antibodies from each lot were active. Exhibit D27 also indicates D133-3 antibodies in lots 010, 020 and 021 each met MBL’s quality control standards. All of these factors convince me that D-133 antibodies from each of lots 010, 020 and 021 are the same antibodies with the same physical and functional properties.
94. My laboratory also purchased aliquots of another J105 antibody, antibody #14-2799ID from eBioscience, Inc., based in San Diego, California. Ex. D33, 14-2799 Purchase

Receipts; Ex. 33, 14-2799 Datasheet (eBioscience). The datasheet for the eBioscience 14-2799 antibody (Exhibit D34) also refers to the Iwai 2002 paper (Exhibit D31) as the source of the J105 hybridoma clone used to produce this antibody. The functional binding properties I observed for this J105 antibody were generally the same as those I observed for the D133-3 antibodies that were tested. Based on this information, I believe the J105/14-2799 antibodies from eBioscience are the same antibodies as the J105/D133-3 antibody purchased from MBL.

95. The reference and test antibodies used in experiments (other than the anti-Fc capture antibodies) were blinded to me and the Biacore operator. The blinding table is shown below. Two numbers were used to blind J105 from eBioscience: the 0808 blinding number was used in the experiment that evaluated J105 for competition against nivolumab; the 0227 blinding number was used in the experiment that compared the binding of J105 from eBioscience against the J105 from Medical & Biological Laboratories Co., Ltd. to further obscure the source of the antibodies being tested.

Blinding Number	Antibody
0112	J105 (MBL #D133-3)
0227, 0808	J105 (eBioscience #14-2799)
0907	nivolumab (5C4)
1114	J116 (eBioscience #14-9989)
1208	J110 (MBL #D132-3)

96. I performed binding kinetics studies of the nivolumab/5C4 (0907), J105/D133-3 (0112), J105/14-2799 (0227, 0808), J116 (1114) and J110 (1208) antibodies. The studies were conducted to determine whether each of these antibodies can bind to each of three forms of PD-1 and, if so, the kinetics of the binding interaction. The studies were conducted using both “direct” and “indirect” capture designs.

97. In the indirect capture assays, anti-human Fc-specific antibody or anti-mouse Fc specific antibody (see paragraph 84) was covalently coupled to the chip by amine coupling, with a kit from Biacore, according to Biacore's standard procedures. After the chip was washed, as per standard Biacore procedures, a solution containing the test antibody was flowed over the surface of the chip until no additional change in RU was obtained. At this point, the PD-1 antigen was then injected into the Biacore instrument and the change in RU (if any) was measured. If the test antibody bound the PD-1 antigen, the Biacore instrument recorded a change in the RU value. This change in RU was fitted to a curve by a Biacore algorithm, which outputs a graph of RU as a function of time that is superimposed on the actual changes in RU measured by the instrument. The quality of the "fit" of the calculated data to the measured data is evaluated by a Chi Squared function. Only data with Chi Squared values of greater than one were included in these studies.
98. In the direct capture assays, the PD-1 protein was covalently linked to the chip using the same amine coupling procedure mentioned above in paragraph 97 and, after washing steps recommended by Biacore, buffer was flowed over the chip until no change in RU was observed. After this was observed, the binding of each antibody was performed by flowing a solution containing the antibody over the surface of the chip and the change in RU (if any) was measured. If the test antibody bound the PD-1 antigen, the Biacore instrument recorded a change in RU. This change was fitted to a curve by a Biacore algorithm and the fit evaluated, as above.
99. Controls were used in the studies of binding kinetics to ensure that the instrument was running properly and that any binding observed was attributable to a specific binding

between the antibody and antigen, and not to non-specific interactions between the reagents. The following controls were used in these experiments:

- (i) a buffer control to correct for non-specific background signal attributable to the reagents;
- (ii) controls to demonstrate that each antibody does not bind to an irrelevant antigen including human CD28, human kappa light chain, human CTLA-4, and human ICOS-FC; and
- (iii) an antibody control (i.e., a species-specific IgG) to demonstrate that binding is not due to non-specific interactions of IgG with the chip surface, or with the different forms of the PD-1 proteins used in testing.

100. Binding was evaluated using three different forms of the extracellular domain of the human PD-1 protein. These three forms of PD-1 were: (i) a commercially available wild-type PD-1 protein from MednaBio (Human PD1-10xHis, Catalog No. E1951) (“wild type PD1”), (ii) a human PD-1 variant with one substitution, in which a serine residue occurs at the 62nd residue of the sequence of this variant rather than the cysteine residue that is present in the corresponding location in the wild-type sequence (“C62S PD-1”)¹, and (iii) a human PD-1 variant with two substitutions, in which in addition to a cysteine-to-serine substitution at the 62nd residue of this variant, a leucine residue occurs at the 101st residue of the variant rather than the alanine residue that is present in the corresponding location

¹ Position 62 of the amino acid sequence C62S variant of human PD-1 corresponds to position 93 of the human wild-type sequence reported in Q15116 in UniProtKB. See **Annex B**.

in the wild-type sequence (“A101L PD-1”).² I obtained the C62S PD-1 and A101L PD-1 proteins from Merck.

101. I was informed by a representative of MednaBio (Rebecca Silver) that the human PD-1-10xHis product sold by MednaBio consists of residues 21-167 of the human PD-1 protein sequence having accession number Q15116. See Ex. D35.
102. The amino acid sequences of the three human PD-1 proteins used in the binding experiments are provided in **Annex B**.
103. **Table 1**, below, provides the “yes-no” binding results for both “direct” and “indirect” binding experiments, indicating whether I observed binding between each antibody and form of PD-1. A “yes” indicates that the antibody was observed to bind to the PD-1 protein; a “no” indicates that no binding was observed. **Table 2**, below, summarizes the “yes-no” binding results for each of the control antigens. The results of the binding kinetics studies performed on each antibody-antigen pair in **Table 1** are summarized in **Table 3**, below. **Table 3** also provides dissociation constants, $K_D \times 10^{-9}$ (M), for each discrete binding assay performed. Where multiple tests were performed, the reported values are separated by commas.

² Position 101 of the amino acid sequence of the A101L variant of human PD-1 corresponds to position 132 of the human PD-1 sequence reported in Q15116 in UniProtKB. See **Annex B**.

Table 1: Binding of nivolumab, J105/D133-3, J105/14-2799, J110, and J116 to PD-1						
	Wild Type PD-1		C62S PD-1		A101L PD-1	
Antibody	Direct	Indirect	Direct	Indirect	Direct	Indirect
nivolumab	Yes	Yes	Yes	Yes	No	No
J105/D133-3	Yes	Yes	Yes	Yes	Yes	Yes
J105/14-2799	Yes	Yes	Yes	Yes	Yes	Yes
J110	Yes	Yes	Yes	Yes	Yes	Yes
J116	Yes	Yes	Yes	Yes	Yes	Yes

Table 2: Binding of nivolumab, J105/D133-3, J105/14-2799, J110, and J116 to Controls				
Antibody	CTLA4	Kappa LC	CD28	ICOS-Fc
nivolumab	No	No	No	No
J105/D133-3	No	No	No	No
J105/14-2799	No	No	No	No
J110	No	No	No	No
J116	No	No	No	No
Anti-CTLA4	Yes	NT*	NT	NT
Anti-Kappa LC	NT	Yes	NT	NT
Anti-CD28	NT	NT	Yes	NT
Anti-ICOS-Fc	NT	NT	NT	Yes
*NT = not tested				

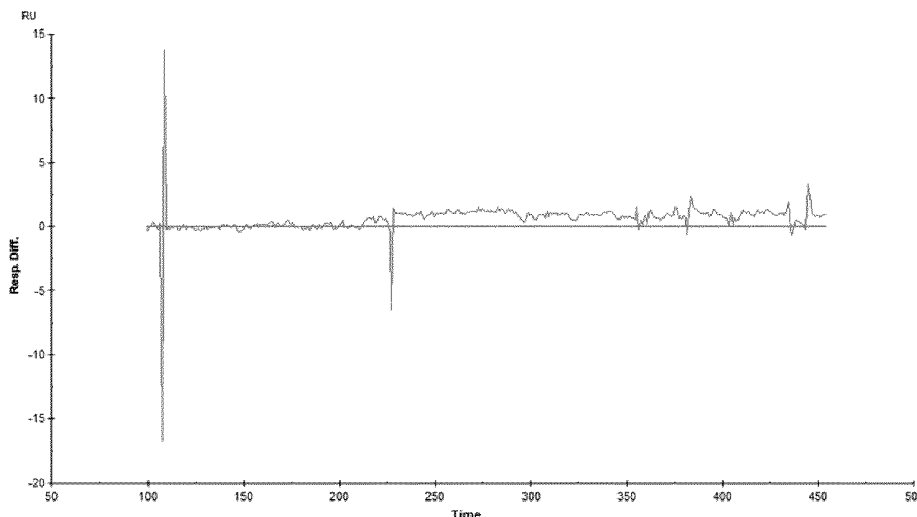
Table 3: Binding Affinity of J105/D133-3, J105/14-2799, nivolumab, J110, and J116 to PD-1*						
	Wild Type PD1		C62S PD-1		A101L PD-1	
Antibody	Direct	Indirect	Direct	Indirect	Direct	Indirect
nivolumab	0.42 ± 0.20 (0.14, 0.43, 0.49, 0.62)	1.9, 5.1	0.47 ± 0.19 (0.21, 0.49, 0.54, 0.65)	3.3 ± 2.2 (1.1, 2.5, 2.9, 2.9, 7.1)	DNB**	DNB**
J105/D133-3	0.64, 4.4	4.8, 7.9	1.0, 3.2	8.3, 13.2	0.09 ± 0.08 (0.004, 0.08, 0.09, 0.19)	7.4 ± 1.5 (6.0, 7.2, 9.1)
J105/14-2799	1.1 ± 0.41 (0.44, 0.88, 1.2, 1.3, 1.5)	3.6 ± 0.65 (3.1, 3.1, 3.7, 4.5)	1.1 ± 0.87 (0.32, 0.60, 0.80, 1.2, 2.6)	12.5 ± 5.1 (7.0, 7.5, 10.3, 12.8, 13.2, 14.8, 22.1)	0.28 ± 0.14 (0.15, 0.18, 0.20, 0.22, 0.32, 0.36, 0.54)	6.7 ± 2.2 (4.0, 5.7, 5.9, 6.5, 7.3, 10.7)
J110	0.89, 2.4	2.0, 5.8	0.83 ± 0.12 (0.70, 0.87, 0.91)	2.8 ± 0.86 (1.8, 2.2, 3.5, 3.5)	0.51 ± 0.19 (0.39, 0.41, 0.73)	1.4 ± 0.97 (0.67, 1.0, 1.2, 2.9)
J116	2.3	4.1, 6.1	1.9 ± 0.76 (1.2, 1.7, 2.7)	4.4 ± 4.8 (0.24, 1.8, 1.9, 1.9, 3.5, 7.3, 14.1)	1.2, 3.3	0.40, 0.46
* Dissociation constants (K _D) for each binding experiment performed are provided in nM (10 ⁻⁹ M). ** DNB = does not bind.						

