(19) DANMARK

(10) **DK/EP 3157956 T3**

Patent- og Varemærkestyrelsen

(12) Oversættelse af europæisk patentskrift

(51) Int.Cl.: *C 07 K 16/28 (2006.01)* *A 01 K 67/027 (2006.01)* *C 07 K 14/705 (2006.01)* *C 12 N 15/85 (2006.01)*

(45) Oversættelsen bekendtgjort den: **2020-04-27**

(80) Dato for Den Europæiske Patentmyndigheds bekendtgørelse om meddelelse af patentet: **2020-02-05**

(86) Europæisk ansøgning nr.: **15738177.3**

(86) Europæisk indleveringsdag: **2015-06-19**

(87) Den europæiske ansøgnings publiceringsdag: **2017-04-26**

(86) International ansøgning nr.: **US2015036649**

(87) Internationalt publikationsnr.: **WO2015196051**

(30) Prioritet: **2014-06-19 US 201462014181 P** **2014-12-02 US 201462086518 P** **2015-03-25 US 201562138221 P**

(84) Designerede stater: **AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO RS SE SI SK SM TR**

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(54) Benævnelse: **Ikke-humane dyr med et humaniseret gen med programmeret celledød 1**

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DESCRIPTION

BACKGROUND

[0001] Although an intense focus of medical research and development has been devoted to cancer immunotherapy and significant improvements have been made, cancer remains a major challenge in the healthcare industry worldwide. This major challenge is due, in part, to the ability of cancer cells to evade the monitoring mechanisms of the immune system, which is partly the result of inhibition and/or down-regulation of anti-tumor immunity. Still, development of *in vivo* systems to optimally determine the therapeutic potential of new cancer therapies that are designed to activate and/or promote anti-tumor immunity and determine the molecular aspects of how cancer cells provide inhibitory signals to immune cells (e.g., T cells) is lacking. Such systems provide a source for assays for assessing the therapeutic efficacy of candidate agents that promote an anti-tumor environment *in vivo.*

[0002] Iwai, et al. (International immunology 17.2 (2004): 133-144) relates to PD-1 blockade inhibiting hematogenous spread of poorly immunogenic tumor cells by enhanced recruitment of effector T cells. Selby, et al. (Journal of Clinical Oncology 31.15 Suppl. (2013): 3061-3061) relates to the antitumor activity of concurrent blockade of immune checkpoint molecules CTLA-4 and PD-1 in preclinical models.

SUMMARY

[0003] The present disclosure encompasses the recognition that it is desirable to engineer non-human animals to permit improved systems for identifying and developing new therapeutics that can be used for the treatment of cancer. The present disclosure also encompasses the recognition that it is desirable to engineer non-human animals to permit improved systems for identifying and developing new therapeutics that can be used to treat autoimmune (or inflammatory) diseases, disorders or conditions. Further, the present disclosure also encompasses the recognition that non-human animals having a humanized Pdcdl gene and/or otherwise expressing, containing, or producing a human or humanized PD-1 polypeptide are desirable, for example for use in identifying and developing cancer therapeutics that up-regulate anti-tumor immunity. Non-human animals described herein may provide improved *in vivo* systems for the identification and development of combination therapies that include targeting PD-1.

[0004] The invention provides a mouse whose genome comprises a humanized Programmed cell death 1 (Pdcdl) gene at an endogenous Pdcdl locus, wherein said mouse expresses a humanized Programmed cell death 1 (PD-1) polypeptide from said humanized Pdcdl gene, which humanized PD-1 polypeptide has an amino acid sequence that is at least 95% identical to SEQ ID NO: 6 and comprises a human portion and an endogenous portion, wherein the human portion comprises amino acids 26-169 of a human PD-1 polypeptide and the endogenous portion comprises the intracellular portion of an endogenous mouse PD-1 polypeptide.

[0005] The invention also provides an isolated mouse cell or tissue whose genome comprises a humanized Pdcdl gene at an endogenous Pdcdl locus, wherein said humanized Pdcdl gene encodes a humanized PD-1 polypeptide, which humanized PD-1 polypeptide has an amino acid sequence that is at least 95% identical to SEQ ID NO: 6 and comprises a human portion and an endogenous portion, wherein the human portion comprises amino acids 26-169 of a human PD-1 polypeptide and the endogenous portion comprises the intracellular portion of an endogenous mouse PD-1 polypeptide, wherein the humanized Pdcdl gene is operably linked to a mouse Pdcdl promoter, and wherein the mouse cell is optionally a mouse embryonic stem cell.

[0006] The invention further provides a mouse embryo generated from a mouse embryonic stem cell of the invention.

[0007] The invention additionally provides a method of making a mouse, wherein: (A) the genome of the mouse comprises a Pdcdl gene that encodes a PD-1 polypeptide having a human portion and an endogenous portion, wherein the Pdcdl gene is operably linked to a mouse Pdcdl promoter, the method comprising modifying the genome of a mouse so that it comprises a humanized Pdcdl gene at an endogenous Pdcdl locus, wherein said mouse expresses a humanized PD-1 polypeptide from said humanized Pdcdl gene, which humanized PD-1 polypeptide has an amino acid sequence that is at least 95% identical to SEQ ID NO: 6 and comprises a human portion and an endogenous portion, wherein the human portion comprises amino acids 26-169 of a human PD-1 polypeptide and the endogenous portion comprises the intracellular portion of an endogenous mouse PD-1 polypeptide; or (B) the mouse expresses a PD-1 polypeptide from an endogenous Pdcdl gene, wherein the PD-1 polypeptide comprises a human sequence, the method comprising (a) inserting a human genomic fragment of a human Pdcdl gene into an endogenous mouse Pdcdl gene at an endogenous Pdcdl locus in a mouse embryonic stem cell to form a humanized Pdcdl gene that encodes a humanized PD-1 polypeptide, wherein said humanized PD-1 polypeptide has an amino acid sequence that is at least 95% identical to SEQ ID NO: 6 and comprises a human portion and an endogenous portion, wherein the human portion comprises amino acids 26-169 of a human PD-1 polypeptide and the endogenous portion comprises the intracellular portion of an endogenous mouse PD-1 polypeptide; (b) obtaining the mouse embryonic stem cell generated in (a); and, (c) creating a mouse using the mouse embryonic stem cell of (b).

[0008] The invention also provides a method of assessing the pharmacokinetic properties of a drug targeting human PD-1, comprising: administering the drug to a mouse of the invention; and performing an assay to determine one or more pharmacokinetic properties of the drug targeting human PD-1 wherein the drug is optionally an anti-PD-1 antibody.

[0009] The invention further provides a mouse tumor model obtained by: a) providing a mouse of the invention; and b) implanting one or more tumor cells in the mouse of (a); thereby obtaining said mouse tumor model.

[0010] Also disclosed herein is a non-human animal having a genome comprising a Pdcdl gene that includes genetic material from two different species (e.g., a human and a non-human). In some aspects, the Pdcdl gene of a non-human animal as described herein encodes a PD-1 polypeptide that contains human and non-human portions, wherein the human and non-human portions are linked together and form a functional PD-1 polypeptide. In some aspects, the Pdcdl gene of a non-human animal as described herein encodes a PD-1 polypeptide that contains an extracellular domain, in whole or in part, of a human PD-1 polypeptide.

[0011] Also disclosed herein is a non-human animal that expresses a PD-1 polypeptide, which PD-1 polypeptide comprises a human portion and an endogenous portion. In some aspects, a PD-1 polypeptide described herein is translated in a cell of the non-human animal with a non-human signal peptide; in some certain aspects, a rodent signal peptide.

[0012] In some aspects, an endogenous portion comprises an intracellular portion of an endogenous PD-1 polypeptide. In some aspects, an endogenous portion further comprises a transmembrane portion of an endogenous PD-1 polypeptide. In some aspects, an endogenous portion has an amino acid sequence that is at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95% identical to a corresponding amino acid sequence of a mouse PD-1 polypeptide that appears in Figure 8. In some aspects, an endogenous portion has an amino acid sequence that is substantially identical to a corresponding amino acid sequence of a mouse PD-1 polypeptide that appears in Figure 8. In some aspects, an endogenous portion has an amino acid sequence that is identical to a corresponding amino acid sequence of a mouse PD-1 polypeptide that appears in Figure 8.

[0013] In some aspects, a human portion comprises amino acids 35-145, 27-145, 27-169, 26-169 or 21-170 of a human PD-1 polypeptide. In some aspects, a human portion comprises an amino acid sequence that is at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95% identical to a corresponding amino acid sequence of a human PD-1 polypeptide that appears in Figure 8. In some aspects, a human portion comprises an amino acid sequence that is substantially identical to a corresponding amino acid sequence of a human PD-1 polypeptide that appears in Figure 8. In some aspects, a human portion comprises an amino acid sequence that is identical to a corresponding amino acid sequence of a human PD-1 polypeptide that appears in Figure 8.

[0014] In some aspects, a PD-1 polypeptide, which comprises a human portion and an endogenous portion, is encoded by an endogenous Pdcdl gene. In some certain aspects, an endogenous Pdcdl gene comprises endogenous Pdcdl exons 1, 4 and 5. In some certain aspects, an endogenous Pdcdl gene further comprises an endogenous Pdcdl exon 3 in whole or in part. In some certain aspects, an endogenous Pdcdl gene comprises SEQ ID NO:21. In some certain aspects, an endogenous Pdcdl gene comprises SEQ ID NO:22. In some certain aspects, an endogenous Pdcdl gene comprises SEQ ID NO:21 and SEQ ID NO:22.

[0015] In some aspects, a PD-1 polypeptide expressed by a non-human animal as described herein has an amino acid sequence that is at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95% identical to SEQ ID NO:6. In some aspects, a PD-1 polypeptide expressed by a non-human animal as described herein has an amino acid sequence that is substantially identical to SEQ ID NO:6. In some aspects, a PD-1 polypeptide expressed by a non-human animal as described herein has an amino acid sequence that is identical to SEQ ID NO:6.

[0016] Also disclosed herein is a humanized Pdcdl locus comprising one or more exons of a non-human Pdcdl gene operably linked to one or more exons, in whole or in part, of a human Pdcdl gene. In some aspects, a humanized Pdcdl locus further comprises 5' and 3' non-human Pdcdl untranslated regions (UTRs) flanking the one or more exons of a human Pdcdl gene. In some aspects, a humanized Pdcdl locus is under the control of a rodent promoter; in some certain aspects, an endogenous rodent promoter.

[0017] In some aspects, a humanized Pdcdl locus comprises non-human Pdcdl exons 1, 3, 4 and 5 operably linked to a human Pdcdl exon 2. In some aspects, a humanized Pdcdl locus comprises non-human Pdcdl exons 1, 4 and 5, a human Pdcdl exon 2 and further comprises a Pdcdl exon 3, which Pdcdl exon 3 comprises a human portion and a non-human portion, and wherein said non-human and human exons are operably linked. In some aspects, a human portion of a Pdcdl exon 3 includes nucleotides that encode a PD-1 stalk sequence. In some aspects, a human portion of a Pdcdl exon 3 includes about 71 bp of a human Pdcdl exon 3. In some aspects, a non-human portion of a Pdcdl exon 3 includes nucleotides that encode a transmembrane sequence. In some aspects, a non-human portion of a Pdcdl exon 3 includes about 91 bp of a rodent Pdcdl exon 3.

[0018] Also disclosed herein is a non-human animal comprising a Pdcdl gene that comprises an endogenous portion and a human portion, where the endogenous and human portions are operably linked to a rodent Pdcdl promoter. In some aspects, the rodent Pdcdl promoter is an endogenous rodent Pdcdl promoter.

[0019] In some aspects, an endogenous portion comprises endogenous Pdcdl exons 1, 4 and 5. In some aspects, an endogenous portion further comprises endogenous Pdcdl exon 3 in whole or in part. In some aspects, exons 1, 3 in whole or in part, 4 and 5 of an endogenous Pdcdl gene are at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95% identical to the corresponding exons 1, 3 in whole or in part, 4 and 5 of an endogenous Pdcdl gene that appears in Figure 8. In some aspects, exons 1, 3 in whole or in part, 4 and 5 of an endogenous Pdcdl gene are at substantially identical to the corresponding exons 1, 3 in whole or in part, 4 and 5 of an endogenous Pdcdl gene that appears in Figure 8. In some aspects, exons 1, 3 in whole or in part, 4 and 5 of an endogenous Pdcdl gene are at identical to the corresponding exons 1, 3 in whole or in part, 4 and 5 of an endogenous Pdcdl gene that appears in Figure 8.

[0020] In some aspects, a human portion encodes amino acids 21-170, 26-169, 27-169, 27-145 or 35-145 of a human PD-1 polypeptide.

[0021] In some aspects, a human portion comprises exon 2 of a human Pdcdl gene. In some aspects, a human portion further comprises a human Pdcdl exon 3 in whole or in part. In some aspects, human Pdcdl exons 2 and 3, in whole or in part, are at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95% identical to the corresponding exons 2 and 3, in whole or in part, of a human Pdcdl gene that appears in Figure 8. In some aspects, human Pdcdl exons 2 and 3, in whole or in part, are substantially identical to the corresponding exons 2 and 3, in whole or in part, of a human Pdcdl gene that appears in Figure 8. In some aspects, human Pdcdl exons 2 and 3, in whole or in part, are identical to the corresponding exons 2 and 3, in whole or in part, of a human Pdcdl gene that appears in Figure 8. In some aspects, a human portion comprises a sequence that is codon-optimized for expression in a non-human animal; in some aspects, expression in a rodent; in some certain aspects, expression in a mouse; in some certain aspects, expression in a rat.

[0022] In some aspects, a human portion comprises a sequence that is at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95% identical to SEQ ID NO:23. In some aspects, a human portion comprises a sequence that is substantially identical to SEQ ID NO:23. In some aspects, a human portion comprises a sequence that is identical to SEQ ID NO:23. In some aspects, a human portion comprises SEQ ID NO:23.

[0023] Also disclosed herein is a PD-1 polypeptide produced (or generated) by a non-human animal as described herein. In some certain aspects, a PD-1 polypeptide produced by a non-human animal as described herein comprises an amino acid sequence that is at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95% identical to SEQ ID NO:6. In some certain aspects, a PD-1 polypeptide produced by a non-human animal as described herein comprises an amino acid sequence that is substantially identical to SEQ ID NO:6. In some certain aspects, a PD-1 polypeptide produced by a non-human animal as described herein comprises an amino acid sequence that is identical to SEQ ID NO:6.

[0024] Also disclosed herein is an isolated cell or tissue from a non-human animal as described herein. Also disclosed herein is an isolated cell or tissue that comprises a Pdcdl gene as described herein. In some aspects, a cell is a lymphocyte. In some aspects, a cell is selected from a B cell, dendritic cell, macrophage, monocyte (e.g., an activated monocyte), NK cell, and T cell (e.g., an activated T cell). In some aspects, a tissue is selected from adipose, bladder, brain, breast, bone marrow, eye, heart, intestine, kidney, liver, lung, lymph node, muscle, pancreas, plasma, serum, skin, spleen, stomach, thymus, testis, ovum, and a combination thereof.

[0025] Also disclosed herein is a non-human embryonic stem cell whose genome comprises a Pdcdl gene as described herein. In some aspects, a non-human embryonic stem cell is a mouse embryonic stem cell and is from a 129 strain, C57BL/6 strain or a BALB/c strain. In some aspects, a non-human embryonic stem cell is a mouse embryonic stem cell and is from a 129 strain, C57BL/6 strain or a mixture thereof. In some aspects, a non-human embryonic stem cell is a mouse embryonic stem cell and is from a mixture of 129 and C57BL/6 strains.

[0026] In some aspects, a non-human embryonic stem cell has a genome comprising a Pdcdl gene that comprises SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO: 22 or a combination thereof.

[0027] Also disclosed herein is the use of a non-human embryonic stem cell as described herein to make a non-human animal. In some certain aspects, a non-human embryonic stem cell is a mouse embryonic stem cell and is used to make a mouse comprising a Pdcdl gene as described herein. In some certain aspects, a non-human embryonic stem cell is a rat embryonic stem cell and is used to make a rat comprising a Pdcdl gene as described herein.

[0028] Also disclosed herein is a non-human embryo comprising, made from, obtained from, or generated from a non-human embryonic stem cell comprising a Pdcdl gene as described herein. In some certain aspects, a non-human embryo is a rodent embryo; in some aspects, a mouse embryo; in some aspects, a rat embryo.

[0029] Also disclosed herein is the use of a non-human embryo as described herein to make a non-human animal. In some certain aspects, a non-human embryo is a mouse embryo and is used to make a mouse comprising a Pdcdl gene as described herein. In some certain aspects, a non-human embryo is a rat embryo and is used to make a rat comprising a Pdcdl gene as described herein.

[0030] Also disclosed herein is a targeting vector (or nucleic acid construct) as described herein. Also disclosed herein is a targeting vector (or nucleic acid construct) that comprises a humanized Pdcdl gene as described herein. Also disclosed herein is a targeting vector (or nucleic acid construct) that comprises a Pdcdl gene that encodes a PD-1 polypeptide that comprises a human extracellular domain in whole or in part; in some certain aspects a PD-1 polypeptide that comprises amino acids 21-170, 26-169, 27-169, 27-145 or 35-145 of a human PD-1 polypeptide.

[0031] In some aspects, a targeting vector (or nucleic acid construct) comprises one or more exons, in whole or in part, of a non-human Pdcdl gene operably linked to one or more exons, in whole or in part, of a human Pdcdl gene. In some aspects, a targeting vector (or nucleic acid construct) comprises 5' and 3' non-human Pdcdl untranslated regions (UTRs) flanking the one or more exons of a human Pdcdl gene. In some aspects, a targeting vector (or nucleic acid construct) comprises one or more selection markers. In some aspects, a targeting vector (or nucleic acid construct) comprises one or more site-specific recombination sites. In some aspects, a targeting vector (or nucleic acid construct) comprises a human Pdcdl exon 2. In some aspects, a targeting vector (or nucleic acid construct) comprises a human Pdcdl exon 2 and a human Pdcdl exon 3 in whole or in part.

[0032] Also disclosed herein is use of a targeting vector (or nucleic acid construct) as described herein to make a modified non-human embryonic stem cell. Also disclosed herein is use of a targeting vector (or nucleic acid construct) as described herein to make a modified non-human embryo. Also disclosed herein is use of a targeting vector (or nucleic acid construct) as described herein to make a non-human animal.

[0033] Also disclosed herein is a method of making a non-human animal that expresses a PD-1 polypeptide from an endogenous Pdcdl gene, wherein the PD-1 polypeptide comprises a human sequence, the method comprising (a) inserting a genomic fragment into an endogenous Pdcdl gene in a rodent embryonic stem cell, said genomic fragment comprising a nucleotide sequence that encodes a human PD-1 polypeptide in whole or in part; (b) obtaining the rodent embryonic stem cell generated in (a); and, creating a rodent using the rodent embryonic stem cell of (b).

[0034] In some aspects, a human sequence comprises amino acids 35-145, 27-145, 27-169, 26-169 or 21-170 of a human PD-1 polypeptide.

[0035] In some aspects, a nucleotide sequence comprises human Pdcdl exon 2. In some aspects, a nucleotide sequence further comprises human Pdcdl exon 3 in whole or in part. In some aspects, a nucleotide sequence comprises one or more selection markers. In some aspects, a nucleotide sequence comprises one or more site-specific recombination sites.

[0036] Also disclosed herein is a method of making a non-human animal whose genome comprises a Pdcdl gene that encodes a PD-1 polypeptide having a human portion and an endogenous portion, which portions are operably linked to a rodent Pdcdl promoter, the method comprising modifying the genome of a non-human animal so that it comprises a Pdcdl gene that encodes a PD-1 polypeptide having a human portion and an endogenous portion, which portions are operably linked to a rodent Pdcdl promoter, thereby making said non-human animal.

[0037] In some aspects, a rodent Pdcdl promoter is an endogenous rodent Pdcdl promoter.

[0038] In some aspects, a human portion comprises amino acids 35-145, 27-145, 27-169, 26-169 or 21-170 of a human PD-1 polypeptide.

[0039] In some aspects, a Pdcdl gene is modified to include human Pdcdl exon 2. In some aspects, a Pdcdl gene is modified to include human Pdcdl exon 2 and human Pdcdl exon 3 in whole or in part.

[0040] In some aspects, modifying the genome of a non-human animal is performed in a non-human embryonic stem cell followed by generating a non-human animal with said non-human embryonic stem cell. In some certain aspects, the non-human embryonic stem cell is a rodent embryonic stem cell; in some aspects, a mouse embryonic stem cell; in some aspects, a rat embryonic stem cell.

[0041] Also disclosed herein is a non-human animal obtainable by methods as described herein.

[0042] Also disclosed herein is a method of reducing tumor growth in a non-human animal, the method comprising the steps of administering a drug targeting human PD-1 to a non-human animal whose genome comprises a Pdcdl gene that encodes a PD-1 polypeptide having a human portion and an endogenous portion, which portions are operably linked to a rodent Pdcdl promoter; the administering being performed under conditions and for a time sufficient that tumor growth is reduced in the non-human animal.

[0043] Also disclosed herein is a method of killing tumor cells in a non-human animal, the method comprising the steps of administering a drug targeting human PD-1 to a non-human animal whose genome comprises a Pdcdl gene that encodes a PD-1 polypeptide having a human portion and an endogenous portion, which portions are operably linked to a rodent Pdcdl promoter; the administering being performed under conditions and for a time sufficient that the drug mediates killing of the tumor cells in the non-human animal.

[0044] Also disclosed herein is a method of assessing the pharmacokinetic properties of a drug targeting human PD-1, the method comprising the steps of administering the drug to a non-human animal whose genome comprises a Pdcdl gene that encodes a PD-1 polypeptide having a human portion and an endogenous portion, which portions are operably linked a rodent Pdcdl promoter; and performing an assay to determine one or more pharmacokinetic properties of the drug targeting human PD-1.

[0045] In many aspects, a non-human animal as described herein is a rodent whose genome includes a Pdcdl gene that encodes a PD-1 polypeptide having a human portion and an endogenous portion, which portions are operably linked to a rodent Pdcdl promoter. In many aspects, a rodent Pdcdl promoter is an endogenous rodent Pdcdl promoter. In many aspects, a human portion comprises amino acids 35-145, 27-145, 27-169, 26-169 or 21-170 of a human PD-1 polypeptide.

[0046] In some aspects, a drug targeting human PD-1 is a PD-1 antagonist. In some aspects, a drug targeting human PD-1 is a PD-1 agonist. In some aspects, a drug targeting human PD-1 is an anti-PD-1 antibody. In some aspects, a drug targeting human PD-1 is administered intravenously, intraperitoneally, or subcutaneously.

[0047] Also disclosed herein is a non-human animal tumor model, which non-human animal expresses a PD-1 polypeptide comprising a human portion and an endogenous portion.

[0048] Also disclosed herein is a non-human animal tumor model, which non-human animal has a genome comprising a Pdcdl gene that comprises an endogenous portion and a human portion, wherein the endogenous and human portions are operably linked to a non-human animal Pdcdl promoter.

[0049] Also disclosed herein is a non-human animal tumor model obtained by (a) providing a non-human animal whose genome comprises a Pdcdl gene that includes an endogenous portion and a human portion, which endogenous and human portions are operatively linked to a non-human animal Pdcdl promoter; and (b) implanting one or more tumor cells in the rodent of (a); thereby providing said non-human animal tumor model.

[0050] In some aspects, a non-human animal tumor model described herein is a rodent tumor model. In some aspects, a non-human animal Pdcdl promoter is a rodent Pdcdl promoter.

[0051] Also disclosed herein is a method for identification or validation of a drug or vaccine, the method comprising the steps of delivering a drug or vaccine to a non-human animal whose genome includes a Pdcdl gene that encodes a PD-1 polypeptide, which PD-1 polypeptide comprises a human portion and an endogenous portion, and monitoring one or more of the immune response to the drug or vaccine, the safety profile of the drug or vaccine, or the effect on a disease, disorder or condition. In some aspects, monitoring the safety profile includes determining if the non-human animal exhibits a side effect or adverse reaction as a result of delivering the drug or vaccine. In some aspects, a side effect or adverse reaction is selected from morbidity, mortality, alteration in body weight, alteration of the level of one or more enzymes (e.g., liver), alteration in the weight of one or more organs, loss of function (e.g., sensory, motor, organ, etc.), increased susceptibility to one or more diseases, alterations to the genome of the non-human animal, increase or decrease in food consumption and complications of one or more diseases. In some aspects, the disease, disorder or condition is induced in the non-human animal. In some aspects, the disease, disorder or condition induced in the non-human animal is associated with a disease, disorder or condition suffered by one or more human patients in need of treatment. In some certain aspects, the drug is an antibody.

