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DESCRIPTION

CROSS REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of priority of U.S. Provisional Application No. 61/881,261, filed September 23, 2013.

BACKGROUND

[0002] The immune system is composed of several different cell types that are involved in multiple highly regulated processes and together generate immune responses that are effective in eliminating foreign proteins. Further, these same immune cells have been found to possess a self-awareness property by virtue of, *inter alia*, regulatory membrane proteins that regulate cell-to-cell interactions. Such communication is critical for the survival of such organisms, as these same proteins are suggested to be an important determinant of transplant engraftment. However, no *in vivo* system exists to determine the molecular aspects of human immune cell-to-cell interactions and its regulation. Such a system provides a source for assays in human hematopoietic and immune system related functions *in vivo*, identification of novel therapies and vaccines.

SUMMARY OF INVENTION

[0003] The present invention is based on the recognition that it is desirable to engineer non-human animals to permit improved engraftment of human hematopoietic stem cells. The present invention is also based on the recognition that non-human animals having a humanized SIRP α gene and/or otherwise expressing, containing, or producing a human or humanized SIRP α protein are desirable, for example for use in engraftment of human hematopoietic stem cells.

[0004] The invention provides a rodent whose genome comprises a humanized SIRP α gene, wherein the humanized SIRP α gene is operably linked to a rodent SIRP α promoter at an endogenous rodent SIRP α locus, and expresses a humanized SIRP α protein in the rodent, and wherein the humanized SIRP α protein comprises (i) an extracellular portion that is at least 95% identical to amino acid residues 28-362 of a human SIRP α protein of SEQ. ID 4 and (ii) the intracellular portion of a rodent SIRP α protein.

[0005] The invention further provides an isolated rodent cell or tissue whose genome comprises a humanized SIRP α gene, wherein the humanized SIRP α gene is operably linked to a rodent SIRP α promoter at the endogenous rodent SIRP α locus, and encodes a humanized SIRP α protein, and wherein the humanized SIRP α protein comprises (i) an extracellular portion

that is at least 95% identical to amino acid residues 28-362 of a human SIRP α protein of SEQ. ID 4, and (ii) the intracellular portion of a rodent SIRP α protein.

[0006] The invention additionally provides a method of making a rodent, comprising: (a) inserting a human SIRP α nucleic acid sequence into an endogenous rodent SIRP α locus in a rodent embryonic stem (ES) cell to form a humanized SIRP α gene, wherein the humanized SIRP α gene is operably linked to a rodent SIRP α promoter at the endogenous rodent SIRP α locus and encodes a humanized SIRP α protein, and wherein the humanized SIRP α protein comprises (i) an extracellular portion that is at least 95% identical to amino acid residues 28-362 of the human SIRP α protein of SEQ. ID 4, and (ii) the intracellular portion of a rodent SIRP α protein, thereby obtaining a modified rodent ES cell comprising the humanized SIRP α gene; and (b) making a rodent using the modified ES cell obtained in (a).

[0007] The invention also provides a method of assessing the therapeutic efficacy of a drug targeting human cells, comprising: (a) providing a rodent of the invention into which one or more human cells have been transplanted; (b) administering a drug candidate to the rodent; and (c) monitoring the human cells in the rodent to determine the therapeutic efficacy of the drug candidate.

[0008] The invention further provides a nucleic acid targeting vector, comprising: a human genomic DNA fragment comprising exons 2, 3 and 4 of a human SIRP α gene, flanked by a 5' homology arm comprising a rodent genomic DNA fragment upstream of exon 2 of a rodent SIRP α gene, and a 3' homology arm comprising a rodent genomic DNA fragment downstream of exon 4 of the rodent SIRP α gene; wherein integration of the human genomic DNA fragment into the genome of a rodent cell based on homologous recombination results in a replacement of exons 2, 3 and 4 of the rodent SIRP α gene at an endogenous rodent SIRP α locus with exons 2, 3 and 4 of the human SIRP α gene to form a humanized SIRP α gene, wherein said humanized SIRP α gene is operably linked to the rodent SIRP α promoter at said endogenous rodent SIRP α locus, and encodes a humanized SIRP α protein comprising an extracellular portion of the human SIRP α protein encoded by said human SIRP α gene and an intracellular portion of the rodent SIRP α protein encoded by said rodent SIRP α gene, and wherein the rodent is selected from a mouse or a rat.

[0009] Described herein is a non-human animal expressing a SIRP α polypeptide comprising an extracellular portion of a human SIRP α protein and intracellular portion of a mouse SIRP α protein.

[0010] An extracellular portion of a human SIRP α protein may comprise amino acids corresponding to residues 28-362 of a human SIRP α protein that appears in SEQ ID NO: 4.

[0011] An extracellular portion of a human SIRP α protein may share a percent identity of at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% with a corresponding extracellular portion of a human SIRP α protein that appears in Table 3. An extracellular portion of a human

SIRP α protein may share 100% identity (or identical) with a corresponding extracellular portion of a human SIRP α protein that appears in Table 3.

[0012] Also described herein is a non-human animal that does not also express an endogenous non-human SIRP α protein. The non-human animal may be a rodent which does not also express an endogenous rodent SIRP α protein. The non-human animal may be a mouse that does not also express an endogenous mouse SIRP α protein having a sequence that appears in Table 3.

[0013] Further described herein is a non-human animal comprising a SIRP α gene that comprises exons 2, 3 and 4 of a human SIRP α gene operably linked to a non-human SIRP α promoter.

[0014] A SIRP α gene of a non-human animal described herein may comprise exons 1, 5, 6, 7 and 8 of an endogenous non-human SIRP α gene.

[0015] A non-human animal described herein may be a rodent. A rodent may be selected from a mouse or a rat.

[0016] Further described herein is a SIRP α polypeptide encoded by the gene of a non-human animal as described herein.

[0017] Also described herein is a cell or tissue isolated from a non-human animal as described herein. A cell may be selected from a lymphocyte (e.g., a B or T cell), a myeloid cell (e.g., a macrophage, a neutrophil, a granulocyte, a myeloid dendritic cell, and a mast cell), and a neuron. A tissue may be 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/or a combination thereof.

[0018] Additionally described herein is an isolated mouse cell or tissue whose genome includes a SIRP α gene that encodes the extracellular portion of a human SIRP α protein linked to the intracellular portion of a mouse SIRP α protein. The SIRP α gene described herein may be operably linked to a mouse SIRP α promoter. A SIRP α gene described herein may comprise exons 2, 3, and 4 of a human SIRP α gene.

[0019] Further described herein is a non-human embryonic stem (ES) cell whose genome comprises a SIRP α gene as described herein. The ES cell may comprise exons 2, 3 and 4 of a human SIRP α gene operably linked to a non-human SIRP α promoter. The ES cell may be a rodent ES cell. A non-human embryonic stem cell described herein may be a mouse or rat embryonic stem cell.

[0020] Also described herein is a non-human embryo comprising, made from, obtained from, or generated from a non-human embryonic stem cell comprising a SIRP α gene as described herein. The non-human embryo may be is a rodent embryo. The rodent embryo as described

herein may be a mouse or rat embryo.

[0021] Further described herein is a method of making a non-human animal that expresses a SIRP α protein from an endogenous SIRP α locus, wherein the SIRP α protein comprises a human sequence, the method comprising targeting an endogenous SIRP α locus in a non-human ES cell with a genomic fragment comprising a nucleotide sequence that encodes a human SIRP α protein in whole or in part; obtaining a modified non-human ES cell comprising an endogenous SIRP α locus that comprises said human sequence; and, creating a non-human animal using said modified ES cell.

[0022] As described herein, said nucleotide sequence may comprise exons 2, 3 and 4 of a human SIRP α gene. Said nucleotide sequence may comprise exons 2, 3 and 4 of a human SIRP α gene having a sequence at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% identical to a human SIRP α gene that appears in Table 3.

[0023] Said nucleotide sequence may encode amino acid residues 28-362 of a human SIRP α protein. Said nucleotide sequence may encode amino acid residues 28-362 of a human SIRP α protein having a sequence at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 98% identical to a human SIRP α protein that appears in Table 3.

[0024] Additionally described herein is a method of providing a mouse whose genome includes a SIRP α gene that encodes the extracellular portion of a human SIRP α protein linked to the intracellular portion of a mouse SIRP α protein, the method comprising modifying the genome of a mouse so that it comprises a SIRP α gene that encodes the extracellular portion of a human SIRP α protein linked to the intracellular portion of a mouse SIRP α protein thereby providing said mouse. The SIRP α gene may be a SIRP α gene as described herein. The SIRP α gene may comprise exons 2, 3, and 4 of a human SIRP α gene.

[0025] Also described herein is a method of engrafting human cells into a mouse, the method comprising steps of providing a mouse whose genome comprises a SIRP α gene that encodes the extracellular portion of a human SIRP α protein linked to the intracellular portion of a mouse SIRP α protein, and transplanting one or more human cells into the mouse. The method may further comprise as a step assaying engraftment of the one or more human cells in the mouse. The step of assaying may comprise comparing the engraftment of the one or more human cells to the engraftment in one or more wild-type mice. The step of assaying may comprise comparing the engraftment of the one or more human cells to the engraftment in one or more mice whose genome does not comprise a SIRP α gene that encodes the extracellular portion of a human SIRP α protein linked to the intracellular portion of a mouse SIRP α protein.

[0026] The human cells may be hematopoietic stem cells. The human cells may be transplanted intravenously. The human cells may be transplanted intraperitoneally. The human cells may be transplanted subcutaneously.

[0027] Further described herein is a method comprising the steps of providing one or more cells whose genome includes a SIRP α gene that encodes the extracellular portion of a human SIRP α protein linked to the intracellular portion of a mouse SIRP α protein, incubating the one or more cells with a labeled substrate, and measuring phagocytosis of the labeled substrate by the one or more cells. The cells may be mouse cells.

[0028] The substrate may be fluorescently labeled. The substrate may be labeled with an antibody. The substrate may be one or more red blood cells. The substrate may be one or more bacterial cells.

[0029] Additionally described herein is a method comprising the steps of providing a mouse whose genome includes a SIRP α gene that encodes the extracellular portion of a human SIRP α protein linked to the intracellular portion of a mouse SIRP α protein, exposing the mouse to an antigen, and measuring phagocytosis of the antigen by one or more cells of the mouse. The step of exposing may comprise exposing the mouse to an antigen that is fluorescently labeled. The step of exposing may comprise exposing the mouse to one or more cells that comprise the antigen. The step of exposing may comprise exposing the mouse to one or more human cells comprising the antigen. The step of exposing may comprise exposing the mouse to one or more bacterial cells comprising the antigen.

[0030] A SIRP α gene described herein may comprise exons 2, 3, and 4 of a human SIRP α gene. An extracellular portion of a human SIRP α protein described herein may comprise amino acids corresponding to residues 28-362 of a human SIRP α protein that appears in Table 3. A SIRP α gene described herein may be operably linked to a mouse SIRP α promoter.

[0031] Also described herein is a non-human animal obtainable by methods as described herein. Non-human animals described herein may not detectably express an extracellular portion of an endogenous SIRP α protein.