104. As shown in **Table 3**, the studies of binding kinetics by direct capture demonstrate that the J105/D133-3 antibody binds with high affinity to wild-type PD-1 (0.64, 4.4 nM), C62S PD-1 (1.0, 3.2 nM), and to A101L PD-1 (0.09 ± 0.08 nM). **Table 3** also shows that, in studies of binding kinetics by indirect capture, J105/D133-3 binds with high affinity to wild-type PD-1 (4.8, 7.9 nM), C62S PD-1 (8.3, 13.2 nM), and to A101L PD-1 (7.4 ± 1.5 nM).
105. The studies of binding kinetics also confirm that the J105/D133-3 antibody and the J105/14-2799 antibody, which is from the same J105 hybridoma clone as the J105/D133-3 antibody (Exs. D30, D35), bind at the same high level of affinity to wild type PD-1, C62S PD-1, and A101L PD-1.
106. The studies of binding kinetics also demonstrate that the nivolumab antibody binds with high affinity to wild type PD-1 (0.42 ± 0.20 nM by direct capture; 1.9-5.1 nM by indirect

capture) and C62S PD-1 (0.47 ± 0.19 nM by direct capture; 3.3 ± 2.2 nM by indirect capture), but does not bind to A101L PD-1. A sensorgram showing the binding assay of the nivolumab antibody (0907) to the A101L variant of human PD-1 (“mutant hPD1”) is reproduced below:

K3A-10212013-06

Yes-No binding of 0907 to mutant hPD1: Direct Capture



Chip = 25 RU mutant hPD1 captured directly
0907 applied at 100 nM

10-21-13

107. The binding results for the nivolumab antibody indicate that the amino acid residue at position 101 of the A101L PD-1 variant is involved in binding of the nivolumab antibody to human PD-1. This is demonstrated by the abolition of binding by the nivolumab antibody to the A101L PD-1 variant. Position 101 of the A101L sequence corresponds to position 132 of the wild type human PD-1 sequence listed in Q15116. See **Annex B** and Ex. D20 (UniProtKB Q15116).

108. The experimental results also indicate that the nivolumab and J105/D133-3 antibodies bind to different epitopes on human PD-1. This is shown by the different binding patterns seen with the J105/D133-3 and nivolumab antibodies relative to the three human PD-1 proteins used in the testing. Specifically, the J105/D133-3 antibody bound to all three forms of the human PD-1 protein while the nivolumab antibody bound only to the wild-type and C62S forms of human PD-1.
109. Finally, the studies of binding kinetics demonstrate that the J116 and J110 antibodies bind to each of wild type PD-1, C62S PD-1, and A101L PD-1 proteins with high affinity.

B. Simultaneous Binding Studies

110. Using the reagents identified in paragraph 84, I performed simultaneous binding studies to determine if the nivolumab and J105/D133-3 antibodies can simultaneously bind to human PD-1, or if the binding of one of these antibodies prevents the other antibody from simultaneously binding to human PD-1. I also evaluated simultaneous binding of the nivolumab antibody with each of the J105/14-2799, J116 and J110 antibodies.
111. I performed simultaneous binding assays using the wild-type MednaBio human PD-1 protein, as well as with the C62S PD-1 variant. Simultaneous binding assays were not conducted using the A101L PD-1 variant because nivolumab does not bind to this PD-1 variant. See paragraph 106 above.
112. The binding studies described below tested the ability of a human Fc-containing antibody (i.e., nivolumab) and murine J105/D133-3, J105/14-2799, J116 and J110 to bind simultaneously to each of the wild-type and C62S forms of human PD-1.
113. In simultaneous binding assays by indirect capture using J105/D133-3 and J105/14-2799 (which are both mouse antibodies with Fc regions), a step was included in which a non-specific blocking antibody (e.g., a non-immune mouse IgG, used at 2 μ M concentrations

or higher) was repeatedly added after the first test antibody was injected into the instrument. This step was done to occupy any open binding sites on the chip (i.e., unoccupied anti-mouse Fc antibody molecules that had been coupled to the chip). This step was unnecessary in indirect binding experiments testing simultaneous binding between human and murine antibodies because the anti-mouse Fc or anti-human Fc antibody coupled to the chip will only bind its corresponding species of antibody (i.e., mouse or human), not both. The inability of the anti-human Fc antibodies to cross-react with mouse IgG, and of anti-mouse Fc antibodies to cross-react with human IgG, was included in the descriptions of these products, provided by the manufacturer. Exs. D36, D37. The absence of such non-specific binding was confirmed in control studies.

114. Controls used in the simultaneous binding assays by indirect capture addressed the possibility that any binding to the chip surface that occurred after the capture of human PD-1 was non-specific. As one control, instead of introducing a second antibody that binds to PD-1 with high affinity, control human IgG or control non-immune murine IgG (see ¶ 84) was introduced at high concentrations (2 μ M or higher). As a second, negative control, the same antibody used to capture the human PD-1 protein to the chip surface was injected a second time. Increases of more than about 2 RU (which could be considered “background noise” in the signal) were not observed when either of these control steps were performed.
115. Positive controls for binding were performed in a number of the simultaneous binding assays by indirect capture when significant levels of binding of the second antibody was not detected. In these controls, a third antibody, typically J110, was introduced to determine if the human PD-1 captured by the first antibody retained was still available to

- be bound by another antibody. In nearly all of these studies, J110 showed significant levels of binding to the captured PD-1 protein, suggesting that the failure of the second antibody to bind was not due to a loss or inactivation of the captured PD-1 protein.
116. Controls used in the simultaneous binding assays by direct capture first addressed the possibility that any binding of the first or second antibody to the chip surface containing covalently bound human PD-1 was non-specific. Instead of introducing an antibody that binds to PD-1 with high affinity, either control human or control murine IgG was introduced at high concentrations (2 μ M or higher). No more than about 2 RU of non-specific binding of these control IgGs was observed.
117. As a second, negative control for the simultaneous binding assays by direct capture, instead of the introduction of a second antibody, the chip surface linked to the human PD-1 protein was saturated with the first antibody and then that same first antibody was introduced again after a brief wash in a solution containing control IgG. Binding of the same antibody after the wash returned the RU signal to within 2 RU of the signal originally reached at saturation. These results show that binding to the captured PD-1 protein, achieved by the initial procedure to reach saturation, could not be exceeded by repeated applications of that first antibody.
118. Positive controls for binding were performed in some of the direct simultaneous binding assays in which significant levels of binding of the second antibody was not detected. For these controls, a third antibody, typically J110, was injected to determine if the human PD-1 bound to the chip surface retained antibody-binding activity. In these studies, J110 showed significant levels of binding to the PD-1 protein, suggesting that the

lack of response seen after addition of the second antibody was not due to inactivation of the PD-1 protein.

1. Nivolumab-J105/D133-3 Simultaneous Binding Assay

119. Indirect and direct capture simultaneous binding assays were performed using nivolumab and J105/D133-3 antibodies.
120. In the indirect capture assays, nivolumab (“first antibody”) was first captured on the anti-human Fc chip, typically to levels of 150-250 RU. Then, either the wild type or C62S human PD-1 (“antigen”) was flowed over the captured nivolumab antibody, and finally the J105/D133-3 antibody (“second antibody”) was flowed over the nivolumab-human PD-1 complex. This assay was also conducted with the antibodies added in the opposite order (i.e., J105/D133-3 captured first on an anti-mouse Fc chip, then wild type or C62S human PD-1 was flowed across the captured J105/D133-3 antibody, and finally nivolumab was flowed over the J105/D133-3-human PD-1 complex).
121. Direct simultaneous binding assays were also performed using the nivolumab and J105/D133-3 antibodies. In this experiment, either wild type or C62S human PD-1 was first coupled to the chip using Biacore standard procedure for amine coupling described in paragraph 97. Then, nivolumab was flowed over the captured human PD-1, and finally the J105/D133-3 antibody was flowed over the human PD-1-nivolumab complex. This assay was also conducted with the antibodies added in the opposite order, where the J105/D133-3 antibody was flowed over the captured wild type or C62S human PD-1, and then nivolumab was flowed over the human PD-1-J105/D133-3 complex.
122. The results of experiments evaluating the simultaneous binding of nivolumab vs. J105/D133-3 in the indirect capture assay demonstrate that nivolumab and J105/D133-3

antibodies do not bind PD-1 simultaneously. The data from these experiments are compiled in **Table 4**, below:

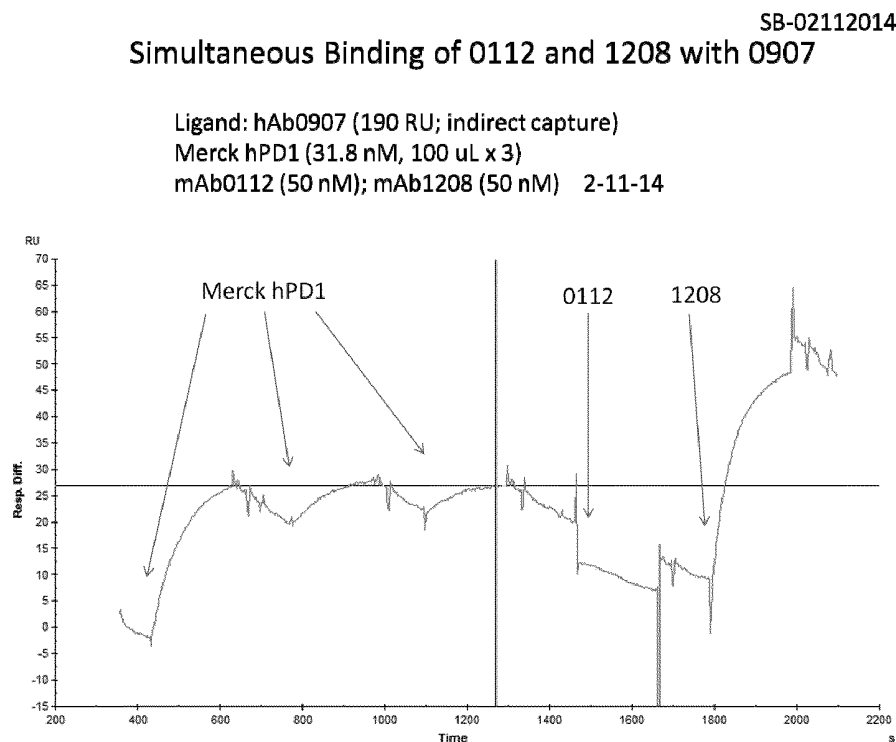
Table 4: Simultaneous Binding Assays by Indirect Capture of nivolumab and J105/D133-3					
Sensorgram #	First Antibody	Antigen	RU Change	Second Antibody	RU Change
Nivolumab and J105/D133-3 to C62S PD-1					
SB-01232014-07	nivolumab	C62S PD-1	48	J105/D133-3	0
SB-02112014-01	nivolumab	C62S PD-1	26	J105/D133-3	0
SB-10092013-02	J105/D133-3	C62S PD-1	87	nivolumab	0
SB-01232014-01	J105/D133-3	C62S PD-1	23	nivolumab	0
SB-04042014-06	J105/D133-3	C62S PD-1	42	nivolumab	0
Nivolumab and J105/D133-3 to PD-1 (wild-type)					
SB-04022014-01	nivolumab	PD-1 (w.t.)	44	J105/D133-3	0
SB-04022014-02	nivolumab	PD-1 (w.t.)	56	J105/D133-3	0
SB-04032014-01	J105/D133-3	PD-1 (w.t.)	32	nivolumab	6
SB-04042014-01	J105/D133-3	PD-1 (w.t.)	62	nivolumab	0
SB-04082014-03	J105/D133-3	PD-1 (w.t.)	38	nivolumab	10