[0052] Also disclosed herein is use of a non-human animal as described herein in the development of a drug or vaccine for use in medicine, such as use as a medicament.

[0053] Also disclosed herein is use of a non-human animal as described herein in the manufacture of a medicament for the treatment of cancer, neoplasm, an infectious disease, an inflammatory disease, disorder or condition, or an autoimmune disease, disorder or condition.

[0054] In various aspects, a Pdcdl gene described herein encodes a PD-1 polypeptide having a human portion and an endogenous portion, which portions are operably linked to a rodent Pdcdl promoter. In various aspects, a rodent promoter is an endogenous rodent promoter. In various aspects, a human portion comprises a human Pdcdl exon 2. In various aspects, a human portion comprises a human Pdcdl exon 2 and further comprises a human Pdcdl exon 3 in whole or in part.

[0055] In various aspects, a non-human animal described herein does not detectably express a full-length endogenous non-human PD-1 polypeptide. In various aspects, a non-human animal described herein does not detectably express an extracellular portion of an endogenous PD-1 polypeptide. In various aspects, a non-human animal described herein does not detectably express an N-terminal immunoglobulin V domain of an endogenous PD-1 polypeptide.

[0056] In various aspects, a non-human animal described herein is a rodent; in some aspects, a mouse; in some aspects, a rat. In some aspects, a mouse described herein is selected from the group consisting of a 129 strain, a BALB/C strain, a C57BL/6 strain, and a mixed 129xC57BL/6 strain; in some certain aspects, 50% 129 and 50% C57BL/6; in some certain aspects, 25% 129 and 75% C57BL/6.

[0057] As used in this application, the terms "about" and "approximately" are used as equivalents. Any numerals used in this application with or without about/approximately are meant to cover any normal fluctuations appreciated by one of ordinary skill in the relevant art.

[0058] Other features, objects, and advantages described herein are apparent in the detailed description of certain aspects that follows. It should be understood, however, that the detailed description, while indicating certain aspects described herein, is given by way of illustration only, not limitation. Various changes and modifications may become apparent to those skilled in the art from the detailed description.

BRIEF DESCRIPTION OF THE DRAWING

[0059] The Drawing included herein, which is composed of the following Figures, is for illustration purposes only and not for limitation.

Figure 1 shows a diagram, not to scale, of the genomic organization of a non-human (e.g., mouse) and human Programmed cell death 1 (Pdcdl) genes. Exons and untranslated regions (UTRs) are numbered beneath each exon and above each UTR.

Figure 2 shows a diagram, not to scale, of an exemplary method for humanization of a non-human Programmed cell death 1 (Pdcdl) gene. Selected nucleotide junction locations are marked with a line below each junction. Sequences of these selected nucleotide junctions are indicated by SEQ ID NOs.

Figure 3 shows a diagram, not to scale, of the genomic organization of a mouse and human Programmed cell death 1 (Pdcdl) genes indicating the approximate locations of probes used in an assay described in Example 1.

Figure 4 shows exemplary histograms of T cells gated on CD19 and CD8 isolated from a wild-type mouse and a mouse heterozygous for humanization of an endogenous Pdcdl gene as described in Example 1 that express mouse and/or humanized PD-1. Stimulated and unstimulated cell populations are indicated, as are cells stained with an isotype control.

Figure 5 shows exemplary tumor growth curves over 21 days in mice homozygous for humanization of an endogenous Pdcdl gene as described in Example 1. Control: antibody not specific for PD-1, a-hPD-1 Ab: antibody specific for human PD-1. Arrows indicate the days for antibody treatment. The number of tumor-free mice on day 21 is shown for each treatment group.

Figure 6 shows exemplary real-time PCR analysis of CD8b, CD3, IFN-g and PD-1 mRNA expression in spleens in mice homozygous for humanization of an endogenous Pdcdl gene as described in Example 1 after treatment with anti-PD-1 antibody. A, mean of five mice per group. B, expression levels for individual mice in each treatment group. Control: antibody not specific for PD-1; α-PD-1: anti-PD-1 antibody.

Figure 7 shows exemplary tumor growth curves over 60 days in mice homozygous for humanization of an endogenous Pdcdl gene as described in Example 1 that were administered 0.3 - 25 mg/kg of anti-hPD-1 antibody or 25 mg/kg of control antibody (antibody not specific for PD-1). Arrows indicate the days of antibody treatment. The number of tumor-free mice on day 60 is shown for each treatment group.

Figure 8 sets forth exemplary murine, human and humanized Pdcdl and PD-1 sequences, and an exemplary human nucleic acid sequence for humanization of a non-human Pdcdl gene. For mRNA sequences, bold font indicates coding sequence and consecutive exons, where indicated, are separated by alternating underlined text; for humanized mRNA sequences, human sequences are contained within parentheses. For protein sequences, signal peptides are underlined, extracellular sequences are bold font, immunoglobulin V domain sequences are within parentheses, and intracellular sequences are italicized; and for humanized protein sequences, non-human sequences are indicated in regular font, and human sequences are indicated in bold font.

DEFINITIONS

[0060] This invention is not limited to particular methods and experimental conditions described herein, as such methods and conditions may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular aspects only, and is not intended to be limiting, since the scope of the present invention is defined by the claims.

[0061] Unless defined otherwise, all terms and phrases used herein include the meanings that the terms and phrases have attained in the art, unless the contrary is clearly indicated or clearly apparent from the context in which the term or phrase is used. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, particular methods and materials are now described.

[0062] The term *"approximately",* as applied herein to one or more values of interest, refers to a value that is similar to a stated reference value. In certain aspects, the term *"approximately"* or *"about"* refers to a range of values that fall within 25%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less in either direction (greater than or less than) of the stated reference value unless otherwise stated or otherwise evident from the context (except where such number would exceed 100% of a possible value).

[0063] The term *"biologically active"* includes a characteristic of any agent that has activity in a biological system, *in vitro* or *in vivo* (e.g., in an organism). For instance, an agent that, when present in an organism, has a biological effect within that organism, is considered to be biologically active. In particular aspects, where a protein or polypeptide is biologically active, a portion of that protein or polypeptide that shares at least one biological activity of the protein or polypeptide is typically referred to as a *"biologically active"* portion.

[0064] The term *"comparable"* includes to two or more agents, entities, situations, sets of conditions, etc. that may not be identical to one another but that are sufficiently similar to permit comparison between them so that conclusions may reasonably be drawn based on differences or similarities observed. Those of ordinary skill in the art will understand, in context, what degree of identity is required in any given circumstance for two or more such agents, entities, situations, sets of conditions, etc. to be considered comparable.

[0065] The term *"conservative",* e.g., as in a conservative amino acid substitution, includes substitution of an amino acid residue by another amino acid residue having a side chain R group with similar chemical properties (e.g., charge or hydrophobicity). In general, a conservative amino acid substitution will not substantially change the functional properties of interest of a protein, for example, the ability of a receptor to bind to a ligand. Examples of groups of amino acids that have side chains with similar chemical properties include: aliphatic side chains such as glycine, alanine, valine, leucine, and isoleucine; aliphatic-hydroxyl side chains such as serine and threonine; amide-containing side chains such as asparagine and glutamine; aromatic side chains such as phenylalanine, tyrosine, and tryptophan; basic side chains such as lysine, arginine, and histidine; acidic side chains such as aspartic acid and glutamic acid; and, sulfur-containing side chains such as cysteine and methionine. Conservative amino acids substitution groups include, for example, valine/leucine/isoleucine, phenylalanine/tyrosine, lysine/arginine, alanine/valine, glutamate/aspartate, and asparagine/glutamine. In some aspects, a conservative amino acid substitution can be a substitution of any native residue in a protein with alanine, as used in, for example, alanine scanning mutagenesis. In some aspects, a conservative substitution is made that has a positive value in the PAM250 log-likelihood matrix disclosed in Gonnet et al. (1992) Exhaustive Matching of the Entire Protein Sequence Database, Science 256:1443-45. In some aspects, the substitution is a moderately conservative substitution wherein the substitution has a nonnegative value in the PAM250 log-likelihood matrix.

[0066] The term *"control"* includes the art-understood meaning of a "control" being a standard against which results are compared. Typically, controls are used to augment integrity in experiments by isolating variables in order to make a conclusion about such variables. In some aspects, a control is a reaction or assay that is performed simultaneously with a test reaction or assay to provide a comparator. As used herein, a *"control"* may include a *"control animal".* A *"control animal"* may have a modification as described herein, a modification that is different as described herein, or no modification (i.e., a wild-type animal). In one experiment, the "test" (i.e., the variable being tested) is applied. In the second experiment, the "control," the variable being tested is not applied. In some aspects, a control is a historical control (i.e., of a test or assay performed previously, or an amount or result that is previously known). In some aspects, a control is or comprises a printed or otherwise saved record. A control may be a positive control or a negative control.

[0067] The term *"disruption"* includes the result of a homologous recombination event with a DNA molecule (e.g., with an endogenous homologous sequence such as a gene or gene locus). In some aspects, a disruption may achieve or represent an insertion, deletion, substitution, replacement, missense mutation, or a frame-shift of a DNA sequence(s), or any combination thereof. Insertions may include the insertion of entire genes or fragments of genes, e.g., exons, which may be of an origin other than the endogenous sequence (e.g., a heterologous sequence). In some aspects, a disruption may increase expression and/or activity of a gene or gene product (e.g., of a protein encoded by a gene). In some aspects, a disruption may decrease expression and/or activity of a gene or gene product. In some aspects, a disruption may alter sequence of a gene or an encoded gene product (e.g., an encoded protein). In some aspects, a disruption may truncate or fragment a gene or an encoded gene product (e.g., an encoded protein). In some aspects, a disruption may extend a gene or an encoded gene product; in some such aspects, a disruption may achieve assembly of a fusion protein. In some aspects, a disruption may affect level but not activity of a gene or gene product. In some aspects, a disruption may affect activity but not level of a gene or gene product. In some aspects, a disruption may have no significant effect on level of a gene or gene product. In some aspects, a disruption may have no significant effect on activity of a gene or gene product. In some aspects, a disruption may have no significant effect on either level or activity of a gene or gene product.

[0068] The terms *"determining", "measuring", "evaluating", "assessing", "assaying"* and *"analyzing"* are used interchangeably to refer to any form of measurement, and include determining if an element is present or not. These terms include both quantitative and/or qualitative determinations. Assaying may be relative or absolute. *"Assaying for the presence of"* can be determining the amount of something present and/or determining whether or not it is present or absent.

[0069] The term *"dosing regimen"* or *"therapeutic regimen"* includes a set of unit doses, in some aspects, more than one, that are administered individually to a subject, typically separated by periods of time. In some aspects, a given therapeutic agent has a recommended dosing regiment, which may involve one or more doses. In some aspects, a dosing regimen comprises a plurality of doses each of which are separated from one another by a time period of the same length; in some aspects, a dosing regimen comprises a plurality of doses and at least two different time periods separating individual doses.

[0070] The phrase *"endogenous locus"* or *"endogenous gene"* includes a genetic locus found in a parent or reference organism. In some aspects, the endogenous locus has a sequence found in nature. In some aspects, the endogenous locus is a wild type locus. In some aspects, the reference organism is a wild-type organism. In some aspects, the reference organism is an engineered organism. In some aspects, the reference organism is a laboratory-bred organism (whether wild-type or engineered).

[0071] The phrase *"endogenous promoter"* includes a promoter that is naturally associated, e.g., in a wild-type organism, with an endogenous gene.

[0072] The term *"heterologous"* includes an agent or entity from a different source. For example, when used in reference to a polypeptide, gene, or gene product or present in a particular cell or organism, the term clarifies that the relevant polypeptide, gene, or gene product: 1) was engineered by the hand of man; 2) was introduced into the cell or organism (or a precursor thereof) through the hand of man (e.g., via genetic engineering); and/or 3) is not naturally produced by or present in the relevant cell or organism (e.g., the relevant cell type or organism type).

[0073] The term *"host cell"* includes a cell into which a heterologous (e.g., exogenous) nucleic acid or protein has been introduced. Persons of skill upon reading this disclosure will understand that such terms refer not only to the particular subject cell, but also is used to refer to the progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term *"host cell".* In some aspects, a host cell is or comprises a prokaryotic or eukaryotic cell. In general, a host cell is any cell that is suitable for receiving and/or producing a heterologous nucleic acid or protein, regardless of the Kingdom of life to which the cell is designated. Exemplary cells include those of prokaryotes and eukaryotes (single-cell or multiple-cell), bacterial cells (e.g., strains of E. coli, Bacillus spp., Streptomyces spp., etc.), mycobacteria cells, fungal cells, yeast cells (e.g., S. cerevisiae, S. pombe, P. pastoris, P. methanolica, etc.), plant cells, insect cells (e.g., SF-9, SF-21, baculovirus-infected insect cells, Trichoplusia ni, etc.), non-human animal cells, human cells, or cell fusions such as, for example, hybridomas or quadromas. In some aspects, the cell is a human, monkey, ape, hamster, rat, or mouse cell. In some aspects, the cell is eukaryotic and is selected from the following cells: CHO (e.g., CHO K1, DXB-11 CHO, Veggie-CHO), COS (e.g., COS-7), retinal cell, Vero, CV1, kidney (e.g., HEK293, 293 EBNA, MSR 293, MDCK, HaK, BHK), HeLa, HepG2, WI38, MRC 5, Colo205, HB 8065, HL-60, (e.g., BHK21), Jurkat, Daudi, A431 (epidermal), CV-1, U937, 3T3, L cell, C127 cell, SP2/0, NS-0, MMT 060562, Sertoli cell, BRL 3A cell, HT1080 cell, myeloma cell, tumor cell, and a cell line derived from an aforementioned cell. In some aspects, the cell comprises one or more viral genes, e.g., a retinal cell that expresses a viral gene (e.g., a PER.C6™ cell). In some aspects, a host cell is or comprises an isolated cell. In some aspects, a host cell is part of a tissue. In some aspects, a host cell is part of an organism.

[0074] The term *"humanized"* includes nucleic acids or proteins whose structures (i.e., nucleotide or amino acid sequences) include portions that correspond substantially or identically with structures of a particular gene or protein found in nature in a non-human animal, and also include portions that differ from that found in the relevant particular non-human gene or protein and instead correspond more closely with comparable structures found in a corresponding human gene or protein. In some aspects, a *"humanized"* gene is one that encodes a polypeptide having substantially the amino acid sequence as that of a human polypeptide (e.g., a human protein or portion thereof - e.g., characteristic portion thereof). To give but one example, in the case of a membrane receptor, a *"humanized"* gene may encode a polypeptide having an extracellular portion, in whole or in part, having an amino acid sequence as that of a human extracellular portion and the remaining sequence as that of a non-human (e.g., mouse) polypeptide. In some aspects, a humanized gene comprises at least a portion of a DNA sequence of a human gene. In some aspect, a humanized gene comprises an entire DNA sequence of a human gene. In some aspects, a humanized protein comprises a sequence having a portion that appears in a human protein. In some aspects, a humanized protein comprises an entire sequence of a human protein and is expressed from an endogenous locus of a non-human animal that corresponds to the homolog or ortholog of the human gene.

[0075] The term *"identity"*, e.g., as in connection with a comparison of sequences, includes identity as determined by a number of different algorithms known in the art that can be used to measure nucleotide and/or amino acid sequence identity. In some aspects, identities as described herein are determined using a ClustalW v. 1.83 (slow) alignment employing an open gap penalty of 10.0, an extend gap penalty of 0.1, and using a Gonnet similarity matrix (MACVECTOR™ 10.0.2, MacVector Inc., 2008).

[0076] The term *"isolated"* includes a substance and/or entity that has been (1) separated from at least some of the components with which it was associated when initially produced (whether in nature and/or in an experimental setting), and/or (2) designed, produced, prepared, and/or manufactured by the hand of man. Isolated substances and/or entities may be separated from about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or more than about 99% of the other components with which they were initially associated. In some aspects, isolated agents are about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or more than about 99% pure. A substance is *"pure"* if it is substantially free of other components. In some aspects, as will be understood by those skilled in the art, a substance may still be considered *"isolated"* or even *"pure"*, after having been combined with certain other components such as, for example, one or more carriers or excipients (e.g., buffer, solvent, water, etc.); in such aspects, percent isolation or purity of the substance is calculated without including such carriers or excipients. To give but one example, in some aspects, a biological polymer such as a polypeptide or polynucleotide that occurs in nature is considered to be *"isolated"* when: a) by virtue of its origin or source of derivation is not associated with some or all of the components that accompany it in its native state in nature; b) it is substantially free of other polypeptides or nucleic acids of the same species from the species that produces it in nature; or c) is expressed by or is otherwise in association with components from a cell or other expression system that is not of the species that produces it in nature. Thus, for instance, in some aspects, a polypeptide that is chemically synthesized or is synthesized in a cellular system different from that which produces it in nature is considered to be an *"isolated"* polypeptide. Alternatively or additionally, in some aspects, a polypeptide that has been subjected to one or more purification techniques may be considered to be an *"isolated"* polypeptide to the extent that it has been separated from other components: a) with which it is associated in nature; and/or b) with which it was associated when initially produced.

[0077] The phrase *"non-human animal"* includes any vertebrate organism that is not a human. In some aspects, a non-human animal is a cyclostome, a bony fish, a cartilaginous fish (e.g., a shark or a ray), an amphibian, a reptile, a mammal, and a bird. In some aspects, a non-human mammal is a primate, a goat, a sheep, a pig, a dog, a cow, or a rodent. In some aspects, a non-human animal is a rodent such as a rat or a mouse.

[0078] The phrase *"nucleic acid"* includes any compound and/or substance that is or can be incorporated into an oligonucleotide chain. In some aspects, a *"nucleic acid"* is a compound and/or substance that is or can be incorporated into an oligonucleotide chain via a phosphodiester linkage. As will be clear from context, in some aspects, *"nucleic acid"* includes individual nucleic acid residues (e.g., nucleotides and/or nucleosides); in some aspects, *"nucleic acid"* includes an oligonucleotide chain comprising individual nucleic acid residues. In some aspects, a *"nucleic acid"* is or comprises RNA; in some aspects, a *"nucleic acid"* is or comprises DNA. In some aspects, a *"nucleic acid"* is, comprises, or consists of one or more natural nucleic acid residues. In some aspects, a *"nucleic acid"* is, comprises, or consists of one or more nucleic acid analogs. In some aspects, a nucleic acid analog differs from a *"nucleic acid"* in that it does not utilize a phosphodiester backbone. For example, in some aspects, a *"nucleic acid"* is, comprises, or consists of one or more *"peptide nucleic acids",* which are known in the art and have peptide bonds instead of phosphodiester bonds in the backbone, are considered within the scope described herein. Alternatively or additionally, in some aspects, a *"nucleic acid"* has one or more phosphorothioate and/or 5'-N-phosphoramidite linkages rather than phosphodiester bonds. In some aspects, a *"nucleic acid"* is, comprises, or consists of one or more natural nucleosides (e.g., adenosine, thymidine, guanosine, cytidine, uridine, deoxyadenosine, deoxythymidine, deoxyguanosine, and deoxycytidine). In some aspects, a *"nucleic acid"* is, comprises, or consists of one or more nucleoside analogs (e.g., 2-aminoadenosine, 2-thiothymidine, inosine, pyrrolo-pyrimidine, 3-methyl adenosine, 5-methylcytidine, C-5 propynyl-cytidine, C-5 propynyl-uridine, 2-aminoadenosine, C5-bromouridine, C5-fluorouridine, C5-iodouridine, C5-propynyl-uridine, C5-propynyl-cytidine, C5-methylcytidine, 2-aminoadenosine, 7-deazaadenosine, 7-deazaguanosine, 8-oxoadenosine, 8-oxoguanosine, O(6)-methylguanine, 2-thiocytidine, methylated bases, intercalated bases, and combinations thereof). In some aspects, a *"nucleic acid"* comprises one or more modified sugars (e.g., 2'-fluororibose, ribose, 2'-deoxyribose, arabinose, and hexose) as compared with those in natural nucleic acids. In some aspects, a *"nucleic acid"* has a nucleotide sequence that encodes a functional gene product such as an RNA or protein. In some aspects, a *"nucleic acid"* includes one or more introns. In some aspects, a *"nucleic acid"* is prepared by one or more of isolation from a natural source, enzymatic synthesis by polymerization based on a complementary template (*in vivo* or *in vitro*), reproduction in a recombinant cell or system, and chemical synthesis. In some aspects, a *"nucleic acid"* is at least 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 20, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 600, 700, 800, 900, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 5000 or more residues long. In some aspects, a *"nucleic acid"* is single stranded; in some aspects, a *"nucleic acid"* is double stranded. In some aspects, a *"nucleic acid"* has a nucleotide sequence comprising at least one element that encodes, or is the complement of a sequence that encodes, a polypeptide. In some aspects, a *"nucleic acid"* has enzymatic activity.

[0079] The phrase *"operably linked"* includes a juxtaposition wherein the components described are in a relationship permitting them to function in their intended manner. A control sequence *"operably linked"* to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under conditions compatible with the control sequences. *"Operably linked"* sequences include both expression control sequences that are contiguous with the gene of interest and expression control sequences that act in trans or at a distance to control the gene of interest. The term *"expression control sequence"* includes polynucleotide sequences, which are necessary to effect the expression and processing of coding sequences to which they are ligated. *"Expression control sequences"* include: appropriate transcription initiation, termination, promoter and enhancer sequences; efficient RNA processing signals such as splicing and polyadenylation signals; sequences that stabilize cytoplasmic mRNA; sequences that enhance translation efficiency (i.e., Kozak consensus sequence); sequences that enhance protein stability; and when desired, sequences that enhance protein secretion. The nature of such control sequences differs depending upon the host organism. For example, in prokaryotes, such control sequences generally include promoter, ribosomal binding site, and transcription termination sequence, while in eukaryotes, typically, such control sequences include promoters and transcription termination sequence. The term *"control sequences"* is intended to include components whose presence is essential for expression and processing, and can also include additional components whose presence is advantageous, for example, leader sequences and fusion partner sequences.

[0080] The term *"patient"* or *"subject"* includes any organism to which a provided composition is or may be administered, e.g., for experimental, diagnostic, prophylactic, cosmetic, and/or therapeutic purposes. Typical patients include animals (e.g., mammals such as mice, rats, rabbits, non-human primates, and/or humans). In some aspects, a patient is a non-human animal. In some aspects, a patient (e.g., a non-human animal patient) may have a modification as described herein, a modification that is different as described herein or no modification (i.e., a wild-type non-human animal patient). In some aspects, a non-human animal is suffering from or is susceptible to one or more disorders or conditions. In some aspects, a non-human animal displays one or more symptoms of a disorder or condition. In some aspects, a non-human animal has been diagnosed with one or more disorders or conditions.

[0081] The term *"polypeptide"* includes any polymeric chain of amino acids. In some aspects, a polypeptide has an amino acid sequence that occurs in nature. In some aspects, a polypeptide has an amino acid sequence that does not occur in nature. In some aspects, a polypeptide has an amino acid sequence that contains portions that occur in nature separately from one another (i.e., from two or more different organisms, for example, human and non-human portions). In some aspects, a polypeptide has an amino acid sequence that is engineered in that it is designed and/or produced through action of the hand of man.

