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(54) Title: METHOD OF PREVENTING ORGAN TRANSPLANT REJECTIONS USING AGONISTS TO THE PD-1 CHECKPOINT PATHWAY

(57) Abstract: Described are methods for treating or preventing solid organ transplant rejection in a subject comprising administering to the subject an agent that activates the PD-1 pathway. The agent may be an anti-PD-1 antibody, a multimerized PD-L1, a multimerized PD-L2, or a small chemical molecule, for example.

METHOD OF PREVENTING ORGAN TRANSPLANT REJECTIONS USING AGONISTS TO THE PD-1 CHECKPOINT PATHWAY

REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Patent Application No. 62/249,499, filed on November 2, 2015, both of which are hereby incorporated by reference for all purposes as if fully set forth herein.

BACKGROUND OF THE INVENTION

[0001] Tumor-specific cytotoxic T cells migrate to a tumor, where they specifically recognize and bind to antigens presented on tumor cells. Upon engagement with tumor cells, cytotoxic T cells attack the tumor and may induce apoptosis or programmed cell death of the tumor cell. Programmed cell death-1 (PD-1) is an immune "checkpoint" receptor that inhibits the T-cell response and plays a key role in modulating T-cell function. One way tumors evade normal immune attack is through exploitation of the PD-1 checkpoint pathway via the PD-1 receptor, a key regulator of T-cell activity, by converting active T cells to inactive T cells. The presence of tumors has been associated with tumor expression of PD-1 ligand (PD-L). Tumors are believed to evade normal immune attack by exploiting the PD-1 immune checkpoint pathway. Both PD-L1 and PD-L2 bind to the PD-1 receptor on activated T cells, which inhibits the T cells and T-cell attack. Pembrolizumab (anti-PD-1) is a commercially available therapeutic agent used to treat patients with metastatic melanoma and other advanced cancers.

[0002] Other ways cancers evade the body's immune response is by exploiting the CTLA-4 immune checkpoint pathway, a regulator of T-cell activity. Interaction of CTLA-4 on the T cell

with its ligands (CD80 [also known as B7-1] and CD86) on the antigen presenting cell (APC) leads to T-cell inhibition. T-cell expression of CTLA-4 inhibits the anti-tumor response by restricting T-cell activation and proliferation. Ipilimumab (anti-CTLA-4) is a commercially available therapeutic agent used to treat patients with metastatic melanoma. The PD-1 and CTLA-4 checkpoint pathways provide targets for possible therapeutic applications but they are not thoroughly understood. These pathways are actively being studied by scientists in hopes of creating therapies that enhance the quality of human life.

SUMMARY OF THE INVENTION

[0003] One embodiment of the present invention is a method for treating or preventing solid organ transplant rejection in a subject comprising administering to the subject an agent that activates the PD-1 pathway. A suitable subject is human, is an organ transplant recipient, wherein the method treats or prevents solid organ transplant rejection in the subject administered the isolated agent compared to a reference subject that did not receive the isolated agent, for example. A suitable solid organ transplant is a kidney transplant, but could be any solid organ transplant. Suitable agents used in the present invention includes an anti-PD-1 antibody, a multimerized PD-L1, a multimerized PD-L2, a small chemical molecule, or a combination thereof, for example. Other suitable agents include an agonist anti-PD-1 antibody; a peptide that activates PD-1 signaling; a small chemical molecule agonist that binds to and activates PD-1 signaling; an aptamer, a nucleic acid sequence, or a combination thereof.

[0004] Unless defined otherwise, all technical and scientific terms used herein have the meaning commonly understood by a person skilled in the art to which this invention belongs. The following references provide one of skill with a general definition of many of the terms used

in this invention: Singleton et al., Dictionary of Microbiology and Molecular Biology (2nd ed. 1994); The Cambridge Dictionary of Science and Technology (Walker ed., 1988); The Glossary of Genetics, 5th Ed., R. Rieger et al. (eds.), Springer Verlag (1991); and Hale & Marham, The Harper Collins Dictionary of Biology (1991). As used herein, the following terms have the meanings ascribed to them below, unless specified otherwise.

[0005] The term "activity" refers to the ability of a gene to perform its function such as PD-1, functioning as an immune checkpoint, plays an important role in down regulating the immune system by preventing the activation of T-cells, which in turn reduces autoimmunity and promotes self-tolerance.

[0006] The term "antibody," as used in this disclosure, refers to an immunoglobulin or a fragment or a derivative thereof, and encompasses any polypeptide comprising an antigen-binding site, regardless of whether it is produced *in vitro* or *in vivo*. The term includes, but is not limited to, polyclonal, monoclonal, monospecific, polyspecific, non-specific, humanized, single-chain, chimeric, synthetic, recombinant, hybrid, mutated, and grafted antibodies. Unless otherwise modified by the term "intact," as in "intact antibodies," for the purposes of this disclosure, the term "antibody" also includes antibody fragments such as Fab, $F(ab')_2$, Fv, scFv, Fd, dAb, and other antibody fragments that retain antigen-binding function, *i.e.*, the ability to bind, for example, PD-L1, specifically. Typically, such fragments would comprise an antigen-binding domain.

[0007] The terms "antigen-binding domain," "antigen-binding fragment," and "binding fragment" refer to a part of an antibody molecule that comprises amino acids responsible for the specific binding between the antibody and the antigen. In instances, where an antigen is large, the antigen-binding domain may only bind to a part of the antigen. A portion of the antigen

molecule that is responsible for specific interactions with the antigen-binding domain is referred to as “epitope” or “antigenic determinant.” An antigen-binding domain typically comprises an antibody light chain variable region (V_L) and an antibody heavy chain variable region (V_H), however, it does not necessarily have to comprise both. For example, a so-called Fd antibody fragment consists only of a V_H domain, but still retains some antigen-binding function of the intact antibody.

[0008] Binding fragments of an antibody are produced by recombinant DNA techniques, or by enzymatic or chemical cleavage of intact antibodies. Binding fragments include Fab, Fab', F(ab')2, Fv, and single-chain antibodies. An antibody other than a "bispecific" or "bifunctional" antibody is understood to have each of its binding sites identical. Digestion of antibodies with the enzyme, papain, results in two identical antigen-binding fragments, known also as "Fab" fragments, and a "Fc" fragment, having no antigen-binding activity but having the ability to crystallize. Digestion of antibodies with the enzyme, pepsin, results in the F(ab')2 fragment in which the two arms of the antibody molecule remain linked and comprise two-antigen binding sites. The F(ab')2 fragment has the ability to crosslink antigen. "Fv" when used herein refers to the minimum fragment of an antibody that retains both antigen-recognition and antigen-binding sites. "Fab" when used herein refers to a fragment of an antibody that comprises the constant domain of the light chain and the CHI domain of the heavy chain.

[0009] By "agent" is meant any small molecule chemical compound, antibody, nucleic acid molecule, or polypeptide, or fragments thereof.

[0010] By “ameliorate” is meant decrease, suppress, attenuate, diminish, arrest, or stabilize the development or progression of a disease or transplant rejection.

[0011] By "alteration" is meant a change (increase or decrease) in the expression levels or activity of a gene or polypeptide as detected by standard art known methods such as those described herein. As used herein, an alteration includes a 10% change in expression levels, preferably a 25% change, more preferably a 40% change, and most preferably a 50% or greater change in expression levels. "

[0012] By "analog" is meant a molecule that is not identical, but has analogous functional or structural features. For example, a polypeptide analog retains the biological activity of a corresponding naturally-occurring polypeptide, while having certain biochemical modifications that enhance the analog's function relative to a naturally occurring polypeptide. Such biochemical modifications could increase the analog's protease resistance, membrane permeability, or half-life, without altering, for example, ligand binding. An analog may include an unnatural amino acid.

[0013] By “anti-PD-1 antibody” is meant an antibody that selectively binds PD-1.

[0014] By “disease” is meant any condition or disorder that damages or interferes with the normal function of a cell, tissue, or organ. Examples of diseases include pancreatic cancer.