123. The absence of simultaneous binding by the nivolumab and J105/D133-3 antibodies is shown by the lack of an increase in the RU (far right column) when the second antibody of the antibody pair was added after the first antibody had been captured and the human PD-1 had been captured by the first antibody. I note that all but two of the RU values for the second antibody are zero, indicating none of the added second antibody bound to the human PD-1 protein when it was being bound by the first antibody. I also note this result was observed regardless of the order of addition of the nivolumab and J105/D133-3 antibodies, and was also observed for both wild-type human PD-1 and C62S PD-1.
124. In addition, the two non-zero increases in RU seen when the second antibody (nivolumab) was flowed over the chip are very small relative to the magnitude of the change in RU when the first antibody (J105/D133-3) was flowed over the chip. Specifically, these values are 6 RU and 10 RU for the second antibody after RU increases

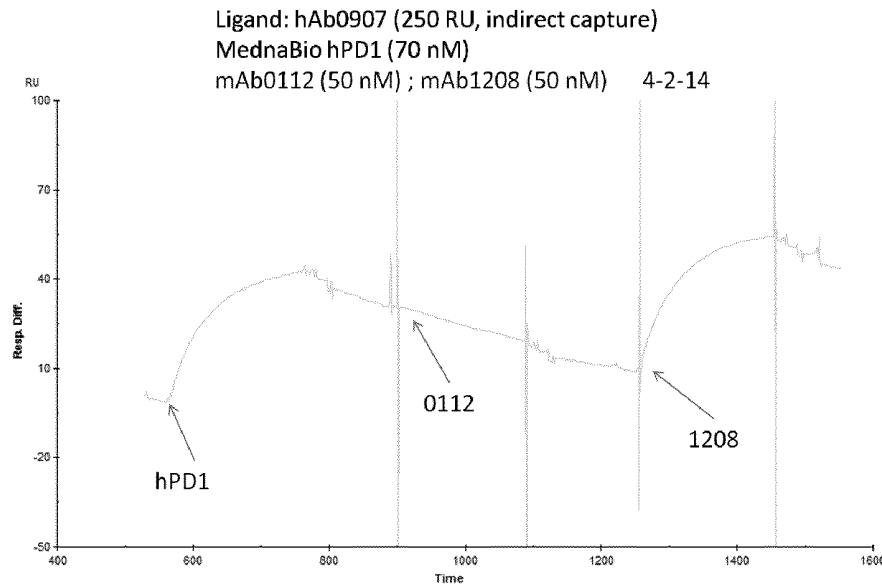
of 32 and 38, respectively, when the PD-1 was bound by the first antibody. If in these cases the nivolumab antibody were able to bind to the captured PD-1 in an unhindered manner, the change in RU values recorded upon its addition would have been considerably higher. For example, after the first antibody showed an RU increase of 38, binding of the second antibody should result in an RU increase of 30-40 RU, if unhindered binding occurred.

125. The indirect capture assay data show that the nivolumab and J105/D133-3 antibodies cannot simultaneously bind to either wild-type human PD-1 or the C62S variant of human PD-1.
126. The slight increases in RU measured in two of the indirect capture assays when the second antibody was injected are not inconsistent with my conclusion that there is no simultaneous binding observed for the nivolumab and J105/D-133-3 antibodies to human PD-1 in the indirect capture assays. First, the increases in RU are small relative to the RU observed for the saturating injections of the first antibody (i.e., 6 RU vs. 32, and 10 RU vs. 38). Second, these slight RU increases were only observed in experiments that used the wild-type PD-1 protein, and were not observed in experiments using the C62S PD-1 variant. The C62S variant omits a cysteine residue that can cause aggregation of the wild-type form of the human PD-1 protein. I believe the slight RU increases can be attributed to aggregates of the wild-type PD-1 protein in these assays, which could present two binding sites instead of one per molecule of PD-1 or could affect the epitopes in ways that might affect binding by one or both of the antibodies.
127. The sensorgrams below show the results of simultaneous binding experiments by indirect capture in which nivolumab was the first antibody added, then PD-1 was introduced, and

finally the J105/D133-3 antibody (0112) was added to the nivolumab (0907)-PD-1 complex. In these experiments, a third antibody, J110 (1208), was used as a positive control, and showed RU increases comparable to the level seen with the first antibody-PD-1 interaction.



Simultaneous Binding of 0112 with 0907



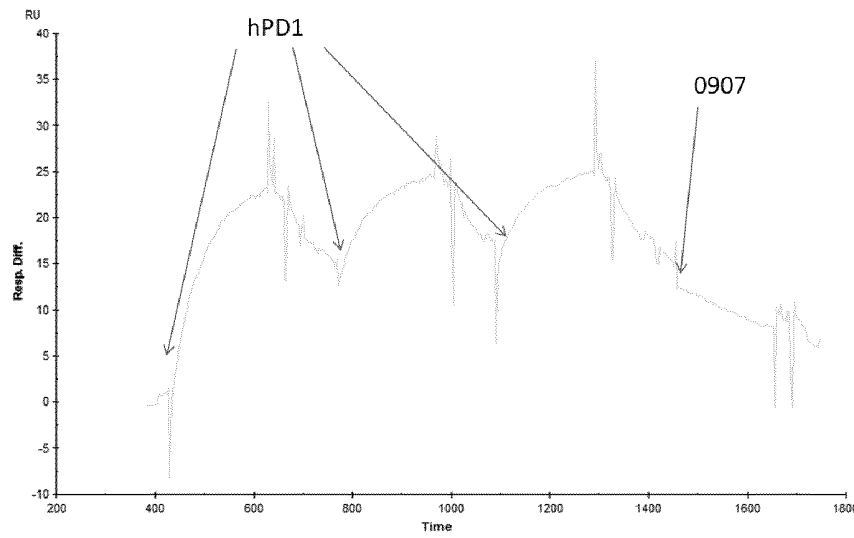
128. The sensorgrams below show the results of simultaneous binding experiments by indirect capture in which the J105/D133-3 antibody (0112) was added first and used to capture PD-1. Next, the PD-1 protein was introduced (“hPD-1”), and finally the nivolumab antibody (0907) was added to the J105/D133-3-PD-1 complex:

Simultaneous Binding of 0907 with 0112

SB-01232014-01

Ligand: mAb0112 (120 RU, indirect capture)

Merck hPD1 (79.5 nM, 100 uL x3), hAb0907 (20 nM) 1-23-14



SB-04042014-01

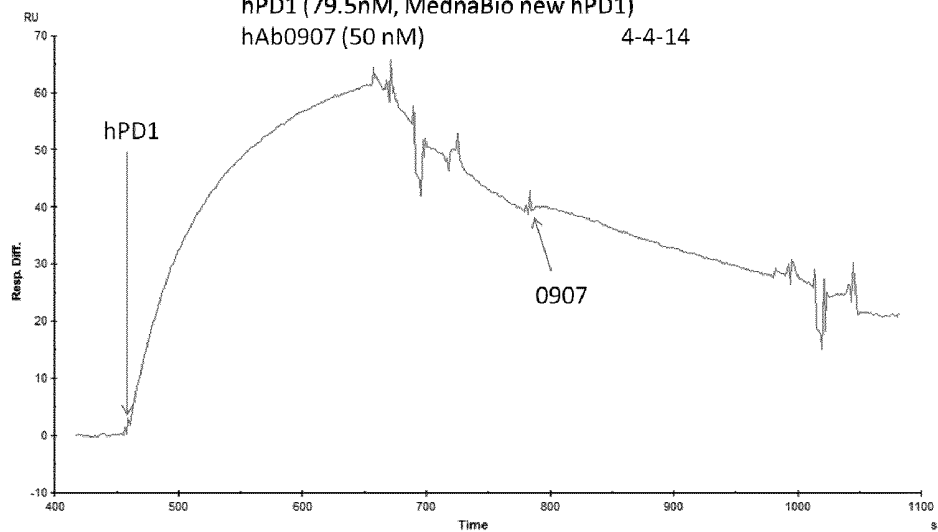
Simultaneous Binding of 0907 with 0112

Ligand: mAb0112 (390 RU, indirect capture)

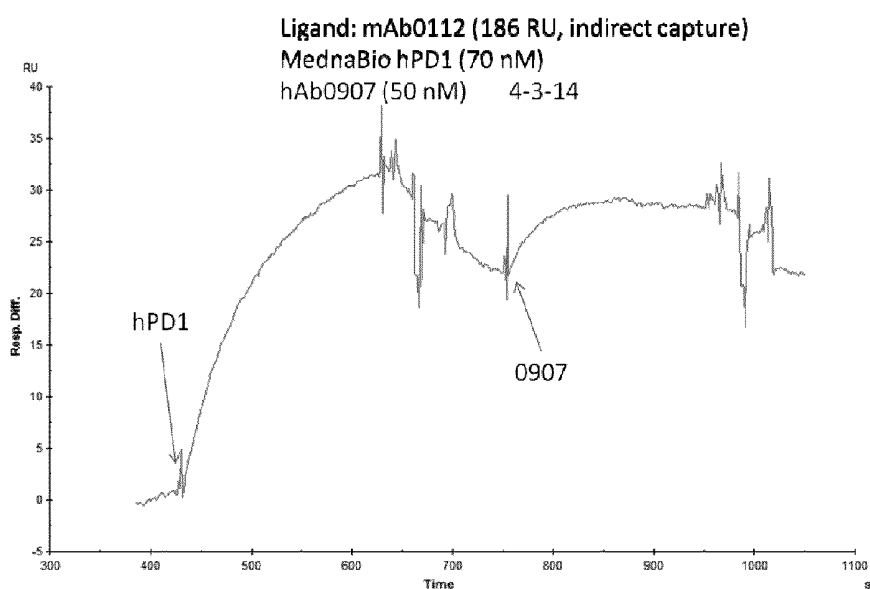
hPD1 (79.5nM, MednaBio new hPD1)

hAb0907 (50 nM)

4-4-14



Simultaneous Binding of 0907 with 0112



129. In the first of these 3 sensorgrams, I note that the PD-1 protein was introduced 3 times. This was done to saturate the available binding sites presented by the J105/D133-3 antibody on the chip surface, and shows that the zero binding results observed following injection of the second antibody, nivolumab (0907), were not due to a failure of the PD-1 protein to saturate available binding sites. Repeated introduction of PD-1 prior to introduction of nivolumab (0907) did not substantially increase the highest RU level reached, indicating that level was very close to saturating. The small drop in RU values recorded by the Biacore instrument in this first sensorgram upon the addition of the nivolumab antibody (0907) illustrates the rapid change that occasionally occurs upon changing solutions, due to small differences in the compositions of the solutions used. Nevertheless, the same results were observed in this study as in the two other studies in which PD-1 was introduced at close to saturating concentrations but only once.