[0032] Further described herein are methods 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 as described herein, 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 or condition. Monitoring the safety profile may include determining if the non-human animal exhibits a side effect or adverse reaction as a result of delivering the drug or vaccine. A side effect or adverse reaction may be 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.

[0033] Additionally described herein is use of a non-human animal described herein in the development of a drug or vaccine for use in medicine, such as use as a medicament.

[0034] Further described herein is use of a non-human animal described herein to assess the efficacy of a therapeutic drug targeting human cells. A non-human animal described herein may be transplanted with human cells, and a drug candidate targeting such human cells is administered to the animal. The efficacy of the drug is determined by monitoring the human cells in the non-human animal after the administration of the drug.

[0035] Non-human animals described herein may be rodents, preferably a mouse or a rat.

[0036] 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.

[0037] Other features, objects, and advantages of the present invention are apparent in the detailed description that follows. It should be understood, however, that the detailed description, while indicating embodiments of the present invention, is given by way of illustration only, not limitation. Various changes and modifications within the scope of the invention will become apparent to those skilled in the art from the detailed description.

BRIEF DESCRIPTION OF THE DRAWING

[0038] The drawing included herein is for illustration purposes only not for limitation.

Figure 1 shows a diagram, not to scale, of an endogenous murine SIRP α gene (top) with each exon numbered. A humanized endogenous SIRP α gene (bottom) is shown containing exons 2-4 of a human SIRP α gene and a neomycin selection cassette (Ub-Neo) flanked by site-specific recombinase recognition sites (e.g., *loxP*). The targeted insertion of exons 2-4 of a human SIRP α gene results in an endogenous gene that expresses a humanized SIRP α gene having an extracellular region corresponding to a human SIRP α protein.

Figure 2 shows an overlay of SIRP α expression of wild type and mice heterozygous for a humanized SIRP α gene.

Figure 3 shows the percent of CD45⁺ cells in different strains of mice engrafted with human CD34⁺ cells.

Figure 4 shows the percent of CD45⁺CD3⁺ cells in different strains of mice engrafted with human CD34⁺ cells.

Figure 5 shows the percent of CD45⁺CD19⁺ cells in different strains of mice engrafted with human CD34⁺ cells.

Figure 6 shows that Ab 1 suppressed growth of Raji tumors in a dose-dependent manner in hCD34⁺ engrafted SIRP α BRG mice. Raji tumor volume was measured on days 3, 6, 9, 13, 16,

20, 23, 27, 30 and 34 post tumor implantation. Data for individual animals (**Panels A-D**) is presented. hCD34+ engrafted SIRPα BRG mice were administered 2×10^6 Raji tumor cells subcutaneously on Day 0. Control groups received no antibody (vehicle control) (**Panel A**). For experimental groups, on Day 0 mice were treated with an IP dose of a non-binding control Ab (control Ab 5) at 0.4 mg/kg (**Panel B**), or Ab 1 at 0.4 mg/kg (**Panel C**) or 0.04 mg/kg (**Panel D**), followed by twice weekly doses for the length of the study. The composite data for all individual test groups are shown in Figure 7.

Figure 7 shows that Ab 1 significantly suppressed growth of Raji tumors compared to controls in hCD34+ engrafted SIRPα BRG mice. Data represents the composite data from n=4-5 mice per group as shown in Figure 6. Data are expressed as mean (SEM) and were analyzed using analysis of variance (ANOVA) and post hoc tests to probe significant effects (Tukey's for two-way ANOVA). One mouse in the vehicle control group, Control Ab 5 group, and Ab 1 0.4mg/kg group was excluded from this composite graph due to early death in order to analyze data by two-way ANOVA.

Figure 8 shows that Ab 1 did not affect body weight in hCD34+ engrafted SIRPα BRG mice. Body weights were measured on days 3, 6, 9, 13, 16, 20, 23, 27, 30 and 34 post tumor implantation. Data for individual animals (**Panels A-D**) was measured. hCD34+ engrafted SIRPα BRG mice were administered 2×10^6 Raji tumor cells subcutaneously on Day 0. Control groups received no antibody (vehicle control) (**Panel A**). For experimental groups, on Day 0 mice were treated with an IP dose of the IgG1 non-binding Control Ab 5 at 0.4 mg/kg (**Panel B**) or Ab 1 at 0.4 mg/kg (**Panel C**) or 0.04 mg/kg (**Panel D**), followed by twice weekly doses for the length of the study.

DEFINITIONS

[0039] This invention is not limited to particular methods, and experimental conditions described, 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 embodiments only, and is not intended to be limiting, since the scope of the present invention is defined by the claims.

[0040] 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.

[0041] 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).

[0042] The term "*biologically active*" as used herein refers to 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.

[0043] The term "*comparable*", as used herein, refers 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 there between 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.

[0044] The term "*conservative*" as used herein to describe a conservative amino acid substitution refers to 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 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.

[0045] The term "*disruption*" as used herein refers to 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. 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.

[0046] The phrase "*endogenous locus*" or "*endogenous gene*" as used herein refers to a genetic locus found in a parent or reference organism prior to introduction of a disruption, deletion, replacement, alteration, or modification as described herein. In some aspects, the endogenous locus has a sequence found in nature. In some aspects, the endogenous locus is wild type. 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).

[0047] The phrase "*endogenous promoter*" refers to a promoter that is naturally associated, e.g., in a wild-type organism, with an endogenous gene.

[0048] The term "*heterologous*" as used herein refers to 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).

[0049] The term "*host cell*", as used herein, refers to 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" as used herein. 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.C6TM 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.

[0050] The term "*humanized*", is used herein in accordance with its art-understood meaning to refer to 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. A "humanized" gene may be 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 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. A humanized gene may comprise at least a portion of a DNA sequence of a human gene. A humanized gene may comprise an entire DNA sequence of a human gene. A humanized protein may comprise a sequence having a portion that appears in a human protein. A humanized protein may comprise 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.

[0051] The term "*identity*" as used herein in connection with a comparison of sequences, refers to 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 (MACVECTORTM 10.0.2, MacVector Inc., 2008).

[0052] The term "*isolated*", as used herein, refers to 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. As used herein, 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; 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.

[0053] The phrase "*non-human animal*" as used herein refers to any vertebrate organism that is not a human. In some aspects, a non-human animal is acyclostome, 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.

[0054] The phrase "*nucleic acid*", as used herein, in its broadest sense, refers to 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" refers to individual nucleic acid residues (e.g., nucleotides and/or nucleosides); in some aspects, "nucleic acid" refers to 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 of the present invention. 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, nucleic acids are 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, 200, 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.

[0055] The phrase "*operably linked*", as used herein, refers to 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" as used herein refers to 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.

[0056] The term "*polypeptide*", as used herein, refers to 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 is engineered in that it is designed and/or produced through action of the hand of man..

[0057] The term "*recombinant*", as used herein, is intended to refer to polypeptides (e.g., signal-regulatory proteins 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) 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 results 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*.

[0058] The term "*replacement*" is used herein to refer to 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 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 protein that has a similar function as a protein encoded by the endogenous sequence (e.g., the endogenous genomic sequence encodes a SIRP α protein, and the DNA fragment encodes one or more human SIRP α proteins). 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.

[0059] The phrase "*signal-regulatory protein*" or "SIRP" as used herein refers to a signal-regulatory protein receptor, e.g., a SIRP α receptor. SIRP genes include a plasma membrane receptor that is expressed on the surface of a cell and serves as a regulatory protein involved in interactions between membrane surface proteins on leukocytes. Within the SIRP genes, polymorphic variants have been described in human subjects. By way of illustration, nucleotide and amino acid sequences of a human and mouse SIRP genes are provided in Table 1. Persons of skill upon reading this disclosure will recognize that one or more endogenous SIRP receptor genes in a genome (or all) can be replaced by one or more heterologous SIRP genes (e.g., polymorphic variants, subtypes or mutants, genes from another species, humanized forms, etc.).

[0060] A "*SIRP-expressing cell*" as used herein refers to a cell that expresses a signal-regulatory protein receptor. In some aspects, a SIRP-expressing cell expresses a signal-regulatory protein receptor on its surface. In some aspects, a SIRP protein expressed on the surface of the cell in an amount sufficient to mediate cell-to-cell interactions via the SIRP protein expressed on the surface of the cell. Exemplary SIRP-expressing cells include neurons, lymphocytes, myeloid cells, macrophages, neutrophils, and natural killer (NK) cells. SIRP-expressing cells regulate the interaction of immune cells to regulate the immune response to various foreign antigens or pathogens. Non-human animals described herein may demonstrate immune cell regulation via humanized SIRP receptors expressed on the surface of one more cells of the non-human animal.

[0061] The term "*substantially*" as used herein refers to 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.

[0062] The phrase "*substantial homology*" as used herein refers to 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

[0063] 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., Basic local alignment search tool, J. Mol. Biol., 215(3): 403-410, 1990; Altschul, et al., 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, 1997; Baxevanis, et al., Bioinformatics : A Practical Guide to the Analysis of Genes and Proteins, Wiley, 1998; and Misener, et al., (eds.), Bioinformatics Methods and Protocols (Methods in Molecular Biology, Vol. 132), Humana Press, 1999. 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.

[0064] The phrase "*substantial identity*" as used herein refers to 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., Basic local alignment search tool, J. Mol. Biol., 215(3): 403-410, 1990; Altschul, et al., Methods in Enzymology; Altschul et al., Nucleic Acids Res. 25:3389-3402, 1997; Baxevanis et al., Bioinformatics : A Practical Guide to the Analysis of Genes and Proteins, Wiley, 1998; and Misener, et al., (eds.), Bioinformatics Methods and Protocols (Methods in Molecular Biology, Vol. 132), Humana Press, 1999. 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.

[0065] The phrase "*targeting vector*" or "*targeting construct*" as used herein refers to 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.

[0066] The term "*variant*", as used herein, refers to 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 one 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.

[0067] The term "*vector*", as used herein, refers to a nucleic acid molecule capable of transporting another nucleic acid to which it is associated. In some aspect, 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."

[0068] The term "*wild-type*", as used herein, has its art-understood meaning that refers to 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

[0069] Described herein are among other things, improved and/or engineered non-human animals having humanized genetic material encoding a signal-regulatory protein (e.g., SIRPs) for assays in transplant engraftment, activation of phagocytosis and signal transduction. It is contemplated that such non-human animals provide an improvement in transplant engraftment of human cells. Therefore, the present disclosure is particularly useful for maintaining human hematopoietic cells in non-human animals. In particular, the present disclosure encompasses the humanization of a rodent SIRP α gene resulting in expression of a humanized protein on the plasma membrane surface of cells of the non-human animal. Such humanized proteins have the capacity to recognize engrafted human cells via engagement of humanized SIRP α proteins and ligands present on the surface of the engrafted human cells. Non-human animals are described herein that are capable of receiving transplanted human hematopoietic cells;

such non-human mammals may develop and/or have an immune system comprising human cells. Humanized SIRP α proteins may have sequence corresponding to amino acid residues 28 - 362 of a human SIRP α protein. Non-human animals described herein may comprise an endogenous SIRP α gene that contains genetic material from the non-human animal and a heterologous species (e.g., a human). Non-human animals described herein may comprise a humanized SIRP α gene, wherein the humanized SIRP α gene comprises exons 2, 3, and 4 of a human SIRP α gene.