[0015] By "effective amount" is meant the amount of a required to ameliorate the symptoms of a disease relative to an untreated patient. The effective amount of active compound(s) used to practice the present invention for therapeutic treatment of a disease varies depending upon the manner of administration, the age, body weight, and general health of the subject. Ultimately, the attending physician or veterinarian will decide the appropriate amount and dosage regimen. Such amount is referred to as an "effective" amount.

[0016] The term “express” refers to the ability of a gene to express the gene product including for example its corresponding mRNA or protein sequence (s).

[0017] By "fragment" is meant a portion of a polypeptide or nucleic acid molecule. This portion contains, preferably, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the entire length of the reference nucleic acid molecule or polypeptide. A fragment may contain 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1000 nucleotides or amino acids.

[0018] "Hybridization" means hydrogen bonding, which may be Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen bonding, between complementary nucleobases. For example, adenine and thymine are complementary nucleobases that pair through the formation of hydrogen bonds.

[0019] "Immunoassay" is an assay that uses an antibody to specifically bind an antigen (*e.g.*, a marker). The immunoassay is characterized by the use of specific binding properties of a particular antibody to isolate, target, and/or quantify the antigen.

[0020] The term, "obtaining" as in "obtaining an agent" includes synthesizing, purchasing, or otherwise acquiring the agent.

[0021] The term "mAb" refers to monoclonal antibody. Antibodies of the invention comprise without limitation whole native antibodies, bispecific antibodies; chimeric antibodies; Fab, Fab', single chain V region fragments (scFv), fusion polypeptides, and unconventional antibodies.

[0022] The term "PD-1", or Programmed cell death protein 1, also known as PD-1 and CD279 (cluster of differentiation 279), is a protein that in humans is encoded by the *PDCD1* gene. PD-1 is a cell surface receptor that belongs to the immunoglobulin superfamily and is expressed on T cells and pro-B cells. PD-1 binds two ligands, PD-L1 andPD-L2. (NAD^+). The Indoleamine-2, 3-dioxygenase protein may also be referred to as IDO1 or IDO.

[0023] The term "PD-L1" refers to one of the major ligands of PD-1 called program death ligand 1. The term "anti PD-L1" refers to one or more entity, such as an antibody for example, that bind to PD-L1.

[0024] The terms "polypeptide," "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is an analog or mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers. Polypeptides can be modified, *e.g.*, by the addition of carbohydrate residues to form glycoproteins. The terms "polypeptide," "peptide" and "protein" include glycoproteins, as well as non-glycoproteins.

[0025] By "reduces" is meant a negative alteration of at least 10%, 25%, 50%, 75%, or 100%.

[0026] A "reference" refers to a standard or control conditions such as a sample (human cells) or a subject that is a free, or substantially free, of an agent such agent that activates the PD-1 pathway.

[0027] A "reference sequence" is a defined sequence used as a basis for sequence comparison. A reference sequence may be a subset of or the entirety of a specified sequence; for example, a segment of a full-length cDNA or gene sequence, or the complete cDNA or gene sequence. For polypeptides, the length of the reference polypeptide sequence will generally be at least about 16 amino acids, preferably at least about 20 amino acids, more preferably at least about 25 amino acids, and even more preferably about 35 amino acids, about 50 amino acids, or about 100 amino acids. For nucleic acids, the length of the reference nucleic acid sequence will generally be at least about 50 nucleotides, preferably at least about 60 nucleotides, more

preferably at least about 75 nucleotides, and even more preferably about 100 nucleotides or about 300 nucleotides or any integer thereabout or there between.

[0028] As used herein, the term “sensitivity” is the percentage of subjects with a particular disease.

[0029] As used herein, the term “specificity” is the percentage of subjects correctly identified as having a particular disease i.e., normal or healthy subjects. For example, the specificity is calculated as the number of subjects with a particular disease as compared to non-cancer subjects (e.g., normal healthy subjects).

[0030] By "specifically binds" is meant a compound or antibody that recognizes and binds a polypeptide of the invention, but which does not substantially recognize and bind other molecules in a sample, for example, a biological sample, which naturally includes a polypeptide of the invention.

[0031] As used herein, the term "subject" is intended to refer to any individual or patient to which the method described herein is performed. Generally the subject is human, although as will be appreciated by those in the art, the subject may be an animal. Thus other animals, including mammals such as rodents (including mice, rats, hamsters and guinea pigs), cats, dogs, rabbits, farm animals including cows, horses, goats, sheep, pigs, etc., and primates (including monkeys, chimpanzees, orangutans and gorillas) are included within the definition of subject.

[0032] Nucleic acid molecules useful in the methods of the invention include any nucleic acid molecule that encodes a polypeptide of the invention or a fragment thereof. Such nucleic acid molecules need not be 100% identical with an endogenous nucleic acid sequence, but will typically exhibit substantial identity. Polynucleotides having “substantial identity” to an

endogenous sequence are typically capable of hybridizing with at least one strand of a double-stranded nucleic acid molecule. Nucleic acid molecules useful in the methods of the invention include any nucleic acid molecule that encodes a polypeptide of the invention or a fragment thereof. Such nucleic acid molecules need not be 100% identical with an endogenous nucleic acid sequence, but will typically exhibit substantial identity. Polynucleotides having "substantial identity" to an endogenous sequence are typically capable of hybridizing with at least one strand of a double-stranded nucleic acid molecule. By "hybridize" is meant pair to form a double-stranded molecule between complementary polynucleotide sequences (e.g., a gene described herein), or portions thereof, under various conditions of stringency. (See, e.g., Wahl, G. M. and S. L. Berger (1987) Methods Enzymol. 152:399; Kimmel, A. R. (1987) Methods Enzymol. 152:507).

[0033] For example, stringent salt concentration will ordinarily be less than about 750 mM NaCl and 75 mM trisodium citrate, preferably less than about 500 mM NaCl and 50 mM trisodium citrate, and more preferably less than about 250 mM NaCl and 25 mM trisodium citrate. Low stringency hybridization can be obtained in the absence of organic solvent, e.g., formamide, while high stringency hybridization can be obtained in the presence of at least about 35% formamide, and more preferably at least about 50% formamide. Stringent temperature conditions will ordinarily include temperatures of at least about 30° C, more preferably of at least about 37° C, and most preferably of at least about 42° C. Varying additional parameters, such as hybridization time, the concentration of detergent, e.g., sodium dodecyl sulfate (SDS), and the inclusion or exclusion of carrier DNA, are well known to those skilled in the art. Various levels of stringency are accomplished by combining these various conditions as needed. In a preferred: embodiment, hybridization will occur at 30° C in 750 mM NaCl, 75 mM trisodium citrate, and

1% SDS. In a more preferred embodiment, hybridization will occur at 37° C in 500 mM NaCl, 50 mM trisodium citrate, 1% SDS, 35% formamide, and 100 .mu.g/ml denatured salmon sperm DNA (ssDNA). In a most preferred embodiment, hybridization will occur at 42° C in 250 mM NaCl, 25 mM trisodium citrate, 1% SDS, 50% formamide, and 200 μg/ml ssDNA. Useful variations on these conditions will be readily apparent to those skilled in the art.

[0034] For most applications, washing steps that follow hybridization will also vary in stringency. Wash stringency conditions can be defined by salt concentration and by temperature. As above, wash stringency can be increased by decreasing salt concentration or by increasing temperature. For example, stringent salt concentration for the wash steps will preferably be less than about 30 mM NaCl and 3 mM trisodium citrate, and most preferably less than about 15 mM NaCl and 1.5 mM trisodium citrate. Stringent temperature conditions for the wash steps will ordinarily include a temperature of at least about 25° C, more preferably of at least about 42° C, and even more preferably of at least about 68° C. In a preferred embodiment, wash steps will occur at 25° C in 30 mM NaCl, 3 mM trisodium citrate, and 0.1% SDS. In a more preferred embodiment, wash steps will occur at 42 C in 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS. In a more preferred embodiment, wash steps will occur at 68° C in 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS. Additional variations on these conditions will be readily apparent to those skilled in the art. Hybridization techniques are well known to those skilled in the art and are described, for example, in Benton and Davis (Science 196:180, 1977); Grunstein and Hogness (Proc. Natl. Acad. Sci., USA 72:3961, 1975); Ausubel et al. (Current Protocols in Molecular Biology, Wiley Interscience, New York, 2001); Berger and Kimmel (Guide to Molecular Cloning Techniques, 1987, Academic Press, New York); and Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, New York.