130. Simultaneous binding studies using the nivolumab and J105/D133-3 antibodies were also performed using a direct capture assay, in which the wild-type or C62S PD-1 protein was coupled to the sensor chip, and the two antibodies flowed sequentially over the chip (bound to human PD-1). **Table 5**, below, summarizes the results of these experiments.

Table 5: Simultaneous Binding Assays by Direct Capture of nivolumab and J105/D133-3						
Sensorgram #	Antigen	First Antibody	Max RU	Second Antibody	RU Change	Max RU
nivolumab and J105/D133-3 to C62S PD-1						
SB-11212013-01	C62S PD-1	nivolumab	30	J105/D133-3	0	30
SB-11272013-01	C62S PD-1	nivolumab	15	J105/D133-3	9	23
SB-12022013-01	C62S PD-1	nivolumab	40	J105/D133-3	17	49
SB-01082014-10	C62S PD-1	nivolumab	65	J105/D133-3	16	78
SB-03112014-09	C62S PD-1	nivolumab	19	J105/D133-3	12	26
SB-02072014-03	C62S PD-1	J105/D133-3	41	nivolumab	9	52
SB-03112014-01	C62S PD-1	J105/D133-3	26	nivolumab	3	31
SB-03112014-02	C62S PD-1	J105/D133-3	23	nivolumab	3	28
SB-03252014-06	C62S PD-1	J105/D133-3	32	nivolumab	9	43
SB-03252014-07	C62S PD-1	J105/D133-3	39	nivolumab	9	52
SB-03252014-08	C62S PD-1	J105/D133-3	38	nivolumab	12	49
SB-03252014-09	C62S PD-1	J105/D133-3	42	nivolumab	16	58
nivolumab and J105/D133-3 to PD-1 (wild-type)						
SB-04022014-07	PD-1 (w.t.)	nivolumab	23	J105/D133-3	16	30
SB-04082014-02	PD-1 (w.t.)	nivolumab	19	J105/D133-3	12	20
SB-04092014-02	PD-1 (w.t.)	nivolumab	17	J105/D133-3	11	17
SB-04022014-06	PD-1 (w.t.)	J105/D133-3	32	nivolumab	6	36
SB-04082014-01	PD-1 (w.t.)	J105/D133-3	26	nivolumab	6	26
SB-04092014-01	PD-1 (w.t.)	J105/D133-3	22	nivolumab	5	24

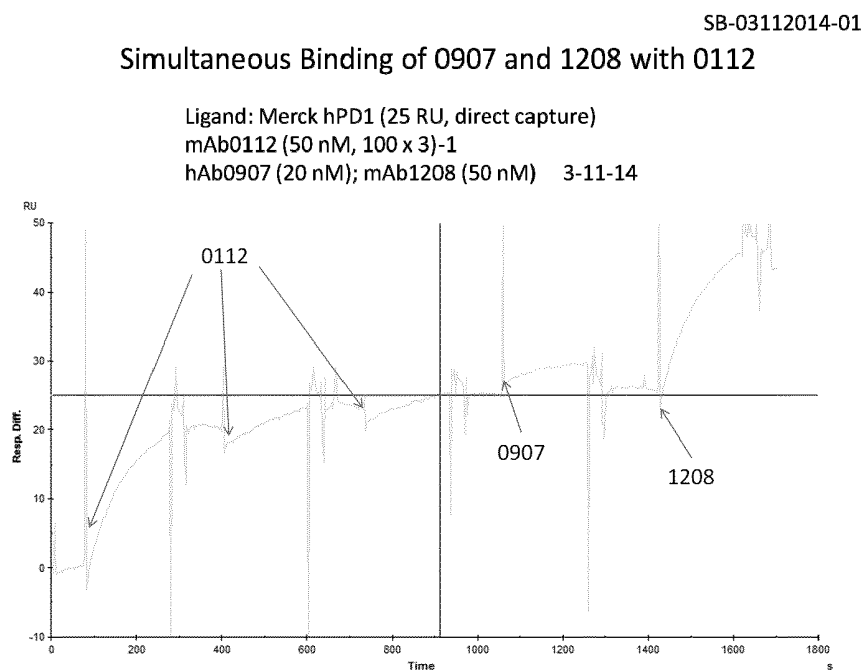
131. The direct binding results show that in a substantial number of the runs (10 out of 18), the addition of the second antibody to the reaction environment resulted in only a small increase in the RU measured relative to the highest RU observed when the first antibody was added (i.e., [Ab1 Max RU / Ab2 Change in RU]: 30/0, 65/16, 26/3, 23/3, 32/9, 39/9, 38/12, 32/6, 26/6, 22/5). In the remaining eight runs, the increase in RU for the second

antibody was larger but still significantly lower than the RU seen with the addition of the first antibody (e.g., 15/9, 40/17, 19/12, 42/12, 25/16, 23/16, 19/12, 17/11). In every case, the RU increase observed for the second antibody was substantially below the RU at saturating amounts of the first antibody.

132. Part of the RU increase seen when the second antibody was added in the direct capture assays could be caused by experimental conditions, such as insufficient saturation of the surface with the first antibody or wild-type PD-1 aggregates that present multiple binding sites. I do not believe these variables would have contributed significantly to the measured RU.
133. Another source for the observed RU increase could be simultaneous binding by the second antibody that was added. Simultaneous binding could occur if the distinct epitope on PD-1 recognized by the second antibody remains partially exposed, or becomes partially exposed during the experiment. The former possibility might occur if the first antibody partially blocks access of the second antibody to PD-1 on the chip surface. The second possibility might occur if binding of the PD-1 to the chip surface induced conformational changes that exposed binding sites on the PD-1 protein for the second antibody that ordinarily are inaccessible to the second antibody when the first antibody has bound to its epitope.
134. The magnitude of the RU increases observed for the second antibody in these direct assays did not exceed the magnitude of the RU increases observed for the first antibody. This result is inconsistent with the second antibody binding in an unhindered manner to a distinct site on the PD-1 protein. If unhindered second antibody binding were occurring, the RU increases would be consistently comparable to the RU increases observed with

the addition of the first antibody, as is seen when the J110 (1208) antibody was added as a positive control antibody. The experimental data instead suggests that the second antibody's access to its binding site was at least partially blocked by the binding of the first antibody.

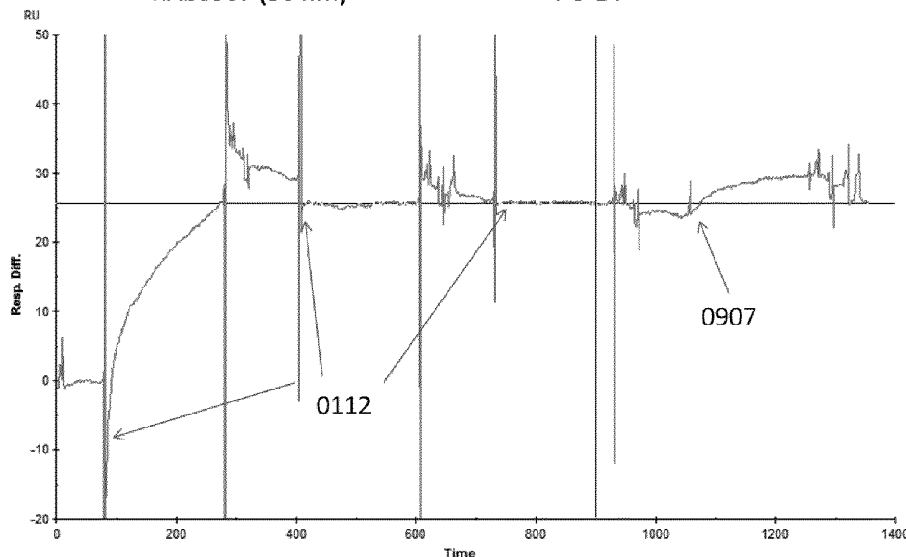
135. The sensorgrams below show results from simultaneous binding experiments by direct capture in which the J105/D133-3 antibody (0112) was the first antibody exposed to the PD-1 protein coupled to the surface, and the nivolumab antibody (0907) was then added to the J105/D133-3–PD-1 complex. The first sensorgram (SB-03112014-01) also shows a significant RU increase when a third antibody (J116/1208) was added.



Simultaneous Binding of 0907 with 0112

Ligand: new MednaBio hPD1 (25 RU, direct capture)
 mAb0112 (50 nM, 100 x 3)
 hAb0907 (50 nM)

4-8-14



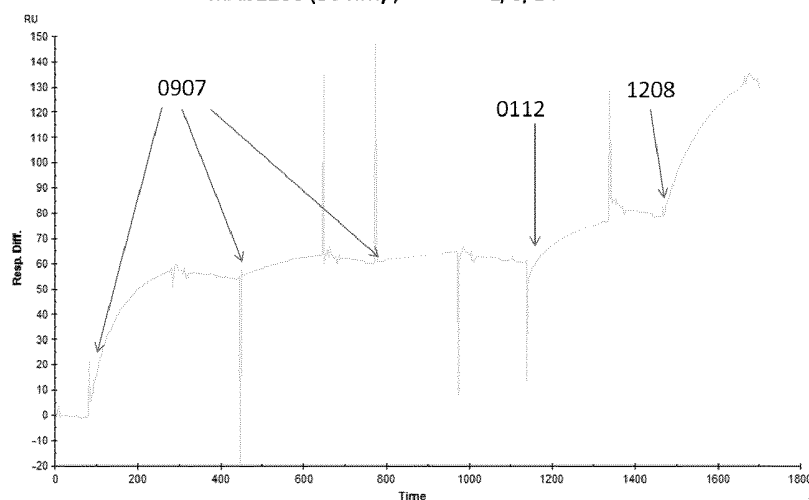
136. The sensorgram below shows the results for a simultaneous binding experiment by direct capture in which the nivolumab antibody was the first antibody added. J105/D133-3 antibody was then added to the nivolumab–PD-1 complex, after which a third antibody (J110 / 1208) was injected. Again, the addition of the third antibody caused an RU increase comparable to that seen with the saturating amount of nivolumab, which indicates nivolumab and J110 are able to simultaneously bind to the human PD-1 to an equivalent extent.
137. In this direct capture experiment, the second antibody (J105/D133-3) (0112) binds to a much lesser extent than the first antibody, nivolumab (0907), and to a much lesser extent than the third antibody, J110 (1208). As I explained in paragraphs 132-134, if the RU increases were due to simultaneous binding, access of the second antibody (J105/D133-3)

to its epitope was at least partially restricted by the earlier binding of the first antibody (nivolumab).