[0082] The term *"recombinant",* is intended to include polypeptides (e.g., PD-1 polypeptides as described herein) that are designed, engineered, prepared, expressed, created or isolated by recombinant means, such as polypeptides expressed using a recombinant expression vector transfected into a host cell, polypeptides isolated from a recombinant, combinatorial human polypeptide library (Hoogenboom H. R., (1997) TIB Tech. 15:62-70; Azzazy H., and Highsmith W. E., (2002) Clin. Biochem. 35:425-445; Gavilondo J. V., and Larrick J. W. (2002) BioTechniques 29:128-145; Hoogenboom H., and Chames P. (2000) Immunology Today 21:371-378), antibodies isolated from an animal (e.g., a mouse) that is transgenic for human immunoglobulin genes (see e.g., Taylor, L. D., et al. (1992) Nucl. Acids Res. 20:6287-6295; Kellermann S-A., and Green L. L. (2002) Current Opinion in Biotechnology 13:593-597; Little M. et al. (2000) Immunology Today 21:364-370; Murphy, A.J., et al. (2014) Proc. Natl. Acad. Sci. U.S.A. 111(14):5153-5158) or polypeptides prepared, expressed, created or isolated by any other means that involves splicing selected sequence elements to one another. In some aspects, one or more of such selected sequence elements is found in nature. In some aspects, one or more of such selected sequence elements is designed *in silico.* In some aspects, one or more such selected sequence elements result from mutagenesis (e.g., *in vivo* or *in vitro*) of a known sequence element, e.g., from a natural or synthetic source. For example, in some aspects, a recombinant polypeptide is comprised of sequences found in the genome of a source organism of interest (e.g., human, mouse, etc.). In some aspects, a recombinant polypeptide has an amino acid sequence that resulted from mutagenesis (e.g., *in vitro* or *in vivo,* for example in a non-human animal), so that the amino acid sequences of the recombinant polypeptides are sequences that, while originating from and related to polypeptides sequences, may not naturally exist within the genome of a non-human animal *in vivo.*

[0083] The term *"replacement"* includes a process through which a *"replaced"* nucleic acid sequence (e.g., a gene) found in a host locus (e.g., in a genome) is removed from that locus, and a different, *"replacement"* nucleic acid is located in its place. In some aspects, the replaced nucleic acid sequence and the replacement nucleic acid sequences are comparable to one another in that, for example, they are homologous to one another and/or contain corresponding elements (e.g., protein-coding elements, regulatory elements, etc.). In some aspects, a replaced nucleic acid sequence includes one or more of a promoter, an enhancer, a splice donor site, a splice receiver site, an intron, an exon, an untranslated region (UTR); in some aspects, a replacement nucleic acid sequence includes one or more coding sequences. In some aspects, a replacement nucleic acid sequence is a homolog of the replaced nucleic acid sequence. In some aspects, a replacement nucleic acid sequence is an ortholog of the replaced sequence. In some aspects, a replacement nucleic acid sequence is or comprises a human nucleic acid sequence. In some aspects, including where the replacement nucleic acid sequence is or comprises a human nucleic acid sequence, the replaced nucleic acid sequence is or comprises a rodent sequence (e.g., a mouse or rat sequence). The nucleic acid sequence so placed may include one or more regulatory sequences that are part of source nucleic acid sequence used to obtain the sequence so placed (e.g., promoters, enhancers, 5'- or 3'-untranslated regions, etc.). For example, in various aspects, the replacement is a substitution of an endogenous sequence with a heterologous sequence that results in the production of a gene product from the nucleic acid sequence so placed (comprising the heterologous sequence), but not expression of the endogenous sequence; the replacement is of an endogenous genomic sequence with a nucleic acid sequence that encodes a polypeptide that has a similar function as a polypeptide encoded by the endogenous sequence (e.g., the endogenous genomic sequence encodes a PD-1 polypeptide, and the DNA fragment encodes one or more human PD-1 polypeptides). In various aspects, an endogenous gene or fragment thereof is replaced with a corresponding human gene or fragment thereof. A corresponding human gene or fragment thereof is a human gene or fragment that is an ortholog of, or is substantially similar or the same in structure and/or function, as the endogenous gene or fragment thereof that is replaced.

[0084] The phrase *"Programmed cell death 1 protein"* or *"PD-1 protein"* includes a type I transmembrane protein that belongs to the CD28/CTLA-4 family of T cell regulators. The protein structure of a PD-1 protein includes an extracellular amino-terminal immunoglobulin V domain, a transmembrane domain and a carboxyl-terminal intracellular tail, which intracellular tail contains an immunoreceptor tyrosine-based inhibitory motif (ITIM) and an immunoreceptor tyrosine-based switch motif. PD-1 is expressed on the cell surface and interacts with PD-L1 and PD-L2, members of the B7 family immune-regulatory ligands (Collins, M. et al. (2005) Genome Biol. 6:223). PD-1 is expressed in, *inter alia,* activated T cells, B cells, macrophages, monocytes, mast cells, and also in many tumors. PD-1 has been shown to be involved in negative regulation of immune response and, in particular, negative regulation of T cell responses. By way of illustration, nucleotide and amino acid sequences of mouse and human Pdcdl genes, which encode PD-1 proteins, are provided in Figure 8. Persons of skill upon reading this disclosure will recognize that one or more endogenous Pdcdl genes in a genome (or all) can be replaced by one or more heterologous Pdcdl genes (e.g., polymorphic variants, subtypes or mutants, genes from another species, humanized forms, etc.).

[0085] A *"PD-1-expressing cell"* includes a cell that expresses a PD-1 type I membrane protein. In some aspects, a PD-1-expressing cell expresses a PD-1 type I membrane protein on its surface. In some aspects, a PD-1 protein is expressed on the surface of the cell in an amount sufficient to mediate cell-to-cell interactions. Exemplary PD-1-expressing cells include B cells, macrophages and T cells. PD-1-expressing cells regulate various cellular processes via the interaction of PD-1 expressed on the surface of immune cells (e.g., T and B cells) and play a role in determining the differentiation and fate of such cells. In some aspects, non-human animals described herein demonstrate regulation of various cellular processes (as described herein) via humanized PD-1 proteins expressed on the surface of one more cells of the non-human animal. In some aspects, non-human animals described herein demonstrate negative regulation of signaling through T cell receptors (TCRs) via humanized PD-1 proteins expressed on the surface of one or more cells of the non-human animal. In some aspects, non-human animals demonstrate negative regulation of immune responses via humanized PD-1 proteins expressed on the surface of one or more cells of the non-human animal.

[0086] The term *"reference"* includes a standard or control agent, cohort, individual, population, sample, sequence or value against which an agent, animal, cohort, individual, population, sample, sequence or value of interest is compared. In some aspects, a reference agent, cohort, individual, population, sample, sequence or value is tested and/or determined substantially simultaneously with the testing or determination of the agent, cohort, individual, population, sample, sequence or value of interest. In some aspects, a reference agent, cohort, individual, population, sample, sequence or value is a historical reference, optionally embodied in a tangible medium. In some aspects, a reference may refer to a control. As used herein, a *"reference"* may include a *"reference animal".* A *"reference animal"* may have a modification as described herein, a modification that is different as described herein or no modification (i.e., a wild-type animal). Typically, as would be understood by those skilled in the art, a reference agent, animal, cohort, individual, population, sample, sequence or value is determined or characterized under conditions comparable to those utilized to determine or characterize the agent, animal (e.g., a mammal), cohort, individual, population, sample, sequence or value of interest.

[0087] The term *"substantially"* includes the qualitative condition of exhibiting total or near-total extent or degree of a characteristic or property of interest. One of ordinary skill in the biological arts will understand that biological and chemical phenomena rarely, if ever, go to completion and/or proceed to completeness or achieve or avoid an absolute result. The term *"substantially"* is therefore used herein to capture the potential lack of completeness inherent in many biological and chemical phenomena.

[0088] The phrase *"substantial homology"* includes a comparison between amino acid or nucleic acid sequences. As will be appreciated by those of ordinary skill in the art, two sequences are generally considered to be *"substantially homologous"* if they contain homologous residues in corresponding positions. Homologous residues may be identical residues. Alternatively, homologous residues may be non-identical residues will appropriately similar structural and/or functional characteristics. For example, as is well known by those of ordinary skill in the art, certain amino acids are typically classified as *"hydrophobic"* or *"hydrophilic"* amino acids, and/or as having *"polar"* or *"non-polar"* side chains. Substitution of one amino acid for another of the same type may often be considered a *"homologous"* substitution. Typical amino acid categorizations are summarized in Table 1 and 2.

TABLE 1

Alanine	Ala	A	Nonpolar	Neutral	1.8
Arginine	Arg	R	Polar	Positive	-4.5
Asparagine	Asn	N	Polar	Neutral	-3.5
Aspartic acid	Asp	D	Polar	Negative	-3.5
Cysteine	Cys	C	Nonpolar	Neutral	2.5
Glutamic acid	Glu	E	Polar	Negative	-3.5
Glutamine	Gln	Q	Polar	Neutral	-3.5
Glycine	Gly	G	Nonpolar	Neutral	-0.4
Histidine	His	H	Polar	Positive	-3.2
Isoleucine	Ile	I	Nonpolar	Neutral	4.5
Leucine	Leu	L	Nonpolar	Neutral	3.8
Lysine	Lys	K	Polar	Positive	-3.9
Methionine	Met	M	Nonpolar	Neutral	1.9
Phenylalanine	Phe	F	Nonpolar	Neutral	2.8
Proline	Pro	P	Nonpolar	Neutral	-1.6
Serine	Ser	S	Polar	Neutral	-0.8
Threonine	Thr	T	Polar	Neutral	-0.7
Tryptophan	Trp	W	Nonpolar	Neutral	-0.9
Tyrosine	Tyr	Y	Polar	Neutral	-1.3
Valine	Val	V	Nonpolar	Neutral	4.2

TABLE 2

Ambiguous Amino Acids	3- Letter	1-Letter
Asparagine or aspartic acid	Asx	B
Glutamine or glutamic acid	Glx	Z
Leucine or Isoleucine	Xle	J
Unspecified or unknown amino acid	Xaa	X

[0089] As is well known in this art, amino acid or nucleic acid sequences may be compared using any of a variety of algorithms, including those available in commercial computer programs such as BLASTN for nucleotide sequences and BLASTP, gapped BLAST, and PSI-BLAST for amino acid sequences. Exemplary such programs are described in Altschul et al. (1990) Basic local alignment search tool, J. Mol. Biol., 215(3): 403-410; Altschul et al. (1997) Methods in Enzymology; Altschul et al., "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs", Nucleic Acids Res. 25:3389-3402; Baxevanis et al. (1998) Bioinformatics: A Practical Guide to the Analysis of Genes and Proteins, Wiley; and Misener et al. (eds.) (1999) Bioinformatics Methods and Protocols (Methods in Molecular Biology, Vol. 132), Humana Press. In addition to identifying homologous sequences, the programs mentioned above typically provide an indication of the degree of homology. In some aspects, two sequences are considered to be substantially homologous if at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more of their corresponding residues are homologous over a relevant stretch of residues. In some aspects, the relevant stretch is a complete sequence. In some aspects, the relevant stretch is at least 9, 10, 11, 12, 13, 14, 15, 16, 17 or more residues. In some aspects, the relevant stretch includes contiguous residues along a complete sequence. In some aspects, the relevant stretch includes discontinuous residues along a complete sequence. In some aspects, the relevant stretch is at least 10, 15, 20, 25, 30, 35, 40, 45, 50, or more residues.

[0090] The phrase *"substantial identity"* includes a comparison between amino acid or nucleic acid sequences. As will be appreciated by those of ordinary skill in the art, two sequences are generally considered to be *"substantially identical"* if they contain identical residues in corresponding positions. As is well known in this art, amino acid or nucleic acid sequences may be compared using any of a variety of algorithms, including those available in commercial computer programs such as BLASTN for nucleotide sequences and BLASTP, gapped BLAST, and PSI-BLAST for amino acid sequences. Exemplary such programs are described in Altschul et al. (1990) Basic local alignment search tool, J. Mol. Biol., 215(3): 403-410; Altschul et al., Methods in Enzymology; Altschul et al. (1997) Nucleic Acids Res. 25:3389-3402; Baxevanis et al. (1998) Bioinformatics: A Practical Guide to the Analysis of Genes and Proteins, Wiley; and Misener et al., (eds.) (1999) Bioinformatics Methods and Protocols (Methods in Molecular Biology, Vol. 132), Humana Press. In addition to identifying identical sequences, the programs mentioned above typically provide an indication of the degree of identity. In some aspects, two sequences are considered to be substantially identical if at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more of their corresponding residues are identical over a relevant stretch of residues. In some aspects, the relevant stretch is a complete sequence. In some aspects, the relevant stretch is at least 10, 15, 20, 25, 30, 35, 40, 45, 50, or more residues.

[0091] The phrase *"targeting vector"* or *"targeting construct"* includes a polynucleotide molecule that comprises a targeting region. A targeting region comprises a sequence that is identical or substantially identical to a sequence in a target cell, tissue or animal and provides for integration of the targeting construct into a position within the genome of the cell, tissue or animal via homologous recombination. Targeting regions that target using site-specific recombinase recognition sites (e.g., *loxP* or *Frt* sites) are also included. In some aspects, a targeting construct described herein further comprises a nucleic acid sequence or gene of particular interest, a selectable marker, control and or regulatory sequences, and other nucleic acid sequences that allow for recombination mediated through exogenous addition of proteins that aid in or facilitate recombination involving such sequences. In some aspects, a targeting construct described herein further comprises a gene of interest in whole or in part, wherein the gene of interest is a heterologous gene that encodes a protein, in whole or in part, that has a similar function as a protein encoded by an endogenous sequence. In some aspects, a targeting construct described herein further comprises a humanized gene of interest, in whole or in part, wherein the humanized gene of interest encodes a protein, in whole or in part, that has a similar function as a protein encoded by the endogenous sequence.

[0092] The phrase *"therapeutically effective amount"* includes an amount that produces the desired effect for which it is administered. In some aspects, the term refers to an amount that is sufficient, when administered to a subject (e.g., an animal) suffering from or susceptible to a disease, disorder, and/or condition in accordance with a therapeutic dosing regimen, to treat the disease, disorder, and/or condition. In some aspects, a therapeutically effective amount is one that reduces the incidence and/or severity of, and/or delays onset of, one or more symptoms of the disease, disorder, and/or condition. Those of ordinary skill in the art will appreciate that the term *"therapeutically effective amount"* does not in fact require successful treatment be achieved in a particular individual. Rather, a therapeutically effective amount may be that amount that provides a particular desired pharmacological response in a significant number of subjects when administered to subjects in need of such treatment. In some aspects, reference to a therapeutically effective amount may be a reference to an amount as measured in one or more specific tissues (e.g., a tissue affected by the disease, disorder or condition) or fluids (e.g., blood, saliva, serum, sweat, tears, urine, etc.). Those of ordinary skill in the art will appreciate that, in some aspects, a therapeutically effective amount of a particular agent or therapy may be formulated and/or administered in a single dose. In some aspects, a therapeutically effective agent may be formulated and/or administered in a plurality of doses, for example, as part of a dosing regimen.

[0093] The term *"treatment"* (also *"treat"* or *"treating"),* in its broadest sense includes any administration of a substance (e.g., provided compositions) that partially or completely alleviates, ameliorates, relives, inhibits, delays onset of, reduces severity of, and/or reduces incidence of one or more symptoms, features, and/or causes of a particular disease, disorder, and/or condition. In some aspects, such treatment may be administered to a subject who does not exhibit signs of the relevant disease, disorder and/or condition and/or of a subject who exhibits only early signs of the disease, disorder, and/or condition. Alternatively or additionally, in some aspects, treatment may be administered to a subject who exhibits one or more established signs of the relevant disease, disorder and/or condition. In some aspects, treatment may be of a subject who has been diagnosed as suffering from the relevant disease, disorder, and/or condition. In some aspects, treatment may be of a subject known to have one or more susceptibility factors that are statistically correlated with increased risk of development of the relevant disease, disorder, and/or condition.

[0094] The term *"variant"* includes an entity that shows significant structural identity with a reference entity, but differs structurally from the reference entity in the presence or level of one or more chemical moieties as compared with the reference entity. In many aspects, a *"variant"* also differs functionally from its reference entity. In general, whether a particular entity is properly considered to be a *"variant"* of a reference entity is based on its degree of structural identity with the reference entity. As will be appreciated by those skilled in the art, any biological or chemical reference entity has certain characteristic structural elements. A *"variant",* by definition, is a distinct chemical entity that shares one or more such characteristic structural elements. To give but a few examples, a small molecule may have a characteristic core structural element (e.g., a macrocycle core) and/or one or more characteristic pendent moieties so that a variant of the small molecule is one that shares the core structural element and the characteristic pendent moieties but differs in other pendent moieties and/or in types of bonds present (single vs. double, E vs. Z, etc.) within the core, a polypeptide may have a characteristic sequence element comprised of a plurality of amino acids having designated positions relative to one another in linear or three-dimensional space and/or contributing to a particular biological function, a nucleic acid may have a characteristic sequence element comprised of a plurality of nucleotide residues having designated positions relative to on another in linear or three-dimensional space. For example, a *"variant polypeptide"* may differ from a reference polypeptide as a result of one or more differences in amino acid sequence and/or one or more differences in chemical moieties (e.g., carbohydrates, lipids, etc.) covalently attached to the polypeptide backbone. In some aspects, a *"variant polypeptide"* shows an overall sequence identity with a reference polypeptide that is at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, or 99%. Alternatively or additionally, in some aspects, a *"variant polypeptide"* does not share at least one characteristic sequence element with a reference polypeptide. In some aspects, the reference polypeptide has one or more biological activities. In some aspects, a *"variant polypeptide"* shares one or more of the biological activities of the reference polypeptide. In some aspects, a *"variant polypeptide"* lacks one or more of the biological activities of the reference polypeptide. In some aspects, a *"variant polypeptide"* shows a reduced level of one or more biological activities as compared with the reference polypeptide. In many aspects, a polypeptide of interest is considered to be a *"variant"* of a parent or reference polypeptide if the polypeptide of interest has an amino acid sequence that is identical to that of the parent but for a small number of sequence alterations at particular positions. Typically, fewer than 20%, 15%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% of the residues in the variant are substituted as compared with the parent. In some aspects, a *"variant"* has 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 substituted residue as compared with a parent. Often, a *"variant"* has a very small number (e.g., fewer than 5, 4, 3, 2, or 1) number of substituted functional residues (i.e., residues that participate in a particular biological activity). Furthermore, a *"variant"* typically has not more than 5, 4, 3, 2, or 1 additions or deletions, and often has no additions or deletions, as compared with the parent. Moreover, any additions or deletions are typically fewer than about 25, about 20, about 19, about 18, about 17, about 16, about 15, about 14, about 13, about 10, about 9, about 8, about 7, about 6, and commonly are fewer than about 5, about 4, about 3, or about 2 residues. In some aspects, the parent or reference polypeptide is one found in nature. As will be understood by those of ordinary skill in the art, a plurality of variants of a particular polypeptide of interest may commonly be found in nature, particularly when the polypeptide of interest is an infectious agent polypeptide.

[0095] The term *"vector"* includes a nucleic acid molecule capable of transporting another nucleic acid to which it is associated. In some aspects, vectors are capable of extra-chromosomal replication and/or expression of nucleic acids to which they are linked in a host cell such as a eukaryotic and/or prokaryotic cell. Vectors capable of directing the expression of operatively linked genes are referred to herein as *"expression vectors."*

[0096] The term *"wild-type"* includes an entity having a structure and/or activity as found in nature in a *"normal"* (as contrasted with mutant, diseased, altered, etc.) state or context. Those of ordinary skill in the art will appreciate that wild- type genes and polypeptides often exist in multiple different forms (e.g., alleles).

DETAILED DESCRIPTION OF CERTAIN ASPECTS

[0097] Also described herein are, among other things, improved and/or engineered non-human animals having humanized genetic material encoding a Programmed cell death 1 (Pdcdl) gene for determining the therapeutic efficacy of Pdcdl modulators (e.g., an anti-PD-1 antibody) for the treatment of cancer, and assays in T cell responses and signal transduction. It is contemplated that such non-human animals provide an improvement in determining the therapeutic efficacy of PD-1 modulators and their potential for PD-1 blockade. Therefore, the present disclosure is particularly useful for the development of anti-PD-1 therapies for the treatment of various cancers, as well as for augmenting immune responses to treat and/or remove viral infection in non-human animals. In particular, the present disclosure encompasses the humanization of a murine *Pdcdl* gene resulting in expression of a humanized PD-1 protein on the surface of cells of the non-human animal. Such humanized PD-1 proteins have the capacity to provide a source of human PD-1^+ cells for determining the efficacy of anti-PD-1 therapeutics to promote anti-tumor immune responses. In some aspects, non-human animals described herein demonstrate augmented immune responses via blockade of PD-1 signaling through the humanized PD-1 protein expressed on the surface of cells of the non-human animal. In some aspects, humanized PD-1 proteins have a sequence corresponding to the N-terminal immunoglobulin V domain, in whole or in part, of a human PD-1 protein. In some aspects, humanized PD-1 proteins have a sequence corresponding to the intracellular tail of a murine PD-1 protein; in some aspects, a sequence corresponding to the transmembrane domain and intracellular tail of a murine PD-1 protein. In some aspects, humanized PD-1 proteins have a sequence corresponding to amino acid residues 21-170 (or 26-169, 27-169, or 27-145, or 35-145) of a human PD-1 protein. In some aspects, non-human animals described herein comprise an endogenous Pdcdl gene that contains genetic material from the non-human animal and a heterologous species (e.g., a human). In some aspects, non-human animals described herein comprise a humanized Pdcdl gene, wherein the humanized Pdcdl gene comprises exon 2 and exon 3, in whole or in part, of a human *PDCD1* gene. In some certain aspects, non-human animals described herein comprise a humanized Pdcdl gene, wherein the humanized Pdcdl gene compries 883 bp of a human *PDCD1* gene corresponding to exon 2 and the first 71 bp of exon 3 (i.e., encoding the stalk) of a human *PDCD1* gene.

[0098] Various aspects are described in detail in the following sections. The use of sections is not meant to limit the invention. Each section can apply to any aspect of the invention. In this application, the use of "or" means "and/or" unless stated otherwise.

Programmed cell death 1 (Pdcdl) gene

[0099] Pdcdl (also referred to as CD279) was originally discovered as an upregulated gene in a T cell hybridoma that was undergoing apoptosis (Ishida, Y. et al. (1992) EMBO J. 11(11):3887-3895). The Pdcdl gene consists of 5 exons that encode PD-1, which is a type I membrane protein (referred to as PD-1) that includes an N-terminal immunoglobulin V (IgV) domain, a stalk (~20 amino acids in length), a transmembrane domain, and an intracellular tail that contains both an immunoreceptor tyrosine inhibitory motif (ITIM) and an immunoreceptor tyrosine switch motif (ITSM). PD-1 is expressed on many cell types such as, for example, B cells, dendritic cells, activated monocytes, natural killer (NK) cells and activated T cells (Keir, M.E., et al. (2008) Annu. Rev. Immunol. 26:677-704). Various splice variants of PD-1 have also been reported and vary based on which exon is lacking (Nielsen, C. et al. (2005) Cell. Immunol. 235:109-116). Indeed, certain splice variants have been observed as a causitive factor in autoimmune diseases (Wan, B. et al. (2006) J. Immunol. 177(12):8844-8850). Further, Pdcdl-deficient mice have been reported to develop autoimmune conditions (Nishimura, H. et al. (1998) Intern. Immunol. 10(10):1563-1572; Nishimura, H. et al. (1999) Immunity 11:141-151; Nishimura, H. et al. (2001) Science 291:319-322), which have lead the way to solidifying PD-1 as a negative regulator of activated lymphocytes and serves to protect against the development of autoimmune disease. Interestingly, tumors have been discovered to use PD-1 signaling to evade surveillance by the immune system. Therefore, PD-1 and at least one of its ligands (i.e., PD-L1) are currently being explored as targets for cancer therapy by promotion of anti-tumor activity in tumor microenvironments via PD-1 blockade (see e.g., Pedoeem, A. et al. (2014) Clin. Immunol. 153:145-152; and Philips, G.K. and Atkins, M. (2014) Intern. Immunol. 8 pages).

[0100] A more thorough and detailed understanding of PD-1-mediated functions and the PD-1 pathway is needed to develop practical targeted therapies for future cancer treatment.