[0035] By "substantially identical" is meant a polypeptide or nucleic acid molecule exhibiting at least 50% identity to a reference amino acid sequence (for example, any one of the amino acid sequences described herein) or nucleic acid sequence (for example, any one of the nucleic acid sequences described herein). Preferably, such a sequence is at least 60%, more preferably 80% or 85%, and more preferably 90%, 95% or even 99% identical at the amino acid level or nucleic acid to the sequence used for comparison.

[0036] Sequence identity is typically measured using sequence analysis software (for example, Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, Wis. 53705, BLAST, BESTFIT, GAP, or PILEUP/PRETTYBOX programs). Such software matches identical or similar sequences by assigning degrees of homology to various substitutions, deletions, and/or other modifications. Conservative substitutions typically include substitutions within the following groups: glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid, asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine. In an exemplary approach to determining the degree of identity, a BLAST program may be used, with a probability score between e^{-3} and e^{-100} indicating a closely related sequence.

[0037] Ranges provided herein are understood to be shorthand for all of the values within the range. For example, a range of 1 to 50 is understood to include any number, combination of numbers, or sub-range from the group consisting 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50.

[0038] As used herein, the terms “treat,” treating,” “treatment,” and the like refer to reducing or ameliorating a disorder and/or symptoms associated therewith. It will be appreciated

that, although not precluded, treating a disorder or condition does not require that the disorder, condition or symptoms associated therewith be completely eliminated.

[0039] Unless specifically stated or obvious from context, as used herein, the term "or" is understood to be inclusive. Unless specifically stated or obvious from context, as used herein, the terms "a", "an", and "the" are understood to be singular or plural.

[0040] Unless specifically stated or obvious from context, as used herein, the term "about" is understood as within a range of normal tolerance in the art, for example within 2 standard deviations of the mean. About can be understood as within 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.1%, 0.05%, or 0.01% of the stated value. Unless otherwise clear from context, all numerical values provided herein are modified by the term about.

[0041] The recitation of a listing of chemical groups in any definition of a variable herein includes definitions of that variable as any single group or combination of listed groups. The recitation of an embodiment for a variable or aspect herein includes that embodiment as any single embodiment or in combination with any other embodiments or portions thereof.

[0042] Any compositions or methods provided herein can be combined with one or more of any of the other compositions and methods provided herein.

[0043] As used herein, the terms "prevent," "preventing," "prevention," "prophylactic treatment" and the like refer to reducing the probability of developing a disorder or condition in a subject, who does not have, but is at risk of or susceptible to developing a disorder or condition.

[0044] Such treatment (surgery and/or chemotherapy) will be suitably administered to subjects, particularly humans, suffering from, having, susceptible to, or at risk for pancreatic cancer or disease, disorder, or symptom thereof. Determination of those subjects "at risk" can be made by any objective or subjective determination by a diagnostic test or opinion of a subject or

health care provider (e.g., genetic test, enzyme or protein marker, a marker, family history, and the like).

BRIEF DESCRIPTION OF THE DRAWINGS

[0045] Figure 1 illustrates histologic sections of cutaneous squamous cell carcinoma in a kidney transplant recipient labeled H&E, PD-L1, PD-1, PD-L2, CD8/Ki67, and TIA-1.

[0046] Figure 2 illustrates CT scan images demonstrating regression of cutaneous squamous cell carcinoma lung metastases in a kidney transplant recipient. Arrowheads indicate sites of metastases. The CT scans were taken pre-therapy and 8 months after initiating pembrolizumab (anti-PD-1).

[0047] Figure 3A-3H illustrates histologic sections of the explanted kidney from a kidney transplant recipient who was treated with anti-PD-1 for metastatic cutaneous squamous cell carcinoma.

DETAILED DESCRIPTION OF THE INVENTION

[0048] All references, including publications, patent applications, and patents, cited herein are hereby incorporated by reference to the same extent as if each reference were individually and specifically indicated to be incorporated by reference and were set forth in its entirety herein. The use of the terms “a” and “an” and “the” and similar referents in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. The terms “comprising,” “having,” “including,” and “containing” are to be construed as open-ended terms (i.e., meaning “including, but not limited to,”) unless otherwise noted. Recitation of

ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., "such as") provided herein, is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the invention.

[0049] Preferred embodiments of this invention are described herein, including the best mode known to the inventors for carrying out the invention. Variations of those preferred embodiments may become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventors expect skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

[0050] The present invention relates to the surprising discovery of using agonist of the PD-1 checkpoint pathway to enhance the maintenance of solid organ transplants in solid organ transplant recipients. The discovery was made when pembrolizumab (anti-PD-1) was administered to a kidney transplant patient on chronic immunosuppressive medications who

developed metastatic cutaneous squamous cell carcinoma. Fig. 2 illustrates regression of cutaneous squamous cell carcinoma lung metastases after administration of pembrolizumab to this patient. Fig. 3 illustrates histologic changes that occurred after the medicament induced rejection of the kidney allograft. These findings suggest the importance of PD-1 and its ligands in influencing allograft rejection, and is the first case to directly demonstrate the relevance of the PD-1 pathway in maintaining adaptive tolerance to solid organ transplants in humans.

[0051] Not being held to a particular theory, adaptive immune responses to self and non-self antigens, including those against antigens in solid organ allografts, are largely regulated by a series of molecular signals that control T cell activation. Following the interaction between a T cell receptor and its cognate peptide (signal 1), a co-stimulatory signal (signal 2) is required to trigger T cell proliferation, acquisition of effector function, and migration to sites of antigen expression. The prototypical immune checkpoint molecule, cytotoxic T-lymphocyte-associated protein-4 (CTLA-4) is expressed on T cells, and functions by binding to the co-stimulatory ligands B7-1 and B7-2 with higher affinity than their co-stimulatory receptor, CD28, effectively hijacking signal 2 and attenuating T cell function. In normal hosts, immune checkpoints like CTLA-4 prevent immune activation against self-antigens that might otherwise lead to autoimmunity. PD-1, a second immune checkpoint, is also important in maintaining self-tolerance as well as mitigating collateral damage of tissues involved in active immune responses. In general, CTLA-4 likely operates in lymph nodes, where it functions by modulating activation during the initial (priming) phase of an immune response. PD-1, by contrast, likely functions within peripheral tissues and, in the case of cancer, within the tumor microenvironment, during the effector phase of a T cell response. Although clinical trials testing drugs targeting the CTLA-4 and PD-1 pathways have demonstrated remarkable anti-tumor activity of these agents, they

have excluded patients on chronic immunosuppressive drug regimens, both for autoimmune disease and solid organ transplants.

[0052] The first report of a solid organ transplant recipient (SOTR) receiving immune checkpoint blockade therapy involved two patients with metastatic melanoma who had previously undergone kidney transplantation. Renal allografts from both patients appeared to have been unaffected by administration of ipilimumab (anti-CTLA-4). Both patients experienced an anti-tumor response to therapy. Similarly, allograft tolerance remained intact in two patients who had undergone liver transplantation and received ipilimumab for metastatic melanoma.