SB-01082014-10

Simultaneous Binding of 0112 and 1208 with 0907

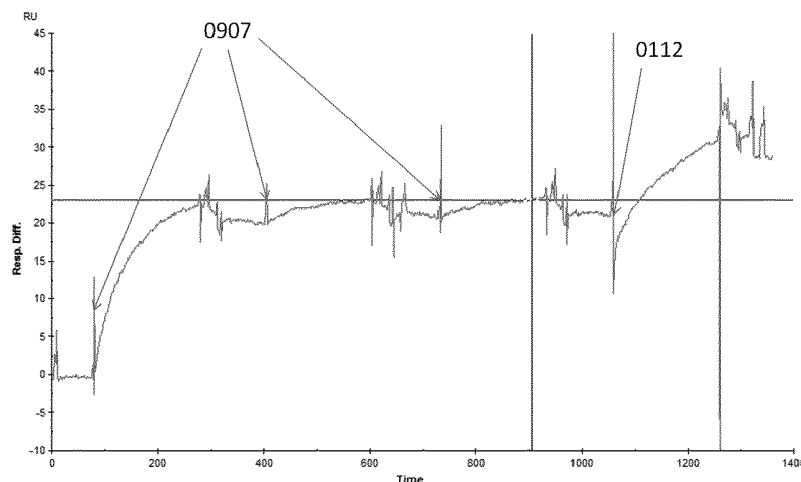
Ligand: Merck hPD1 (30 RU, Direct Capture)
hAb0907 (20 nM), mAb0112 (100 nM),
mAb1208 (50 nM) ; 1/8/14



SB-04022014-07

Simultaneous Binding of 0907 with 0112

Ligand: MednaBio hPD1 (25 RU, direct capture)
hAb0907 (20 nM, 100 x 3)
mAb0112 (50 nM) 4-2-14



138. The results of the direct capture studies, when considered with the results from the indirect capture studies, are consistent with the conclusion that the nivolumab and J105/D133-3 antibodies cannot simultaneously bind in an unrestricted manner to human PD-1. In the indirect capture experiments, there was in all but two instances 100% inhibition of binding of the second antibody when the first antibody had bound to the human PD-1. In the direct capture experiments, there was a significant amount of inhibition of binding of the second antibody to human PD-1 once the first antibody had bound to the PD-1.
139. Based on the entirety of these results, I believe the J105/D133-3 antibody (J105 from MBL) cross-competes with the nivolumab antibody (5C4) for binding to PD-1 within the meaning of “cross-competes” in the EP ‘336 patent because in both indirect and direct binding assays, the binding to PD-1 of either of these antibodies completely or substantially inhibited binding of the other antibody added as the second antibody.

2. Nivolumab-J105/14-2799 Simultaneous Binding Assays

140. Simultaneous binding assays were also performed using the nivolumab and J105/14-2799 antibodies.
141. As noted above, the labels 0227 and 0808 refer to the same antibody – a murine antibody produced by the J105 hybridoma clone obtained from eBioscience, Inc.
142. Assays of simultaneous binding by indirect capture using the J105/14-2799 antibody were performed in the same manner as the indirect capture experiments performed using the J105/D133-3 assays described above in paragraph 113. The same controls were used for the nivolumab/0227-0808 indirect assays as were used for the nivolumab-J105/D133-3 indirect assays.

143. The results of indirect simultaneous binding studies using the nivolumab and J105/14-2799 antibodies are compiled in **Table 6** below.

Table 6: Simultaneous Binding Assays by Indirect Capture of nivolumab and J105/14-2799					
Sensorgram #	First Antibody	Antigen	RU Change	Second Antibody	RU Change
Nivolumab and J105/14-2799 to C62S PD-1					
SB-11212013-05	nivolumab	C62S PD-1	18	J105/14-2799	3
SB-11222013-01	nivolumab	C62S PD-1	19	J105/14-2799	3
SB-01232014-08	nivolumab	C62S PD-1	50	J105/14-2799	0
SB-02112014-02	nivolumab	C62S PD-1	26	J105/14-2799	0
SB-09262013-02	J105/14-2799	C62S PD-1	39	nivolumab	0
SB-10092013-01	J105/14-2799	C62S PD-1	67	nivolumab	0
SB-10252013-01	J105/14-2799	C62S PD-1	28	nivolumab	3
SB-12032013-01	J105/14-2799	C62S PD-1	13	nivolumab	9
SB-01232014-03	J105/14-2799	C62S PD-1	18	nivolumab	0
SB-01232014-05	J105/14-2799	C62S PD-1	19	nivolumab	0
SB-02242014-01	J105/14-2799	C62S PD-1	11	nivolumab	0
SB-02242014-03	J105/14-2799	C62S PD-1	19	nivolumab	0
SB-02242014-05	J105/14-2799	C62S PD-1	17	nivolumab	0
SB-02272014-01	J105/14-2799	C62S PD-1	17	nivolumab	0
SB-02272014-02	J105/14-2799	C62S PD-1	17	nivolumab	0
SB-02272014-03	J105/14-2799	C62S PD-1	17	nivolumab	2
SB-02272014-04	J105/14-2799	C62S PD-1	20	nivolumab	3
SB-02272014-05	J105/14-2799	C62S PD-1	11	nivolumab	4
SB-02272014-06	J105/14-2799	C62S PD-1	10	nivolumab	3
SB-02272014-07	J105/14-2799	C62S PD-1	10	nivolumab	4
SB-02272014-08	J105/14-2799	C62S PD-1	10	nivolumab	4
Nivolumab and J105/14-2799 to PD-1 (w.t.)					
SB-04022014-03	nivolumab	PD-1 (w.t.)	42	J105/14-2799	0
SB-04032014-03	J105/14-2799	PD-1 (w.t.)	23	nivolumab	2
SB-04042014-03	J105/14-2799	PD-1 (w.t.)	56	nivolumab	0
SB-04082014-05	J105/14-2799	PD-1 (w.t.)	42	nivolumab	0

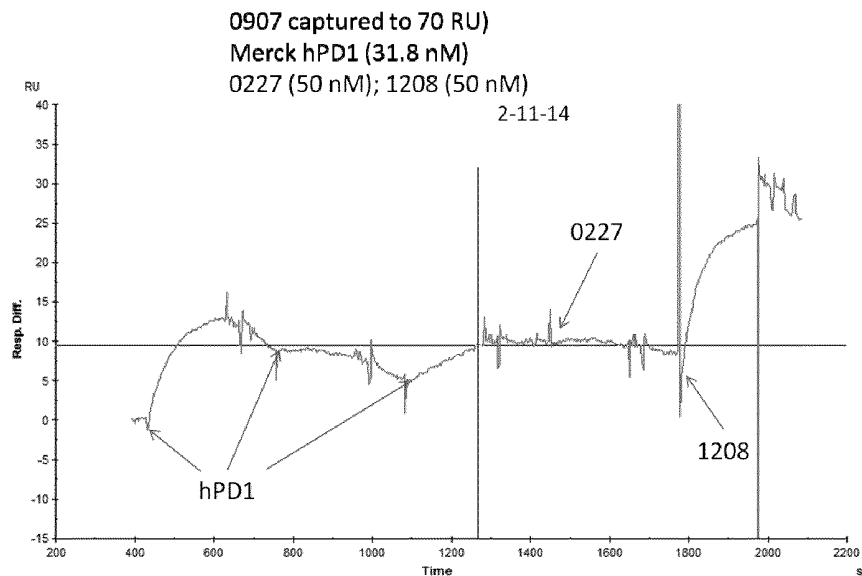
144. The results from the simultaneous binding studies by indirect capture using the nivolumab and J105/14-2799 antibodies are very similar to the results observed for the indirect capture simultaneous binding experiments with the nivolumab and J105/D133-3 antibodies. Both sets of binding results show that the second antibody added to the

reaction environment did not bind simultaneously with the first added antibody. This was true regardless of the order of addition of the antibodies (i.e., nivolumab first then J105/14-2799 or J105/14-2799 first, then nivolumab).

145. In certain of the assays, the increase in RU observed was non-zero. In these assays, however, the increases were a small fraction of the increase in RU observed for the first antibody (i.e., 18/3 28/3, 13/9, 17/2, 20/3, 11/4, 10/3, 10/4, 10/4, 23/2). These non-zero RU values are not consistent with unrestricted simultaneous binding of the two antibodies to human PD-1.
146. The sensorgram below shows the result of a simultaneous binding experiment by indirect capture in which the nivolumab antibody (0907) was first captured, then the human PD-1 protein was introduced, and then the J105/14-2799 antibody (0227) was added to the nivolumab–PD-1 complex. Finally, a third antibody (J110 / 1208) was added. As the sensorgram shows, there was no binding by the second antibody, but the third antibody, J110 (1208) bound robustly.

SB-02112014-02

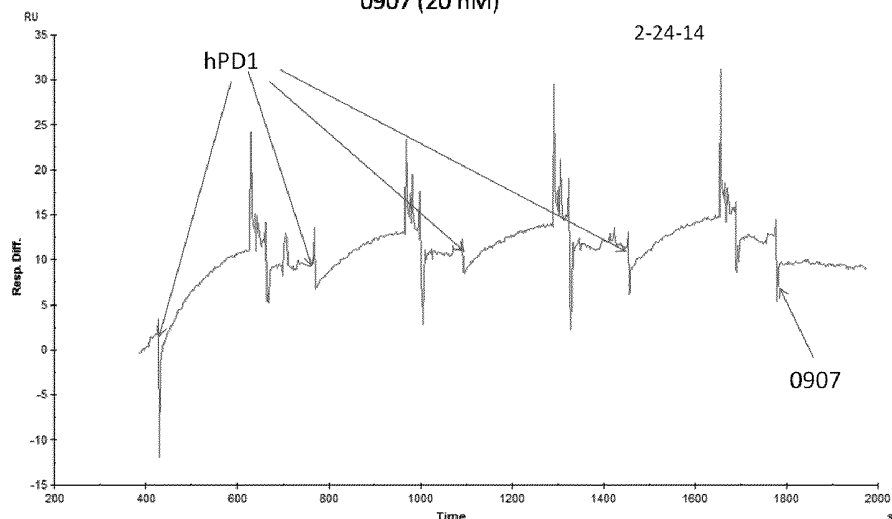
Simultaneous binding of 0227 and 1208 to Merck hPD1 captured with 0907
Indirect capture



147. The sensorgram below shows the results of a simultaneous binding experiment by indirect capture in which the J105/14-2799 antibody was first captured, then human PD-1 was introduced, and then the nivolumab antibody was added to the J105/14-2799–PD-1 complex. As the sensorgram shows, there was no increase in RU when the nivolumab antibody was added.