Pdcd1 and PD-1 Sequences

[0101] Exemplary murine, human and humanized Pdcdl and PD-1 sequences are set forth in Figure 8. An exemplary human nucleic acid sequence for humanization of a non-human Pdcdl gene is also set forth in Figure 8.

Humanized Pdcdl Non-Human Animals

[0102] Non-human animals are provided that express humanized PD-1 proteins on the surface of cells of the non-human animals resulting from a genetic modification of an endogenous locus (e.g., a Pdcdl locus) of the non-human animal that encodes a PD-1 protein. Suitable examples described herein include rodents, in particular, mice.

[0103] A humanized Pdcdl gene, in some aspects, comprises genetic material from a heterologous species (e.g., humans), wherein the humanized Pdcdl gene encodes a PD-1 protein that comprises the encoded portion of the genetic material from the heterologous species. In some aspects, a humanized Pdcdl gene described herein comprises genomic DNA of a heterologous species that encodes the extracellular portion of a PD-1 protein that is expressed on the plasma membrane of a cell. Non-human animals, embryos, cells and targeting constructs for making non-human animals, non-human embryos, and cells containing said humanized Pdcdl gene are also provided.

[0104] In some aspects, an endogenous Pdcdl gene is deleted. In some aspects, an endogenous Pdcdl gene is altered, wherein a portion of the endogenous Pdcdl gene is replaced with a heterologous sequence (e.g., a human *PDCD1* sequence, in whole or in part). In some aspects, all or substantially all of an endogenous Pdcdl gene is replaced with a heterologous gene (e.g., a human *PDCD1* gene). In some aspects, a portion of a heterologous Pdcdl gene is inserted into an endogenous non-human Pdcdl gene at an endogenous Pdcdl locus. In some aspects, the heterologous gene is a human gene. In some aspects, the modification or humanization is made to one of the two copies of the endogenous Pdcdl gene, giving rise to a non-human animal that is heterozygous with respect to the humanized Pdcdl gene. In other aspects, a non-human animal is provided that is homozygous for a humanized Pdcdl gene.

[0105] In various aspects, a non-human animal contains a human *PDCD1* gene, in whole or in part, at an endogenous non-human Pdcdl locus. Thus, such non-human animals can be described as having a heterologous Pdcdl gene. The replaced, inserted, modified or altered Pdcdl gene at the endogenous Pdcdl locus or a protein expressed from such gene can be detected using a variety of methods including, for example, PCR, Western blot, Southern blot, restriction fragment length polymorphism (RFLP), or a gain or loss of allele assay. In some aspects, the non-human animal is heterozygous with respect to the humanized Pdcdl gene.

[0106] In various aspects, a humanized Pdcdl gene includes a Pdcdl gene that has a second exon having a sequence at least 50% (e.g., 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more) identical to a second exon that appears in a human *PDCD1* gene of Figure 8.

[0107] In various aspects, a humanized Pdcdl gene includes a Pdcdl gene that has a second exon having a sequence that is substantially identical to a second exon that appears in a human *PDCD1* gene of Figure 8.

[0108] In various aspects, a humanized Pdcdl gene includes a Pdcdl gene that has a second exon having a sequence that is identical to a second exon that appears in a human *PDCD1* gene of Figure 8.

[0109] In various aspects, a humanized Pdcdl gene includes a Pdcdl gene that has a third exon having a sequence at least 50% (e.g., 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more) identical to a third exon that appears in a humanized Pdcdl mRNA sequence of Figure 8.

[0110] In various aspects, a humanized Pdcdl gene includes a Pdcdl gene that has a third exon having a sequence that is substantially identical to a third exon that appears in a humanized Pdcdl mRNA sequence of Figure 8.

[0111] In various aspects, a humanized Pdcdl gene includes a Pdcdl gene that has a third exon having a sequence that is identicial to a third exon that appears in a humanized Pdcdl mRNA sequence of Figure 8.

[0112] In various aspects, a humanized Pdcdl gene includes a Pdcdl gene that comprises a sequence at least 50% (e.g., 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more) identical to SEQ ID NO:21 or SEQ ID NO:23.

[0113] In various aspects, a humanized Pdcdl gene includes a Pdcdl gene that comprises a sequence that is substantially identical to SEQ ID NO:21 or SEQ ID NO:23.

[0114] In various aspects, a humanized Pdcdl gene includes a Pdcdl gene that comprises a sequence that is identical to SEQ ID NO:21 or SEQ ID NO:23.

[0115] In various aspects, a humanized Pdcdl gene includes a Pdcdl gene that has a second exon and a portion of a third exon each having a sequence at least 50% (e.g., 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more) identical to a second exon and a portion of a third exon that appear in a human *PDCD1* gene of Figure 8.

[0116] In various aspects, a humanized Pdcdl gene includes a Pdcdl gene that has a first, fourth and fifth exon each having a sequence at least 50% (e.g., 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more) identical to a first, fourth and fifth exon that appear in a mouse *Pdcdl* gene of Figure 8.

[0117] In various aspects, a humanized Pdcdl gene includes a Pdcdl gene that has a first, a portion of a third, a fourth and a fifth exon each having a sequence at least 50% (e.g., 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more) identical to a first, a portion of a third, a fourth and a fifth exon that appear in a mouse *Pdcdl* gene of Figure 8.

[0118] In various aspects, a humanized Pdcdl gene includes a Pdcdl gene that has a 5' untranslated region and a 3' untranslated region each having a sequence at least 50% (e.g., 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more) identical to a 5' untranslated region and a 3' untranslated region that appear in a mouse *Pdcdl* gene of Figure 8.

[0119] In various aspects, a humanized Pdcdl gene includes a Pdcdl gene that has a nucleotide coding sequence (e.g., a cDNA sequence) at least 50% (e.g., 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more) identical to a nucleotide coding sequence that appears in a humanized Pdcdl nucleotide coding sequence of Figure 8.

[0120] In various aspects, a humanized Pdcdl mRNA sequence comprises a sequence that is at least 50% (e.g., 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more) identical to a humanized mRNA sequence that appears in Figure 8.

[0121] In various aspects, a humanized Pdcdl gene encodes a PD-1 polypeptide having an amino acid sequence at least 50% (e.g., 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more) identical to an amino acid sequence that appears in a PD-1 polypeptide sequence of Figure 8.

[0122] In various aspects, a humanized PD-1 protein produced by a non-human animal described herein has an extracellular portion having an amino acid sequence that is at least 50% (e.g., 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more) identical to an extracellular portion of a human PD-1 protein that appears in Figure 8.

[0123] In various aspects, a humanized PD-1 protein produced by a non-human animal described herein has an extracellular portion, which extracellular portion comprises an amino acid sequence that is at least 50% (e.g., 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more) identical to amino acid residues 21-170 that appear in a human or humanized PD-1 protein of Figure 8.

[0124] In various aspects, a humanized PD-1 protein produced by a non-human animal described herein has an extracellular portion, which extracellular portion comprises an amino acid sequence that is substantially identical to amino acid residues 21-170 that appear in a human or humanized PD-1 protein of Figure 8.

[0125] In various aspects, a humanized PD-1 protein produced by a non-human animal described herein has an extracellular portion, which extracellular portion comprises an amino acid sequence that is identical to amino acid residues 21-170 that appear in a human or humanized PD-1 protein of Figure 8.

[0126] In various aspects, a humanized PD-1 protein produced by a non-human animal described herein has an extracellular portion, which extracellular portion comprises an amino acid sequence that is at least 50% (e.g., 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more) identical to amino acid residues 26-169 that appear in a human or humanized PD-1 protein of Figure 8.

[0127] In various aspects, a humanized PD-1 protein produced by a non-human animal described herein has an extracellular portion, which extracellular portion comprises an amino acid sequence that is substantially identical to amino acid residues 26-169 that appear in a human or humanized PD-1 protein of Figure 8.

[0128] In various aspects, a humanized PD-1 protein produced by a non-human animal described herein has an extracellular portion, which extracellular portion comprises an amino acid sequence that is identical to amino acid residues 26-169 that appear in a human or humanized PD-1 protein of Figure 8.

[0129] In various aspects, a humanized PD-1 protein produced by a non-human animal described herein has an extracellular portion, which extracellular portion comprises an amino acid sequence that is at least 50% (e.g., 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more) identical to amino acid residues 27-169 that appear in a human or humanized PD-1 protein of Figure 8.

[0130] In various aspects, a humanized PD-1 protein produced by a non-human animal described herein has an extracellular portion, which extracellular portion comprises an amino acid sequence that is substantially identical to amino acid residues 27-169 that appear in a human or humanized PD-1 protein of Figure 8.

[0131] In various aspects, a humanized PD-1 protein produced by a non-human animal described herein has an extracellular portion, which extracellular portion comprises an amino acid sequence that is identical to amino acid residues 27-169 that appear in a human or humanized PD-1 protein of Figure 8.

[0132] In various aspects, a humanized PD-1 protein produced by a non-human animal described herein has an extracellular portion, which extracellular portion comprises an amino acid sequence that is at least 50% (e.g., 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more) identical to amino acid residues 27-145 that appear in a human or humanized PD-1 protein of Figure 8.

[0133] In various aspects, a humanized PD-1 protein produced by a non-human animal described herein has an extracellular portion, which extracellular portion comprises an amino acid sequence that is substantially identical to amino acid residues 27-145 that appear in a human or humanized PD-1 protein of Figure 8.

[0134] In various aspects, a humanized PD-1 protein produced by a non-human animal described herein has an extracellular portion, which extracellular portion comprises an amino acid sequence that is identical to amino acid residues 27-145 that appear in a human or humanized PD-1 protein of Figure 8.

[0135] In various aspects, a humanized PD-1 protein produced by a non-human animal described herein has an extracellular portion, which extracellular portion comprises an amino acid sequence that is at least 50% (e.g., 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more) identical to amino acid residues 35-145 that appear in a human or humanized PD-1 protein of Figure 8.

[0136] In various aspects, a humanized PD-1 protein produced by a non-human animal described herein has an extracellular portion, which extracellular portion comprises an amino acid sequence that is substantially identical to amino acid residues 35-145 that appear in a human or humanized PD-1 protein of Figure 8.

[0137] In various aspects, a humanized PD-1 protein produced by a non-human animal described herein has an extracellular portion, which extracellular portion comprises an amino acid sequence that is identical to amino acid residues 35-145 that appear in a human or humanized PD-1 protein of Figure 8.

[0138] In various aspects, a humanized PD-1 protein produced by a non-human animal described herein has an N-terminal immunoglobulin V domain having an amino acid sequence that is at least 50% (e.g., 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more) identical to an N-terminal immunoglobulin V domain of a human or humanized PD-1 protein that appears in Figure 8.

[0139] In various aspects, a humanized PD-1 protein produced by a non-human animal described herein has an N-terminal immunoglobulin V domain having an amino acid sequence that is substantially identical to an N-terminal immunoglobulin V domain that appears in a human or humanized PD-1 protein of Figure 8.

[0140] In various aspects, a humanized PD-1 protein produced by a non-human animal described herein has an N-terminal immunoglobulin V domain having an amino acid sequence that is identical to an N-terminal immunoglobulin V domain that appears in a human or humanized PD-1 protein of Figure 8.

[0141] In various aspects, a humanized PD-1 protein produced by a non-human animal described herein has a transmembrane domain having a sequence that is at least 50% (e.g., 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more) identical to a transmembrane domain of a mouse PD-1 protein that appears in

Figure 8.

[0142] In various aspects, a humanized PD-1 protein produced by a non-human animal described herein has an intracellular tail having a sequence that is at least 50% (e.g., 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more) identical to an intracellular tail of a mouse PD-1 protein that appears in Figure 8.

[0143] In various aspects, a humanized PD-1 protein produced by a non-human animal described herein has an amino acid sequence that is at least 50% (e.g., 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more) identical to amino acid residues 27-169 (or 26-169) that appear in a human PD-1 protein of Figure 8.

[0144] In various aspects, a humanized PD-1 protein produced by a non-human animal described herein has an amino acid sequence that is substantially identical to amino acid residues 27-169 (or 26-169) that appear in a human PD-1 protein of Figure 8.

[0145] In various aspects, a humanized PD-1 protein produced by a non-human animal described herein has an amino acid sequence that is identical to amino acid residues 27-169 (or 26-169) that appear in a human PD-1 protein of Figure 8.

[0146] In various aspects, a humanized PD-1 protein produced by a non-human animal described herein has an amino acid sequence that is at least 50% (e.g., 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more) identical to an amino acid sequence of a humanized PD-1 protein that appears in Figure 8.

[0147] In various aspects, a humanized PD-1 protein produced by a non-human animal described herein has an amino acid sequence that is substantially identical to an amino acid sequence of a humanized PD-1 protein that appears in Figure 8.

[0148] In various aspects, a humanized PD-1 protein produced by a non-human animal described herein has an amino acid sequence that is identical to an amino acid sequence of a humanized PD-1 protein that appears in Figure 8.

[0149] Compositions and methods for making non-human animals that express a humanized PD-1 protein, including specific polymorphic forms, allelic variants (e.g., single amino acid differences) or alternatively spliced isoforms, are provided, including compositions and methods for making non-human animals that express such proteins from a human promoter and a human regulatory sequence. In some aspects, compositions and methods for making non-human animals that express such proteins from an endogenous promoter and an endogenous regulatory sequence are also provided. In some certain aspects, endogenous promoters and endogenous regulatory sequences are endogenous rodent promoters and endogenous rodent regulatory sequences. The methods include inserting the genetic material encoding a human PD-1 protein in whole or in part at a precise location in the genome of a non-human animal that corresponds to an endogenous Pdcdl gene thereby creating a humanized Pdcdl gene that expresses a PD-1 protein that is human in whole or in part. In some aspects, the methods include inserting genomic DNA corresponding to exon 2 and exon 3, in whole or in part, of a human *PDCD1* gene into an endogenous Pdcdl gene of the non-human animal thereby creating a humanized gene that encodes a PD-1 protein that contains a human portion containing amino acids encoded by the inserted exons.

[0150] Where appropriate, the coding region of the genetic material or polynucleotide sequence(s) encoding a human (or humanized) PD-1 protein in whole or in part may be modified to include codons that are optimized for expression from cells in the non-human animal (e.g., see U.S. Patent No.'s 5,670,356 and 5,874,304). Codon optimized sequences are synthetic sequences, and preferably encode the identical polypeptide (or a biologically active fragment of a full length polypeptide which has substantially the same activity as the full length polypeptide) encoded by the non-codon optimized parent polynucleotide. In some aspects, the coding region of the genetic material encoding a human (or humanized) PD-1 protein, in whole or in part, may include an altered sequence to optimize codon usage for a particular cell type (e.g., a rodent cell). For example, the codons of the genomic DNA corresponding to exon 2 and a portion of exon 3 (e.g., 71 bp) of a human *PDCD1* gene to be inserted into an endogenous Pdcdl gene of a non-human animal (e.g., a rodent) may be optimized for expression in a cell of the non-human animal. Such a sequence may be described as a codon-optimized sequence.

[0151] A humanized Pdcdl gene approach employs a relatively minimal modification of the endogenous gene and results in natural PD-1-mediated signal transduction in the non-human animal, in various aspects, because the genomic sequence of the Pdcdl sequences are modified in a single fragment and therefore retain normal functionality by including necessary regulatory sequences. Thus, in such aspects, the Pdcdl gene modification does not affect other surrounding genes or other endogenous Pdcd1-interacting genes (e.g., PD-L1, PD-L2, etc.). Further, in various aspects, the modification does not affect the assembly of a functional PD-1 transmembrane protein on the cell membrane and maintains normal effector functions via binding and subsequent signal transduction through the cytoplasmic portion of the protein which is unaffected by the modification.

[0152] A schematic illustration (not to scale) of the genomic organization of an endogenous murine *Pdcdl* gene and a human *PDCD1* gene is provided in Figure 1. An exemplary method for humanizing an endogenous murine *Pdcdl* gene using a genomic fragment containing exon 2 and a portion of exon 3 of a human *PDCD1* gene is provided in Figure 2. As illustrated, an 883 bp genomic DNA fragment containing exon 2 and a portion of exon 3 (e.g., the first 71 bp) of a human *PDCD1* gene is inserted into the place of a 900 bp sequence of an endogenous murine *Pdcdl* gene locus by a targeting construct. The 883 bp human DNA fragment may be cloned directly from human DNA or synthesized from a source sequence (e.g., Genbank accession no. NM_005018.2). This genomic DNA includes the portion of the gene that encodes substantially all of the extracellular portion (e.g., amino acid residues 27-169 or 26-169) of a human PD-1 protein responsible for ligand binding.

[0153] A non-human animal (e.g., a mouse) having a humanized Pdcdl gene at the endogenous

Pdcdl locus can be made by any method known in the art. For example, a targeting vector can be made that introduces a human Pdcdl gene in whole or in part with a selectable marker gene. Figure 2 illustrates a targeting vector that contains an endogenous *Pdcdl* locus of a mouse genome comprising an insertion of an 883 bp human DNA fragment that includes exon 2 and the first 71 bp of exon 3 of a human *PDCD1* gene. As illustrated, the targeting construct contains a 5' homology arm containing sequence upstream of exon 2 of an endogenous murine *Pdcdl* gene (~61.7 Kb), followed by a drug selection cassette (e.g., a neomycin resistance gene flanked on both sides by loxP sequences; ~5 Kb), a genomic DNA fragment containing exon 2 and the first 71 bp of exon 3 of a human Pdcdl gene (883 bp), and a 3' homology arm containing the remaining sequence of an endogenous murine exon 3 (i.e., portion which encodes a transmembrane portion of a PD-1 protein), exon 4 and exon 5 of an endogenous murine *Pdcdl* gene (~84 Kb). The targeting construct contains a self-deleting drug selection cassette (e.g., a neomycin resistance gene flanked by loxP sequences; see U.S. Patent No.'s 8,697,851, 8,518,392 and 8,354,389). Upon electroporation in embryonic stem cells, a modified endogenous *Pdcdl* gene is created that exchanges 900 bp of an endogenous wild-type *Pdcdl* gene with 883 bp of a human *PDCD1* gene (i.e., exon 2 and the first 71 bp of exon 3), which is contained in the targeting vector. A humanized Pdcdl gene is created resulting in a cell or non-human animal that expresses a humanized PD-1 protein that contains amino acids encoded by the 883 bp human DNA fragment (i.e., exon 2 and 71 bp of exon 3 of a human *PDCD1* gene). The drug selection cassette is removed in a development-dependent manner, i.e., progeny derived from mice whose germ line cells containing the humanized Pdcdl gene described above will shed the selectable marker from differentiated cells during development (see bottom of Figure 2).

[0154] Although aspects employing a humanized Pdcdl gene in a mouse (i.e., a mouse with a *Pdcdl* gene that encodes a PD-1 protein that includes a human portion and a mouse portion) are extensively discussed herein, other non-human animals that comprise a humanized Pdcdl gene are also provided. In some aspects, such non-human animals comprise a humanized Pdcdl gene operably linked to a rodent *Pdcdl* promoter. In some aspects, such non-human animals comprise a humanized Pdcdl gene operably linked to an endogenous Pdcdl promoter; in some aspects, an endogenous rodent Pdcdl promoter. In some aspects, such non-human animals express a humanized PD-1 protein from an endogenous locus, wherein the humanized PD-1 protein comprises amino acid residues 21-170 (or 26-169, or 27-169, 27-145 or 35-145) of a human PD-1 protein. Such non-human animals include any of those which can be genetically modified to express a PD-1 protein as disclosed herein, including, e.g., mammals, e.g., mouse, rat, rabbit, pig, bovine (e.g., cow, bull, buffalo), deer, sheep, goat, chicken, cat, dog, ferret, primate (e.g., marmoset, rhesus monkey), etc. For example, for those non-human animals for which suitable genetically modifiable ES cells are not readily available, other methods are employed to make a non-human animal comprising the genetic modification. Such methods include, e.g., modifying a non-ES cell genome (e.g., a fibroblast or an induced pluripotent cell) and employing somatic cell nuclear transfer (SCNT) to transfer the genetically modified genome to a suitable cell, e.g., an enucleated oocyte, and gestating the modified cell (e.g., the modified oocyte) in a non-human animal under suitable conditions to form an embryo.

[0155] Methods for modifying a non-human animal genome (e.g., a pig, cow, rodent, chicken, etc. genome) include, e.g., employing a zinc finger nuclease (ZFN) or a transcription activator-like effector nuclease (TALEN) to modify a genome to include a humanized Pdcdl gene.

[0156] In some aspects, a non-human animal described herein is a mammal. In some aspects, a non-human animal described herein is a small mammal, e.g., of the superfamily Dipodoidea or Muroidea. In some aspects, a genetically modified animal described herein is a rodent. In some aspects, a rodent described herein is selected from a mouse, a rat, and a hamster. In some aspects, a rodent described herein is selected from the superfamily Muroidea. In some aspects, a genetically modified animal described herein is from a family selected from Calomyscidae (e.g., mouse-like hamsters), Cricetidae (e.g., hamster, New World rats and mice, voles), Muridae (true mice and rats, gerbils, spiny mice, crested rats), Nesomyidae (climbing mice, rock mice, white-tailed rats, Malagasy rats and mice), Platacanthomyidae (e.g., spiny dormice), and Spalacidae (e.g., mole rates, bamboo rats, and zokors). In some certain aspects, a genetically modified rodent described herein is selected from a true mouse or rat (family Muridae), a gerbil, a spiny mouse, and a crested rat. In some certain aspects, a genetically modified mouse described herein is from a member of the family Muridae. In some aspect, a non-human animal described herein is a rodent. In some certain aspects, a rodent described herein is selected from a mouse and a rat. In some aspects, a non-human animal described herein is a mouse.

[0157] In some aspects, a non-human animal described herein is a rodent that is a mouse of a C57BL strain selected from C57BL/A, C57BL/An, C57BL/GrFa, C57BL/KaLwN, C57BL/6, C57BL/6J, C57BL/6ByJ, C57BL/6NJ, C57BL/10, C57BL/10ScSn, C57BL/10Cr, and C57BL/Ola. In some certain aspects, a mouse described herein is a 129 strain selected from the group consisting of a strain that is 129P1, 129P2, 129P3, 129X1, 129S1 (e.g., 129S1/SV, 129S1/SvIm), 129S2, 129S4, 129S5, 129S9/SvEvH, 129/SvJae, 129S6 (129/SvEvTac), 129S7, 129S8, 129T1, 129T2 (see, e.g., Festing et al., 1999, Mammalian Genome 10:836; Auerbach, W. et al., 2000, Biotechniques 29(5):1024-1028, 1030, 1032). In some certain aspects, a genetically modified mouse described herein is a mix of an aforementioned 129 strain and an aforementioned C57BL/6 strain. In some certain aspects, a mouse described herein is a mix of aforementioned 129 strains, or a mix of aforementioned BL/6 strains. In some certain aspects, a 129 strain of the mix as described herein is a 129S6 (129/SvEvTac) strain. In some aspects, a mouse described herein is a BALB strain, e.g., BALB/c strain. In some aspects, a mouse described herein is a mix of a BALB strain and another aforementioned strain.

[0158] In some aspects, a non-human animal described herein is a rat. In some certain aspects, a rat described herein is selected from a Wistar rat, an LEA strain, a Sprague Dawley strain, a Fischer strain, F344, F6, and Dark Agouti. In some certain aspects, a rat strain as described herein is a mix of two or more strains selected from the group consisting of Wistar, LEA, Sprague Dawley, Fischer, F344, F6, and Dark Agouti.