[0053] In contrast, pembrolizumab (anti-PD-1) likely contributed to allograft rejection in the current report. T cell-mediated rejection (TCMR) occurs rarely >10 years post-transplant. In addition, the severity of the TCMR suggests that it was triggered by PD-1 blockade rather than discontinuation of immunosuppression. Although preclinical studies have suggested the importance of PD-1 and its ligands in influencing allograft rejection, our case is the first to directly demonstrate the relevance of the PD-1 pathway in maintaining adaptive tolerance to solid organ transplants in humans. These findings underscore the functional differences between CTLA-4 and PD-1, the latter more heavily influencing immunomodulation within peripheral tissues.

[0054] Our patient's explanted allograft demonstrated changes consistent with advanced cell mediated rejection. (Figure 3) The lack of C4d staining in the peritubular capillaries as well as the lack of HLA antibody suggest an absence of anti-donor humoral activity and argues against a component of antibody-mediated rejection. Although no histological or serological samples were available from the time at which allograft dysfunction was initially detected, if donor-specific

antibodies were present at the time of rejection, it is likely that the inflammation associated with the surgical nephrectomy would have resulted in an increase in antibody affinity as well as the persistence of antibodies associated with rejection. Taken together, our findings do not support a substantial humoral component of graft rejection in this case.

[0055] The present invention further suggests that, in the setting of immune checkpoint blockade, allograft destruction is likely mediated by T cells. The relative roles of PD-1 and CTLA-4 in this process remain unclear; however, the lack of organ rejection in a small number of anti-CTLA-4 treated patients compared with the organ rejection seen in this anti-PD-1 treated patient further emphasize the difference between these two checkpoints in immune regulation. The differing roles of the CTLA-4 and PD-1 pathways in transplant tolerance has potential relevance to clinical management of melanomas in SOTR patients. Both anti-CTLA-4 (ipilimumab) and anti-PD-1 (nivolumab and pembrolizomab) are FDA-approved for treatment of advanced melanoma. The higher response rate and lower toxicity of anti-PD-1 relative to anti-CTLA-4 underpins a shift toward anti-PD-1 treatment of melanoma in the first line. However, we propose that anti-CTLA-4 should be considered before anti-PD-1 for treatment of melanoma in SOTRs because of the different effects on adaptive transplant tolerance.

[0056] The present invention demonstrates a critical role for the PD-1 pathway both in malignancies arising in the setting of long-term immunosuppression and in maintaining adaptive immune tolerance to transplanted organs. Importantly, these findings further suggest that PD-1 pathway agonists might be useful in the prevention of allograft rejection. This patient's robust anti-tumor response as well as the patient's acute allograft rejection illustrate the complexity of the interactions between immune checkpoint molecules, alloantigens, and cancer neoantigens, thus making clinical outcomes to various immune activators difficult to predict.

[0057] Embodiments of the disclosure concern methods and/or compositions for treating and/or preventing organ transplant rejections in which modulation, preferably the activation, of thePD-1 pathway is directly or indirectly related. In certain embodiments, individuals with a solid organ transplant are treated with an agent that is an activator of the PD-1 pathway.

[0058] In certain embodiments, the level to which an agent activates the PD-1 pathway may be any level so long as it provides amelioration of at least one symptom of the organ transplant rejection. The level of expression may increase by at least 2, 3, 4, 5, 10, 25, 50, 100, 1000, or more fold expression compared to the level of expression in a standard, or reference, in at least some cases. An individual may monitor the activity of the PD-1 pathway using standard methods in the art, such as northern assays or quantitative PCR, for example.

[0059] An individual known to have an organ transplant rejection, suspected of having an organ transplant rejection, or at risk for having an organ transplant rejection may be provided an effective amount of an agent that activates the PD-1 pathway, including an agonist anti-PD1 antibody. Those at risk for PD may be those individuals having one or more genetic factors, may be of advancing age, and/or may have a family history, for example.

[0060] In particular embodiments of the disclosure, an individual is given an agent for that treats or prevents organ transplant rejection in addition to the one or more of the agents that activates the PD-1 pathway. When combination therapy is employed with one or more agents that activates the PD-1 pathway, the additional therapy may be given prior to, at the same time as, and/or subsequent to an agent that activates the PD-1 pathway.

Pharmaceutical Preparations

[0061] Pharmaceutical compositions of the present invention comprise an effective amount of one or more agents that activates the PD-1 pathway such as agonist anti PD-1 antibody,

dissolved or dispersed in a pharmaceutically acceptable carrier. The phrases "pharmaceutical or pharmacologically acceptable" refers to molecular entities and compositions that do not produce an adverse, allergic or other untoward reaction when administered to an animal, such as, for example, a human, as appropriate. The preparation of a pharmaceutical composition that comprises at least one agent that activates the PD-1 pathway or an additional active ingredient will be known to those of skill in the art in light of the present disclosure, as exemplified by Remington: The Science and Practice of Pharmacy, 21st Ed. Lippincott Williams and Wilkins, 2005, incorporated herein by reference. Moreover, for animal (*e.g.*, human) administration, it will be understood that preparations should meet sterility, pyrogenicity, general safety and purity standards as required by FDA Office of Biological Standards.

[0062] As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, surfactants, antioxidants, preservatives (*e.g.*, antibacterial agents, antifungal agents), isotonic agents, absorption delaying agents, salts, preservatives, drugs, drug stabilizers, gels, binders, excipients, disintegration agents, lubricants, sweetening agents, flavoring agents, dyes, such like materials and combinations thereof, as would be known to one of ordinary skill in the art (see, for example, Remington's Pharmaceutical Sciences, 18th Ed. Mack Printing Company, 1990, pp. 1289-1329, incorporated herein by reference). Except insofar as any conventional carrier is incompatible with the active ingredient, its use in the pharmaceutical compositions is contemplated.

[0063] The agent that activates the PD-1 pathway may comprise different types of carriers depending on whether it is to be administered in solid, liquid or aerosol form, and whether it need to be sterile for such routes of administration as injection. The present compositions can be administered intravenously, intradermally, transdermally, intrathecally, intraarterially,

intraperitoneally, intranasally, intravaginally, intrarectally, topically, intramuscularly, subcutaneously, mucosally, orally, topically, locally, inhalation (*e.g.*, aerosol inhalation), injection, infusion, continuous infusion, localized perfusion bathing target cells directly, *via* a catheter, *via* a lavage, in cremes, in lipid compositions (*e.g.*, liposomes), or by other method or any combination of the forgoing as would be known to one of ordinary skill in the art (see, for example, Remington's Pharmaceutical Sciences, 18th Ed. Mack Printing Company, 1990, incorporated herein by reference).

[0064] The agent that activates the PD-1 pathway may be formulated into a composition in a free base, neutral or salt form. Pharmaceutically acceptable salts, include the acid addition salts, *e.g.*, those formed with the free amino groups of a proteinaceous composition, or which are formed with inorganic acids such as for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric or mandelic acid. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as for example, sodium, potassium, ammonium, calcium or ferric hydroxides; or such organic bases as isopropylamine, trimethylamine, histidine or procaine. Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective. The formulations are easily administered in a variety of dosage forms such as formulated for parenteral administrations such as injectable solutions, or aerosols for delivery to the lungs, or formulated for alimentary administrations such as drug release capsules and the like.

[0065] Further in accordance with the present disclosure, the composition of the present invention suitable for administration is provided in a pharmaceutically acceptable carrier with or without an inert diluent. The carrier should be assimilable and includes liquid, semi-solid, *i.e.*,

pastes, or solid carriers. Except insofar as any conventional media, agent, diluent or carrier is detrimental to the recipient or to the therapeutic effectiveness of the composition contained therein, its use in administrable composition for use in practicing the methods of the present invention is appropriate. Examples of carriers or diluents include fats, oils, water, saline solutions, lipids, liposomes, resins, binders, fillers and the like, or combinations thereof. The composition may also comprise various antioxidants to retard oxidation of one or more component. Additionally, the prevention of the action of microorganisms can be brought about by preservatives such as various antibacterial and antifungal agents, including but not limited to parabens (e.g., methylparabens, propylparabens), chlorobutanol, phenol, sorbic acid, thimerosal or combinations thereof. In accordance with the present invention, the composition is combined with the carrier in any convenient and practical manner, *i.e.*, by solution, suspension, emulsification, admixture, encapsulation, absorption and the like. Such procedures are routine for those skilled in the art.