Simultaneous binding of 0907 to hPD1 captured by 0808
Indirect capture

0808 Captured to 80 RU
Merck hPD1 (31.8 nM)
0907 (20 nM)



148. Simultaneous binding experiments by direct capture using the J105 antibody (J105/14-2799) and the nivolumab antibody were performed in the manner previously described in paragraph 121. The data from these experiments are compiled in **Table 7**, below. The controls used in the direct capture binding studies for the nivolumab and J105/14-2799 antibodies were the same as those used for the direct capture binding studies involving the nivolumab and J105/D133-3 antibodies above at paragraph 116.

Table 7: Simultaneous Binding Assays by Direct Capture of nivolumab and J105/14-2799						
Sensorgram #	Antigen	First Antibody	RU Max	Second Antibody	RU Change	RU Max
nivolumab and J105/14-2799 to C62S PD-1						
SB-01082014-05	C62S PD-1	nivolumab	74	J105/14-2799	4	76
SB-01082014-07	C62S PD-1	nivolumab	72	J105/14-2799	6	78
SB-01082014-08	C62S PD-1	nivolumab	70	J105/14-2799	12	81
SB-03112014-11	C62S PD-1	nivolumab	20	J105/14-2799	7	28
SB-11212013-02	C62S PD-1	nivolumab	30	J105/14-2799	2	32
SB-11272013-02	C62S PD-1	nivolumab	17	J105/14-2799	8	22
SB-01082014-09	C62S PD-1	nivolumab	70	J105/14-2799	17	83
SB-01082014-06	C62S PD-1	nivolumab	70	J105/14-2799	11	80
SB-03112014-10	C62S PD-1	nivolumab	19	J105/14-2799	8	29
SB-03132014-05	C62S PD-1	nivolumab	48	J105/14-2799	9	56
SB-03132014-06	C62S PD-1	nivolumab	61	J105/14-2799	9	68
SB-01082014-01	C62S PD-1	J105/14-2799	52	nivolumab	15	68
SB-01082014-02	C62S PD-1	J105/14-2799	51	nivolumab	16	68
SB-02172014-02	C62S PD-1	J105/14-2799	45	nivolumab	6	48
SB-02212014-02	C62S PD-1	J105/14-2799	42	nivolumab	5	44
SB-02212014-03	C62S PD-1	J105/14-2799	17	nivolumab	1	17
SB-03072014-01	C62S PD-1	J105/14-2799	24	nivolumab	8	30
SB-03072014-02	C62S PD-1	J105/14-2799	23	nivolumab	4	24
SB-03182014-11	C62S PD-1	J105/14-2799	24	nivolumab	2	23
SB-03182014-12	C62S PD-1	J105/14-2799	23	nivolumab	2	24
SB-02072014-04	C62S PD-1	J105/14-2799	47	nivolumab	10	53
SB-02172014-01	C62S PD-1	J105/14-2799	45	nivolumab	8	48
SB-03112014-05	C62S PD-1	J105/14-2799	27	nivolumab	2	27
SB-03112014-06	C62S PD-1	J105/14-2799	27	nivolumab	3	27
SB-03262014-06	C62S PD-1	J105/14-2799	42	nivolumab	10	37
SB-03262014-07	C62S PD-1	J105/14-2799	41	nivolumab	12	52
SB-03262014-08	C62S PD-1	J105/14-2799	31	nivolumab	10	40
SB-03262014-09	C62S PD-1	J105/14-2799	35	nivolumab	14	40
SB-03132014-01	C62S PD-1	J105/14-2799	49	nivolumab	13	62
SB-03132014-02	C62S PD-1	J105/14-2799	61	nivolumab	20	77
SB-03182014-03	C62S PD-1	J105/14-2799	22	nivolumab	6	26
SB-03182014-04	C62S PD-1	J105/14-2799	22	nivolumab	8	29
SB-03182014-05	C62S PD-1	J105/14-2799	22	nivolumab	3	24
SB-03182014-06	C62S PD-1	J105/14-2799	22	nivolumab	6	27
SB-03182014-09	C62S PD-1	J105/14-2799	22	nivolumab	3	23

SB-03182014-10	C62S PD-1	J105/14-2799	22	nivolumab	5	25
Nivolumab and J105/14-2799 to PD-1 (w.t.)						
SB-02212014-01	PD-1 (w.t.)	J105/14-2799	17	nivolumab	0	14

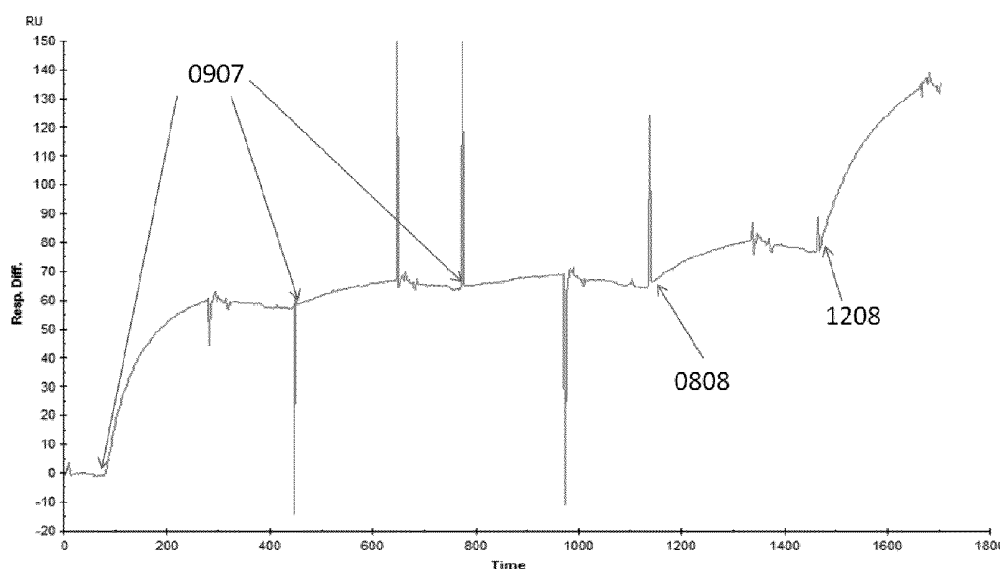
149. Similar to the results for the direct binding assays using the nivolumab and J105/D133-3 antibodies, the direct simultaneous binding assays for the J105/14-2799 and nivolumab antibodies show that the two antibodies do not bind PD-1 at levels comparable to the levels they reach when the other antibody is not present. In many runs, there was only a slight RU increase following the addition of the second antibody indicating that the second antibody did not bind to a significant extent after the first antibody had bound to the human PD-1. In other runs, the increase in the RU was substantially less than the increase in RU seen after addition of the first antibody. Again, restricted binding was not due to inactivation of the PD-1 protein on the chip, as a third antibody, J110 (1208) bound to the PD-1-coated chip as robustly as did the first antibody, nivolumab (0907).

SB-01082014-08

Simultaneous binding of 0808 and 1208 after saturation with 0907
Direct capture on hPD1 chip

Merck hPD1 on chip at 30 RU
0907 (20 nM), 0808 (40 nM), 1208 (50 nM)

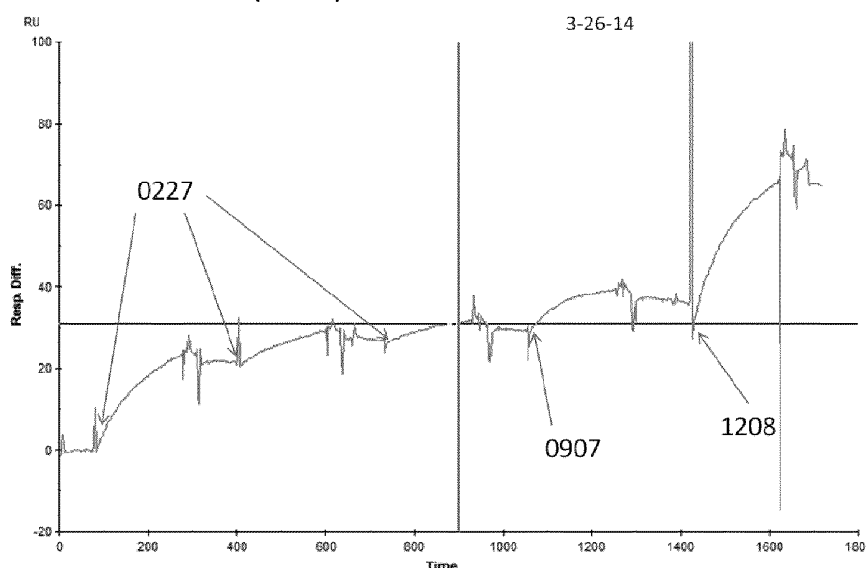
(1-8-14)



150. The sensorgram below shows the results of simultaneous binding experiments by direct capture in which the J105/14-2799 (0227) antibody was first captured, then the human PD-1 protein was introduced, then the nivolumab antibody (0907) was added to the J105/14-2799–PD-1 complex, and finally a third control antibody (J110 / 1208) was added.

Simultaneous binding of 0907 to Merck hPD1 after saturation with 0227
Direct capture on hPD1 chip

Merck hPD1 on chip to 25 RU
0227 (50 nM); 0907 (20 nM);
mAb1208 (50 nM)



151. The simultaneous binding results by direct capture in which the nivolumab antibody was added after the J105/14-2799 antibody had bound to PD-1, when considered with the results from the indirect binding studies, are consistent with the conclusion that the nivolumab and J105/14-2799 antibodies inhibit each other's ability to bind to human PD-1 to a significant extent.
152. Based on these results, I believe the J105/14-2799 antibody cross-competes with the nivolumab antibody (5C4) because in both indirect and direct binding models, the binding to PD-1 of either of these antibodies added as the first antibody completely inhibited or substantially inhibited the binding of the other antibody added as the second antibody.