[0070] Various aspects of the invention 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.

Signal-Regulatory Protein (SIRP) Gene Family

[0071] Signal regulatory proteins (SIRPs) constitute a family of cell surface glycoproteins which are expressed on lymphocytes, myeloid cells (including macrophages, neutrophils, granulocytes, myeloid dendritic cells, and mast cells) and neurons (e.g., see Barclay and Brown, 2006, Nat Rev Immunol 6, 457-464). There are several reported SIRP genes and they can be categorized by their respective ligands and types of signaling in which they are involved. SIRP α (also referred to as CD172A, SHPS1, P84, MYD-1, BIT and PTPNS1) is expressed on immune cells of the myeloid lineage and functions as an inhibitory receptor via an immunoreceptor tyrosine-based inhibitory motif (ITIM). SIRP α expression has also been observed on neurons. Reported ligands for SIRP α include, most notably, CD47, but also include surfactant proteins A and D. SIRP β (also referred to as CD172b) is expressed on macrophages and neutrophils, however, no known ligands have been reported. SIRP β contains a short cytoplasmic region in comparison to SIRP α and is known to associate with a signaling component known as DNAX activation protein 12 (DAP12). Thus, SIRP β is thought to be an activating receptor. SIRP γ (also referred to as CD172g and SIRP β 2) is expressed on lymphocytes and natural killer cells and also binds to CD47, however, no signaling function has been reported as the cytoplasmic tail only contains four amino acids and lacks a sequence that would facilitate association with DAP12. Another member, SIRP δ , has been described and exists as a soluble receptor.

[0072] The role of SIRP α , in particular, has been investigated in respect of its inhibitory role in the phagocytosis of host cells by macrophages. For example, CD47 binding to SIRP α on macrophages, triggers inhibitory signals that negatively regulates phagocytosis. Alternatively, positive signaling effects mediated through SIRP α binding have been reported (Shultz et al., 1995, J Immunol 154, 180-91).

SIRP α Sequences

[0073] Exemplary SIRP α sequences for human and mouse are set forth in Table 3. For cDNA sequences, consecutive exons are separated by alternating underlined text. For protein sequences, signal peptides are underlined and transmembrane and cytoplasmic sequences are italicized.

TABLE 3

<p>Mouse SIRPα cDNA NM_007547.3</p>	<p>GCGCTCGGCCGGGCGCCCTCGCGCTGGCCTCGCGACGGCTC CGCACAGCCCCGCACTCGCTCTGCGAGCTGTCCCCGCTCGCGCT TGCTCTCCGATCTCCGTCCCCGCTCCCTCTCCCTCTTCCTCTCC CCCTCTTTCCTTCTCCCTCGCTATCCGCTCCCCCGCCCCCGTGC CTCTGGCTCTGCGCCTGGCTCCCTCGGGTCCGCTCCCCCTTTCCC GCCGGCCTGGCCCCGGCGTCACGCTCCCGGAGTCTCCCCGCTCG GCGGCGTCTCATTGTGGGAGGGGGTCAGATCACCCCGCCGGG CGGTGGCGCTGGGGGGCAGCGGAGGGGGAGGGGCCCTTAGTC GTTGCCCCGCGCCGCCCGCCGCTGCCGAGCGCGCTCACCGC CGCTCTCCCTCCTTGCTCTGCAGCCGCGGCCCATGGAGCCCCG CGCCCCGGCCCCCTGGCCGCTAGGGCCGCTGCTGCTCTGCCTG CTGCTCTCCGCGTCTGTTTCTGTACAGGAGCCACGGGGAAGG <u>AACTGAAGGTGACTCAGCCTGAGAAATCAGTGTCTGTTGCTG</u> <u>CTGGGGATTGACCGTTCTGAACTGCACTTTGACCTCCTTGTT</u> <u>GCCGGTGGGACCCATTAGGTGGTACAGAGGAGTAGGGCCAAG</u> <u>CCGGCTGTTGATCTACAGTTTCGAGGAGAATACGTTCTCTGA</u> <u>ATTAGAAATGTTTCAGATACTACTAAGAGAAACAATATGGAC</u> <u>TTTTCCATCCGTATCAGTAATGTCACCCCAGCAGATGCTGGCA</u> <u>TCTACTACTGTGTGAAGTTCCAGAAAGGATCATCAGAGCCTG</u> <u>ACACAGAAATACAATCTGGAGGGGGGAACAGAGGTCTATGTAC</u> <u>TCGCCAAACCTTCTCCACCGGAGGTATCCGGCCCAGCAGACA</u> <u>GGGGCATACCTGACCAGAAAGTGAACCTCACCTGCAAGTCTC</u> <u>ATGGCTTCTCTCCCCGGAATATCACCTGAAGTGGTTCAAAGA</u> <u>TGGGCAAGAACTCCACCCCTTGGAGACCACCGTGAACCCTAG</u> <u>TGGAAAGAATGTCTCCTACAACATCTCCAGCACAGTCAGGGT</u> <u>GGTACTAAACTCCATGGATGTTAATTCTAAGGTCATCTGCGAG</u> <u>GTAGCCACATCACCTTGGATAGAAGCCCTCTTCGTGGGATTG</u> <u>CTAACCTGTCTAACTTCATCCGAGTTTCACCCACCGTGAAGGT</u> <u>CACCCAACAGTCCCCGACGTCAATGAACCAGGTGAACCTCAC</u> <u>CTGCCGGGCTGAGAGGTTCTACCCCGAGGATCTCCAGCTGATC</u> <u>TGGCTGGAGAATGGAACGTATCACGGAATGACACGCCCAAG</u> <u>AATCTCACAAGAACACGGATGGGACCTATAATTACACAAGC</u> <u>TTGTTCTGGTGAACCTCATCTGCTCATAGAGAGGACGTGGTGT</u> <u>TCACGTGCCAGGTGAAGCACGACCAACAGCCAGCGATCACCC</u> <u>GAAACCATACCGTGCTGGGATTTGCCCACTCGAGTGATCAAG</u> <u>GGAGCATGCAAACCTTCCCTGATAATAATGCTACCCACAAC</u> <u>GGAATGTCTTCATCGGTGTGGGCGTGGCGTGTGCTTTGCTCGT</u> <u>AGTCCTGCTGATGGCTGCTCTACCTCCTCCGGATCAAACAG</u> <u>AAGAAAGCCAAGGGGTCAACATCTTCCACACCGTTGCACGAG</u></p>
	<p><u>CCCGAGAAGAACGCCAGGGAAATAACCCAGATCCAGGACAC</u> <u>AAATGACATCAACGACATCACATACGCAGACCTGAATCTGCC</u> <u>CAAAGAGAAGAAGCCCCGACCCCGGGCCCCCTGAGCCTAACAA</u> <u>CCACACAGAATATGCAAGCATTGAGACAGGCAAAGTGCCTAG</u> <u>GCCAGAGGATACCCTCACCTATGCTGACCTGGACATGGTCCA</u> <u>CCTCAGCCGGGCACAGCCAGCCCCAAGCCTGAGCCATCTTTC</u> <u>TCAGAGTATGCTAGTGTCCAGGTCCAGAGGAAGTGAATGGGG</u> <u>CTGTGGTCTGTACTAGGCCCCATCCCCACAAGTTTTCTTGTCCT</u> <u>ACATGGAGTGGCCATGACGAGGACATCCAGCCAGCCAATCCT</u></p>

	<p> <u>GTCCCCAGAAGGCCAGGTGGCACGGGTCCTAGGACCAGGGGT</u> <u>AAGGGTGGCCTTTGTCTTCCCTCCGTGGCTCTTCAACACCTCT</u> <u>GGGCACCCACGTCCCCCTCTTCCGGAGGCTGGGTGTTGCAGAA</u> <u>CCAGAGGGCGAACTGGAGAAAGCTGCCTGGAATCCAAGAAGT</u> <u>GTTGTGCCTCGGCCATCACTCGTGGGTCTGGATCCTGGTCTT</u> <u>GGCAACCCAGGTGCGTCCTTGATGTTCCAGAGCTTGGTCTT</u> <u>CTGTGTGGAGAAGAGCTCACCATCTCTACCCAACCTTGAGCTT</u> <u>GGGACCAGACTCCCTTTAGATCAAACCGCCCCATCTGTGGAA</u> <u>GAACACACCAGAAGTCAGCAAGTTTTCAGCCAACAGTGCTG</u> <u>GCCTCCCCACCTCCCAGGCTGACTAGCCCTGGGGAGAAGGAA</u> <u>CCCTCTCCTCCTAGACCAGCAGAGACTCCCTGGGCATGTTT</u> <u>TGTGGCCCCACCTCCCTTCCAGTCCCAGCTTGCTTCCCTCCAGCT</u> <u>AGCACTAACTCAGCAGCATCGCTCTGTGGACGCCTGTAAATTA</u> <u>TTGAGAAATGTGAAGTGTGCAGTCTTAAAGCTAAGGTTAG</u> <u>AAAATTTGATTTATGCTGTTTAGTTGTTGTTGGGTTTCTTTCT</u> <u>TTTTAATTTCTTTTCTTTTGTATTTTTTCTTTCCCTTAAAA</u> <u>CAACAGCAGCAGCATCTTGGCTCTTGTGATGTGTGAATGGT</u> <u>TGGGTCTTGTGAAGTCTGAGGTCTAACAGTTTATTGTCTGGA</u> <u>AGGATTTTCTTACAGCAGAAACAGATTTTTTCAAATTCCCAG</u> <u>AATCCTGAGGACCAAGAAGGATCCCTCAGCTGCTACTTCCAG</u> <u>CACCCAGCGTCACTGGGACGAACCAGGCCCTGTTCTTACAAG</u> <u>GCCACATGGCTGGCCCTTGCCTCCATGGCTACTGTGGTAAGT</u> <u>GCAGCCTTGTCTGACCCAATGCTGACCTAATGTTGGCCATTCC</u> <u>ACATTGAGGGGACAAGGTCAGTGATGCCCCCTTCACTCACA</u> <u>AGCACTTCAGAGGCATGCAGAGAGAAGGGCACTCGGCCAGC</u> <u>TCTCTGAGGTAAATCAGTGCAAGGAGGAGTCCGTTTTTTGCCAG</u> <u>CAAACCTCAGCAGGATCACACTGGAACAGAACCTGGTCATAC</u> <u>CTGTGACAACACAGCTGTGAGCCAGGGCAAACCACCCACTGT</u> <u>CACTGGCTCGAGAGTCTGGGCAGAGGCTCTGACCCTCCACCCT</u> <u>TTAAACTGGATGCCGGGGCCTGGCTGGGCCCAATGCCAAGTG</u> <u>GTTATGGCAACCCTGACTATCTGGTCTTAACATGTAGCTCAGG</u> <u>AAGTGGAGGGCGCTAATGTCCCCAATCCCTGGGGATTCTGATT</u> <u>CCAGCTATTCATGTAAGCAGAGCCAACCTGCCTATTTCTGTAG</u> <u>GTGCGACTGGGATGTTAGGAGCACAGCAAGGACCCAGCTCTG</u> <u>TAGGGCTGGTGACCTGATACTTCTCATAATGGCATCTAGAAGT</u> <u>TAGGCTGAGTTGGCTCACTGGCCCAGCAAACCAGAACTGT</u> <u>CTTTGTCCGGGCCATGTTCTTGGGCTGTCTTCTAATTCCAAAG</u> <u>GGTTGGTTGGTAAAGCTCCACCCCTTCTCCTCTGCCTAAAGA</u> <u>CATCACATGTGTATACACACACGGGTGTATAGATGAGTTAAA</u> <u>AGAATGTCTCGCTGGCATCCTAATTTTGTCTTAAGTTTTTTTG</u> <u>GAGGGAGAAAGGAACAAGGCAAGGGAAGATGTGTAGCTTTG</u> <u>GCTTTAACCAGGCAGCCTGGGGGCTCCCAAGCCTATGGAACC</u> <u>CTGGTACAAAGAAGAGAACAGAAGCGCCCTGTGAGGAGTGG</u> </p>
	<p> <u>GATTTGTTTTTCTGTAGACCAGATGAGAAGGAAACAGGCCCT</u> <u>GTTTTGTACATAGTTGCAACTTAAATTTTTTGGCTTGCAAAAT</u> <u>ATTTTTGTAATAAAGATTTCTGGGTAACAATAAAAAAAAAAA</u> <u>AAAAAA (SEQ ID NO: 1)</u> </p>
<p> Mouse SIRPα Protein NP_031573.2 </p>	<p> <u>MEPAGPAPGRLGPLLLCLLLSASCFTGATGKELKVTQPEKSVSV</u> <u>AAGDSTVLNCTLTSLLPVGPWRVYRGVGPSRLLIYSFAGEYVPRI</u> <u>RNVSDTTKRNNMDFSRISNVT PADAGIYYCVKFQKGSSEPDEI</u> <u>QSGGGTEVYVLAKPSPPEVSGPADRGIPDQKVNFCKSHGFSPRN</u> <u>ITLKWFKDQGELHPLETTVNPSGKNVSYNISSTVRVVLNSMDVN</u> <u>SKVICEVAHITLDRSPLRGIANLSNFIRVSPTVKVTQQSPTSMNQV</u> <u>NLTCRAERFYPEDLQLIWLENGNVS RNDTPKNLTKN TDGTYNYT</u> <u>SLFLVNSSAHREDVVF TCQVKHDQQPAITRNHTVLGF AHSSDQG</u> <u>SMQTFPDNNATHNWNVFIGVGVACALLVLLMAALYLLRIKQKKAK</u> <u>CTCTCTDIIEDEYNADFTKGDNTNININITYADIMDEKVKPADAD</u> </p>