[0066] In a specific embodiment of the present invention, the composition is combined or mixed thoroughly with a semi-solid or solid carrier. The mixing can be carried out in any convenient manner such as grinding. Stabilizing agents can be also added in the mixing process in order to protect the composition from loss of therapeutic activity, *i.e.*, denaturation in the stomach. Examples of stabilizers for use in an the composition include buffers, amino acids such as glycine and lysine, carbohydrates such as dextrose, mannose, galactose, fructose, lactose, sucrose, maltose, sorbitol, mannitol, *etc.*

[0067] In further embodiments, the present invention may concern the use of a pharmaceutical lipid vehicle compositions that include an agent that activates the PD-1 pathway, one or more lipids, and an aqueous solvent. As used herein, the term "lipid" will be defined to

include any of a broad range of substances that is characteristically insoluble in water and extractable with an organic solvent. This broad class of compounds are well known to those of skill in the art, and as the term "lipid" is used herein, it is not limited to any particular structure. Examples include compounds which contain long-chain aliphatic hydrocarbons and their derivatives. A lipid may be naturally occurring or synthetic (*i.e.*, designed or produced by man). However, a lipid is usually a biological substance. Biological lipids are well known in the art, and include for example, neutral fats, phospholipids, phosphoglycerides, steroids, terpenes, lysolipids, glycosphingolipids, glycolipids, sulphatides, lipids with ether and ester-linked fatty acids and polymerizable lipids, and combinations thereof. Of course, compounds other than those specifically described herein that are understood by one of skill in the art as lipids are also encompassed by the compositions and methods of the present invention.

[0068] One of ordinary skill in the art would be familiar with the range of techniques that can be employed for dispersing a composition in a lipid vehicle. For example, the agent that activates the PD-1 pathway may be dispersed in a solution containing a lipid, dissolved with a lipid, emulsified with a lipid, mixed with a lipid, combined with a lipid, covalently bonded to a lipid, contained as a suspension in a lipid, contained or complexed with a micelle or liposome, or otherwise associated with a lipid or lipid structure by any means known to those of ordinary skill in the art. The dispersion may or may not result in the formation of liposomes.

[0069] The actual dosage amount of a composition of the present invention administered to an animal patient can be determined by physical and physiological factors such as body weight, severity of condition, the type of disease being treated, previous or concurrent therapeutic interventions, idiopathy of the patient and on the route of administration. Depending upon the dosage and the route of administration, the number of administrations of a preferred dosage

and/or an effective amount may vary according to the response of the subject. The practitioner responsible for administration will, in any event, determine the concentration of active ingredient(s) in a composition and appropriate dose(s) for the individual subject.

[0070] In certain embodiments, pharmaceutical compositions may comprise, for example, at least about 0.1% of an active compound. In other embodiments, the an active compound may comprise between about 2% to about 75% of the weight of the unit, or between about 25% to about 60%, for example, and any range derivable therein. Naturally, the amount of active compound(s) in each therapeutically useful composition may be prepared is such a way that a suitable dosage will be obtained in any given unit dose of the compound. Factors such as solubility, bioavailability, biological half-life, route of administration, product shelf life, as well as other pharmacological considerations will be contemplated by one skilled in the art of preparing such pharmaceutical formulations, and as such, a variety of dosages and treatment regimens may be desirable.

[0071] In other non-limiting examples, a dose may also comprise from about 1 microgram/kg/body weight, about 5 microgram/kg/body weight, about 10 microgram/kg/body weight, about 50 microgram/kg/body weight, about 100 microgram/kg/body weight, about 200 microgram/kg/body weight, about 350 microgram/kg/body weight, about 500 microgram/kg/body weight, about 1 milligram/kg/body weight, about 5 milligram/kg/body weight, about 10 milligram/kg/body weight, about 50 milligram/kg/body weight, about 100 milligram/kg/body weight, about 200 milligram/kg/body weight, about 350 milligram/kg/body weight, about 500 milligram/kg/body weight, to about 1000 mg/kg/body weight or more per administration, and any range derivable therein. In non-limiting examples of a derivable range from the numbers listed herein, a range of about 5 mg/kg/body weight to about 100 mg/kg/body

weight, about 5 microgram/kg/body weight to about 500 milligram/kg/body weight, etc., can be administered, based on the numbers described above.

A. Alimentary Compositions and Formulations

[0072] In one embodiment of the present disclosure, an agent that activates the PD-1 pathway is formulated to be administered *via* an alimentary route. Alimentary routes include all possible routes of administration in which the composition is in direct contact with the alimentary tract. Specifically, the pharmaceutical compositions disclosed herein may be administered orally, buccally, rectally, or sublingually. As such, these compositions may be formulated with an inert diluent or with an assimilable edible carrier, or they may be enclosed in hard- or soft- shell gelatin capsule, or they may be compressed into tablets, or they may be incorporated directly with the food of the diet.

[0073] In certain embodiments, the active compounds may be incorporated with excipients and used in the form of ingestible tablets, buccal tables, troches, capsules, elixirs, suspensions, syrups, wafers, and the like (Mathiowitz et al., 1997; Hwang et al., 1998; U.S. Pat. Nos. 5,641,515; 5,580,579 and 5,792, 451, each specifically incorporated herein by reference in its entirety). The tablets, troches, pills, capsules and the like may also contain the following: a binder, such as, for example, gum tragacanth, acacia, cornstarch, gelatin or combinations thereof; an excipient, such as, for example, dicalcium phosphate, mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate or combinations thereof; a disintegrating agent, such as, for example, corn starch, potato starch, alginic acid or combinations thereof; a lubricant, such as, for example, magnesium stearate; a sweetening agent, such as, for example, sucrose, lactose, saccharin or combinations thereof; a flavoring agent, such as, for example peppermint, oil of wintergreen, cherry flavoring, orange flavoring, etc. When the

dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar, or both. When the dosage form is a capsule, it may contain, in addition to materials of the above type, carriers such as a liquid carrier. Gelatin capsules, tablets, or pills may be enterically coated. Enteric coatings prevent denaturation of the composition in the stomach or upper bowel where the pH is acidic. See, *e.g.*, U.S. Pat. No. 5,629,001. Upon reaching the small intestines, the basic pH therein dissolves the coating and permits the composition to be released and absorbed by specialized cells, *e.g.*, epithelial enterocytes and Peyer's patch M cells. A syrup of elixir may contain the active compound sucrose as a sweetening agent methyl and propylparabens as preservatives, a dye and flavoring, such as cherry or orange flavor. Of course, any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the active compounds may be incorporated into sustained-release preparation and formulations.

[0074] For oral administration the compositions of the present disclosure may alternatively be incorporated with one or more excipients in the form of a mouthwash, dentifrice, buccal tablet, oral spray, or sublingual orally- administered formulation. For example, a mouthwash may be prepared incorporating the active ingredient in the required amount in an appropriate solvent, such as a sodium borate solution (Dobell's Solution). Alternatively, the active ingredient may be incorporated into an oral solution such as one containing sodium borate, glycerin and potassium bicarbonate, or dispersed in a dentifrice, or added in a therapeutically- effective amount to a composition that may include water, binders, abrasives, flavoring agents, foaming

agents, and humectants. Alternatively the compositions may be fashioned into a tablet or solution form that may be placed under the tongue or otherwise dissolved in the mouth.

[0075] Additional formulations which are suitable for other modes of alimentary administration include suppositories. Suppositories are solid dosage forms of various weights and shapes, usually medicated, for insertion into the rectum. After insertion, suppositories soften, melt or dissolve in the cavity fluids. In general, for suppositories, traditional carriers may include, for example, polyalkylene glycols, triglycerides or combinations thereof. In certain embodiments, suppositories may be formed from mixtures containing, for example, the active ingredient in the range of about 0.5% to about 10%, and preferably about 1% to about 2%.