Methods Employing Non-Human Animals Having Humanized Pdcdl Genes

[0159] Investigation into PD-1 function has employed the use of various Pdcdl mutant and transgenic non-human animals (e.g., see Nishimura, H. et al. (1998) Intern. Immunol. 10(10):1563-1572; Nishimura, H. et al. (1999) Immunity 11:141-151; Nishimura, H. et al. (2001) Science 291:319-322; Iwai, Y. et al. (2004) Intern. Immunol. 17(2):133-144; Keir, M. E. et al. (2005) J. Immunol. 175:7372-7379; Keir, M. E. et al. (2007) J. Immunol. 179:5064-5070; Carter, L.L. et al. (2007) J. Neuroimmunol. 182:124-134; Chen, L. et al. (2007) Europ. Soc. Organ Transplant. 21:21-29; Okazaki, T. et al. (2011) J. Exp. Med. 208(2):395-407; U.S. Patent No. 7,414,171; and European Patent No. 1 334 659 B1). Such mutant and transgenic animals have been useful in determining the molecular aspects of PD-1 expression, function and regulation of various cellular processes. However, they are not without limitation. For example, PD-1-deficient mice generated by knock-in of a human PD-1 cDNA into exon 1 of a mouse *Pdcdl* gene did not express human PD-1 even after stimulation with PMA (Carter, L.L. et al., supra). Further, considerable phenotypic differences among PD-1 mutant animals in different genetic backgrounds has complicated investigation, especially when attempting to assign various functions and/or regulatory activities to PD-1. Still, other transgenic animals have been created that overexpress PD-1 (Chen, L. et al., supra). Such animals have displayed different expression patterns of the transgene, which can reasonably be attributed to construct design. Further, due to the use of the same source genetic material (i.e., mouse), PD-1 overexpression may have corresponded to endogenous PD-1 rather than transgenic PD-1 due to possible position effects of the transgene. While PD-1 transgenic mice have proved useful in elucidating some PD-1-mediated biological function, they have demonstrated variability in the results obtained, which are based, at least in part, from the different approaches employed to make them. Therefore, current *in vivo* systems exploiting PD-1-mediated biology are incomplete. The molecular aspects of PD-1-mediated biological function and signaling pathways has not been exploited in transgenic mice to its fullest potential.

[0160] Non-human animals described herein provide an improved *in vivo* system and source of biological materials (e.g., cells) expressing human (or humanized) PD-1 that are useful for a variety of assays. In various aspects, non-human animals described herein are used to develop therapeutics that target PD-1 and/or modulate PD-1 signaling (e.g., interfering with interactions with PD-L1 and/or PD-L2). In various aspects, non-human animals described herein are used to identify, screen and/or develop candidate therapeutics (e.g., antibodies) that bind human PD-1. In various aspects, non-human animals described herein are used to screen and develop candidate therapeutics (e.g., antibodies) that block interaction of human PD-1 with human PD-L1 and/or human PD-L2. In various aspects, non-human animals described herein are used to determine the binding profile of antagonists and/or agonists of a humanized PD-1 on the surface of a cell of a non-human animal as described herein; in some aspects, non-human animals described herein are used to determine the epitope or epitopes of one or more candidate therapeutic antibodies that bind human PD-1.

[0161] In various aspects, non-human animals described herein are used to determine the pharmacokinetic profiles of anti-PD-1 antibodies. In various aspects, one or more non-human animals described herein and one or more control or reference non-human animals are each exposed to one or more candidate therapeutic anti-PD-1 antibodies at various doses (e.g., 0.1 mg/kg, 0.2 mg/kg, 0.3 mg/kg, 0.4 mg/kg, 0.5 mg/kg, 1 mg/kg, 2 mg/kg, 3 mg/kg, 4 mg/kg, 5 mg/mg, 7.5 mg/kg, 10 mg/kg, 15 mg/kg, 20 mg/kg, 25 mg/kg, 30 mg/kg, 40 mg/kg, or 50 mg/kg or more). Candidate therapeutic antibodies may be dosed via any desired route of administration including parenteral and non-parenteral routes of administration. Parenteral routes include, e.g., intravenous, intraarterial, intraportal, intramuscular, subcutaneous, intraperitoneal, intraspinal, intrathecal, intracerebro ventricular, intracranial, intrapleural or other routes of injection. Non-parenteral routes include, e.g., oral, nasal, transdermal, pulmonary, rectal, buccal, vaginal, ocular. Administration may also be by continuous infusion, local administration, sustained release from implants (gels, membranes or the like), and/or intravenous injection. Blood is isolated from non-human animals (humanized and control) at various time points (e.g., 0 hr, 6 hr, 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, or up to 30 or more days). Various assays may be performed to determine the pharmacokinetic profiles of administered candidate therapeutic antibodies using samples obtained from non-human animals as described herein including, but not limited to, total IgG, anti-therapeutic antibody response, agglutination, etc.

[0162] In various aspects, non-human animals described herein are used to measure the therapeutic effect of blocking or modulating PD-1 signaling and the effect on gene expression as a result of cellular changes. In various aspects, a non-human animal described herein or cells isolated therefrom are exposed to a candidate therapeutic that binds a humanized PD-1 protein (or a human portion of a PD-1 protein) on the surface of a cell of the non-human animal and, after a subsequent period of time, analyzed for effects on PD-1-dependent processes, for example, adhesion, apoptosis, cytokine production, inflammation, proliferation, self-tolerance and viral infection (or responses).

[0163] Non-human animals described herein express humanized PD-1 protein, thus cells, cell lines, and cell cultures can be generated to serve as a source of humanized PD-1 for use in binding and functional assays, e.g., to assay for binding or function of a PD-1 antagonist or agonist, particularly where the antagonist or agonist is specific for a human PD-1 sequence or epitope or, alternatively, specific for a human PD-1 sequence or epitope that associates with PD-L1 and/or PD-L2. In various aspects, PD-1 epitopes bound by candidate therapeutic antibodies can be determined using cells isolated from non-human animals described herein. In various aspects, a humanized PD-1 protein expressed by a non-human animal as described herein may comprise a variant amino acid sequence. Variant human PD-1 proteins (e.g., polymorphisms) associated with autoimmune and infectious diseases have been reported (e.g., see Lee, Y.H. et al. (2014) Z. Rheumatol. PMID: 24942602; Mansur, A. et al. (2014) J. Investig. Med. 62(3):638-643; Nasi, M. et al. (2013) Intern. J. Infect. Dis. 17:e845-e850; Piskin, I.E. et al. (2013) Neuropediatrics 44(4):187-190; Carter, L.L. et al. (2007) J. Neuroimmunol. 182(1-2):124-134; Wan, B. et al. (2006) J. Immunol. 177(12):8844-8850). Exemplary human PD-1 variants include those listed in the SNP GeneView webpage from NCBI and are summarized in Table 3. In various aspects, non-human animals described herein express a humanized PD-1 protein variant. In various aspects, the variant is polymorphic at an amino acid position associated with ligand binding. In various aspects, non-human animals described herein are used to determine the effect of ligand binding through interaction with a polymorphic variant of human PD-1. In some certain aspects, non-human animals described herein express a human PD-1 variant that appears in Table 3.

TABLE 3

Chromosome position	mRNA position	Variant ID No.	Allele	Amino acid	Codon position	Amino acid position
241851110	883	rs372765600	A	Gln [Q]	2	272
			G	Arg [R]	2	272
241851118	875	rs368411538	T	Asp [D]	3	269
			C	Asp [D]	3	269
241851121	872	rs2227981	A	Ala [A]	3	268
			C	Ala [A]	3	268
			G	Ala [A]	3	268
			T	Ala [A]	3	268
241851135	858	rs146642159	T	Cys [C]	1	264
			C	Arg [R]	1	264
241851138	855	rs143359677	A	Thr [T]	1	263
			G	Ala [A]	1	263
241851160	833	rs141228784	T	Ser [S]	3	255
			C	Ser [S]	3	255
241851163	830	rs200434733	C	Pro [P]	3	254
			T	Pro [P]	3	254
241851171	822	rs201961957	A	Ile [I]	1	252
			G	Val [V]	1	252
241851188	805	rs201540918	T	Met [M]	2	246
			C	Thr [T]	2	246
241851190	803	rs201481671	A	Gln [Q]	3	245
			G	Gln [Q]	3	245
241851210	783	rs137861407	A	Met [M]	1	239
			G	Val [V]	1	239
241851220	773	rs370462869	A	Pro [P]	3	235
			G	Pro [P]	3	235
241851237	756	rs147213978	C	Arg [R]	1	230
			T	Trp [W]	1	230
241851264	729	rs373940258	A	Met [M]	1	221
			G	Val [V]	1	221
241851274	719	rs373831349	G	Pro [P]	3	217
			T	Pro [P]	3	217
241851279	714	rs376257658	A	Met [M]	1	216

Chromosome position	mRNA position	Variant ID No.	Allele	Amino acid	Codon position	Amino acid position
			G	Val [V]	1	216
241851281	712	rs2227982	T	Val [V]	2	215
			C	Ala [A]	2	215
241851954	690	rs148456597	A	Thr [T]	1	208
			C	Pro [P]	1	208
241851961	683	rs146821282	G	Thr [T]	3	205
			T	Thr [T]	3	205
			C	Thr [T]	3	205
241852204	654	rs144217487	A	Thr [T]	1	196
			G	Ala [A]	1	196
241852205	653	rs141119263	T	Ala [A]	3	195
			C	Ala [A]	3	195
241852209	649	rs200312345	A	Gln [Q]	2	194
			G	Arg [R]	2	194
241852258	600	rs55667829	T	Leu [L]	1	178
			C	Leu [L]	1	178
241852271	587	rs377191240	T	Val [V]	3	173
			C	Val [V]	3	173
241852310	548	rs370660750	G	Pro [P]	3	160
			C	Pro [P]	3	160
241852644	481	rs138031190	A	Gln [Q]	2	138
			T	Leu [L]	2	138
241852658	467	rs374762232	A	Gln [Q]	3	133
			G	Gln [Q]	3	133
241852661	464	rs41400345	A	Ala [A]	3	132
			G	Ala [A]	3	132
241852691	434	rs367833850	T	Leu [L]	3	122
			C	Leu [L]	3	122
241852697	428	rs186074812	T	Thr [T]	3	120
			C	Thr [T]	3	120
241852715	410	rs141299049	A	Arg [R]	3	114
			G	Arg [R]	3	114
241852716	409	rs55679128	A	Gln [Q]	2	114
			G	Arg [R]	2	114

Chromosome position	mRNA position	Variant ID No.	Allele	Amino acid	Codon position	Amino acid position
241852720	405	rs200323895	A	Thr [T]	1	113
			G	Ala [A]	1	113
241852729	396	rs190602950	A	Met [M]	1	110
			G	Val [V]	1	110
241852730	395	rs370268595	T	Ser [S]	3	109
			C	Ser [S]	3	109
241852743	382	rs368009835	G	Gly [G]	2	105
			A	Asp [D]	2	105
241852746	379	rs138016578	A	His [H]	2	104
			G	Arg [R]	2	104
241852750	375	rs56124337	A	Arg [R]	1	103
			G	Gly [G]	1	103
241852751	374	rs55637807	T	Asn [N]	3	102
			C	Asn [N]	3	102
241852755	370	rs371902970	T	Leu [L]	2	101
			C	Pro [P]	2	101
241852788	337	rs144257658	T	Val [V]	2	90
			G	Gly [G]	2	90
241852808	317	rs55804130	T	Pro [P]	3	83
			C	Pro [P]	3	83
241852817	308	rs373755187	A	Ala [A]	3	80
			T	Ala [A]	3	80
			C	Ala [A]	3	80
241852860	265	rs28615468	C	Thr [T]	2	66
			A	Asn [N]	2	66
241852866	259	rs142434414	G	Gly [G]	2	64
			T	Val [V]	2	64
241852877	248	rs181904226	A	Ser [S]	3	60
			G	Ser [S]	3	60
241852892	233	rs55993679	T	Ser [S]	3	55
			C	Ser [S]	3	55
241852904	221	rs373582646	G	Thr [T]	3	51
			C	Thr [T]	3	51
241852910	215	rs141718335	T	Asn [N]	3	49

Chromosome position	mRNA position	Variant ID No.	Allele	Amino acid	Codon position	Amino acid position
			C	Asn [N]	3	49
241852922	203	rs374726495	T	Thr [T]	3	45
			C	Thr [T]	3	45
241852928	197	rs147586902	C	Val [V]	3	43
			G	Val [V]	3	43
241852930	195	rs368829632	A	Met [M]	1	43
			G	Val [V]	1	43
241852951	174	rs373081859	G	Ala [A]	1	36
			A	Thr [T]	1	36
241852952	173	rs41444844	G	Pro [P]	3	35
			C	Pro [P]	3	35
241852974	151	rs56234260	T	Leu [L]	2	28
			C	Pro [P]	2	28
241858780	127	rs368550965	A	Gln [Q]	2	20
			G	Arg [R]	2	20
241858800	107	rs370111035	A	Ala [A]	3	13
			G	Ala [A]	3	13
241858808	99	rs142544044	A	Ile [I]	1	11
			G	Val [V]	1	11

[0164] Cells from non-human animals described herein can be isolated and used on an ad hoc basis, or can be maintained in culture for many generations. In various aspects, cells from a non-human animal described herein are immortalized (e.g., via use of a virus) and maintained in culture indefinitely (e.g., in serial cultures).

[0165] In various aspects, cells and/or non-human animals described herein are used in various immunization regimens to determine the PD-1-mediated functions in the immune response to an antigen. In some aspects, candidate therapeutics that bind to, or block one or more functions of, human (or humanized) PD-1 are characterized in a non-human animal described herein. Suitable measurements include various cellular assays, proliferation assays, serum immunoglobulin analysis (e.g., antibody titer), cytotoxicity assays, characterization of ligand-receptor interactions (e.g., immunoprecipitation assays). In some aspects, non-human animals described herein are used to characterize the PD-1-mediated functions regulating an immune response to an antigen. In some aspects, the antigen is associated with an autoimmune disease, disorder or condition. In some aspects, the antigen is associated with an inflammatory disease, disorder or condition. In some aspects, the antigen is a test antigen (e.g., ovalbumin or OVA). In some aspects, the antigen is a target associated with a disease or condition suffered by one or more human patients in need of treatment.

[0166] In various aspects, non-human animals described herein are used in serum assays for determining titers of autoantibody production for testing the pharmaco-toxicological aspects of candidate therapeutics that target human PD-1. In some aspects, autoantibody production in non-human animals described herein results from one or more autoimmune diseases, disorders or conditions induced in the non-human animal.

[0167] In various aspects, non-human animals described herein are used for challenge with one or more antigens to determine the therapeutic potential of compounds or biological agents to modulate PD-1-dependent regulation of an immune response, including but not limited to, the specific T cell-dependent and B cell-dependent responses to a given antigen.

[0168] In various aspects, cells and/or non-human animals described herein are used in a survival and/or proliferation assay (e.g., employing B or T cells) to screen and develop candidate therapeutics that modulate human PD-1 signaling. Activation or loss of PD-1 plays an important role in the regulation of T cell responses, and regulation of self-tolerance by PD-1 may result from the activation of specific epitopes of the extracellular domain of PD-1, therefore, candidate PD-1 modulators (e.g., antagonists or agonists) maybe identified, characterized and developed using cells of non-human animals described herein and/or a non-human animal as described herein. In some aspects, cells and/or non-human animals described herein are used in survival or death assay(s) to determine the effect on proliferation or apoptosis of a specific cell(s) (e.g., cancer cells) in the presence and absence of PD-1.

[0169] In various aspects, cells and/or non-human animals described herein are used in xenotransplantation of heterologous (e.g., human) cells or tissue to determine the PD-1-mediated functions in the physiological (e.g., immune) response to the transplanted human cells or tissue. In some aspects, candidate therapeutics that bind, or block one or more functions of, human PD-1 are characterized in a non-human animal described herein. Suitable measurements include various cellular assays, proliferation assays, serum immunoglobulin analysis (e.g., antibody titer), cytotoxicity assays, and characterization of ligand-receptor interactions (immunoprecipitation assays). In some aspects, non-human animals described herein are used to characterize the PD-1-mediated functions regulating an immune response to an antigen. In some aspects, the antigen is associated with a neoplasm. In some aspects, the antigen is associated with an autoimmune disease, disorder or condition. In some aspects, the antigen is associated with an inflammatory disease, disorder or condition. In some aspects, the antigen is a target associated with a disease or condition suffered by one or more human patients in need of treatment.

[0170] In various aspects, non-human animals described herein are used in transplantation or adoptive transfer experiments to determine the therapeutic potential of compounds or biological agents to modulate PD-1-dependent regulation of new lymphocytes and their immune function. In various aspects, non-human animals described herein are transplanted with human T cells; in some aspects, naive T cells; in some aspects, activated T cells.

[0171] In various aspects, cells of non-human animals described herein are used to in T cell assays to determine the therapeutic potential of compounds or biological agents to modulate PD-1-dependent regulation of T cell-dependent response and function. Exemplary T cell assays include, but are not limited to, ELISpot, intracellular cytokine staining, major histocompatibility complex (MHC) restriction, viral suppression assays, cytotoxicity assays, proliferation assays and regulatory T cell suppression assays.

[0172] In various aspects, cells of non-human animals described herein are used in a cell transmigration assay to screen and develop candidate therapeutics that modulate human PD-1. Cell transmigration involves the migration of cells across the endothelium and transmigration assays permit the measurement of interactions with, and transmigration of, the endothelium by leukocytes or tumor cells.

[0173] In various aspects, cells of non-human animals described herein are used in tumor cell growth (or proliferation) assays to determine the therapeutic potential of compounds or biological agents to modulate PD-1-dependent regulation and/or apoptosis of tumor cells.

[0174] In various aspects, cells of non-human animals described herein are used in cytokine production assays to determine the therapeutic potential of compounds or biological agents to modulate PD-1-dependent regulation of cytokine release from T cells. In some aspects, cells of non-human animals described herein are used for detection (and/or measurement) of intracellular cytokine release resulting from interaction of humanized PD-1 with a drug targeting human PD-1 or a PD-1 ligand (e.g., PD-L1 or PD-L2).

[0175] In various aspects, an autoimmune disease, disorder or condition is induced in one or more non-human animals described herein to provide an *in vivo* system for determining the therapeutic potential of compounds or biological agents to modulate PD-1-dependent regulation of one or more functions of the autoimmune disease, disorder or condition. Exemplary autoimmune diseases, disorders or conditions that may be induced in one or more non-human animals described herein include diabetes, experimental autoimmune encephalomyelitis (e.g., a model for multiple sclerosis), rheumatoid arthritis, and systemic lupus erythematosus.

[0176] Non-human animals described herein provide an *in vivo* system for the analysis and testing of a drug or vaccine. In various aspects, a candidate drug or vaccine may be delivered to one or more non-human animals described herein, followed by monitoring of the non-human animals to determine one or more of the immune response to the drug or vaccine, the safety profile of the drug or vaccine, or the effect on a disease or condition. In some aspects, the vaccine targets a virus such as, for example, human immunodeficiency virus or hepatitis virus (e.g. HCV). Exemplary methods used to determine the safety profile include measurements of toxicity, optimal dose concentration, efficacy of the drug or vaccine, and possible risk factors. Such drugs or vaccines may be improved and/or developed in such non-human animals.

[0177] Non-human animals described herein provide an *in vivo* system for assessing the pharmacokinetic properties of a drug targeting human PD-1. In various aspects, a drug targeting human PD-1 may be delivered or administered to one or more non-human animals described herein, followed by monitoring of, or performing one or more assays on, the non-human animals (or cells isolated therefrom) to determine the effect of the drug on the non-human animal. Pharmacokinetic properties include, but are not limited to, how an animal processes the drug into various metabolites (or detection of the presence or absence of one or more drug metabolites, including, toxic metabolites), drug half-life, circulating levels of drug after administration (e.g., serum concentration of drug), anti-drug response (e.g., anti-drug antibodies), drug absorption and distribution, route of administration, routes of excretion and/or clearance of the drug. In some aspects, pharmacokinetic and pharmacodynamic properties of drugs (e.g., PD-1 modulators) are monitored in or through the use of non-human animals described herein.

[0178] Non-human animals described herein provide an *in vivo* system for assessing the on-target toxicity of a drug targeting human PD-1. In various aspects, a drug targeting human PD-1 may be delivered or administered to one or more non-human animals described herein, followed by monitoring of or performing one or more assays on the non-human animals (or cells isolated therefrom) to determine the on-target toxic effect of the drug on the non-human animal. Typically, drugs are intended to modulate one or more functions of their targets. To give but one example, a PD-1 modulator is intended to modulate PD-1-mediated functions (e.g., PD-1 signal transduction) through interacting in some way with the PD-1 molecule on the surface of one or more cells. In some aspects, such a modulator may have an adverse effect that is an exaggeration of the desired pharmacologic action(s) of the modulator. Such effects are termed on-target effects. Exemplary on-target effects include too high of a dose, chronic activation/inactivation, and correct action in an incorrect tissue. In some aspects, on-target effects of a drug targeting PD-1 identified in or through the use of non-human animals described herein are used to determine a previously unknown function(s) of PD-1.

[0179] Non-human animals described herein provide an *in vivo* system for assessing the off-target toxicity of a drug targeting human PD-1. In various aspects, a drug targeting human PD-1 may be delivered or administered to one or more non-human animals described herein, followed by monitoring of or performing one or more assays on the non-human animals (or cells isolated therefrom) to determine the off-target toxic effect of the drug on the non-human animal. Off-target effects can occur when a drug interacts with an unintended target (e.g., cross-reactivity to a common epitope). Such interactions can occur in an intended or unintended tissue. To give but one example, mirror image isomers (enantiomers) of a drug can lead to off-target toxic effects. Further, a drug can inappropriately interact with and unintentionally activate different receptor subtypes. Exemplary off-target effects include incorrect activation/inhibition of an incorrect target regardless of the tissue in which the incorrect target is found. In some aspects, off-target effects of a drug targeting human PD-1 are determined by comparing the effects of administering the drug to non-human animals described herein to one or more reference non-human animals.

[0180] In some aspects, performing an assay includes determining the effect on the phenotype and/or genotype of the non-human animal to which the drug is administered. In some aspects, performing an assay includes determining lot-to-lot variability for a PD-1 modulator (e.g., an antagonist or an agonist). In some aspects, performing an assay includes determining the differences between the effects of a drug targeting PD-1 administered to a non-human animal described herein and a reference non-human animal. In various aspects, reference non-human animals may have a modification as described herein, a modification that is different as described herein (e.g., one that has a disruption, deletion or otherwise non-functional Pdcd1 gene) or no modification (i.e., a wild-type non-human animal).

[0181] Exemplary parameters that may be measured in non-human animals (or in and/or using cells isolated therefrom) for assessing the pharmacokinetic properties, on-target toxicity, and/or off-target toxicity of a drug targeting human PD-1 include, but are not limited to, agglutination, autophagy, cell division, cell death, complement-mediated hemolysis, DNA integrity, drug-specific antibody titer, drug metabolism, gene expression arrays, metabolic activity, mitochondrial activity, oxidative stress, phagocytosis, protein biosynthesis, protein degradation, protein secretion, stress response, target tissue drug concentration, non-target tissue drug concentration, transcriptional activity and the like. In various aspects, non-human animals described herein are used to determine a pharmaceutically effective dose of a PD-1 modulator.

[0182] Non-human animals described herein provide an improved *in vivo* system for the development and characterization of candidate therapeutics for use in cancer. In various aspects, non-human animals described herein may be implanted with a tumor, followed by administration of one or more candidate therapeutics. In some aspects, candidate therapeutics may include a multi-specific antibody (e.g., a bi-specific antibody) or an antibody cocktail; in some aspects, candidate therapeutics include combination therapy such as, for example, administration of mono-specific antibodies dosed sequentially or simultaneously. The tumor may be allowed sufficient time to be established in one or more locations within the non-human animal. Tumor cell proliferation, growth, survival, etc. may be measured both before and after administration with the candidate therapeutic(s). Cytoxicity of candidate therapeutics may also be measured in the non-human animal as desired.

[0183] Non-human animals described herein may be used to develop one or more disease models to evaluate or assess candidate therapeutics and/or therapeutic regimens (e.g., monotherapy, combination therapy, dose range testing, etc.) to effectively treat diseases, disorders or conditions that affect humans. Various disease conditions may be established in non-human animals described herein followed by administration of one or more candidate molecules (e.g., drugs targeting PD-1) so that efficacy of the one or more candidate molecules in a disease condition can determined. In some aspects, disease models include autoimmune, inflammatory and/or neoplastic diseases, disorders or conditions.