	<p> <i>GSISSIRLHEPEKNARETIQIQDINDINDITADLNLFPENKAPAPKAP EPNNHTEYASJETGKVPRPEDTLTYADLDMVHLSRAQPAPKPEPSFS EYASVQVQRK (SEQ ID NO: 2)</i> </p>
Human SIRPα DNA NM_001040022.1	<p> TCCGGCCCCGCACCCACCCCCAAGAGGGGCCTTCAGCTTTGGG GCTCAGAGGCACGACCTCCTGGGGAGGGTTAAAAGGCAGACG CCCCCCCCGCCCCCGCGCCCCCGCGCCCCGACTCCTTCGCCGC CTCCAGCCTCTCGCCAGTGGGAAGCGGGGAGCAGCCGCGCGG CCGGAGTCCGGAGGCGAGGGGAGGTTCGGCCGCAACTTCCCCG GTCCACCTTAAGAGGACGATGTAGCCAGCTCGCAGCGCTGAC CTTAGAAAAACAAGTTTGCGCAAAGTGGAGCGGGGACCCGGC CTCTGGGCAGCCCCGGCGGCGCTTCCAGTGCCTTCCAGCCCTC GCGGGCGGCGCAGCCGCGGCCCATGGAGCCCCGCGGCCCGGC CCCCGGCCGCTCGGGCCGCTGCTCTGCCTGCTGCTCGCCGCG TCCTGCGCCTGGTCAGGAGTGGCGGGTGAGGAGGAGCTGCAG GTGATTCAGCCTGACAAGTCCGTGTTGGTTGCAGCTGGAGAG ACAGCCACTCTGCGCTGCACTGCGACCTCTCTGATCCCTGTGG GGCCCATCCAGTGGTTCAGAGGAGCTGGACCAGGCCGGGAAT TAATCTACAATCAAAAAGAAAGGCCACTTCCCCCGGGTAACAA CTGTTTCAGACCTCACAAAGAGAAACAACATGGACTTTTCCAT CCGCATCGGTAACATCACCCAGCAGATGCCGGCACCTACTA CTGTGTGAAGTTCCGGAAAGGGAGCCCCGATGACGTGGAGTT TAAGTCTGGAGCAGGCACTGAGCTGTCTGTGCGCGCCAAACC CTCTGCCCCCGTGGTATCGGGCCCTGCGGCGAGGGGCCACACCT CAGCACACAGTGAGCTTCACCTGCGAGTCCCACGGCTTCTCAC CCAGAGACATCACCTGAAATGGTTCAAAAATGGGAATGAGC TCTCAGACTTCCAGACCAACGTGGACCCCGTAGGAGAGAGCG TGTCCTACAGCATCCACAGCACAGCCAAGGTGGTGCTGACCC GCGAGGACGTTCACTCTCAAGTCATCTGCGAGGTGGCCACG TCACCTTGCAAGGGGGACCTCTTCGTGGGACTGCCAACTTGTC TGAGACCATCCGAGTTCCACCCACCTTGAGGTTACTCAACAG CCCGTGAGGGCAGAGAACCAGGTGAATGTCACCTGCCAGGTG AGGAAGTTCTACCCCCAGAGACTACAGCTGACCTGGTTGGAG AATGGAAACGTGTCCCGGACAGAAACGGCCTCAACCGTTACA GAGAACAAGGATGGTACCTACAACCTGGATGAGCTGGCTCCTG GTGAATGTATCTGCCCACAGGGATGATGTGAAGCTCACCTGC CAGGTGGAGCATGACGGGCAGCCAGCGGTCAGCAAAAAGCCAT GACCTGAAGGTCTCAGCCCACCCGAAGGAGCAGGGCTCAAAT </p>
	<p> ACCGCCGCTGAGAACACTGGATCTAATGAACGGAACATCTAT ATTGTGGTGGGTGTGGTGTGCACCTTGCTGGTGGCCCTACTGA TGGCGGCCCTCTACCTCGTCCGAATCAGACAGAAGAAAGCCC AGGGCTCCACTTCTTCTACAAGGTTGCATGAGCCCCGAGAAGA ATGCCAGAGAAATAACACAGGACACAAATGATATCACATATG CAGACCTGAACCTGCCCAAGGGGAAGAAGCCTGCTCCCCAGG CTGCGGAGCCCCAACAACACACGAGTATGCCAGCATTGAGA CCAGCCCGCAGCCCGCGTCGGAGGACACCCTCACCTATGCTG ACCTGGACATGGTCCACCTCAACCGGACCCCCAAGCAGCCGG CCCCAAGCCTGAGCCGTCTTCTCAGAGTACGCCAGCGTCCA GGTCCCGAGGAAGTGAATGGGACCGTGTTTGTCTAGCACC CATCTCTACGCGCTTTCTTGTCCACAGGGAGCCGCCGTGATG AGCACAGCCAACCCAGTTCCCGGAGGGCTGGGGCGGTGCAGG CTCTGGGACCCAGGGGCCAGGGTGGCTCTTCTCTCCCCACCCC TCCTTGGCTCTCCAGCACTTCCTGGGCAGCCACGGCCCCCTCC CCCCACATTGCCACATACCTGGAGGCTGACGTTGCCAAACCA GCCAGGGAACCAACCTGGGAAGTGGCCAGAAGTGCCTGGGGT </p>

	<p> CCAAGAACTCTTGTCCTCCGTCATCAUATGIGGGTTTGA AGACCCTCGACTGCCTCCCCGATGCTCCGAAGCCTGATCTCC AGGGTGGGGAGGAGAAAATCCCACCTCCCCTGACCTCCACCA CCTCCACCACCACCACCACCACCACCACCACCCTACCACCAC CACCCAACTGGGGCTAGAGTGGGGAAGATTTCCCTTTAGAT CAAACTGCCCCTTCCATGGAAAAGCTGGAAAAAACTCTGGA ACCCATATCCAGGCTTGGTGAGGTTGCTGCCAACAGTCCTGGC CTCCCCCATCCCTAGGCTAAAGAGCCATGAGTCCTGGAGGAG GAGAGGACCCCTCCCAAAGGACTGGAGACAAAACCTCTGCT TCCTTGGGTCCCTCCAAAGACTCCCTGGGGCCCCAACTGTGTTGC TCCACCCGGACCCATCTCTCCCTTCTAGACCTGAGCTTGCCCC TCCAGCTAGCACTAAGCAACATCTCGCTGTGGACGCCTGTAA ATTACTGAGAAATGTGAAACGTGCAATCTTGAAACTGAGGTG TTAGAAAACCTGATCTGTGGTGTTTTGTTTTGTTTTTTCTTA AAACAACAGCAACGTGATCTTGGCTGTCTGTCTATGTGTTGAAG TCCATGGTTGGGTCTTGTGAAGTCTGAGGTTTAAACAGTTTGT GTCCTGGAGGGATTTTCTTACAGCGAAGACTTGAGTTCCTCCA AGTCCCAGAACCCCAAGAATGGGCAAGAAGGATCAGGTCAGC CACTCCCTGGAGACACAGCCTTCTGGCTGGGACTGACTTGGCC ATGTTCTCAGCTGAGCCACGCGGCTGGTAGTGCAGCCTTCTGT GACCCCGCTGTGGTAAGTCCAGCCTGCCCAGGGCTGCTGAGG GCTGCCTCTTGACAGTGCAGTCTTATCGAGACCCAATGCCTCA GTCTGCTCATCCGTAAAGTGGGGATAGTGAAGATGACACCCC TCCCACCACCTCTCATAAGCACTTTAGGAACACACAGAGGG TAGGGATAGTGGCCCTGGCCGTCTATCCTACCCCTTTAGTGAC CGCCCCCATCCCGGCTTTCTGAGCTGATCCTTGAAGAAGAAAT CTTCATTTCTGCTCTCAAACCCTACTGGGATCAAACCTGGAAT AAATTGAAGACAGCCAGGGGGATGGTGCAGCTGTGAAGCTCG GGCTGATTCCCCCTCTGTCCAGAAAGGTTGGCCAGAGGGTGTG ACCCAGTTACCCCTTAACCCCAACCTTCCAGTCGGGTGTGAG GGCTGACCGGGGCCAGGGCAAGCAGATGTCGCAAGCCCTAT TTATTCAGTCTTCACTATAACTCTTAGAGTTGAGACGCTAATG TTCATGACTCCTGGCCTTGGGATGCCCAAGGGATTTCTGGCTC AGGCTGTAAAAGTAGCTGAGCCATCCTGCCCATCCTGGAGG TCCTACAGGTGAAACTGCAGGAGCTCAGCATAGACCCAGCTC </p>
	<p> TCTGGGGGATGGTCACCTGGTGATTTCAATGATGGCATCCAGG AATTAGCTGAGCCAACAGACCATGTGGACAGCTTTGGCCAGA GCTCCCGTGTGGCATCTGGGAGCCACAGTGACCCAGCCACCT GGCTCAGGCTAGTTCCAAATTCCAAAAGATTGGCTTGTA AAC TTCGTCTCCCTCTCTTTTACCCAGAGACAGCACATACGTGTGC ACACGCATGCACACACACATTCAGTATTTTAAAAGAATGTTTT CTTGGTGCCATTTTCATTTTATTTTATTTTAACTCTTGAGG GGGAAATAAGGGAATAAGGCCAAGGAAGATGTATAGCTTTAG CTTTAGCCTGGCAACCTGGAGAATCCACATACCTTGTGTATTG AACCCAGGAAAAGGAAGAGGTGCAACCAACCCTGCGGAAG GAGCATGGTTTCAGGAGTTTATTTTAAAGACTGCTGGGAAGGA AACAGGCCCATTTTGTATATAGTTGCAACTTAACTTTTTGG CTTGCAAAATATTTTGTATAAAGATTTCTGGGTAATAATGA (SEQ ID NO: 3) </p>
Human SIRPα Protein NP_001035111.1	<p> MEPAGPAPGRLGPLLCLLLAASCAWSGVAGEEELQVIQPKSVL VAAGETATLRCTATSLIPVGPIQWFRGAGPGRELIYNQKEGHFPR VTTVSDLTKRNNMDFSIRIGNITPADAGTYCYVKFRKGSPPDVEF KSGAGTELSVRAPKPSAPVVSGPAARATPQHTVSFTCESHGFSRDR ITLKWFKNGNELSDFTQNVDPVGESVSYSIHSTAKVVLTRDVHS QVICEVAHVTLQGDPLRGTANLSETIRVPPTLEVQQPVRANQV NVTCOVRKFYPORIOLTWLENGNVSRFTETASTVTENKDGTYNW </p>