B. Parenteral Compositions and Formulations

[0076] In further embodiments, an agent that activates the PD-1 pathway may be administered via a parenteral route. As used herein, the term "parenteral" includes routes that bypass the alimentary tract. Specifically, the pharmaceutical compositions disclosed herein may be administered for example, but not limited to intravenously, intradermally, intramuscularly, intraarterially, intrathecally, subcutaneous, or intraperitoneally U.S. Pat. Nos. 6,7537,514, 6,613,308, 5,466,468, 5,543,158; 5,641,515; and 5,399,363 (each specifically incorporated herein by reference in its entirety).

[0077] Solutions of the active compounds as free base or pharmacologically acceptable salts may be prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions may also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms. The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the

extemporaneous preparation of sterile injectable solutions or dispersions (U.S. Patent 5,466,468, specifically incorporated herein by reference in its entirety). In all cases the form must be sterile and must be fluid to the extent that easy injectability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (*i.e.*, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and/or vegetable oils. Proper fluidity may be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

[0078] For parenteral administration in an aqueous solution, for example, the solution should be suitably buffered if necessary and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous, and intraperitoneal administration. In this connection, sterile aqueous media that can be employed will be known to those of skill in the art in light of the present disclosure. For example, one dosage may be dissolved in isotonic NaCl solution and either added hypodermoclysis fluid or injected at the proposed site of infusion, (see for example, "Remington's Pharmaceutical Sciences" 15th Edition, pages 1035-1038 and 1570-1580). Some

variation in dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject. Moreover, for human administration, preparations should meet sterility, pyrogenicity, general safety and purity standards as required by FDA Office of Biologics standards.

[0079] Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. A powdered composition is combined with a liquid carrier such as, *e.g.*, water or a saline solution, with or without a stabilizing agent.

C. Miscellaneous Pharmaceutical Compositions and Formulations

[0080] In other preferred embodiments of the invention, one or more agents that activate the PD-1 pathway may be formulated for administration via various miscellaneous routes, for example, topical (*i.e.*, transdermal) administration, mucosal administration (intranasal, vaginal, *etc.*) and/or inhalation.

[0081] Pharmaceutical compositions for topical administration may include the active compound formulated for a medicated application such as an ointment, paste, cream or powder. Ointments include all oleaginous, adsorption, emulsion and water-solubly based compositions

for topical application, while creams and lotions are those compositions that include an emulsion base only. Topically administered medications may contain a penetration enhancer to facilitate adsorption of the active ingredients through the skin. Suitable penetration enhancers include glycerin, alcohols, alkyl methyl sulfoxides, pyrrolidones and luarocapram. Possible bases for compositions for topical application include polyethylene glycol, lanolin, cold cream and petrolatum as well as any other suitable absorption, emulsion or water-soluble ointment base. Topical preparations may also include emulsifiers, gelling agents, and antimicrobial preservatives as necessary to preserve the active ingredient and provide for a homogenous mixture. Transdermal administration of the present invention may also comprise the use of a "patch". For example, the patch may supply one or more active substances at a predetermined rate and in a continuous manner over a fixed period of time.

[0082] In certain embodiments, the pharmaceutical compositions may be delivered by eye drops, intranasal sprays, inhalation, and/or other aerosol delivery vehicles. Methods for delivering compositions directly to the lungs via nasal aerosol sprays has been described e.g., in U.S. Pat. Nos. 5,756,353 and 5,804,212 (each specifically incorporated herein by reference in its entirety). Likewise, the delivery of drugs using intranasal microparticle resins (Takenaga et al., 1998) and lysophosphatidyl-glycerol compounds (U.S. Pat. No. 5,725, 871, specifically incorporated herein by reference in its entirety) are also well-known in the pharmaceutical arts. Likewise, transmucosal drug delivery in the form of a polytetrafluoroetheylene support matrix is described in U.S. Pat. No. 5,780,045 (specifically incorporated herein by reference in its entirety).

[0083] The term aerosol refers to a colloidal system of finely divided solid of liquid particles dispersed in a liquefied or pressurized gas propellant. The typical aerosol of the present

invention for inhalation will consist of a suspension of active ingredients in liquid propellant or a mixture of liquid propellant and a suitable solvent. Suitable propellants include hydrocarbons and hydrocarbon ethers. Suitable containers will vary according to the pressure requirements of the propellant. Administration of the aerosol will vary according to subject's age, weight and the severity and response of the symptoms.

Kits of the Disclosure

[0084] Any of the compositions described herein may be comprised in a kit. In a non-limiting example, an agent that activates the PD-1 pathway (for example, an agonist anti PD-1 antibody) may be comprised in a kit.

[0085] The kits may comprise a suitably aliquoted agent that activates the PD-1 pathway and, in some cases, one or more additional agents. The component(s) of the kits may be packaged either in aqueous media or in lyophilized form. The container means of the kits will generally include at least one vial, test tube, flask, bottle, syringe or other container means, into which a component may be placed, and preferably, suitably aliquoted. Where there are more than one component in the kit, the kit also will generally contain a second, third or other additional container into which the additional components may be separately placed. However, various combinations of components may be comprised in a vial. The kits of the present invention also will typically include a means for containing an agent that activates the PD-1 pathway and any other reagent containers in close confinement for commercial sale. Such containers may include injection or blow-molded plastic containers into which the desired vials are retained.

[0086] When the components of the kit are provided in one and/or more liquid solutions, the liquid solution is an aqueous solution, with a sterile aqueous solution being particularly

preferred. The one or more agents that activates the PD-1 pathway may be formulated into a syringeable composition. In which case, the container means may itself be a syringe, pipette, and/or other such like apparatus, from which the formulation may be applied to an infected area of the body, injected into an animal, and/or even applied to and/or mixed with the other components of the kit. However, the components of the kit may be provided as dried powder(s). When reagents and/or components are provided as a dry powder, the powder can be reconstituted by the addition of a suitable solvent. It is envisioned that the solvent may also be provided in another container means.

EXAMPLES

Evaluation of Serum Using a Multiplex Bead Assay

[0087] Evaluation of the patient's serum with multiplex bead assays was performed using the Luminex platform (Lifecodes class I and II ID panels, Immucor Gen-Probe, San Diego, CA and Single Antigen Beads, One Lambda, Canoga Park, CA). ELISA for angiotensin II type 1 receptor (AT1R) antibody was performed using EIA-AT1R (One Lambda, Canoga Park, CA). Additionally, flow cytometric crossmatches were performed against precursor endothelial cell targets obtained from two surrogate donors because the actual kidney donor was not available. Although the presence of endothelial antibodies cannot be entirely ruled out because it is unknown whether the surrogates carry the donor target antigens, results were negative. Fig. 1 illustrates the immunoarchitecture of the pre-treatment cutaneous squamous cell carcinoma (cSCC) specimen. Histologic sections demonstrate an infiltrating cSCC with a vigorous immune infiltrate and immune checkpoint expression at the tumor-stroma interface. The cSCC expresses PD-L1 at the interface with the stroma and the host-immune response. PD-L1 expression is also