3. J105/D133-3-J105/14-2799 Simultaneous Binding Assays

153. The murine monoclonal antibodies J105/D133-3 and J105/14-2799 are each produced by the hybridoma clone designated J105. The product datasheets for each of the antibodies identifies the same publication as the source of the J105 hybridoma clone, namely Iwai et al., “Microanatomical localization of PD-1 in human tonsils,” *Immunol. Lett.*, 83(3):215-220 (October 1, 2002). See Exs. D30, D34. The datasheets for each antibody indicate that each antibody binds to the human PD-1 protein when it is expressed on the surface of cells. See Exs. D30, D34.
154. The J105/D133-3 and J105/14-2799 antibodies were tested in simultaneous binding assays with both direct and indirect capture methods to determine if one prevents or interferes with the binding of the other.
155. In the simultaneous binding assays by indirect capture, an anti-mouse Fc antibody was coupled to the sensor chip using Biacore standard procedure for amine coupling described in paragraph 97. A saturating amount of the J105/14-2799 antibody was then captured on the anti-mouse Fc chip. At this point, because a second mouse antibody was being tested for simultaneous binding, a murine antibody that does not bind human PD-1 (control mouse IgG, see ¶ 84) was flowed over the surface of the chip to saturate the remaining binding sites for mouse IgG. Then, the C62S PD-1 was flowed over the captured J105/14-2799 antibody. Finally the J105/D133-3 antibody was flowed over the J105/14-2799 antibody-PD-1 complex. In addition, a second injection of the J105/14-2799 antibody was performed to compare binding of the antibody to itself. The results of these binding assays are presented in Table 8 below:

Table 8: Simultaneous Binding Assays by Indirect Capture of J105/D133-3 and J105/14-2799 to C62S PD-1					
Sensorgram #	First Antibody	Antigen	RU Change	Second Antibody	RU Change
SB-03312014-01	J105/14-2799	C62S PD-1	8	J105/D133-3	3
SB-03312014-02	J105/14-2799	C62S PD-1	7	J105/D133-3	4
SB-04092014-08	J105/14-2799	C62S PD-1	8	J105/D133-3	2
SB-04222014-07	J105/14-2799	C62S PD-1	10	J105/D133-3	0
SB-04222014-08	J105/14-2799	C62S PD-1	10	J105/D133-3	0
SB-04222014-13	J105/14-2799	C62S PD-1	10	J105/D133-3	0
SB-04242014-03	J105/14-2799	C62S PD-1	8	J105/D133-3	1
SB-04222014-03	J105/D133-3	C62S PD-1	2	J105/14-2799	0
SB-04222014-04	J105/D133-3	C62S PD-1	16	J105/14-2799	0
SB-04222014-05	J105/D133-3	C62S PD-1	16	J105/14-2799	0
SB-04222014-06	J105/D133-3	C62S PD-1	14	J105/14-2799	0
SB-04242014-02	J105/D133-3	C62S PD-1	10	J105/14-2799	0
SB-03312014-03	J105/14-2799	C62S PD-1	7	J105/14-2799	5
SB-03312014-04	J105/14-2799	C62S PD-1	7	J105/14-2799	5
SB-03312014-05	J105/14-2799	C62S PD-1	10	J105/14-2799	5
SB-03312014-06	J105/14-2799	C62S PD-1	9	J105/14-2799	5
SB-04092014-09	J105/14-2799	C62S PD-1	9	J105/14-2799	0
SB-04092014-10	J105/14-2799	C62S PD-1	9	J105/14-2799	0
SB-04222014-09	J105/14-2799	C62S PD-1	10	J105/14-2799	0
SB-04222014-10	J105/14-2799	C62S PD-1	9	J105/14-2799	0
SB-04222014-11	J105/14-2799	C62S PD-1	10	J105/14-2799	0
SB-04222014-12	J105/14-2799	C62S PD-1	9	J105/14-2799	0
SB-04222014-14	J105/14-2799	C62S PD-1	10	J105/14-2799	0
SB-04222014-15	J105/14-2799	C62S PD-1	10	J105/14-2799	0
SB-04242014-04	J105/14-2799	C62S PD-1	7	J105/14-2799	0
SB-04222014-01	J105/D133-3	C62S PD-1	12	J105/D133-3	0
SB-04222014-02	J105/D133-3	C62S PD-1	12	J105/D133-3	0
SB-04242014-01	J105/D133-3	C62S PD-1	10	J105/D133-3	1

156. The results of the indirect capture binding experiments show that the J105/14-2799 antibody does not bind simultaneously with the J105/D133-3 antibody or with itself. After the addition of the two J105 antibodies, addition of a third antibody, J110, showed a

measurable increase in RU, which indicates retention of the PD-1 captured initially by the first J105 antibody used.

157. Direct capture binding assays were also performed using the J105/14-2799 and J105/D133-3 antibodies. In these experiments, either the wild-type human PD-1 protein or C62S PD-1 protein was first coupled to the chip using Biacore standard procedure for amine coupling described in paragraph 97. Then, the J105/D133-3 antibody was injected in a saturating amount, followed by injection of the J105/14-2799 antibody. As in the indirect capture binding assay, a second injection of the J105/14-2799 antibody was performed to compare binding of the antibody to itself. **Table 9** below summarizes the results of these experiments.

Table 9: Simultaneous Binding Assays by Direct Capture of J105/D133-3 and J105/14-2799 to C62S PD-1						
Sensorgram #	Antigen	First Antibody	RU Max	Second Antibody	RU Change	RU Max
SB-03252014-01	C62S PD-1	J105/D133-3	36	J105/14-2799	4	28
SB-04092014-14	C62S PD-1	J105/D133-3	27	J105/14-2799	7	30
SB-03252014-03	C62S PD-1	J105/D133-3	31	J105/14-2799	3	41
SB-04092014-15	C62S PD-1	J105/D133-3	27	J105/14-2799	8	29
SB-03262014-01	C62S PD-1	J105/14-2799	25	J105/D133-3	4	25
SB-04092014-16	C62S PD-1	J105/14-2799	31	J105/D133-3	7	28
SB-03202014-01	C62S PD-1	J105/14-2799	20	J105/D133-3	3	9
SB-04092014-19	C62S PD-1	J105/14-2799	31	J105/D133-3	7	28
SB-04092014-13	C62S PD-1	J105/D133-3	29	J105/D133-3	6	28
SB-03252014-04	C62S PD-1	J105/D133-3	31	J105/D133-3	7	32
SB-04092014-18	C62S PD-1	J105/14-2799	30	J105/14-2799	8	30
SB-03262014-04	C62S PD-1	J105/14-2799	27	J105/14-2799	7	27
SB-03202014-02	C62S PD-1	J105/14-2799	20	J105/14-2799	2	20
SB-04092014-20	C62S PD-1	J105/14-2799	30	J105/14-2799	7	30
SB-04092014-21	C62S PD-1	J105/14-2799	36	J105/14-2799	7	35
SB-03192014-11	C62S PD-1	J105/14-2799	22	J105/14-2799	5	22

158. The indirect and direct simultaneous binding experiments demonstrate that the J105/D133-3 and J105/14-2799 antibodies do not bind simultaneously to the C62S

human PD-1 protein. This result is consistent with the two J105 antibodies being produced by the same hybridoma clone.

159. I note that non-zero increases in RU levels were observed in the indirect and direct binding assays even when the second antibody being injected into the Biacore instrument was the same antibody as the first antibody. This illustrates the point I made earlier that relatively small non-zero increases in RU values when a second antibody is introduced into the reaction environment occur for a variety of reasons. Consistent with this interpretation, the final injection of the second J105 antibody in direct capture studies produced a final maximal RU value that was equal to or lower than the initial level achieved by repeated injection of the first antibody in 14 of the 18 studies, and in 3 of the remaining 4 studies the increase in RU was at a level that was insignificant. In the one remaining study, the solution change caused a shift in RU values that made assessment of absolute changes difficult. Both sets of results indicate that the second J105 antibody was not able to bind simultaneously to the human PD-1 protein after the first J105 antibody had bound to that protein.

4. Nivolumab-J116 & J110 Simultaneous Binding Assays

160. In addition to testing the J105/D133-3 and J105/14-2799 antibodies for simultaneous binding to human PD-1 with the nivolumab antibody, I oversaw testing of two other murine monoclonal antibodies specific to the human PD-1 protein for simultaneous binding with the nivolumab antibody. These are an antibody produced by hybridoma clone J110 (see datasheet attached as Exhibit D38) (blinding label 1208) and an antibody produced by hybridoma clone J116 (see datasheet attached as Exhibit D39) (blinding label 1114).

161. The indirect and direct assays were performed for these two murine monoclonal antibodies in a manner analogous to that used to investigate simultaneous binding between the J105/D133-3 and J105/14-2799 antibodies relative to the nivolumab antibody. In both types of assays, the addition of the second antibody caused a significant increase in RU values that was consistent with the second antibody binding simultaneously to the human PD-1 protein with the first antibody. Tables compiling the results of these experiments are reproduced below.

Table 10: Simultaneous Binding Assays by Indirect Capture of nivolumab and J116 or J110					
Sensorgram #	Antibody 1	Antigen	RU Change	Antibody 2	RU Change
nivolumab and J116 to C62S PD-1					
SB-09262013-01	nivolumab	C62S PD-1	82	J116	20
SB-09262013-03	J116	C62S PD-1	28	nivolumab	78
SB-10252013-02	J116	C62S PD-1	17	nivolumab	38
nivolumab and J116 to PD-1 (w.t.)					
SB-04022014-04	nivolumab	PD-1 (w.t.)	44	J116	27
SB-04032014-05	J116	PD-1 (w.t.)	22	nivolumab	66
SB-04082014-07	J116	PD-1 (w.t.)	17	nivolumab	56
nivolumab and J110 to C62S PD-1					
SB-10232013-01	nivolumab	C62S PD-1	20	J110	10
SB-10232013-02	nivolumab	C62S PD-1	28	J110	3
SB-10232013-03	nivolumab	C62S PD-1	60	J110	16
SB-10242013-01	nivolumab	C62S PD-1	27	J110	10
SB-11152013-01	nivolumab	C62S PD-1	21	J110	10
SB-04072014-02	nivolumab	C62S PD-1	41	J110	71
SB-09262013-04	J110	C62S PD-1	32	nivolumab	64
SB-12032013-02	J110	C62S PD-1	22	nivolumab	51
nivolumab and J110 to PD-1 (w.t.)					
SB-04022014-05	nivolumab	PD-1 (w.t.)	45	J110	45
SB-04042014-05	nivolumab	PD-1 (w.t.)	23	J110	41
SB-04072014-01	nivolumab	PD-1 (w.t.)	27	J110	44
SB-04032014-06	J110	PD-1 (w.t.)	27	nivolumab	70
SB-04082014-09	J110	PD-1 (w.t.)	34	nivolumab	78

Table 11: Simultaneous Binding Assays by Direct Capture of nivolumab and J116 or J110						
Sensorgram #	Antigen	Antibody 1	Max RU	Antibody 2	RU Change	RU Max
nivolumab and J116 to C62S PD-1						
SB-11272013-03	C62S PD-1	nivolumab	20	J116	5	24
SB-03062014-01	C62S PD-1	nivolumab	21	J116	6	25
SB-03112014-22	C62S PD-1	J116	21	nivolumab	11	34
SB-03112014-23	C62S PD-1	J116	11	nivolumab	11	18
SB-03202014-10	C62S PD-1	J116	15	J116	2	15
SB-04092014-22	C62S PD-1	J116	21	J116	3	24
nivolumab and J110 to C62S PD-1						
SB-10042013-01	C62S PD-1	nivolumab	30	J110	12	38
SB-11212013-03	C62S PD-1	nivolumab	29	J110	21	45
SB-11212013-04	C62S PD-1	nivolumab	29	J110	22	44
SB-01082014-11	C62S PD-1	nivolumab	64	J110	55	112
SB-01082014-12	C62S PD-1	nivolumab	64	J110	55	109
SB-03132014-07	C62S PD-1	nivolumab	46	J110	40	76
SB-03132014-08	C62S PD-1	nivolumab	58	J110	47	98
SB-10042013-02	C62S PD-1	J110	38	nivolumab	26	67
SB-01222014-01	C62S PD-1	J110	50	nivolumab	44	95
SB-02072014-05	C62S PD-1	J110	39	nivolumab	27	72
SB-03212014-01	C62S PD-1	J110	13	nivolumab	12	25
SB-04102014-02	C62S PD-1	J110	35	nivolumab	22	60
SB-03192014-04	C62S PD-1	J110	20	J110	7	18
SB-03192014-12	C62S PD-1	J110	19	J110	6	17

5. J105-J116 & J110 Simultaneous Binding Assays

162. I also oversaw testing of the J110 and J116 antibodies against the J105 antibody in simultaneous binding assays. The indirect and direct assays were performed for these two murine monoclonal antibodies in a manner analogous to that used to investigate simultaneous binding between the J110 and J116 antibodies relative to the antibody J105, or the J105 antibody relative to itself. In both types of assays, the addition of the second antibody caused a significant increase in RU values that was consistent with the second antibody binding simultaneously to the human PD-1 protein with the first antibody. Tables compiling the results of these experiments are reproduced below.