	<p>MSWLLVNVSAHRDDVKLTQCVEHDGQPAVSKSHDLKVSAPHK EQGSNTAAENTGSNERNIYVVGWVCTLLVALLMAALYLVRIRQKKA QGSTSSTRLHEPEKNAREITQDTNDITYADLNLPKGKKPAPQAAEPN NHTEYASIQTSPQPASEDTLTYADLDMVHLNRTPKQPAPKPEPSFSEY ASVQVPRK (SEQ ID NO: 4)</p>
Humanized SIRPα Protein	<p>MEPAGPAPGRLGPLLLCLLLSASCFCTGVAGEEELQVIQPKSVL VAAGETATLRCTATSLIPVGPIQWFRGAGPGRELIYNQKEGHFPR VTTVSDLTKRNNMDFSRIGNITPADAGTYCCKFRKGSPPDVEF KSGAGTELSVRAKPSAPVVSGPAARATPQHTVSFTCESHGFSRDR ITLKWFKNGNELSDFQTNVDPVGESVSYSHSTAKVVLTRDVDHS QVICEVAHVTLQGDPLRG TANLSETIRVPPTLEVTQQPVRAENQV NVTCQVRKFYPQRLQLTWLENGNVSRTETASTVTENKDGTYNW MSWLLVNVSAHRDDVKLTQCVEHDGQPAVSKSHDLKVSAPHK EQGSNTAADNNATHNWNVFIGVGVACALLVLLMAALYLLRIKQKK AKGSTSSTRLHEPEKNAREITQDTNDINDITYADLNLPKEKKPAPR APEPNNHTEYASITGKVP RPEDTLTYADLDMVHLSRAQPAPKPEPS FSEYASVQVQRK (SEQ ID NO: 5)</p>

Humanized SIRPα Non-Human Animals

[0074] Non-human animals are described that express humanized SIRPα proteins on the surface of immune cells (e.g., myeloid cells) of the non-human animals resulting from a genetic modification of an endogenous locus of the non-human animal that encodes a SIRPα protein. Suitable examples described herein include rodents, in particular, mice.

[0075] A humanized SIRPα gene, described herein may comprise genetic material from a heterologous species (e.g., humans), wherein the humanized SIRPα gene encodes a SIRPα protein that comprises the encoded portion of the genetic material from the heterologous species. A humanized SIRPα gene described herein may comprise genomic DNA of a heterologous species that corresponds to the extracellular portion of a SIRPα 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 SIRPα gene are also described.

[0076] An endogenous SIRPα gene may be deleted. An endogenous SIRPα gene may be altered, wherein a portion of the endogenous SIRPα gene is replaced with a heterologous sequence (e.g., a human SIRPα sequence in whole or in part). All or substantially all of an endogenous SIRPα gene may be replaced with a heterologous gene (e.g., a human SIRPα gene). A portion of a heterologous SIRPα gene may be inserted into an endogenous non-human SIRPα gene at an endogenous SIRPα locus. The heterologous gene may be a human gene. The modification or humanization may be made to one of the two copies of the endogenous SIRPα gene, giving rise to a non-human animal is heterozygous with respect to

the humanized SIRP α gene. A non-human animal is described that is homozygous for a humanized SIRP α gene.

[0077] A non-human animal described herein contains a human SIRP α gene in whole or in part at an endogenous non-human SIRP α locus. Thus, such non-human animals can be described as having a heterologous SIRP gene. The replaced, inserted or modified SIRP α gene at the endogenous SIRP α locus 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. The non-human animal may be heterozygous with respect to the humanized SIRP α gene

[0078] A humanized SIRP α gene described herein may include a SIRP α gene that has a second, third and fourth 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, third and fourth exon that appear in a human SIRP α gene of Table 3.

[0079] A humanized SIRP α gene described herein may include a SIRP α 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 nucleotides 352 - 1114 that appear in a human SIRP α cDNA sequence of Table 3.

[0080] A humanized SIRP α protein produced by a non-human animal described herein may have an extracellular portion 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 extracellular portion of a human SIRP α protein that appears in Table 3.

[0081] A humanized SIRP α protein produced by a non-human animal described herein may have an extracellular portion 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 amino acid residues 28 - 362 that appear in a human SIRP α protein of Table 3.

[0082] A humanized SIRP α protein produced by a non-human animal described herein may have 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 SIRP α protein that appears in Table 3.

[0083] Compositions and methods for making non-human animals that expresses a humanized SIRP α protein, including specific polymorphic forms or allelic variants (e.g., single amino acid differences), are described, including compositions and methods for making non-human animals that expresses such proteins from a human promoter and a human regulatory sequence. Compositions and methods for making non-human animals that expresses such proteins from an endogenous promoter and an endogenous regulatory sequence are also described. The methods include inserting the genetic material encoding a human SIRP α protein in whole or in part at a precise location in the genome of a non-human animal that

corresponds to an endogenous SIRP α gene thereby creating a humanized SIRP α gene that expresses a SIRP α protein that is human in whole or in part. The methods may include inserting genomic DNA corresponding to exons 2 - 4 of a human SIRP α gene into an endogenous SIRP α gene of the non-human animal thereby creating a humanized gene that encodes a SIRP α protein that contains a human portion containing amino acids encoded by the inserted exons.

[0084] A humanized SIRP α gene approach employs a relatively minimal modification of the endogenous gene and may result in natural SIRP α -mediated signal transduction in the non-human animal, because the genomic sequence of the SIRP α sequences are modified in a single fragment and therefore retain normal functionality by including necessary regulatory sequences. Thus, the SIRP α gene modification may not affect other surrounding genes or other endogenous SIRP genes. Further, the modification may not affect the assembly of a functional receptor on the plasma and maintains normal effector functions via binding and subsequent signal transduction through the cytoplasmic portion of the receptor which is unaffected by the modification.

[0085] A schematic illustration (not to scale) of an endogenous murine SIRP α gene and a humanized SIRP α gene is provided in Figure 1. As illustrated, genomic DNA containing exons 2 - 4 of a human SIRP α gene is inserted into an endogenous murine SIRP α gene locus by a targeting construct. This genomic DNA includes comprises the portion of the gene that encodes an extracellular portion (e.g., amino acid residues 28 - 362) of a human SIRP α protein responsible for ligand binding.

[0086] A non-human animal (e.g., a mouse) having a humanized SIRP α gene at the endogenous SIRP α locus can be made by any method known in the art. For example, a targeting vector can be made that introduces a human SIRP α gene in whole or in part with a selectable marker gene. Figure 1 illustrates a mouse genome comprising an insertion of exons 2 - 4 of a human SIRP α . As illustrated, the targeting construct contains a 5' homology arm containing sequence upstream of exon 2 of an endogenous murine SIRP α gene, followed by a genomic DNA fragment containing exons 2 - 4 of a human SIRP α gene, a drug selection cassette (e.g., a neomycin resistance gene flanked on both sides by loxP sequences), and a 3' homology arm containing sequence downstream of exons 4 of an endogenous murine SIRP α gene. Upon homologous recombination, exons 2 - 4 of an endogenous murine SIRP α gene is replaced by the sequence contained in the targeting vector. A humanized SIRP α gene is created resulting in a cell or non-human animal that expresses a humanized SIRP α protein that contains amino acids encoded by exons 2- 4 of a human SIRP α gene. The drug selection cassette may optionally be removed by the subsequent addition of a recombinase (e.g., by Cre treatment).

[0087] In addition to mice having humanized SIRP α genes as described herein, also described herein are other genetically modified non-human animals that comprise humanized SIRP α genes. Such non-human animals may comprise a humanized SIRP α gene operably linked to an endogenous SIRP α promoter. Such non-human animals may express a humanized SIRP α

protein from an endogenous locus, wherein the humanized SIRP α protein comprises amino acid residues 28 - 362 of a human SIRP α protein.

[0088] Such non-human animals may be selected from the group consisting of a mouse, rat, rabbit, pig, bovine (e.g., cow, bull, buffalo), deer, sheep, goat, chicken, cat, dog, ferret, primate (e.g., marmoset, rhesus monkey). For the non-human animals where suitable genetically modifiable ES cells are not readily available, other methods are employed to make a non-human animal comprising genetic modifications as described herein. Such methods include, e.g., modifying a non-ES cell genome (e.g., a fibroblast or an induced pluripotent cell) and employing nuclear transfer to transfer the modified genome to a suitable cell, e.g., an oocyte, and gestating the modified cell (e.g., the modified oocyte) in a non-human animal under suitable conditions to form an embryo.

[0089] A non-human animal described herein may be a mammal. A non-human animal described herein may be a small mammal, e.g., of the superfamily Dipodoidea or Muroidea. A genetically modified animal described herein may be a rodent. A rodent described herein may be selected from a mouse, a rat, and a hamster. A rodent may be selected from the superfamily Muroidea. A genetically modified animal described herein may be 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, with-tailed rats, Malagasy rats and mice), Platacanthomyidae (e.g., spiny dormice), and Spalacidae (e.g., mole rates, bamboo rats, and zokors). A genetically modified rodent described herein may be selected from a true mouse or rat (family Muridae), a gerbil, a spiny mouse, and a crested rat. A genetically modified mouse described herein may be a member of the family Muridae. A non-human animal described herein may be a rodent. A rodent described herein may be selected from a mouse and a rat. In some aspects, a non-human animal described herein may be a mouse.