seen on infiltrating macrophages and rare lymphocytes. The activated CD8+ T cell rich immune infiltrate demonstrates PD-1 on T cells and PD-L2 on myeloid cells (arrows). The infiltrating immune cells are predominantly CD8-positive and co-express Ki-67, consistent with an activated cytotoxic T cell phenotype; CD8 (brown chromagen) and Ki-67 (blue chromagen) double immunostain. TIA-1, indicative of cytotoxic activity, is expressed on CD8+ cells. (H&E, hematoxylin and eosin; TIA-1, T-cell intracytoplasmic antigen-1). Fig. 2 contains CT scan images demonstrating regression of cutaneous squamous cell carcinoma lung metastases in a kidney transplant recipient. Arrowheads indicate sites of metastases. The CT scans were taken pre-therapy and 8 months after initiating pembrolizumab (anti-PD-1). Fig. 3 illustrates histologic sections of the explanted kidney from a kidney transplant recipient who was treated with anti-PD-1 for metastatic cutaneous squamous cell carcinoma including: A) Arteries demonstrate intimal arteritis and focal intimal foam cells, consistent with chronic transplant vasculopathy. B) Strong C4d (a product of the classical complement pathway) immunostaining was present in the artery endothelium. C) Glomerulitis, severe tubular loss, tubulitis, interstitial edema and interstitial inflammation. D) Peritubular capillaries demonstrate capillaritis but negative C4d immunostaining. E and F) Immunostains for PD-L1 and PD-L2, respectively, demonstrate that these molecules are displayed on endothelial cells and infiltrating immune cells associated with glomeruli. G) Infiltrating T-cells expressing PD-1 are geographically associated with cells expressing PD-L1 and PD-L2. H) The infiltrating immune cells are predominantly CD8-positive and co-express Ki-67, consistent with an activated cytotoxic T cell phenotype; CD8 (brown chromagen) and Ki-67 (blue chromagen) double immunostain. A T-cell intracytoplasmic antigen-1 (TIA-1) immunostain was also performed and highlighted the CD8 cell subset,

indicative of cytotoxic activation (not shown). Panel C is 200x original magnification. All other panels, 400x original magnification.

Claims:

1. A method for treating or preventing solid organ transplant rejection in a subject comprising administering to the subject an agent that activates the PD-1 pathway.

2. The method of claim 1, wherein the subject is an organ transplant recipient.

3. The method of claim 1 wherein the method treats or prevents solid organ transplant rejection in the subject that is administered the isolated agent compared to a reference subject that is not administered the isolated agent.

4. The method of claim 3, wherein the reference subject is a solid organ transplant recipient.

5. The method of claim 1 wherein the agent is selected from the group comprising an anti-PD-1 antibody, a multimerized PD-L1, a multimerized PD-L2, a small chemical molecule, or a combination thereof.