[0184] To give but one example, non-human animals described herein provide an improved animal model for prophylactic and/or therapeutic treatment of a tumor or tumor cells. In various aspects, non-human animals described herein may be implanted with one or more tumor cells, followed by administration of one or more candidate therapeutics (e.g., antibodies). In some aspects, administration of one or more candidate therapeutics is performed subsequent to (e.g., minutes or hours but typically on the same day as) implantation of one or more tumor cells and one or more candidate therapeutics are evaluated in non-human animals described herein for efficacy in preventing establishment of a solid tumor and/or growth of tumor cells in said non-human animals. In some aspects, administration of one or more candidate therapeutics is performed subsequent to (e.g., days after) implantation of one or more tumor cells and, in some certain aspects, after a sufficient time such that one or more implanted tumor cells have reached a predetermined size (e.g., volume) in non-human animals described herein; and one or more candidate therapeutics are evaluated for efficacy in treatment of one or more established tumors. Non-human animals may be placed into different treatment groups according to dose so that an optimal dose or dose range that correlates to effective treatment of an established tumor can be determined.

[0185] Candidate molecules can be administered to non-human animal disease models using any method of administration including parenteral and non-parenteral routes of administration. Parenteral routes include, e.g., intravenous, intraarterial, intraportal, intramuscular, subcutaneous, intraperitoneal, intraspinal, intrathecal, intracerebroventricular, intracranial, intrapleural or other routes of injection. Non-parenteral routes include, e.g., oral, nasal, transdermal, pulmonary, rectal, buccal, vaginal, ocular. Administration may also be by continuous infusion, local administration, sustained release from implants (gels, membranes or the like), and/or intravenous injection. When a combination therapy is evaluted in non-human animals described herein, candidate molecules can be administered via the same administration route or via different administration routes. When a dosing regimen is evaluated in non-human animals described herein, candidate molecules may be administered at bimonthly, monthly, triweekly, biweekly, weekly, daily, at variable intervals and/or in escalating concentrations to determine a dosing regimen that demonstrates a desired therapeutic or prophylactic effect in a non-human animal in which one or more disease models has been established.

[0186] Non-human animals described herein provide an improved *in vivo* system for the development and characterization of candidate therapeutics for use in infectious diseases. In various aspects, non-human animals described herein may be infected by injection with a virus (e.g., MHV, HIV, HCV, etc.) or pathogen (e.g., bacteria), followed by administration of one or more candidate therapeutics. In some aspects, candidate therapeutics may include a multi-specific antibody (e.g., a bi-specific antibody) or an antibody cocktail; in some aspects, candidate therapeutics include combination therapy such as, for example, administration of mono-specific antibodies dosed sequentially or simultaneously; in some aspects, candidate therapeutics may include a vaccine. The virus or pathogen may be allowed sufficient time to be established in one or more locations or cells within the non-human animal so that one or more symptoms associated with infection of the virus or pathogen develop in the non-human animal. T cell proliferation and growth may be measured both before and after administration with the candidate therapeutic(s). Further, survival, serum and/or intracellular cytokine analysis, liver and/or spleen histopathology may be measured in non-human animals infected with the virus or pathogen. In some aspects, non-human animals described herein are used to determine the extent of organ damage associated with viral infection. In some aspects, non-human animals described herein are used to determine the cytokine expression profile in various organs of non-human animals infected with a particular virus.

[0187] Non-human animals described herein can be employed to assess the efficacy of a therapeutic drug targeting human cells. In various aspects, a non-human animal described herein is transplanted with human cells, and a drug candidate targeting such human cells is administered to such non-human animal. The therapeutic efficacy of the drug is then determined by monitoring the human cells in the non-human animal after the administration of the drug. Drugs that can be tested in the non-human animals include both small molecule compounds, i.e., compounds of molecular weights of less than 1500 kD, 1200 kD, 1000 kD, or 800 daltons, and large molecular compounds (such as proteins, e.g., antibodies), which have intended therapeutic effects for the treatment of human diseases and conditions by targeting (e.g., binding to and/or acting on) human cells.

[0188] In some aspects, the drug is an anti-cancer drug, and the human cells are cancer cells, which can be cells of a primary cancer or cells of cell lines established from a primary cancer. In these aspects, a non-human animal described herein is transplanted with human cancer cells, and an anti-cancer drug is given to the non-human animal. The efficacy of the drug can be determined by assessing whether growth or metastasis of the human cancer cells in the non-human animal is inhibited as a result of the administration of the drug.

[0189] In specific aspects, the anti-cancer drug is an antibody molecule, which binds an antigen on human cancer cells. In particular aspects, the anti-cancer drug is a bi-specific antibody that binds to an antigen on human cancer cells, and to an antigen on other human cells, for example, cells of the human immune system (or "human immune cells") such as B cells and T cells.

EXAMPLES

[0190] The following examples are provided so as to describe to those of ordinary skill in the art how to make and use methods and compositions of the invention. Unless indicated otherwise, temperature is indicated in Celsius, and pressure is at or near atmospheric.

Example 1. Humanization of an endogenous Programmed cell death 1 (Pdcdl) gene

[0191] This example illustrates exemplary methods of humanizing an endogenous Pdcd1 gene encoding Programmed cell death protein 1 (PD-1) in a non-human mammal such as a rodent (e.g., a mouse). The methods described in this example can be employed to humanize an endogenous Pdcd1 gene of a non-human animal using any human sequence, or combination of human sequences (or sequence fragments) as desired. In this example, an ~ 883 bp human DNA fragment containing exon 2, intron 2, and the first 71 bp of exon 3 of a human *PDCD1* gene that appears in GenBank accesion NM_005018.2 (SEQ ID NO:23) is employed for humanizing an endogenous *Pdcd1* gene of a mouse. A targeting vector for humanization of the genetic material encoding an extracellular N-terminal IgV domain, of an endogenous *Pdcd1* gene was constructed using VELOCIGENE® technology (see, e.g., U.S. Patent No. 6,586,251 and Valenzuela et al., 2003, Nature Biotech. 21(6):652-659).

[0192] Briefly, mouse bacterial artificial chromosome (BAC) clone RP23-93N20 (Invitrogen) was modified to delete the sequence containing exon 2, intron 2 and part of exon 3 of an endogenous *Pdcd1* gene and insert exon 2, intron 2 and part of exon 3 of a human *PDCD1* gene using an ~883 bp human DNA fragment, which encodes amino acids 26-169 of a human PD-1 polypeptide. Endogenous DNA containing exon 1, portion of exon 3 (i.e., that encodes the transmembrane domain), 4 and 5 as well as the 5' and 3' untranslated regions (UTRs) were retained. Sequence analysis of the ~883 bp human DNA fragment confirmed all human *PDCD1* exons (i.e., exon 2 and 71 bp of exon 3) and splicing signals. Sequence analysis revealed that the sequence matched the reference genome and *PDCD1* transcript NM_005018.2.

[0193] In more detail, first, a small bacterial homologous recombination donor was constructed from a synthetic DNA fragment containing the following: [(HindIII)-(mouse upstream 78bp)-(XhoI/NheI restriction enzyme sites)-(human *PDCD1* 883bp)-(mouse downstream 75bp)-(HindIII)]. This fragment was synthesized by Genescript Inc. (Piscataway, NJ) and cloned into an ampicilin-resistant plasmid vector. The XhoI-NheI sites were employed to ligate a ~4,996 bp self-deleting neomycin cassette flanked by recombinase recognition sites (loxP-hUb1-em7-Neo-pA-mPrm1-Crei-loxP; see U.S. Patent No.'s 8,697,851, 8,518,392 and 8,354,389). Subsequent selection employed neomycin. The flanking HindIII sites were used to linearize the targeting vector prior to homologous recombination with mouse BAC clone RP23-93N20. By design, the junction between the Human *PDCD1* 883bp fragment and the mouse downstream 75 bp preserved the open reading frame in exon 3 (Figure 2). The resulting targeting vector contained, from 5' to 3', a 5' homology arm containing ~61.7 kb of mouse genomic DNA from BAC clone RP23-93N20, a self-deleting neomycin cassette flanked by loxP sites, an 883 bp human genomic DNA fragment (containing exon 2 through the first 71 bp of exon 3 of a human Pdcd1 gene) and ~84 kb of mouse genomic DNA from BAC clone RP23-93N20.

[0194] The modified RP23-93N20 BAC clone described above was used to electroporate mouse embryonic stem (ES) cells to create modified ES cells comprising an endogenous *Pdcd1* gene that is humanized from exon 2 through to part of exon 3 (i.e., deletion of 900 bp of the endogenous *Pdcd1* gene and insertion of 883 bp of human sequence). Positively targeted ES cells containing a humanized Pdcd1 gene were identified by an assay (Valenzuela et al., supra) that detected the presence of the human *PDCD1* sequences (e.g., exon 2 and part of exon 3) and confirmed the loss and/or retention of mouse *Pdcd1* sequences (e.g., exon 2 and part of exon 3, and/or exons 1, 4 and 5). Table 4 sets forth the primers and probes that were used to confirm humanization of an endogenous Pdcd1 gene as described above (Figure 3). The nucleotide sequence across the upstream insertion point included the following, which indicates endogenous mouse sequence (contained within the parentheses below with an XhoI restriction site italicized) upstream of the 5' end of self-deleting neomycin cassette of the insertion point linked contiguously to a loxP site (bolded) and cassette sequence present at the insertion point: (TCAAAGGACA GAATAGTAGC CTCCAGACCC TAGGTTCAGT TATGCTGAAG GAAGAGCCCT **CTCGAG)ATAACTTCGT ATAATGTATG CTATACGAAG TTAT**ATGCAT GGCCTCCGCG CCGGGTTTTG GCGCCTCCCG CGGGCGCCCC CCTCCTCACG (SEQ ID NO:19). The nucleotide sequence across the downstream insertion point at the 3' end of the self-deleting neomycin cassette included the following, which indicates cassette sequence (contained within the parentheses below with loxP sequence bolded and an NheI restriction site italicized) contiguous with human Pdcd1 genomic sequence downstream of the insertion point: (CTGGA**ATAAC TTCGTATAAT GTATGCTATA CGAAGTTATG** CTAGTAACTA TAACGGTCCT AAGGTAGCGA *GCTAGC*) AAGAGGCTCT GCAGTGGAGG CCAGTGCCCA TCCCCGGGTG GCAGAGGCCC CAGCAGAGAC TTCTCAATGA CATTCCAGCT GGGGTGGCCC TTCCAGAGCC CTTGCTGCCC GAGGGATGTG AGCAGGTGGC CGGGGAGGCT TTGTGGGGCC ACCCAGCCCC (SEQ ID NO:20). The nucleotide sequence across the downstream insertion point at the 3' end of the human *PDCD1* genomic sequence included the following, which indicates human *PDCD1* sequence contiguous with mouse *Pdcd1* genomic sequence (contained within the parentheses below): CCCTTCCAGA GAGAAGGGCA GAAGTGCCCA CAGCCCACCC CAGCCCCTCA CCCAGGCCAG CCGGCCAGTT CCAAACCCTG (GTCATTGGTA TCATGAGTGC CCTAGTGGGT ATCCCTGTAT TGCTGCTGCT GGCCTGGGCC CTAGCTGTCT TCTGCTCAAC) (SEQ ID NO:21). The nucleotide sequence across the upstream insertion point after deletion of the neomycin cassette (77 bp remaining) included the following, which indicates mouse and human genomic sequence juxtaposed with remaining cassette sequence loxP sequence (contained within the parentheses below with XhoI and NheI restriction sites italicized and loxP sequence in bold): TCAAAGGACA GAATAGTAGC CTCCAGACCC TAGGTTCAGT TATGCTGAAG GAAGAGCCCT (*CTCGAG* **ATAACTTCGT ATAATGTATG CTATACGAAG TTAT**GCTAGT AACTATAACG GTCCTAAGGT AGCGA *GCTAGC*) AAGAG GCTCTGCAGT GGAGGCCAGT GCCCATCCCC GGGTGGCAGA GGCCCCAGCA GAGACTTCTC AATGACATTC CAGCTGGGGT GGCCCTTCCA (SEQ ID NO:22).

[0195] Positive ES cell clones were then used to implant female mice using the VELOCIMOUSE® method (see, e.g., U.S. Pat. No. 7,294,754 and Poueymirou et al., 2007, Nature Biotech. 25(1):91-99) to generate a litter of pups containing an insertion of human *PDCD1* exon 2 and part of human *PDCD1* exon 3 into an endogenous *Pdcd1* gene of a mouse. Mice bearing the humanization of exon 2 and 3 in part (i.e., the 883 bp human DNA fragment) of an endogenous *Pdcd1* gene were again confirmed and identified by genotyping of DNA isolated from tail snips using a modification of allele assay (Valenzuela et al., supra) that detected the presence of the human *PDCD1* gene sequences. Pups are genotyped and cohorts of animals heterozygous for the humanized Pdcd1 gene construct are selected for characterization.

TABLE 4

Name	**Primer**	**Sequence (5'-3')**	
7106 hTU	Forward	CCCAGCAGAGACTTCTCAATGAC	(SEQ ID NO:7)
	Probe	TGGCCCTTCCAGAGCCCTTG	(SEQ ID NO:8)
	Reverse	CGGCCACCTGCTCACATC	(SEQ ID NO:9)
	Forward	GGCATCTCTGTCCTCTAGCTC	(SEQ ID NO:10)
7106 hTD	Probe	AAGCACCCCAGCCCCTCTAGTCTG	(SEQ ID NO:11)
	Reverse	GGGCTGTGGGCACTTCTG	(SEQ ID NO:12)

Name	Primer	Sequence (5'-3')	
	Forward	CCTTCCTCACAGCTCTTTGTTC	(SEQ ID NO:13)
7106 TU	Probe	TCTGCATTTCAGAGGTCCCCAATGG	(SEQ ID NO:14)
	Reverse	GAGCCAGGCTGGGTAGAAG	(SEQ ID NO:15)
	Forward	CGGTGTCCTAGAACTCTATTCTTTG	(SEQ ID NO:16)
7106 TD	Probe	TCCTGGAGACCTCAACAAGATATCCCA	(SEQ ID NO:17)
	Reverse	TGAAACCGGCCTTCTGGTT	(SEQ ID NO:18)

Example 2. Expression of humanized PD-1 on activated T cells

[0196] This Example demonstrates that non-human animals (e.g., rodents) modified to contain a humanized Pdcd1 gene according to Example 1 express a humanized PD-1 protein on the surface of activated lymphocytes. In this Example, activated T cells from mice heterozygous for humanization of an endogenous Pdcd1 gene as described in Example 1 were stained with anti-PD-1 antibodies to determine the expression of PD-1 in stimulated T cells isolated from wild-type and humanized mice.

[0197] Briefly, spleens were harvested and processed from a wild-type mouse and a mouse heterozygous for humanization of an endogenous Pdcd1 gene as described in Example 1 into single cell suspensions by mechanical dissociation. Cells were washed in media (RPMI supplemented with 10% FBS) and re-suspended at $1x10^6$/mL and 200 µL (200,000 cells) were plated in 96-well plates. Cells in selected wells were stimulated with anti-CD3 and anti-CD28 antibodies (both at 1 µg/mL) for 72 hours. Cells were stained for FACS according to manufacturer's specifications with antibodies recognizing CD4, CD8, CD19 and human (clone MIH4, BD Biosciences) or mouse (clone J43, eBioscience) PD1. Stained cells were ran on LSRII flow cytometer and data was analyzed using Flowjo software. $CD8^+$ T cells were gated ($CD19^-$ $CD8^+$) for expression of human and mouse PD1. Exemplary results are shown in Figure 4.

[0198] As shown in Figure 4, mice bearing a humanized Pdcd1 gene as described in Example 1 express a PD-1 polypeptide that comprises a human portion and an endogenous mouse portion. The human portion is detectably expressed via recognition by an antibody that recognizes a fully human PD-1 polypeptide.

Example *3. In vivo* **efficacy of PD-1 modulators**

[0199] This Example demonstrates that non-human animals (e.g., rodents) modified to contain a humanized Pdcd1 gene according to Example 1 can be used in an *in vivo* assay to screen PD-

1 modulators (e.g., anti-PD-1 antibodies) and determine various characterisitics such as, for example, inhibition of tumor growth and/or killing of tumor cells. In this Example, several anti-PD-1 antibodies are screened in mice homozygous for humanization of an endogenous Pdcd1 gene as described in Example 1 to determine the optimal antibody dose that inhibits tumor growth and the extent to which anti-PD-1 antibodies mediate killing of tumor cells.

[0200] Briefly, mice were divided evenly according to body weight into five treatment or control groups for Study 1 (n=5/group), eight treatment or control groups for Study 2 (n=5/group), and five treatment or control groups for Study 3 (n=7/group). At day zero, mice were anesthetized by isoflurane inhalation and then subcutaneously injected with MC38.ova cells in suspension of 100 μL of DMEM into the right flank (Study 1: $5x10^5$; Study 2/3: $1x10^6$). MC38.ova (mouse colon adenocarcinoma) cells were engineered to express chicken ovalbumin in order to increase tumor immunogenicity. For Study 1, treatment groups were intraperitoneally injected with 200 μg of either one of three anti-PD-1 antibodies, or an isotype control antibody with irrelevant specificity on days 3, 7, 10, 14, and 17 of the experiment, while one group of mice was left untreated. For Study 2, treatment groups were intraperitoneally injected with either one of three anti-PD-1 antibodies at 10mg/kg or 5mg/kg per/dose, one anti-PD-1 antibody (Ab B, IgG4) at 10mg/kg per dose, or an isotype control antibody with irrelevant specificity at 10mg/kg on days 3, 7, 10, 14, and 17 of the experiment. For Study 3, treatment groups were intraperitoneally injected with either one of two anti-PD-1 antibodies at 5mg/kg or 2.5mg/kg per/dose, or a control antibody not specific to PD-1 (control) at 5mg/kg on days 3, 7, 10, 14, and 17 of the experiment. Table 5 sets forth experimental dosing and treatment protocol for groups of mice.

[0201] For each of the studies, average tumor volumes determined by caliper measurements and percent survival at Day 14 or 17 and Day 23 or 24 of each experiment for each treatment group were recorded. The number of tumor-free mice were also assessed at the end of the study (Day 42 for Study 1 and Day 31 for Study 2 and Study 3). Mean tumor volume (mm^3) (±SD), percent survival, and number of tumor-free mice were calculated for each study (Tables 7-9). Exemplary tumor growth curves are provided in Figure 5.

[0202] As shown in Table 6 for Study 1, mice treated with Ab A did not develop any detectable tumors during the course of the study. Mice treated with Ab C exhibited a sustained reduced tumor volume as compared to controls at days 17 and 24 of the study; and 3 out of 5 mice were tumor free by the end of the experiment. In contrast, treatment with Ab B did not demonstrate significant efficacy in reducing tumor volume in this study as compared to controls. By day 23 of the study, 1 out of 5 mice died in the group that received Ab B, and 2 out of 5 mice died in the isotype control treatment group. In non-treatment and isotype control groups, some mice exhibited spontaneous regression of tumors (1 out of 5 mice and 2 out of 5 mice, respectively).

[0203] As shown in Table 7 for Study 2, mice treated with Ab A at 10mg/kg did not develop detectable tumors during the course of the study. Groups of mice treated with 10 mg/kg of either Ab C or Ab D exhibited substantially reduced tumor volume as compared to controls at days 17 and 24 of the study. Four out of 5 mice in each group treated with 10mg/kg of either Ab C or Ab D were tumor free at Day 31, whereas in the isotype control treatment group only 1 out of 5 animals was tumor free as a result of spontaneous tumor regression. Ab B tested at 10mg/kg demonstrated substantially reduced tumor volume as compared to controls at days 17 and 24 of the study, but this antibody was the least efficacious anti-PDl antibody with only 2 out of 5 mice surviving at the end of the experiment.

[0204] A dose-dependent response in tumor suppression at the tested doses (5 mg/kg and 10 mg/kg) was observed in groups treated with Ab A, Ab C, and Ab D. Ab A or Ab C therapy at 5 mg/kg was less efficacious, with 4 out of 5 tumor-free mice at the end of experiment on day 31, whereas 5 out of 5 mice remained tumor-free in 10 mg/kg dose group of Ab A. Dunett's test in 2 way ANOVA multiple comparisons revealed that the differences in tumor growth between the group treated with isotype control antibody at 10 mg/kg as reference and the groups treated at 10 mg/kg with Ab A, Ab C or Ab D were statistically significant with p value<0.005. The differences in tumor growth between the group treated with isotype control antibody at 10 mg/kg as reference and the groups treated at 5 mg/kg with Ab A, Ab C or Ab D were also statistically significant with a p value<0.05.

[0205] As shown in Table 8 for Study 3, 6 out or 7 mice treated with Ab A or Ab C at 5mg/kg were tumor free at the end of the experiment, whereas there were no tumor free animals in the isotype control group. One tumor-bearing mouse in the IgG4 control group died on post-implantation day 17. Only 4 out of 7 mice treated with Ab C at 2.5mg/kg remained tumor free at the end of the experiment. The difference in tumor volumes at day 21 between anti-PD-1 antibodies tested and an isotype control group was statistically significant as determined by one-way ANOVA with Dunnett's multiple comparison post-test with p<0.01. All four anti-PD-1 antibodies tested were equally more efficacious at the 5 mg/kg dose than at the 2.5 mg/kg dose.

[0206] As shown in Figure 5, anti-PD-1 antibodies significantly inhibited tumor growth in a prophylactic MC38.ova tumor growth model in PD-1 humanized mice made according to Example 1. Anti-PD-1 Ab therapy at 10 mg/kg promoted tumor regression in all mice (5 out of 5) throughout the course of the experiment, whereas only one out of five animals remained tumor-free in the control group resulting from spontaneous tumor regression. Anti-PD-1 therapy at 5 mg/kg was slightly less efficacious, with four out of five tumor-free mice at the end of the experiment. One-way ANOVA with Dunnett's multiple comparison post-test revealed a significant difference in tumor volumes between anti-PD-1 and control antibody treatments with a p value < 0.05 (5 mg/kg) and p value <0.01 (10 mg/kg).

[0207] In a similar experiment, intact functional PD-1 signaling in PD-1 humanized mice made according to Example 1 was investigated by measuring $CD8^+$ T cells and $CD3^+$ T cells responses and IFNγ production in spleens of tumor-bearing mice treated with anti-PD-1 antibody.

[0208] Briefly, spleen cells were obtained from PD-1 humanized mice (75% C57BL/6/25% 129) treated with anti-PD-1 or control antibody at the end of the experiments on Day 21 (described above). Total RNA was isolated, and real-time PCR was performed on reverse transcribed cDNA using oligonucleotides and taqman probe mix specific for mouse CD8b (forward primer:

GCTCTGGCTG GTCTTCAGTA TG, SEQ ID NO:24; reverse primer: TTGCCGTATG GTTGGTTTGA AC, SEQ ID NO:25; probe: AGCAGCTCTG CCCTCAT, SEQ ID NO:26), mouse CD3ζ (Mm00446171_m1, Applied Biosystems), mouse IFN-γ (Mm01168134_m1, Applied Biosystems), human PD-1 (forward primer: ACTTCCACAT GAGCGTGG, SEQ ID NO:27; reverse primer: GGGCTGTGGG CACTTCTG, SEQ ID NO:28; probe: GCAGATCAAA GAGAGCCTGC, SEQ ID NO:29) and mouse PD-1 (Mm01285676_m1, Applied Biosystems). Samples were normalized relative to expression of mouse cyclophilin B. Exemplary results are provided in Figure 6.

[0209] As shown in Figure 6, administration of anti-hPD-1 antibody induced increased production of $CD8^+$ and $CD3^+$ T cells in spleens of humanized mice (made according to Example 1) bearing MC38.ova tumors. Further, activity of anti-hPD-1 antibody in tumor bearing PD-1 humanized mice was dependent on IFNγ, which confirmed proper signaling through humanized PD-1 on the cell surface. Overall, an increase in T cells and IFNγ as compared to control-treated mice was observed for both treatment groups.

[0210] Human PD-1 mRNA expression was measured with human specific probes designed for the extracellular portion of the PD-1 protein and confirmed proper expression of humanized PD-1 protein on the cell surface. Additionally, measurement of mouse PD-1 mRNA expression with primers designed to detect the extracellular portion of mouse PD-1 failed to produce a product.