[0090] 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. A mouse may be 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 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. A mouse described herein may be a BALB strain, e.g., BALB/c strain. A mouse described herein may be is a mix of a BALB strain and another aforementioned strain.

[0091] A non-human animal described herein may be a rat. A rat described herein may be

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 SIRP α Genes

[0092] SIRP α mutant and transgenic non-human animals (e.g., mice) have been reported (Inagaki et al., 2000, EMBO Journal 19(24):6721-6731; Strowig et al., 2011, Proc. Nat. Acad. Sci. 108(32):13218-13223). Such animals have been employed in a variety of assays to determine the molecular aspects of SIRP α expression, function and regulation. However, they are not without limitation. For example, use of SIRP α mutant mice have been limited due to deleterious health conditions resulting from an inability of cells containing the mutant form of SIRP α to signal. Further, because CD47, a ligand for SIRP α , might be present on the same cell as the mutant form of SIRP α and both proteins are capable of providing intracellular signals, it is not possible to distinguish if such results are from lack of SIRP α signaling or lack of CD47 binding. In the case of human SIRP α transgenic mice, mouse SIRP α is intact and functional. Thus, SIRP α -dependent functions in various biological processes (e.g., engraftment) cannot be clearly attributed to either human SIRP α or mouse SIRP α function alone in these mice as both the human and mouse SIRP α receptors are present and functional.

[0093] Non-human animals described herein may provide an improved *in vivo* system and source of biological materials (e.g., cells) expressing human SIRP α that are useful for a variety of assays. Non-human animals described herein may be used to develop therapeutics that target SIRP α and/or modulate SIRP α -CD47 signaling. Mice of the present disclosure may be used to screen and develop candidate therapeutics (e.g., antibodies) that bind to human SIRP α . Non-human animals described herein may be used to determine the binding profile of antagonists and/or agonists a humanized SIRP α on the surface of a cell of a non-human animal as described herein.

[0094] Non-human animals described herein may be used to measure the therapeutic effect of blocking or modulating SIRP α signal transduction (e.g., phosphorylation) and the effect on gene expression as a result of cellular changes. A non-human animal described herein or cells isolated therefrom may be exposed to a candidate therapeutic that binds to a human SIRP α on the surface of a cell of the non-human animal and, after a subsequent period of time, analyzed for effects on SIRP α -dependent processes, for example, B and/or T cell proliferation, clearance of platelets, and induction of cytokine expression.

[0095] Non-human animals described herein may express humanized SIRP α protein, thus cells, cell lines, and cell cultures can be generated to serve as a source of humanized SIRP α for use in binding and functional assays, e.g., to assay for binding or function of a SIRP α antagonist or agonist, particularly where the antagonist or agonist is specific for a human SIRP α sequence or epitope. A humanized SIRP α protein expressed by a non-human animal as

described herein may comprise a variant amino acid sequence. Variant human SIRP α proteins having variations associated with ligand binding residues have been reported. Non-human animals described herein may express a humanized SIRP α protein variant. The variant may be polymorphic at an amino acid position associated with ligand binding. Non-human animals described herein may be used to determine the effect of ligand binding through interaction with a polymorphic variant of human SIRP α .

[0096] Cells from non-human animals described herein may be isolated and used on an ad hoc basis, or can be maintained in culture for many generations. Cells from a non-human animal described herein may be immortalized and maintained in culture indefinitely (e.g., in serial cultures).

[0097] Cells of non-human animals described herein may be used in a cell migration or spreading assay to screen and develop candidate therapeutics that modulate human SIRP α . Such processes are necessary for many cellular processes including wound healing, differentiation, proliferation and survival.

[0098] Cells of non-human animals described herein may be used in clonal assays for megakaryocytic colony-forming cells for testing the pharmaco-toxicological aspects of candidate therapeutics that target human SIRP α .

[0099] Cells of non-human animals described herein may be used in phagocytosis assays to determine the therapeutic potential of compounds or biological agents to modulate SIRP α -dependent regulation of phagocytosis.

[0100] Non-human animals described herein may provide an *in vivo* system for the analysis and testing of a drug or vaccine. 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. Such drugs or vaccines may be improved and/or developed in such non-human animals.

[0101] Non-human animals described herein may provide improved *in vivo* system elucidating mechanisms of human cell-to-cell interaction through adoptive transfer. Non-human animals described herein may be implanted with a tumor xenograft, followed by a second implantation of tumor infiltrating lymphocytes could be implanted in the non-human animals by adoptive transfer to determine the effectiveness in eradication of solid tumors or other malignancies. Such experiments may be done with human cells due to the exclusive presence of human SIRP α without competition with endogenous SIRP α of the non-human animal. Further, therapies and pharmaceuticals for use in xenotransplantation can be improved and/or developed in such non-human animals.

[0102] Non-human animals described herein may provide an improved *in vivo* system for maintenance and development of human hematopoietic stem cells through engraftment. Non-

human animals described herein may provide improved development and maintenance of human stem cells within the non-human animal. Increased populations of differentiated human B and T cells may be observed in the blood, bone marrow, spleen and thymus of the non-human animal. Non-human animals described herein may provide an increase in the level of engraftment of human cells as compared to non-human animals that express both mouse and human SIRP α .

[0103] Non-human animals described herein may be employed to assess the efficacy of a therapeutic drug targeting human cells. A non-human animal described herein may be transplanted with human cells, and a drug candidate targeting such human cells is administered to such 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 dalton, 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.

[0104] The drug may be an anti-cancer drug, and the human cells 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 may be 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.

[0105] The anti-cancer drug may be an antibody molecule which binds to an antigen on human cancer cells. The anti-cancer drug may be a bispecific 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.

[0106] A non-human animal described herein may be engrafted with human immune cells or cells that differentiate into human immune cells. Such non-human animal with engrafted human immune cells is transplanted with human cancer cells, and is administered with an anti-cancer drug, such as a bispecific antibody that binds to an antigen on human cancer cells and to an antigen on human immune cells (e.g., T-cells). The therapeutic efficacy of the bispecific antibody can be evaluated based on its ability to inhibit growth or metastasis of the human cancer cells in the non-human animal. The non-human animal described herein may be engrafted with human CD34+ hematopoietic progenitor cells which give rise to human immune cells (including T cells, B cells, NK cells, among others). Human B cell lymphoma cells (e.g., Raji cells) are transplanted into such non-human animal with engrafted human immune cells, which is then administered with a bispecific antibody that binds to CD20 (an antigen on normal B cells and certain B cell malignancies) and to the CD3 subunit of the T-cell receptor, to test the ability of the bispecific antibody to inhibit tumor growth in the non-human animal.

EXAMPLES

[0107] 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 described herein, and are not intended to limit the scope of what the inventors regard as their invention. Unless indicated otherwise, temperature is indicated in Celsius, and pressure is at or near atmospheric.

Example 1. Humanization of an endogenous signal-regulatory protein (SIRP) gene.

[0108] This example illustrates exemplary methods of humanizing an endogenous gene encoding signal-regulatory protein alpha (SIRP α) in a non-human mammal such as a rodent (e.g., a mouse). Human SIRP α is known to exist in at least 10 allelic forms. The methods described in this example can be employed to humanize an endogenous SIRP α gene of a non-human animal using any human allele, or combination of human alleles (or allele fragments) as desired. In this example, human SIRP α variant 1 is employed for humanizing an endogenous SIRP α gene of a mouse.

[0109] A targeting vector for humanization of an extracellular region of a SIRP (e.g., SIRP α) gene was constructed using VELOCIGENE[®] technology (see, e.g., U.S. Pat. No. 6,586,251 and Valenzuela et al. (2003) High-throughput engineering of the mouse genome coupled with high-resolution expression analysis, *Nature Biotech.* 21(6):652-659).

[0110] Briefly, mouse bacterial artificial chromosome (BAC) clone bMQ-261H14 was modified to delete the sequence containing exons 2 to 4 of an endogenous SIRP α gene and insert exons 2 to 4 of a human SIRP α gene using human BAC clone CTD-3035H21. The genomic DNA corresponding to exons 2 to 4 of an endogenous SIRP α gene (-8555 bp) was replaced in BAC clone bMQ-261H14 with a -8581 bp DNA fragment containing exons 2 to 4 of a human SIRP α gene from BAC clone CTD-3035H21. Sequence analysis of the human SIRP α allele contained in BAC clone CTD-3035H21 revealed the allele to correspond to human variant 1. A neomycin cassette flanked by loxP sites was added to the end of the -8581 bp human DNA fragment containing exons 2 to 4 of the human SIRP α gene (Figure 1).

[0111] Upstream and downstream homology arms were obtained from mouse BAC DNA at positions 5' and 3' of exons 2 and 4, respectively, and added to the -8581 bp human fragment-neomycin cassette to create the final targeting vector for humanization of an endogenous SIRP α gene, which contained from 5' to 3' a 5' homology arm containing 19 kb of mouse DNA 5' of exon 2 of the endogenous SIRP α gene, a -8581 bp DNA fragment containing exons 2 to 4 of a human SIRP α gene, a neomycin cassette flanked by loxP sites, and a 3' homology arm containing 21 kb of mouse DNA 3' of exon 4 of an endogenous SIRP α gene. Targeted insertion of the targeting vector positioned the neomycin cassette in the fifth intron of a mouse SIRP α gene between exons 4 and 5. The targeting vector was linearized by digesting with Swal and

then used in homologous recombination in bacterial cells to achieve a targeted replacement of exons 2 to 4 in a mouse SIRP α gene with exons 2 to 4 of a human SIRP α gene (Figure 1).