6. The method of claim 1 wherein the agent is an agonist anti-PD-1 antibody.

7. The method of claim 1 where the agent is a peptide that activates PD-1 signaling.

8. The method of claim 1 wherein the isolated agent is an aptamer.

9. The method of claim 1 wherein the agent is a nucleic acid sequence.

10. The method of claim 1 wherein the agent is a small chemical molecule.

11. The method of claim 2 wherein the organ transplant recipient received a solid organ transplant.

12. The method of claim 11, wherein the solid organ transplant is a kidney transplant.

13. The method of claim 1 wherein the subject is human.

FIGURE 1

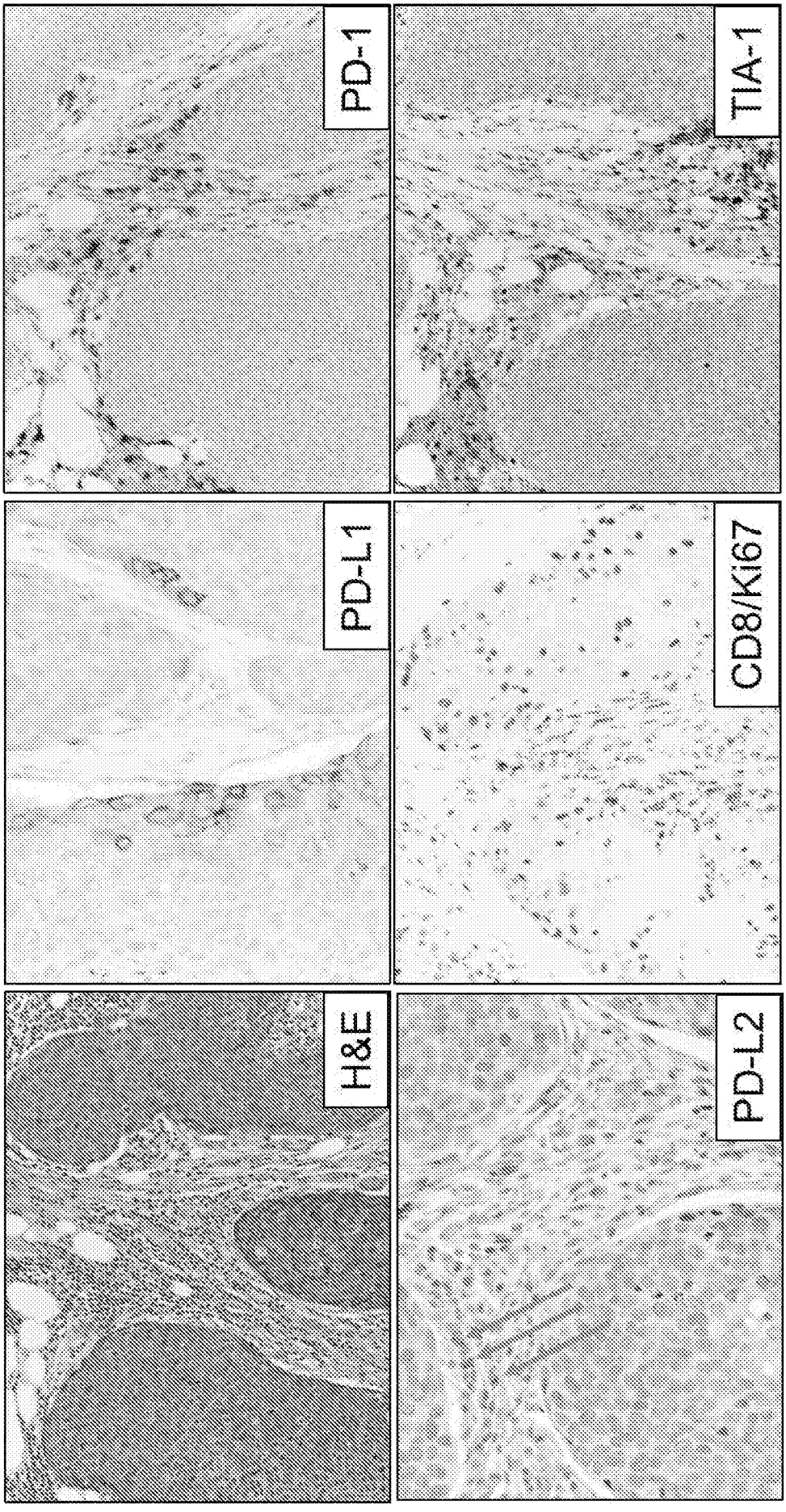


FIGURE 2

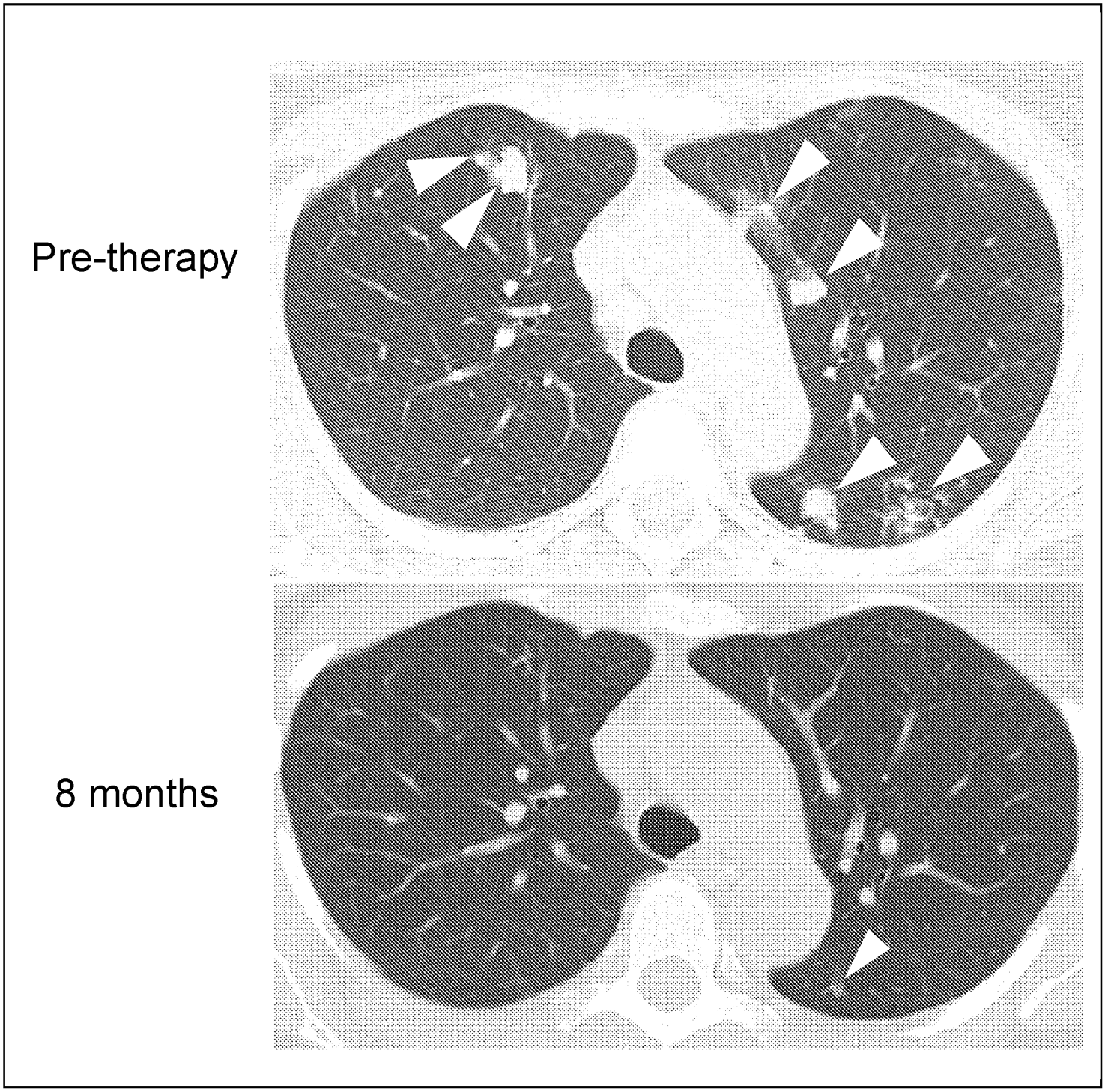
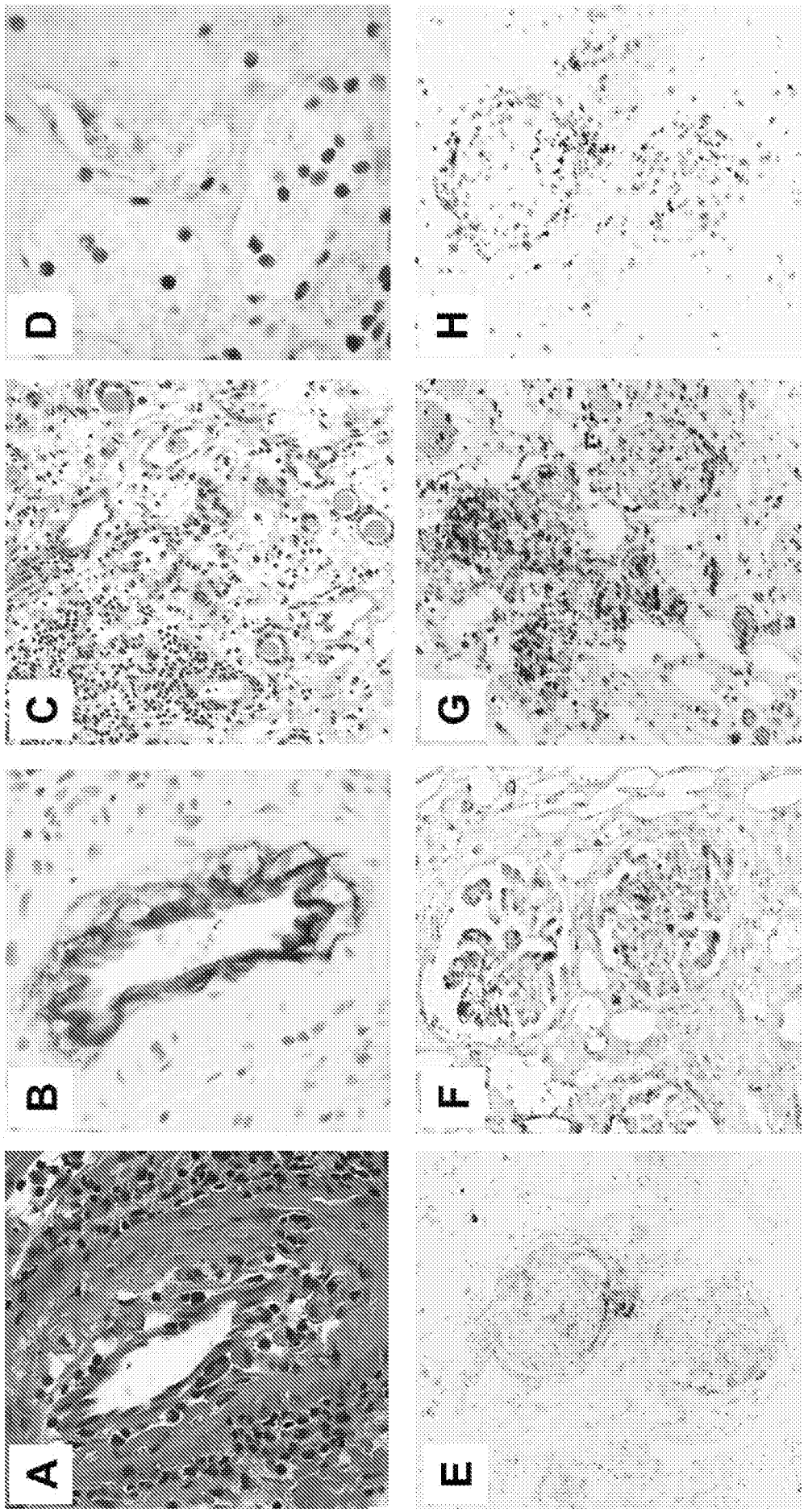


FIGURE 3



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 2016/059660

A. CLASSIFICATION OF SUBJECT MATTER

A61K 39/395 *(2006.01)*
A61K 38/02 *(2006.01)*
A61K 31/7088 *(2006.01)*
A61P 37/06 *(2006.01)*

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K 31/00, 31/7088, 38/00, 38/02, 39/00, 39/395, A61P 37/00, 37/06

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

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

DWPI, Espacenet, Google, NCBI, Patsearch (RUPTO internal), PubMed, RUPTO, USPTO, RMZH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	PENG Wei et al. Dendritic cells transfected with PD-L1 recombinant adenovirus induces T cell suppression and long-term acceptance of allograft transplantation. Cellular Immunology, 2011, 271(1):pp.73-77, abstract, p. 74, p. 75, right col., p. 76	1-4, 9, 11-12 5-8, 10, 13
Y	RIELLA Leonardo V. et al. Role of the PD-1 Pathway in the Immune Response. Am J Transplant., 2012 October; 12(10): pp.2575-2587, p. 2, paragraphs 2-3, p. 5, paragraph 3, p. 9, paragraph 4, p. 7, last paragraph - p. 8, paragraphs 1-2, p. 10, last paragraph - p. 11, paragraph 1	5-7, 13
Y	CHITTASUPHO Chuda et al. Autoimmune therapies targeting costimulation and emerging trends in multivalent therapeutics. Ther Deliv., 2011 July; 2(7):pp. 873-889, p. 4, paragraphs 3-4, p. 9, paragraph 2, p. 11, paragraph 5	8, 10

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

☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
09 December 2016 (09.12.2016)	22 December 2016 (22.12.2016)

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