[0211] Taken together, this Example demonstrates that non-human animals described herein can be used to assess the *in vivo* efficacy of drugs (e.g., an antibody) targeting PD-1, and such animals are useful in discriminating the therapeutic effect of anti-PD-1 antibodies. Morever, non-human animals described herein can be used to assess the extent to which drugs targeting PD-1 can inhibit tumor growth and/or mediate killing of tumor cells. Non-human animals (e.g., mice) described herein demonstrate functional PD-1-signaling and proper PD-1-dependent immune responses via humanized PD-1 as evidenced by expansion of T cells and cytokine expression (e.g., IFN-γ).

TABLE 5

Study #	Antibody	Dosage
1	Isotype Control	200 µg
	No treatment	N/A
	Ab A	200 µg
	Ab B	200 µg
	Ab C	200 µg
2	Isotype Control	10 mg/kg
	Ab A	10 mg/kg
	Ab A	5 mg/kg
	Ab B	10 mg/kg
	Ab C	10 mg/kg
	Ab C	5 mg/kg

Study #	Antibody	Dosage
	Ab D	10 mg/kg
	Ab D	5 mg/kg
3	Isotype Control	5 mg/kg
	Ab A	5 mg/kg
	Ab A	2.5 mg/kg
	Ab C	5 mg/kg
	Ab C	2.5 mg/kg

TABLE 6

Study 1					
Treatment group (n=5)	Mean tumor volume (mm³, ±SD)		Survival (%)		Tumor free mice
	Day 17 200 µg/mouse	Day 23 200 µg/mouse	Day 17 200 µg/mouse	Day 23 200 µg/mouse	Day 42 200 µg/mouse
No treatment	189 (±110)	554 (±317)	100%	100%	1/5
Isotype Control	86 (±114)	515 (±859)	100%	60%	2/5
Ab A	0 (0)	0(0)	1 00%	1 00%	5/5
Ab B	89 (±176)	445 (±889)	100%	80%	3/5
Ab C	14 (±19)	205 (±312)	100%	1 00%	3/5

TABLE 7

Study 2										
Treatment group (n=5)	Mean tumor volume (mm³; ±SD)				Survival (%)				Tumor free Mice	
	Day 17		Day 24		Day 17		Day 24		Day 31	
	5 mg/kg	10 mg/kg	5 mg/kg	10 mg/kg	5 mg/kg	10 mg/kg	5 mg/kg	10 mg/kg	5 mg/kg	10 mg/kg
Isotype Control	N/A	449 (±434)	N/A	824 (±858)	N/A	100%	N/A	60%	N/A	1/5
Ab A	(±38)	(0)	(±233)	(0)	100	100	100	100	4/5	5/5
Ab B	N/A	(±209)	N/A	(±657)	N/A	100	N/A	80	N/A	2/5
Ab C	(±204)	(±28)	(±509)	(±215)	100	100	80	100	4/5	4/5
Ab D	(±160)	(±21)	(±559)	(±150)	100	100	80	100	3/5	4/5

TABLE 8

Study 3										
Treatment group (n=7)	Mean tumor volume (mm^3; ±SD)				Survival (%)				Tumor free mice	
	Days 14		Day 21		Day 14		Day 21		Day 31	
	2.5 mg/kg	5 mg/kg	2.5 mg/kg	5 mg/kg	2.5 mg/kg	5 mg/kg	2.5 mg/kg	5 mg/kg	2.5 mg/kg	5 mg/kg
Isotype Control	N/A	94 (±44)	N/A	405 (±326)	N/A	100	N/A	86	N/A	0/7
Ab A	0(0)	0 (0)	19 (±51)	13 (±35)	100	100	100	100	6/7	6/7
Ab C	41 (±68)	7 (±20)	87 (±123)	16 (±42)	100	100	100	100	4/7	6/7

Example 4. Rodent model of Anti-PD-1 tumor therapy

[0212] This Example demonstrates that non-human animals (e.g., rodents) modified to contain a humanized Pdcdl gene according to Example 1 can be used in a tumor model to determine optimal therapeutic dose(s) of PD-1 modulators (e.g., anti-PD-1 antibodies). In this Example, an anti-PD-1 antibody is administered to mice homozygous for humanization of an endogenous Pdcd1 gene as described in Example 1 to determine the optimal therapeutic dose for treatment of established tumors.

[0213] Briefly, mice containing a humanized Pdcd1 gene (as described in Example 1) were subcutaneously implanted with $1x10^6$ MC38.Ova cells (described above) and subsequently randomized into six treatment groups (n=8 - 9 per group) once tumor volumes reached 80 - 120 mm^3 (day 0). Mice were intraperitoneally administered anti-hPD-1 antibody in an escalating dose range of 0.3-25 mg/kg (i.e., 0.3, 1, 3, 10 or 25 mg/kg) or an isotype control antibody at 25 mg/kg. Antibodies were dosed on days 0, 3, 7, 10 and 13. Tumor volumes were monitored by calipered measurements twice per week for the duration of the experiment (60 days). Exemplary tumor growth curves are provided in Figure 7.

[0214] As shown in Figure 7, none of the mice administered the control antibody were tumor free at the end of the experiment. In constrast, a dose range of 3 - 25 mg/kg anti-hPD-1 antibody resulted in about 44-55% tumor free mice among the different treatment groups. Taken together, this Example demonstrates that non-human animals described herein can be used as a rodent tumor model to determine the optimal dose and/or dose range of drugs (e.g., an antibody) targeting PD-1 to effectively treat established tumors.

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Leu Asn Trp Tyr Arg Met Ser Pro Ser Asn Gln Thr Asp Lys Leu Ala
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Ala Phe Pro Glu Asp Arg Ser Gln Pro Gly Gln Asp Cys Arg Phe Arg

85 90 95

Val Thr Gln Leu Pro Asn Gly Arg Asp Phe His Met Ser Val Val Arg
100 105 110

Ala Arg Arg Asn Asp Ser Gly Thr Tyr Leu Cys Gly Ala Ile Ser Leu
115 120 125

Ala Pro Lys Ala Gln Ile Lys Glu Ser Leu Arg Ala Glu Leu Arg Val
130 135 140

Thr Glu Arg Arg Ala Glu Val Pro Thr Ala His Pro Ser Pro Ser Pro
145 150 155 160

Arg Pro Ala Gly Gln Phe Gln Thr Leu Val Ile Gly Ile Met Ser Ala
165 170 175

Leu Val Gly Ile Pro Val Leu Leu Leu Leu Ala Trp Ala Leu Ala Val
180 185 190

Phe Cys Ser Thr Ser Met Ser Glu Ala Arg Gly Ala Gly Ser Lys Asp
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Asp Thr Leu Lys Glu Glu Pro Ser Ala Ala Pro Val Pro Ser Val Ala
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Tyr Glu Glu Leu Asp Phe Gln Gly Arg Glu Lys Thr Pro Glu Leu Pro
225 230 235 240

Thr Ala Cys Val His Thr Glu Tyr Ala Thr Ile Val Phe Thr Glu Gly
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ctcagactcc ccagacaggc cctggaaccc ccccaccttc tccccagccc tgctcgtggt 240

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ggaccgcagc cagcccggcc aggactgccg cttccgtgtc acacaactgc ccaacgggcg 420

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<400> 29
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liability in this regard.

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PATENTKRAV

1. En mus hvis genom består af et humaniseret programmeret celledøds- 1 (Pdcd1) -gen på en endogen Pdcd1-locus,

hvori denne mus udtrykker et humaniseret programmeret celledød 1 (PD-1) polypeptid fra dette humaniserede Pdcd1-gen, hvis humaniserede PD-1-polypeptid har en aminosyresekvens som er mindst 95 % identisk med SEQ ID NO: 6 og består af en menneskelig del og en endogen del,

hvori den menneskelige del består af aminosyrer 26-169 fra et menneskes PD-1-polypeptid, og den endogene del består af den intracellulære del af et endogent muse-PD-1-polypeptid.

2. Musen fra krav 1, hvori den humaniserede PD-1-polypeptid:

(a) er oversat i en celle fra musen med en muse-signalpeptid; eller

(b) består yderligere af transmembranportionen af det endogene muse-PD-1-polypeptid.

3. En isoleret musecelle eller et isoleret musevæv hvis genom består af humaniseret Pdcd1-gen på en endogen Pdcd1-locus,

hvori dette humaniserede Pdcd1-gen enkoder et humaniseret PD-1-polypeptid, hvis humaniserede PD-1-polypeptid har en aminosyresekvens som er mindst 95 % identisk med SEQ ID NO: 6 og består af en menneskelig del og en endogen del,

hvori den menneskelige del består af aminosyrer 26-169 fra et menneskes PD-1-polypeptid og den endogene del består af den intracellulære del af et endogent muse-PD-1-polypeptid,

hvori det humaniserede Pdcd1 gen er operativt forbundet til en muse-Pdcd1-promotor, og

hvori musecellen eventuelt er en mus´ embryonale stamcelle.

4. Et musefoster skabt på basis af en mus´ embryonale stamcelle ifølge krav 3.

5. En metode til at lave en mus, hvori:

(A) musens genom består af et Pdcd1-gen som enkoder en PD-1-polypeptid som har en menneskelig del og en endogen del, hvori Pdcd1-genet er operativt forbundet til en muse-Pdcd1-promotor, hvor metoden omfatter

modifikation af en mus´ genom så det består af et humaniseret Pdcd1-gen på en endogen Pdcd1-locus,

hvori denne mus udtrykker en humaniseret PD-1-polypeptid fra dette humaniserede Pdcd1-gen, hvis humaniserede PD-1-polypeptid har en aminosyresekvens som er mindst 95 % identisk med SEQ ID NO: 6 og består af en menneskelig del og en endogen del,

hvori den menneskelige del består af aminosyrer 26-169 fra et menneskes PD-1-polypeptid, og den endogene del består af den intracellulære del af en endogen mus´ PD-1-polypeptid; eller

(B) musen udtrykker en PD-1 polypeptid fra et endogent Pdcd1-gen, hvori PD-1-polypeptiden består af en menneskesekvens, hvor metoden omfatter

(a) indsættelse af et menneskeligt genfragment fra et menneskes Pdcd1 i en endogen mus´ Pdcd1-gen på en endogen Pdcd1-locus i en mus´ embryonale stamcelle for at forme et humaniseret Pdcd1-gen, operativt forbundet til en endogen muse-Pdcd1-promotor, som enkoder et humaniseret PD-1-polypeptid,

hvori dette humaniserede PD-1-polypeptid har en aminosyresekvens som er mindst 95 % identisk med SEQ ID NO: 6 og består af en menneskelig del og en endogen del, hvori den menneskelige del består af aminosyrer 26-169 fra et menneskes PD-1-polypeptid og den endogene del består af den intracellulære del af et endogent muse-PD-1-polypeptid;

(b) fremskaffer den museembryonale stamcelle frembragt i (a); og,

(c) frembringer en mus ved hjælp af den museembryonale stamcelle i (b).

6. Metoden ifølge krav 5(A) eller (B), hvori:

det humaniserede PD-1-polypeptid består af den transmembrane sekvens af det endogene muse-PD-1-polypeptid.

7. En metode til at bedømme de farmakokinetiske egenskaber ved et lægemiddel målrettet menneske-PD-1, bestående af:

administration af lægemidlet til en mus ifølge krav 1 eller krav 2; og

udførsel af en analyse for at fastlægge en eller flere farmakokinetiske egenskaber af lægemidlet målrettet menneske-PD-1 hvor i lægemidlet eventuelt er et anti-PD-1-antistof.

8. En musetumormodel fremskaffet ved at:

a) fremvise en mus ifølge krav 1 eller krav 2; og

b) implantere en eller flere tumorceller i musen fra (a);

hvorved fremskaffes denne musetumormodel.

DRAWINGS

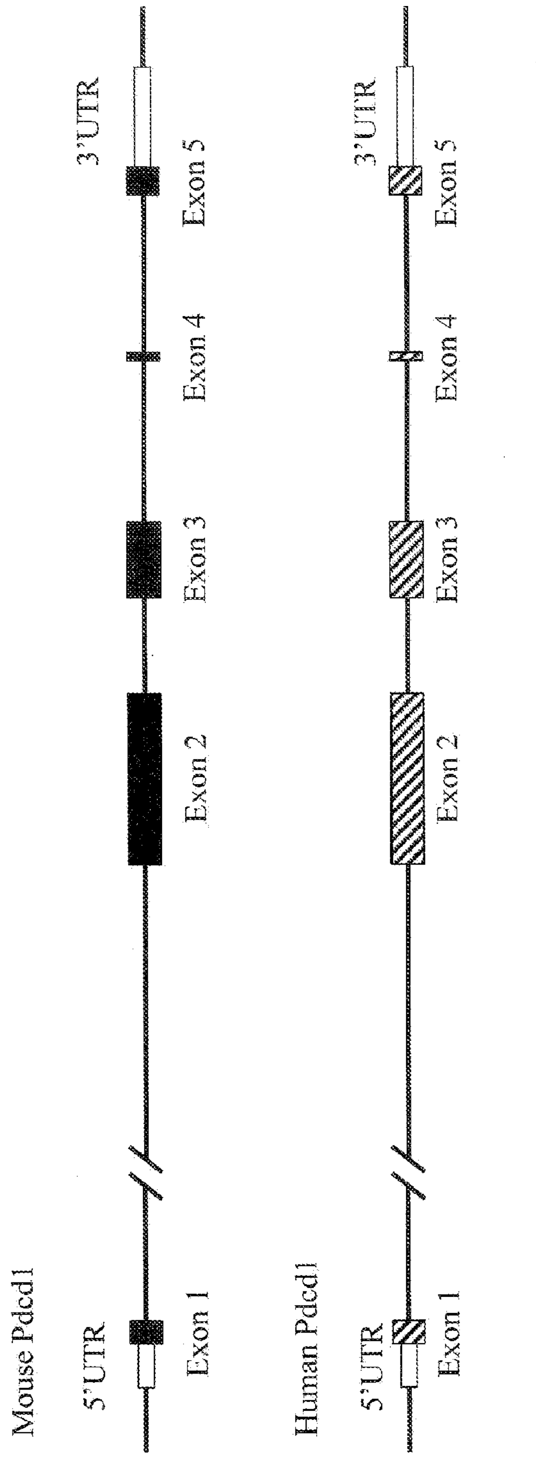
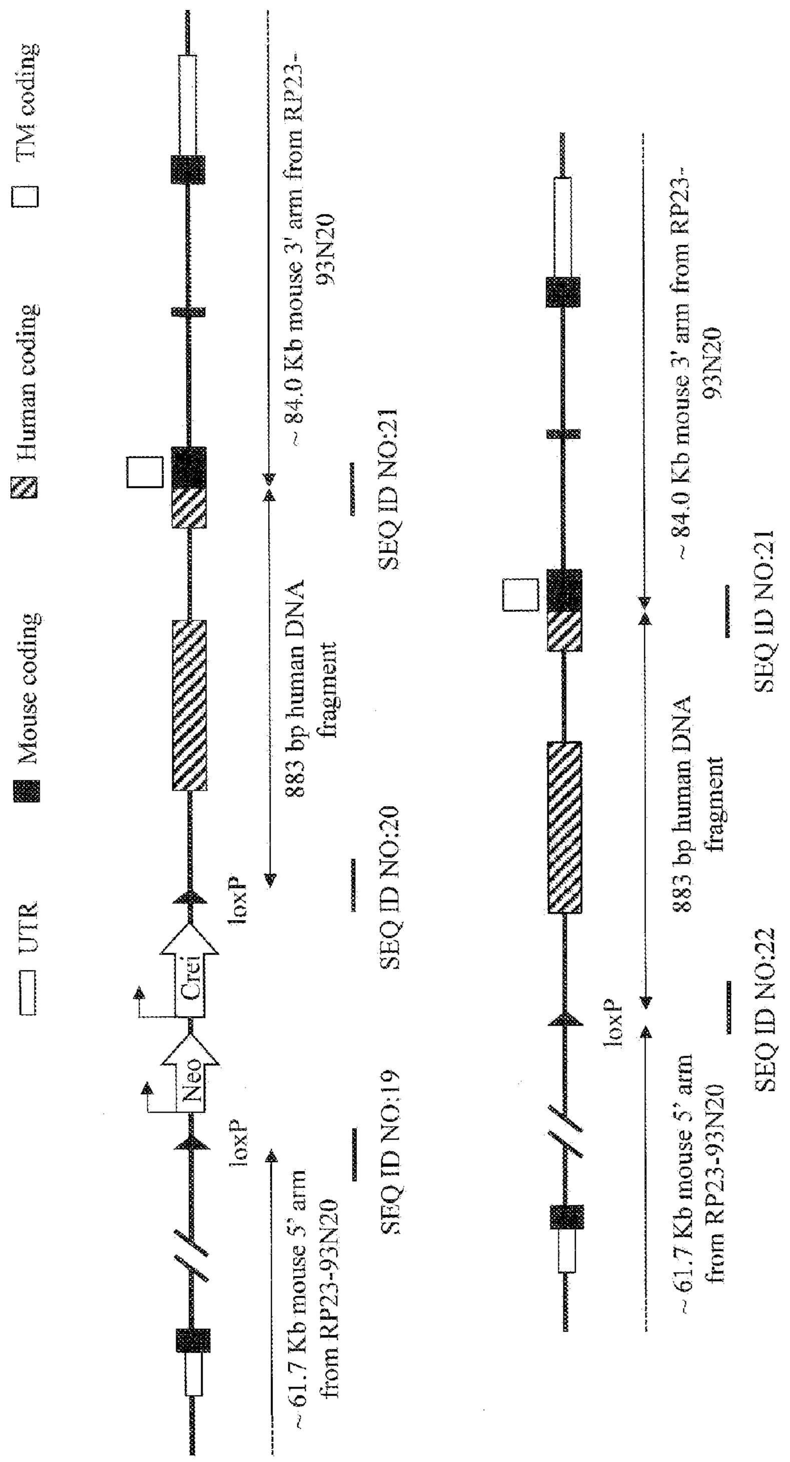


Figure 1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Mouse coding
Human coding
TM coding
Neo
Crei
loxP
loxP
~ 61.7 Kb mouse 5' arm from RP23-93N20
883 bp human DNA fragment
~ 84.0 Kb mouse 3' arm from RP23-93N20
SEQ ID NO:19
SEQ ID NO:20
SEQ ID NO:21
loxP
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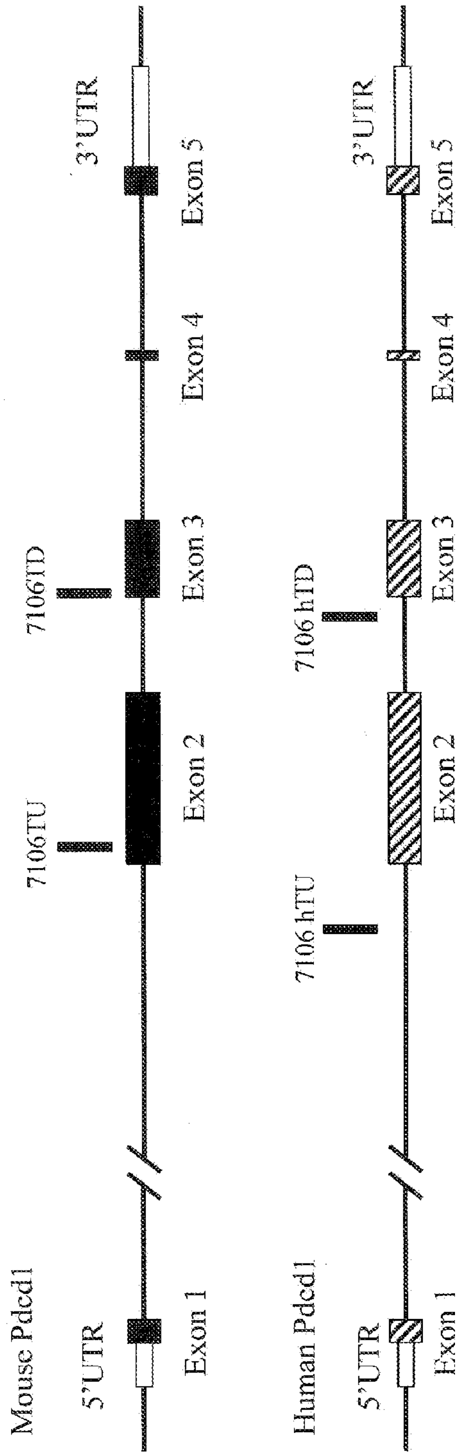
Figure 2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Exon 1
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Exon 3
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Exon 4
Exon 5
3'UTR