[0112] The targeted BAC DNA (described above) was used to electroporate mouse ES cells to create modified ES cells comprising a replacement of exons 2 to 4 in an endogenous mouse SIRP α gene with a genomic fragment comprising exons 2 to 4 of a human SIRP α gene. Positive ES cells containing a genomic fragment comprising exons 2 to 4 of a human SIRP α gene were identified by quantitative PCR using TAQMAN[™] probes (Lie and Petropoulos, 1998. Curr. Opin. Biotechnology 9:43-48). The nucleotide sequence across the upstream insertion point included the following, which indicates endogenous mouse sequence upstream of the insertion point (contained within the parentheses below) linked contiguously to a human SIRP α genomic sequence present at the insertion point: (AGCTCTCCTA CCACTAGACT GCTGAGACCC GCTGCTCTGC TCAGGACTCG ATTTCCAGTA CACAATCTCC CTCTTTGAAA AGTACCACAC ATCCTGGGGT) GCTCTTGCAT TTGTGTGACA CTTTGCTAGC CAGGCTCAGT CCTGGGTTCC AGGTGGGGAC TCAAACACAC TGGCACGAGT CTACATTGGA TATTCTTGGT (SEQ ID NO: 6). The nucleotide sequence across the downstream insertion point at the 5' end of the neomycin cassette included the following, which indicates human SIRP α genomic sequence contiguous with cassette sequence downstream of the insertion point (contained within the parentheses below with loxP sequence italicized): GCTCCCCATT CCTCACTGGC CCAGCCCCTC TTCCCTACTC TTTCTAGCCC CTGCCTCATC TCCCTGGCTG CCATTGGGAG CCTGCCCCAC TGGAAGCCAG (TCGAG *ATAACTTCGTATAATGTATGCTATACGAAGTTAT* ATGCATGGCC TCCGCGCCGG GTTTTGCGC CTCCGCGGG CGCCCCCTC CTCACGGCGA) (SEQ ID NO: 7). The nucleotide sequence across the downstream insertion point at the 3' end of the neomycin cassette included the following, which indicates cassette sequence contiguous with mouse genomic sequence 3' of exon 4 of an endogenous SIRP α gene (contained within the parentheses below): CATTCTCAGT ATTGTTTTGC CAAGTTCTAA TTCCATCAGA CCTCGACCTG CAGCCCCTAG ATAAGTTCGT ATAATGTATG CTATACGAAG TTATGCTAGC (TGTCTCATAG AGGCTGGCGA TCTGGCTCAG GGACAGCCAG TACTGCAAAG AGTATCCTTG TTCATACCTT CTCCTAGTGG CCATCTCCCT GGGACAGTCA) (SEQ ID NO: 8). 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, F0 generation mice that are essentially fully derived from the donor gene-targeted ES cells allowing immediate phenotypic analyses Nature Biotech. 25(1):91-99) to generate a litter of pups containing an insertion of exons 2 to 4 of a human SIRP α gene into an endogenous SIRP α gene of a mouse.

[0113] Targeted ES cells described above were used as donor ES cells and introduced into an 8-cell stage mouse embryo by the VELOCIMOUSE[®] method (supra). Mice bearing the humanization of exons 2 to 4 of an endogenous SIRP α gene were identified by genotyping using a modification of allele assay (Valenzuela et al., supra) that detected the presence of the human SIRP α gene sequences.

[0114] Mice bearing the humanized SIRP α gene construct (i.e., containing human SIRP α

exons 2 to 4 in a mouse SIRP α gene) can be bred to a Cre deleter mouse strain (see, e.g., International Patent Application Publication No. WO 2009/114400) in order to remove any loxed neomycin cassette introduced by the targeting vector that is not removed, e.g., at the ES cell stage or in the embryo. Optionally, the neomycin cassette is retained in the mice.

[0115] Pups are genotyped and a pup heterozygous for the humanized SIRP α gene construct is selected for characterization.

Example 2. Expression of humanized SIRP α in non-human animals.

[0116] This example illustrates the characteristic expression of SIRP α protein on the surface of cells from non-human animals engineered to contain an humanized SIRP α gene construct as described in Example 1 at an endogenous SIRP α locus.

[0117] Briefly, spleens were isolated from wild type (WT) and mice heterozygous for a humanized SIRP α gene. Spleens were then perfused with Collagenase D (Roche Bioscience) and erythrocytes were lysed with ACK lysis buffer according to manufacturer's specifications. Cell surface expression of mouse and human SIRP α was analyzed by FACS using fluorochrome-conjugated anti-CD3 (17A2), anti-CD19 (1D3), anti-CD11b (M1/70), anti-human SIRP α (SE5A5), and anti-mouse SIRP α (P84). Flow cytometry was performed using BD LSRFORTESSA[™]. Exemplary expression of human and mouse SIRP α as detected on the surface of CD11b⁺ monocytes is shown in Figure 2.

[0118] As shown in Figure 2, expression of both mouse and humanized SIRP α were clearly detectable on the surface of CD11b⁺ monocytes from heterozygous mice.

Example 3. Human cell engraftment in humanized SIRP non-human animals.

[0119] This example illustrates an improved engraftment of human hematopoietic stem cells in non-human animals having a humanized SIRP α gene.

[0120] Briefly, Rag2 KO IL2R γ^{null} mice with or without a humanized SIRP α gene were raised under pathogen-free conditions. Newborn mice (2 to 5 days old) were irradiated with 240 cGy and injected intra-hepatically with 1×10^5 CD34⁺ human hematopoietic stem cells. The mice were bled 10 to 12 weeks post engraftment and blood was analyzed by FACS using fluorochrome-conjugated anti-human CD45 (HI30), anti-human CD3 (SK7), anti-human CD19 (HIB 19) and anti-mouse CD45 (30-F11) to check for the reconstitution of the human immune system. The genetic background of the mice is BALB/cTa \times 129/SvJae.

[0121] Exemplary percentages of human CD34⁺ cells in wild type, mice heterozygous for

humanized SIRP α , mice homozygous for humanized SIRP α and BALB-Rag2^{-/-}IL2R γ c^{-/-} (DKO) mice are shown in Figures 3 - 5.

[0122] As shown in this example, mice homozygous for a humanized SIRP α gene demonstrate improved engraftment of human CD34⁺ cells by providing the highest percentage of human CD34⁺ cells in the periphery (e.g., blood) as compared to other strains tested.

[0123] Taken together, these data demonstrate that humanized SIRP α is functional in the mice as described herein through expression on the surface of cells in the mouse and begin capable of supporting the engraftment of human CD34⁺ hematopoietic stem cells.

Example 4. Evaluating the efficacy of Ab 1 on Raji Lymphoma Tumor Growth in BRG Mice.

SUMMARY

[0124] Ab 1 is bispecific antibody (bsAb) that binds to CD3, a T cell antigen associated with the T cell receptor (TCR) complex, and CD20, a B cell surface antigen present on normal B cells and several B cell lineage malignancies. Ab 1 is designed to bridge CD20-expressing cells with cytotoxic T cells by binding to the CD3 subunit of the TCR, resulting in CD20-directed polyclonal T cell killing. CD20 is a clinically validated target for immunotherapy; the chimeric antibody rituximab is approved for treatment of Non Hodgkin Lymphomas (NHL) and Chronic Lymphocytic Leukemia (CLL). Although patients may become refractory to rituximab, loss of expression of CD20 is not typically observed. Therefore, a bispecific antibody bridging CD20-positive tumor cells with cytotoxic T cells represents a potential anti-tumor strategy.

[0125] In this study, the effect of treatment with CD20xCD3 bsAb Ab 1 on human B cell lymphoma (Raji) tumor growth was examined in a mouse tumor model. The model utilized hCD34⁺ engrafted BALB/c-Rag2null IL2rnull (BRG) mice that were humanized for SIRP α . These mice, with human T, B, and NK cells, as well as granulocytes, monocytes, and dendritic cells (DCs), were treated with Ab 1 twice weekly, resulting in significant suppression of Raji tumor growth compared to vehicle control and the non-binding control mAb, Control Ab 5. Ab 1 treatment suppressed tumor growth at both 0.4 mg/kg and 0.04 mg/kg with greater significance than the vehicle control group throughout the treatment period (p<0.0001). No significant weight loss was observed in any treatment group. These results show that Ab 1 targets Raji tumors in mice with human immune cells, resulting in significant tumor suppression.

MATERIALS AND METHODS

Materials**Test Compound and Control Antibody**

[0126] Test compound: Ab 1.

[0127] Control antibody: Control Ab 5.

Reagents

[0128]

Table 4: Reagent List

Reagent	Source	Identification
Raji cells	Regeneron core facility	Raji P 1-4-10 Passage #4
Human CD34+ hematopoietic stem cells (HSC) isolated from human fetal livers	Advanced Biosciences Resource, Inc.	
hPBMCs	Reachbio	Catalog #0500-300, Lot #130322
L-Histidine	Amresco	Catalog #181164-100G, Lot #3363E344
Sucrose	Biosolutions	Catalog #BIO640-07, Lot #0816012
RPMI	Irvine Scientific	Catalog #9160, Lot #9160100803
FBS	Tissue Culture Biologicals	Catalog #101, Lot #107062
Penicillin/Streptomycin/L-Glutamine	Gibco	Catalog #10376-016, Lot #1411480
2-Mercaptoethanol	Gibco	Catalog #21985-023, Lot #762405
Anti-human CD45	Invitrogen	Catalog #MHCD4518, Clone H130
Anti-human NKp46	BD Biosciences	Catalog #558051, Clone 9E2
Anti-human CD19	BD Biosciences	Catalog #555412, Clone HIB 19

Reagent	Source	Identification
Anti-human CD3	Invitrogen	Catalog #MHCD0328, Clone S4.1
Anti-human CD14	BD Biosciences	Catalog #557742, Clone M5E2
Anti-human CD45	BD Biosciences	Catalog #557659, Clone 30-F11
BD Fortessa	BD Biosciences	Special Order Instrument

Test Systems

[0129] The tumor studies presented in this report employed 24-32 week old male and female BALB/c-Rag2null IL2rynull (BRG) immunodeficient mice humanized for the signal regulatory protein alpha (SIRP α) gene. These were generated at Regeneron by embryonic stem (ES) cell targeting (Strowig et al., Proc Natl Acad Sci USA, 108(32): 13218-13223 (2011)). Upon recognition of CD47, SIRP α inhibits clearance of CD47 positive cells by macrophages. Previous studies have shown that BRG mice expressing the human SIRP α transgene have enhanced engraftment of human HSC (Strowig et al., Proc Natl Acad Sci USA, 108(32): 13218-13223 (2011)).

[0130] Newborn SIRP α BRG pups were irradiated and engrafted with hCD34+ hematopoietic progenitor cells derived from fetal liver (Traggiai, et al., Science, 304(5667): 104-107 (2004)). These human HSCs give rise to human T, B, and NK cells, as well as granulocytes, monocytes, and dendritic cells (DCs). Due to the low levels of circulating human B cells, there are low levels of circulating human IgG. Furthermore, these mice do not develop germinal centers, lack lymph nodes and have limited T and B cell replenishment if these cells are depleted. Murine monocytes, DCs, and granulocytes remain present as well. Immune cell composition was confirmed by flow cytometry of blood, and mice were randomized by % human CD45 engraftment prior to use in tumor studies. Mice were implanted with Raji tumor cells at Day 0, and the ability of Ab 1 to block tumor growth over 4 weeks was tested. Body weights and tumor volumes were recorded on days 3, 6, 9, 13, 16, 20, 23, 27, 30 and 34 following implantation.

Experimental Design

Reconstitution of Human Immune System in SIRP α BRG Mice

[0131] Immunodeficient BALB/c Rag2 γ c γ (BRG) human SIRP alpha (SIRP α BRG) mice

were bred in the germ-free isolators in the Regeneron animal facility. Neonate mice were irradiated with one dose of 300cGy, 8-24 h prior to injection of human CD34+ hematopoietic stem cells (HSC) isolated from human fetal livers. The engraftment was allowed to develop for 12-16 weeks and the number of engrafted cells was periodically evaluated by flow cytometry. For the entire duration of the experiment, animals were housed in the Regeneron animal facility under standard conditions in a 12-hour day/night rhythm with access to food and water ad libitum. The number of animals per cage was limited to a maximum of 5 mice.