Table 12: Simultaneous Binding Assays by Indirect Capture of J105 and J110 or J116					
Sensorgram #	Antibody 1	Antigen	RU Change	Antibody 2	RU Change
J105 and J110 to PD-1 (C62S)					
SB-03312014-09	J105/14-2799	C62S PD-1	9	J110	8
SB-03312014-10	J105/14-2799	C62S PD-1	9	J110	8
SB-04092014-12	J105/14-2799	C62S PD-1	9	J110	17
SB-03312014-11	J110	C62S PD-1	11	J105/D133-3	20
SB-03312014-12	J110	C62S PD-1	12	J105/D133-3	15
SB-04092014-03	J110	C62S PD-1	8	J105/D133-3	24
SB-03312014-13	J110	C62S PD-1	12	J105/14-2799	16
SB-03312014-14	J110	C62S PD-1	12	J105/14-2799	15
SB-03312014-15	J110	C62S PD-1	12	J105/14-2799	19
SB-03312014-16	J110	C62S PD-1	12	J105/14-2799	17
SB-04092014-04	J110	C62S PD-1	7	J105/14-2799	20
SB-04092014-05	J110	C62S PD-1	8	J105/14-2799	21
J105 and J116 to PD-1 (C62S)					
SB-03312014-07	J105/14-2799	C62S PD-1	11	J116	6
SB-03312014-08	J105/14-2799	C62S PD-1	9	J116	6
SB-04092014-11	J105/14-2799	C62S PD-1	9	J116	3

Table 13: Simultaneous Binding Assays by Direct Capture of J105 and J110 or J116						
Sensorgram #	Antigen	Antibody 1	Max RU	Antibody 2	RU Change	Max RU
J105/D133-3 or J105/14-2799 and J110 to PD-1 (C62S)						
SB-03112014-03	PD-1 (C62S)	J105/D133-3	23	J110	22	42
SB-03112014-04	PD-1 (C62S)	J105/D133-3	24	J110	22	41
SB-03252014-02	PD-1 (C62S)	J105/D133-3	38	J110	18	49
SB-03252014-05	PD-1 (C62S)	J105/D133-3	33	J110	11	40
SB-03112014-07	PD-1 (C62S)	J105/14-2799	28	J110	21	42
SB-03112014-08	PD-1 (C62S)	J105/14-2799	28	J110	22	42
SB-03132014-03	PD-1 (C62S)	J105/14-2799	47	J110	45	83
SB-03132014-04	PD-1 (C62S)	J105/14-2799	58	J110	54	102
SB-03182014-01	PD-1 (C62S)	J105/14-2799	22	J110	19	31
SB-03182014-02	PD-1 (C62S)	J105/14-2799	23	J110	21	32
SB-03182014-07	PD-1 (C62S)	J105/14-2799	22	J110	18	33
SB-03182014-08	PD-1 (C62S)	J105/14-2799	22	J110	19	34
SB-03202014-03	PD-1 (C62S)	J105/14-2799	20	J110	16	25
SB-03202014-05	PD-1 (C62S)	J105/14-2799	20	J110	17	28
SB-03262014-03	PD-1 (C62S)	J105/14-2799	25	J110	32	41
SB-03262014-05	PD-1 (C62S)	J105/14-2799	28	J110	27	47
SB-03112014-12	PD-1 (C62S)	J110	28	J105/D133-3	20	41
SB-03112014-15	PD-1 (C62S)	J110	22	J105/D133-3	20	38

SB-03112014-13	PD-1 (C62S)	J110	16	J105/14-2799	17	33
SB-03112014-14	PD-1 (C62S)	J110	31	J105/14-2799	17	47
SB-03112014-16	PD-1 (C62S)	J110	13	J105/14-2799	17	33
SB-03112014-17	PD-1 (C62S)	J110	31	J105/14-2799	16	48
J105/D133-3 or J105/14-2799 and J116 to PD-1 (C62S)						
SB-03272014-01	PD-1 (C62S)	J105/14-2799	28	J116	5	32
SB-03272014-02	PD-1 (C62S)	J105/14-2799	30	J116	5	27
SB-03272014-03	PD-1 (C62S)	J116	19	J105/D133-3	22	41
SB-03112014-18	PD-1 (C62S)	J116	17	J105/D133-3	20	34
SB-03202014-06	PD-1 (C62S)	J116	14	J105/D133-3	17	23
SB-03272014-04	PD-1 (C62S)	J116	24	J105/14-2799	23	45
SB-03112014-19	PD-1 (C62S)	J116	17	J105/14-2799	15	34
SB-03272014-05	PD-1 (C62S)	J116	32	J105/14-2799	21	55
SB-03112014-20	PD-1 (C62S)	J116	16	J105/14-2799	9	24
SB-03112014-21	PD-1 (C62S)	J116	16	J105/14-2799	8	24
SB-03202014-07	PD-1 (C62S)	J116	15	J105/14-2799	16	31
SB-03202014-08	PD-1 (C62S)	J116	21	J105/14-2799	17	38

IX. CONCLUSIONS

163. The results of “yes-no” assays of binding indicate that only the nivolumab antibody failed to bind to the A101L variant of human PD-1. The J105, J110, and J116 antibodies bound to this PD-1 variant with high affinity, comparable to affinity values exhibited for both wild-type PD-1 and C62S PD-1. These findings indicate that the J105, J110, and J116 antibodies do not bind to the same epitope as the nivolumab antibody.
164. The indirect capture simultaneous binding studies demonstrate that (a) the J105/D133-3 and J105/14-2799 antibodies did not simultaneously bind to human PD-1 when the nivolumab antibody had already bound to the human PD-1 protein, and (b) the nivolumab antibody did not simultaneously bind to human PD-1 when the J105/D133-3 antibody or the J105/14-2799 antibody had already bound to the human PD-1 protein.
165. The direct capture simultaneous binding studies demonstrate that the J105/D133-3 and the J105/14-2799 monoclonal antibodies could simultaneously bind to human PD-1 with

the nivolumab antibody (5C4) in the direct capture assay, but that the binding of these antibodies was mutually restrictive and therefore significantly reduced. Specifically, RU values for the second antibody were significantly reduced, which is indicative of the first antibody inhibiting binding of the second antibody to the human PD-1 protein when the PD-1 was coupled to the chip surface.

166. More specifically, the direct simultaneous binding results show that:

- (a) the J105/D133-3 and J105/14-2799 antibodies, once bound to the human PD-1 protein coupled to the sensor chip, inhibited the ability of the nivolumab antibody to bind to the human PD-1 protein as indicated by significantly reduced RU values relative to the RU values seen when the first antibody bound to the human PD-1 protein on the chip; and
- (b) the nivolumab antibody, once it was bound to the human PD-1 protein coupled to the sensor chip, inhibited the ability of each of the J105/D133-3 and the J105/14-2799 antibodies to bind to human PD-1 as indicated by significantly reduced RU values relative to the RU values seen when the first antibody bound to the human PD-1 protein on the chip.

167. The simultaneous binding studies I performed show that the J105/D133-3 and J105/14-2799 antibodies each cross-compete for binding to human PD-1 with the nivolumab antibody (5C4).

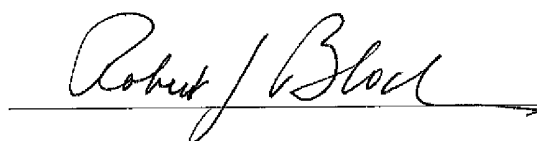
168. The results of the simultaneous binding studies also indicate that the J110 and J116 antibodies do not cross-compete with the nivolumab antibody (5C4) in binding to human PD-1. This is established by the data showing that each of the J110 and J116 antibodies

could bind to human PD-1 while it was simultaneously being bound by the nivolumab antibody in both the direct and indirect capture assays.

169. In summary, based on my analysis of the experiments described above, I believe:

- (a) Each of the J105/D133-3, J105/14-2799, nivolumab, J110 and J116 antibodies bind to wild-type human PD-1 and the C62S variant of human PD-1 with high affinity (i.e., < 5 nanomolar).
- (b) The J105/D133-3, J105/14-2799, J110 and J116 antibodies each bind to the A101L variant of human PD-1 with high affinity (< 5 nanomolar). However, the nivolumab antibody does not bind to this A101L variant. Together, these data demonstrate that the nivolumab antibody binds to a different epitope than each of the J105/D133-3, J105/14-2799, J110, and J116 antibodies.
- (c) Each of the J105/D133-3 and the J105/14-2799 antibodies cross-competes with the nivolumab antibody for binding to PD-1 (i.e., both wild-type human PD-1 and the C62S variant of human PD-1).
- (d) Each of the J105/D133-3, J105/14-2799, nivolumab, J110 and J116 antibodies are not able to bind specifically to the CD28, ICOS or CTLA-4 proteins.
- (e) The J110 antibody and the J116 antibody do not cross-compete with any of the nivolumab, J105/D133-3 or J105/14-2799 antibodies for binding to PD-1 (i.e., wild-type human PD-1 and the C62S variant of human PD-1).

Dated: April 29, 2014



Dr. Robert J. Bloch

Exhibit Number	Exhibit
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D2	Ed Harlow & David Lane, Using Antibodies: A Laboratory Manual (1999) (“Harlow 1999”).
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D28	Declaration of Hiroyuki Yamada
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D34	J105/14-2799 Datasheet (eBioscience)
D35	Email from MednaBio to Bloch re: PD1 Sequence
D36	Datasheet for Mouse IgG Antibody
D37	Datasheet for Human IgG Antibody
D38	J110 Datasheet (MBL)

D39	J116 Datasheet (eBioscience)
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