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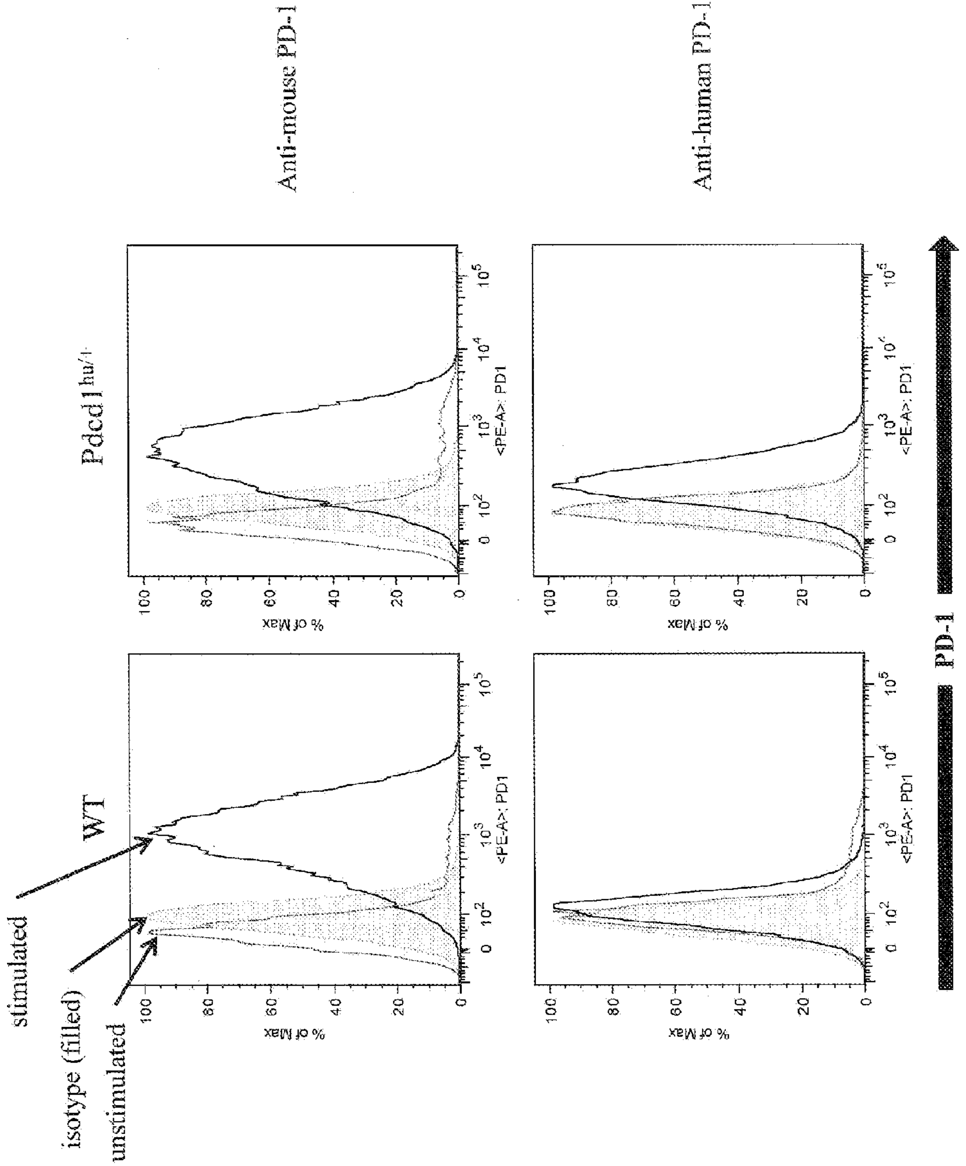
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unstimulated
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Pdcd1 hu/+
Anti-mouse PD-1
Anti-human PD-1
% of Max
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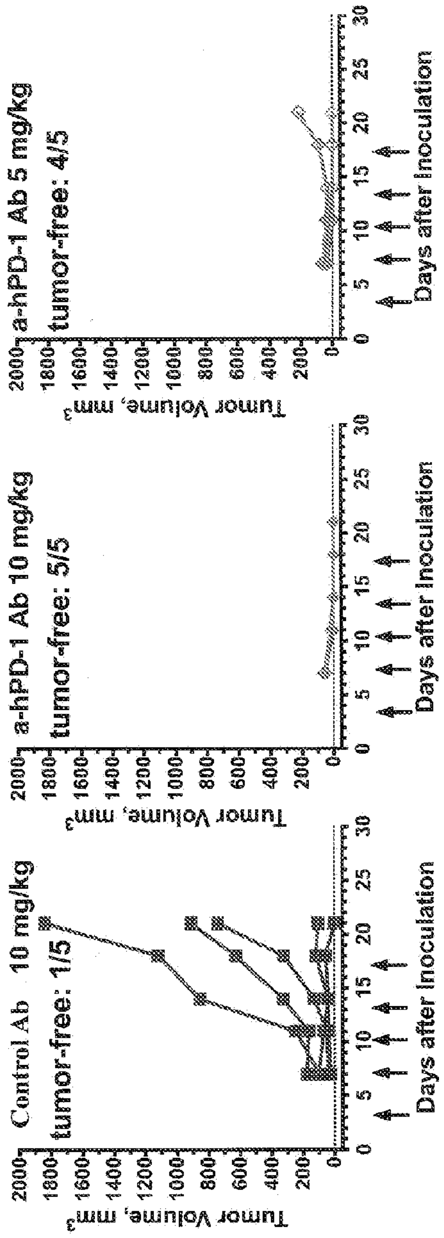
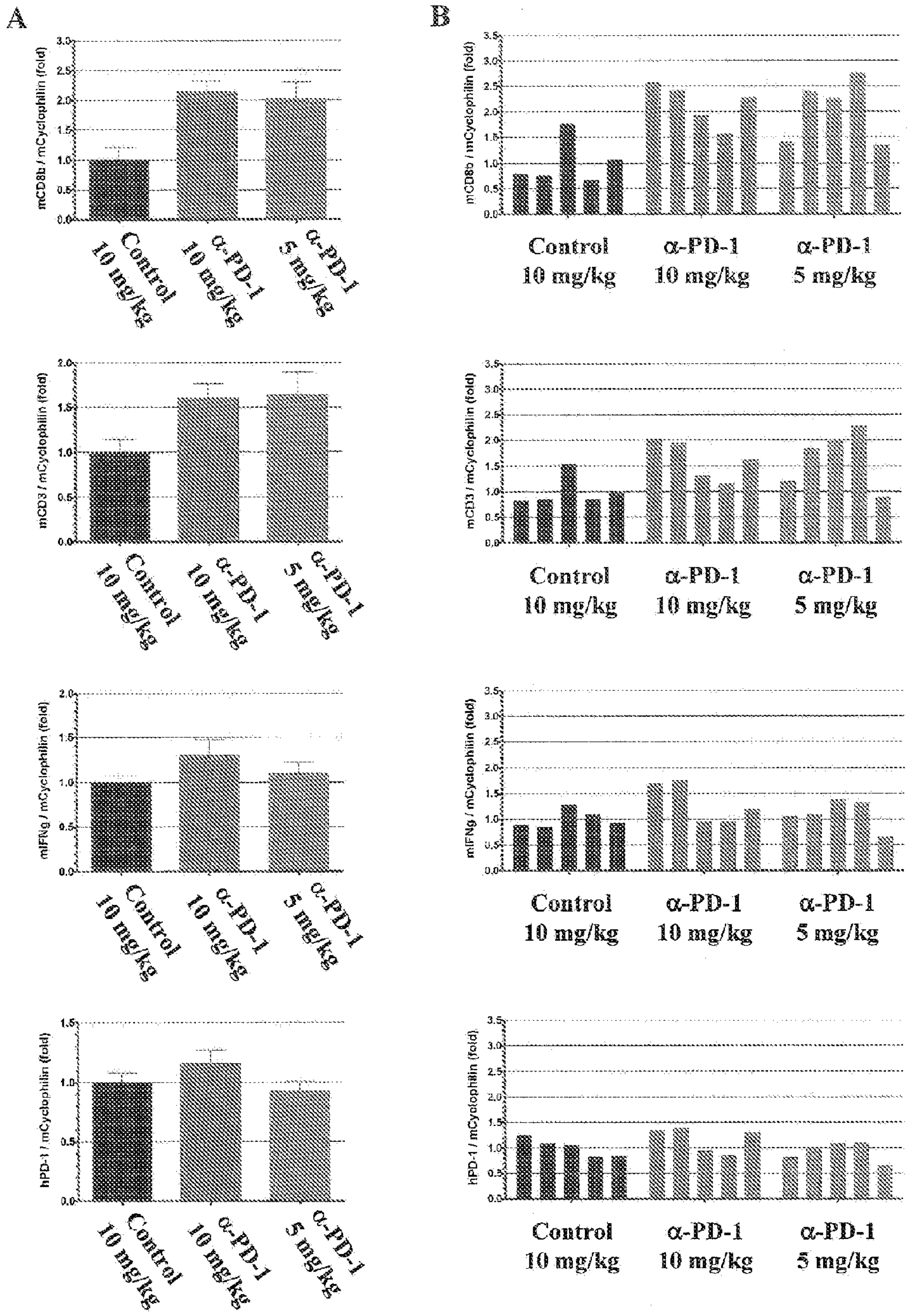
PD-1

Figure 4



Control Ab 10 mg/kg
tumor-free: 1/5
a-hPD-1 Ab 10 mg/kg
tumor-free: 5/5
a-hPD-1 Ab 5 mg/kg
tumor-free: 4/5
Tumor Volume, mm³
Days after Inoculation

Figure 5


A
B
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mIFNg / mCyclophilin (fold)
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Control
10 mg/kg
α-PD-1
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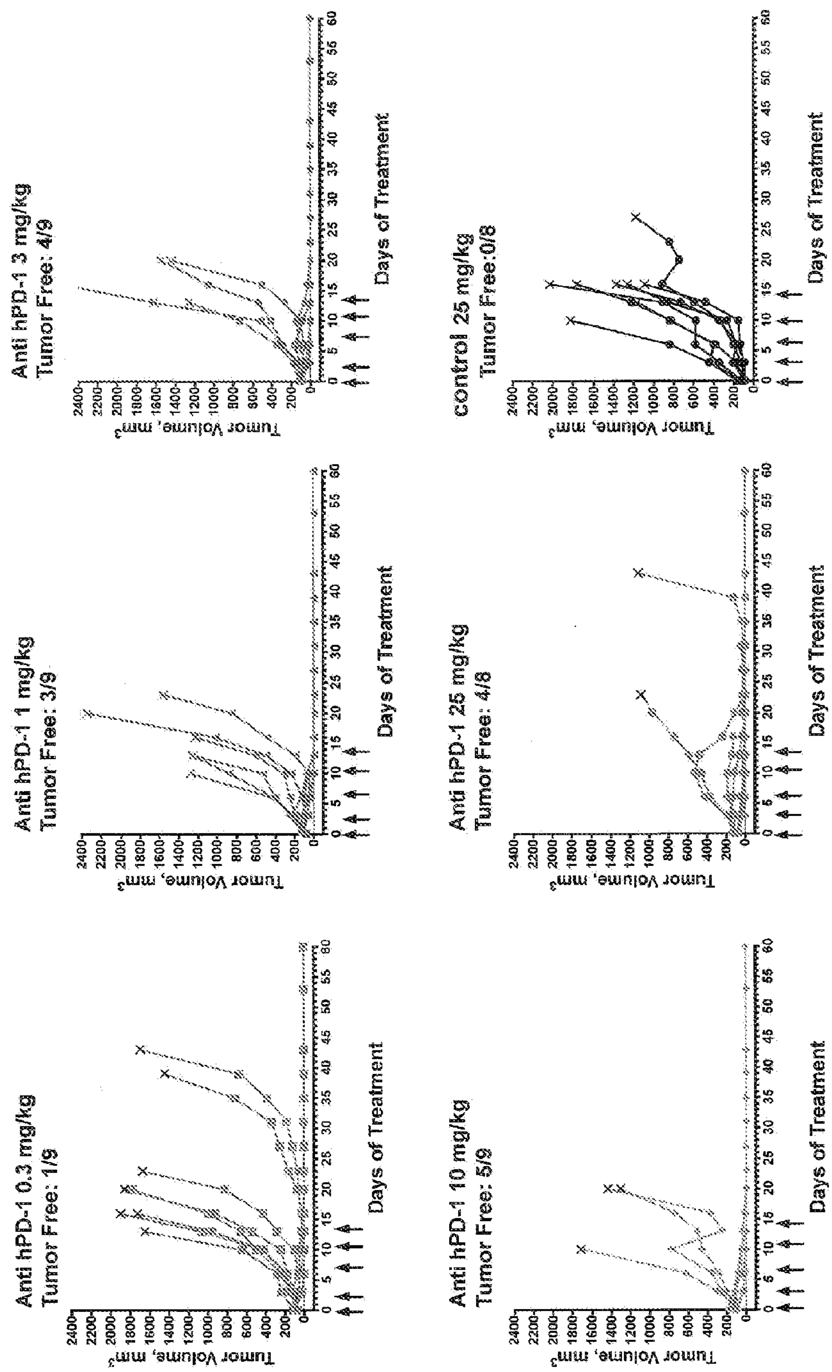
α-PD-1
5 mg/kg

Figure 6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Tumor Free: 1/9
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Tumor Free: 5/9
Anti hPD-1 25 mg/kg
Tumor Free: 4/8
control 25 mg/kg
Tumor Free:0/8
Tumor Volume, mm³
Days of Treatment

Figure 7

Figure 8

Mouse Pdcd1 mRNA (NM_008798.2)
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TGACTCTCCCTACTCCTCCTAGGAACCAAAAGGACAAAGTCCATGTTGACAGCAGGGA
AGGAAAGGGGGATATAACCTTGACGCAAACCAACACTGGGGTGTTAGAATCTCCTCAT
TCACTCTGTCCTGGAGTTGGGTTCTGGCTCTCCTTCACACCTAGGACTCTGAAATGAGC
AAGCACTTCAGACAGTCAGGGTAGCAAGAGTCTAGCTGTCTGGTGGGCACCCAAAATG
ACCAGGGCTTAAGTCCCTTTCCTTTGGTTTAAGCCCGTTATAATTAAATGGTACCAAAA
GCTTTAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA (SEQ ID NO:1)

Mouse PD-1 amino acid (Q02242)
MWVRQVPWSFTWAVLQLSWQ**SGWLLEVPNG(PWRSLTFYPAWLTVSEGANATFTCSL**
SNWSEDLMLNWNRLSPSNQTEKQAAFCNGLSQPVQDARFQIIQLPNRHDFHMNILDT
RRNDSGIYLCGAISLHPKAKIEESPG)AELVVTERILETSTRYPSPSPKPEGRFQGMVIGI
MSALVGIPVLLLLAWAL*AVFCSTSMSEARGAGSKDDTLKEEPSAAPVPSVAYEELDFQGREKT*
PELPTACVHTEYATIVFTEGLGASAMGRRGSADGLQGPRPPRHEDGHCSWPL (SEQ ID NO:2)

Figure 8, continued...

Human Pdcd1 mRNA (NM_005018.2)
AGTTTCCCTTCCGCTCACCTCCGCCTGAGCAGTGGAGAAGGCGGCACTCTGGTGGGGCT
GCTCCAGGC**ATGCAGATCCCACAGGCGCCCTGGCCAGTCGTCTGGGCGGTGCTAC**
AACTGGGCTGGCGGCCAGGATGGTTCTTAGACTCCCCAGACAGGCCCTGGAACCC
CCCCACCTTCTCCCCAGCCCTGCTCGTGGTGACCGAAGGGGACAACGCCACCTTC
ACCTGCAGCTTCTCCAACACATCGGAGAGCTTCGTGCTAAACTGGTACCGCATGA
GCCCCAGCAACCAGACGGACAAGCTGGCCGCCTTCCCCGAGGACCGCAGCCAGC
CCGGCCAGGACTGCCGCTTCCGTGTCACACAACTGCCCAACGGGCGTGACTTCCA
CATGAGCGTGGTCAGGGCCCGGCGCAATGACAGCGGCACCTACCTCTGTGGGGC
CATCTCCCTGGCCCCCAAGGCGCAGATCAAAGAGAGCCTGCGGGCAGAGCTCAG
GGTGACAGAGAGAAGGGCAGAAGTGCCCACAGCCCACCCCAGCCCCTCACCCAG
GCCAGCCGGCCAGTTCCAAACCCTGGTGGTTGGTGTCGTGGGCGGCCTGCTGGG
CAGCCTGGTGCTGCTAGTCTGGGTCCTGGCCGTCATCTGCTCCCGGGCCGCACGA
GGGACAATAGGAGCCAGGCGCACCGGCCAGCCCCTGAAGGAGGACCCCTCAGCC
GTGCCTGTGTTCTCTGTGGACTATGGGGAGCTGGATTTCCAGTGGCGAGAGAAGA
CCCCGGAGCCCCCCGTGCCCTGTGTCCCTGAGCAGACGGAGTATGCCACCATTGT
CTTTCCTAGCGGAATGGGCACCTCATCCCCCGCCCGCAGGGGCTCAGCTGACGGC
CCTCGGAGTGCCCAGCCACTGAGGCCTGAGGATGGACACTGCTCTTGGCCCCTCT
GACCGGCTTCCTTGGCCACCAGTGTTCTGCAGACCCTCCACCATGAGCCCGGGTCAGCG
CATTTCCTCAGGAGAAGCAGGCAGGGTGCAGGCCATTGCAGGCCGTCCAGGGGCTGAG
CTGCCTGGGGGCGACCGGGGCTCCAGCCTGCACCTGCACCAGGCACAGCCCCACCACA
GGACTCATGTCTCAATGCCCACAGTGAGCCCAGGCAGCAGGTGTCACCGTCCCCTACA
GGGAGGGCCAGATGCAGTCACTGCTTCAGGTCCTGCCAGCACAGAGCTGCCTGCGTCC
AGCTCCCTGAATCTCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCCTGCGGCCCGGGGC
TGAAGGCGCCGTGGCCCTGCCTGACGCCCCGGAGCCTCCTGCCTGAACTTGGGGGCTG
GTTGGAGATGGCCTTGGAGCAGCCAAGGTGCCCCTGGCAGTGGCATCCCGAAACGCCC
TGGACGCAGGGCCCAAGACTGGGCACAGGAGTGGGAGGTACATGGGGCTGGGGACTC
CCCAGGAGTTATCTGCTCCCTGCAGGCCTAGAGAAGTTTCAGGGAAGGTCAGAAGAGC
TCCTGGCTGTGGTGGGCAGGGCAGGAAACCCCTCCACCTTTACACATGCCCAGGCAGC
ACCTCAGGCCCTTTGTGGGGCAGGGAAGCTGAGGCAGTAAGCGGGCAGGCAGAGCTG
GAGGCCTTTCAGGCCCAGCCAGCACTCTGGCCTCCTGCCGCCGCATTCCACCCCAGCCC
CTCACACCACTCGGGAGAGGGACATCCTACGGTCCCAAGGTCAGGAGGGCAGGGCTGG
GGTTGACTCAGGCCCCTCCCAGCTGTGGCCACCTGGGTGTTGGGAGGGCAGAAGTGCA
GGCACCTAGGGCCCCCCATGTGCCCACCCTGGGAGCTCTCCTTGGAACCCATTCCTGAA
ATTATTTAAAGGGGTTGGCCGGGCTCCCACCAGGGCCTGGGTGGGAAGGTACAGGCGT
TCCCCCGGGGCCTAGTACCCCCGCCGTGGCCTATCCACTCCTCACATCCACACACTGCA
CCCCCACTCCTGGGGCAGGGCCACCAGCATCCAGGCGGCCAGCAGGCACCTGAGTGGC
TGGGACAAGGGATCCCCCTTCCCTGTGGTTCTATTATATTATAATTATAATTAAATATG
AGAGCATGCTAAGGAAAA (SEQ ID NO:3)

Figure 8, continued…

Human PD-1 amino acid (Q15116)
MQIPQAPWPVVWAVLQLGWR**PGWFLDSPDRPWNP(PTFSPALLVVTEGDNATFTCSFS**
NTSESFVLNWYRMSPSNQTDKLAAFPEDRSQPGQDCRFRVTQLPNGRDFHMSVVRAR
RNDSGTYLCGAISLAPKAQIKESLRAELRVT)ERRAEVPTAHPSPSPRPAGQFQTLVVG
VVGGLLGSLVLLVWVLAVI*CSRAARGTIGARRTGQPLKEDPSAVPVFSVDYGELDFQWREKTP*
EPPVPCVPEQTEYATIVFPSGMGTSSPARRGSADGPRSAQPLRPEDGHCSWPL (SEQ ID NO:4)

Humanized Pdcd1 mRNA
TGAGCAGCGGGGAGGAGGAAGAGGAGACTGCTACTGAAGGCGACACTGCCAGGGGCT
CTGGGC**ATGTGGGTCCGGCAGGTACCCTGGTCATTCACTTGGGCTGTGCTGCAGT**
TGAGCTGGCAATCAGGGTGGCTTCTAG(ACTCCCCAGACAGGCCCTGGAACCCCC
CCACCTTCTCCCCAGCCCTGCTCGTGGTGACCGAAGGGGACAACGCCACCTTCAC
CTGCAGCTTCTCCAACACATCGGAGAGCTTCGTGCTAAACTGGTACCGCATGAGC
CCCAGCAACCAGACGGACAAGCTGGCCGCCTTCCCCGAGGACCGCAGCCAGCCC
GGCCAGGACTGCCGCTTCCGTGTCACACAACTGCCCAACGGGCGTGACTTCCACA
TGAGCGTGGTCAGGGCCCGGCGCAATGACAGCGGCACCTACCTCTGTGGGGCCA
TCTCCCTGGCCCCCAAGGCGCAGATCAAAGAGAGCCTGCGGGCAGAGCTCAGGG
TGACAGAGAGAAGGGCAGAAGTGCCCACAGCCCACCCCAGCCCCTCACCCAGGC
CAGCCGGCCAGTTCCAAACCCTG)GTCATTGGTATCATGAGTGCCCTAGTGGGTAT
CCCTGTATTGCTGCTGCTGGCCTGGGCCCTAGCTGTCTTCTGCTCAACAAGTATG
TCAGAGGCCAGAGGAGCTGGAAGCAAGGACGACACTCTGAAGGAGGAGCCTTCA
GCAGCACCTGTCCCTAGTGTGGCCTATGAGGAGCTGGACTTCCAGGGACGAGAG
AAGACACCAGAGCTCCCTACCGCCTGTGTGCACACAGAATATGCCACCATTGTCT
TCACTGAAGGGCTGGGTGCCTCGGCCATGGGACGTAGGGGCTCAGCTGATGGCC
TGCAGGGTCCTCGGCCTCCAAGACATGAGGATGGACATTGTTCTTGGCCTCTTTG
ACCAGATTCTTCAGCCATTAGCATGCTGCAGACCCTCCACAGAGAGCACCGGTCCGTCC
CTCAGTCAAGAGGAGCATGCAGGCTACAGTTCAGCCAAGGCTCCCAGGGTCTGAGCTA
GCTGGAGTGACAGCCCAGCGCCTGCACCAATTCCAGCACATGCACTGTTGAGTGAGAG
CTCACTTCAGGTTTACCACAAGCTGGGAGCAGCAGGCTTCCCGGTTTCCTATTGTCACA
AGGTGCAGAGCTGGGGCCTAAGCCTATGTCTCCTGAATCCTACTGTTGGGCACTTCTAG
GGACTTGAGACACTATAGCCAATGGCCTCTGTGGGTTCTGTGCCTGGAAATGGAGAGA
TCTGAGTACAGCCTGCTTTGAATGGCCCTGTGAGGCAACCCCAAAGCAAGGGGGTCCA
GGTATACTATGGGCCCAGCACCTAAAGCCACCCTTGGGAGATGATACTCAGGTGGGAA
ATTCGTAGACTGGGGGACTGAACCAATCCCAAGATCTGGAAAAGTTTTGATGAAGACT
TGAAAAGCTCCTAGCTTCGGGGGTCTGGGAAGCATGAGCACTTACCAGGCAAAAGCTC
CGTGAGCGTATCTGCTGTCCTTCTGCATGCCCAGGTACCTCAGTTTTTTTCAACAGCAA
GGAAACTAGGGCAATAAAGGGAACCAGCAGAGCTAGAGCCACCCACACATCCAGGGG
GCACTTGACTCTCCCTACTCCTCCTAGGAACCAAAAGGACAAAGTCCATGTTGACAGC
AGGGAAGGAAAGGGGGATATAACCTTGACGCAAACCAACACTGGGGTGTTAGAATCT
CCTCATTCACTCTGTCCTGGAGTTGGGTTCTGGCTCTCCTTCACACCTAGGACTCTGAAA
TGAGCAAGCACTTCAGACAGTCAGGGTAGCAAGAGTCTAGCTGTCTGGTGGGCACCCA
AAATGACCAGGGCTTAAGTCCCTTTCCTTTGGTTTAAGCCCGTTATAATTAAATGGTAC
CAAAAGCTTTAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
(SEQ ID NO:5)

Figure 8, continued…

Humanized PD-1 amino acid
MWVRQVPWSFTWAVLQLSWQSGWLL**DSPDRPWNP(PTFSPALLVVTEGDNATFTCSFS**
NTSESFVLNWYRMSPSNQTDKLAAFPEDRSQPGQDCRFRVTQLPNGRDFHMSVVRAR
RNDSGTYLCGAISLAPKAQIKESLRAELRVT)ERRAEVPTAHPSPSPRPAGQFQTLVIGI
MSALVGIPVLLLLAWAL*AVFCSTSMSEARGAGSKDDTLKEEPSAAPVPSVAYEELDFQGREKT*
PELPTACVHTEYATIVFTEGLGASAMGRRGSADGLQGPRPPRHEDGHCSWPL (SEQ ID NO:6)

Human 883 bp DNA fragment
AAGAGGCTCTGCAGTGGAGGCCAGTGCCCATCCCCGGGTGGCAGAGGCCCCAGCAGA
GACTTCTCAATGACATTCCAGCTGGGGTGGCCCTTCCAGAGCCCTTGCTGCCCGAGGGA
TGTGAGCAGGTGGCCGGGGAGGCTTTGTGGGGCCACCCAGCCCCTTCCTCACCTCTCTC
CATCTCTCAGACTCCCCAGACAGGCCCTGGAACCCCCCCACCTTCTCCCCAGCCCTGCT
CGTGGTGACCGAAGGGGACAACGCCACCTTCACCTGCAGCTTCTCCAACACATCGGAG
AGCTTCGTGCTAAACTGGTACCGCATGAGCCCCAGCAACCAGACGGACAAGCTGGCCG
CCTTCCCCGAGGACCGCAGCCAGCCCGGCCAGGACTGCCGCTTCCGTGTCACACAACT
GCCCAACGGGCGTGACTTCCACATGAGCGTGGTCAGGGCCCGGCGCAATGACAGCGGC
ACCTACCTCTGTGGGGCCATCTCCCTGGCCCCCAAGGCGCAGATCAAAGAGAGCCTGC
GGGCAGAGCTCAGGGTGACAGGTGCGGCCTCGGAGGCCCCGGGGCAGGGGTGAGCTG
AGCCGGTCCTGGGGTGGGTGTCCCCTCCTGCACAGGATCAGGAGCTCCAGGGTCGTAG
GGCAGGGACCCCCCAGCTCCAGTCCAGGGCTCTGTCCTGCACCTGGGGAATGGTGACC
GGCATCTCTGTCCTCTAGCTCTGGAAGCACCCCAGCCCCTCTAGTCTGCCCTCACCCCT
GACCCTGACCCTCCACCCTGACCCCGTCCTAACCCCTGACCTTTGTGCCCTTCCAGAGA
GAAGGGCAGAAGTGCCCACAGCCCACCCCAGCCCCTCACCCAGGCCAGCCGGCCAGTT
CCAAACCCTG (SEQ ID NO:23)