[0132] Mouse blood was analyzed to determine percent engraftment levels prior to initiating the study. Whole blood was collected into two capillary tubes containing 150 μ L of 2% EDTA (ethylenediaminetetraacetic acid; 15 mg/mL). Red blood cells were lysed using ACK lysing buffer for 3 minutes and the buffer was neutralized with PBS (no calcium or magnesium). Cells were blocked with Fc Block for 5 minutes at 4°C and then stained with human CD45, NKp46, CD19, CD3 and CD14 for 30 minutes at 4°C. Samples were analyzed by 5-laser flow cytometry (BD Fortessa). Percent engraftment was determined as the % human CD45+ cells of total cells.

Raji Tumor Study Procedure in SIRP α BRG Mice

[0133] On day 0, groups of 5 SIRP α BRG mice were administered 2×10^6 Raji tumor cells subcutaneously. On the same day, mice were treated with an intraperitoneal (IP) dose of either Ab 1 (0.4 or 0.04 mg/kg), non-binding control mAb Control Ab 5 (which binds a feline antigen with no cross-reactivity to human CD20 or CD3) at a dose of 0.4 mg/kg or vehicle alone. Mice subsequently received two doses of antibody/week for 4 weeks. Tumor growth was measured with calipers on days 3, 6, 9, 13, 16, 20, 23, 27, 30 and 34. Study groups are summarized in Table 5.

Table 5: Summary of Treatment Groups in SIRP α BRG Mice

Groups	Tumor	Antibody	Dose (mg/kg)	Route	Schedule	# Mice
Control Groups	Raji	No antibody (Vehicle alone)	0	IP	2x/wk	5
	Raji	Control Ab 5	0.4	IP	2x/wk	5
Experimental Groups	Raji	Ab 1	0.4	IP	2x/wk	5
	Raji	Ab 1	0.04	IP	2x/wk	5

Specific Procedures

Preparation of Reagents

[0134] Ab 1 and Control Ab 5 were each diluted to the desired concentration in Vehicle (10mM histidine, 5% sucrose, pH 5.8). Raji cells were obtained from the Regeneron core facility (passage 4) and maintained in culture media: RPMI 1640 +10% FBS + Pen Strep-L-Glu + Mercaptoethanol. Raji cells were diluted to the desired concentration in media.

Statistical Analyses

[0135] Statistical analyses were performed utilizing GraphPad software Prism 5.0 (MacIntosh Version). Statistical significance was determined by two-way ANOVA with Tukey's multiple comparisons post-test. Data from each of the readouts were compared across treatment groups. A threshold of $p < 0.05$ was considered statistically significant, as indicated by *. Mice that died prior to the end of study were removed from the combined tumor growth curve (but not the individual mouse growth curve) graphs as indicated and statistical analysis in order to analyze by two-way ANOVA.

RESULTS

Ab 1 Suppresses Raji Tumor Cell Growth in hCD34+ Engrafted SIRP α BRG Mice

[0136] Ab 1 suppressed Raji tumor growth compared to vehicle control and non-binding control in hCD34+ engrafted SIRP α BRG mice (Figure 6). Newborn SIRP α BRG pups were irradiated and engrafted with hCD34+ fetal liver cells as hematopoietic progenitor cells (Traggiai, et al., Science, 304(5667): 104-107 (2004)), which gave rise to human T, B, and NK cells, as well as granulocytes, monocytes, and DCs. On day 0, hCD34+ engrafted SIRP α BRG mice were administered 2×10^6 Raji tumor cells subcutaneously. On the same day, mice were treated with an intraperitoneal (IP) dose of either Ab 1 (0.4 or 0.04 mg/kg) or the non-binding control mAb Control Ab 5, or vehicle control, followed by twice weekly doses throughout the study.

[0137] Compared to the vehicle control groups and the non-binding control groups, Ab 1 significantly suppressed Raji tumor outgrowth administered at doses of 0.04 mg/kg ($p < 0.0001$) or 0.4 mg/kg ($p < 0.0001$) on day 34 post tumor implantation (Figure 7). Furthermore, the effects of Ab 1 treatment were dose-dependent, with 0.4 mg/kg Ab 1 suppressing growth completely throughout the study, as compared to 0.04 mg/kg Ab 1, which suppressed tumor growth completely by Day 30. Neither Ab 1 nor the non-binding control mAb had a significant effect on mouse body weight throughout the study (Figure 8).

CONCLUSION

[0138] The effect of treatment with Ab 1, a CD20xCD3 bsAb, on Raji tumor growth was examined in a mouse model. Ab 1 was effective in tumor growth suppression in hCD34+ engrafted SIRPα BRG mice with human T, B, and NK cells, as well as granulocytes, monocytes, and DCs. Twice weekly treatment with Ab 1 resulted in significant and dose-dependent suppression of Raji human B cell lymphoma tumor growth compared to vehicle control and non-binding control. No significant weight loss was observed in any treatment group. These results show that Ab 1 targets Raji tumors in mice with human immune cells, resulting in significant tumor growth suppression.

Equivalents

[0139] Having thus described several aspects of at least one aspect of this invention, it is to be appreciated by those skilled in the art that various alterations, modifications, and improvements will readily occur to those skilled in the art. Such alterations, modifications, and improvements are intended to be part of this disclosure. Accordingly, the foregoing description and drawing are by way of example only.

[0140] Use of ordinal terms such as "first," "second," "third," etc., in the claims to modify a claim element does not by itself connote any priority, precedence, or order of one claim element over another or the temporal order in which acts of a method are performed, but are used merely as labels to distinguish one claim element having a certain name from another element having a same name (but for use of the ordinal term) to distinguish the claim elements.

[0141] The articles "a" and "an" as used herein in the specification and in the claims, unless clearly indicated to the contrary, should be understood to include the plural referents. Claims or descriptions that include "or" between one or more members of a group are considered satisfied if one, more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process unless indicated to the contrary or otherwise evident from the context. The invention includes aspects in which exactly one member of the group is present in, employed in, or otherwise relevant to a given product or process. The invention also includes aspects in which more than one, or the entire group members are present in, employed in, or otherwise relevant to a given product or process. Furthermore, it is to be understood that the invention encompasses all variations, combinations, and permutations in which one or more limitations, elements, clauses, descriptive terms, etc., from one or more of the listed claims is introduced into another claim dependent on the same base claim (or, as relevant, any other claim) unless otherwise indicated or unless it would be evident to one of ordinary skill in the art that a contradiction or inconsistency would arise. Where elements are presented as lists, (e.g., in Markush group or similar format) it is to be understood that each subgroup of the elements is also disclosed, and any element(s) can be removed from the group. It should be understood that, in general, where the invention, or

aspects of the invention, is/are referred to as comprising particular elements, features, etc., certain embodiments of the invention or aspects of the invention consist, or consist essentially of, such elements, features, etc. For purposes of simplicity those embodiments have not in every case been specifically set forth in so many words herein. It should also be understood that any embodiment or aspect of the invention can be explicitly excluded from the claims, regardless of whether the specific exclusion is recited in the specification.

[0142] Those skilled in the art will appreciate typical standards of deviation or error attributable to values obtained in assays or other processes described herein.

[0143] Publications, websites and other reference materials referenced herein describe the background of the invention and provide additional detail regarding its practice.

REFERENCES CITED IN THE DESCRIPTION

Cited references

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PATENTKRAV:

1. En gnaver, hvis genom består af
et humaniseret SIRPa-gen,
5 hvori det humaniserede SIRPa-gen er operativt forbundet med en SIRPa-
promotor fra en gnaver ved et endogent SIRPa-locus og udtrykker et humaniseret
SIRPa-protein i gnaveren, og
hvori det humaniserede SIRPa-protein omfatter
 - (i) en ekstracellulær del, der er mindst 95 % identisk med
10 aminosyrerester 28-362 af et humant SIRPa-protein i SEQ. ID 4, og
 - (ii) den intracellulære del af et SIRPa-protein fra en gnaver.
2. Gnaveren ifølge patentkrav 1, hvori den ekstracellulære del af det
humaniserede SIRPa- protein omfatter aminosyrerester 28-362 af det humane
SIRPa-protein.
- 15 3. Gnaveren ifølge patentkrav 1 eller 2, hvori det humaniserede SIRPa-protein
omfatter aminosyresekvensen som angivet i SEQ ID NO: 5.
4. Gnaveren ifølge et hvilket som helst af patentkravene 1-3, hvori gnaveren
ikke udtrykker et SIRPa-protein fra en gnaver.
5. Gnaveren ifølge et hvilket som helst af patentkravene 1-4, hvori gnaveren
20 er en rotte.
6. En isoleret celle eller isoleret væv fra en gnaver, hvis genom omfatter
et humaniseret SIRPa-gen,
hvori det humaniserede SIRPa-gen er operativt forbundet med en SIRPa-
promotor fra en gnaver ved det endogene SIRPa-locus fra en gnaver og koder for
25 et humaniseret SIRPa-protein, og
hvori det humaniserede SIRPa-protein omfatter
 - (i) en ekstracellulær del, der er mindst 95 % identisk med
aminosyrerester 28-362 af et humant SIRPa-protein i SEQ. ID 4, og
 - (ii) den intracellulære del af et SIRPa-protein fra en gnaver.
- 30 7. Den isolerede celle eller det isolerede væv fra en gnaver ifølge patentkrav
6, hvori den ekstracellulære del af det humaniserede SIRPa-protein omfatter
aminosyrerester 28-362 af det humane SIRPa-protein.
8. Den isolerede celle eller det isolerede væv fra en gnaver ifølge et hvilket
som helst af patentkravene 6-7, hvori den isolerede celle eller det isolerede væv
35 fra en gnaver er en isoleret celle eller væv fra en rotte.

9. En fremgangsmåde til fremstilling af en gnaver, omfattende:

(a) indsættelse af en human SIRPα nukleinsyresekvens i et endogent SIRPα-locus fra en gnaver i en embryonal stamcelle (ES) fra en gnaver for at danne et humaniseret SIRPα-gen,

5 hvori det humaniserede SIRPα-gen er operativt forbundet med en SIRPα-promotor fra en gnaver ved det endogene SIRPα-locus fra en gnaver og koder for et humaniseret SIRPα-protein, og

hvor det humaniserede SIRPα-protein omfatter

(i) en ekstracellulær del, der er mindst 95 % identisk med
10 aminosyrerester 28-362 af det humane SIRPα-protein i SEQ. ID 4,
og

(ii) den intracellulære del af et SIRPα-protein fra en gnaver,

hvorved opnås en modificeret ES-celle fra en gnaver, der omfatter det humaniserede SIRPα-gen; og

15 (b) at frembringe en gnaver ved hjælp af den modificerede ES-celle opnået i (a).

10. Fremgangsmåden ifølge patentkrav 9, hvori den ekstracellulære del af det humaniserede SIRPα-protein omfatter aminosyrerester 28-362 af det humane SIRPα-protein.

20 11. Fremgangsmåden ifølge patentkrav 9 eller 10, hvori gnaveren er en rotte.

12. En fremgangsmåde til bedømmelse af den terapeutiske effekt af et lægemiddel rettet mod humane celler, der omfatter:

(a) at levere en gnaver ifølge et hvilket som helst af patentkravene 1-5, i hvilken en eller flere humane celler er blevet transplanteret;

25 (b) at indgive en lægemiddelkandidat til gnaveren; og

(c) at monitorere de humane celler i gnaveren for at bestemme den terapeutiske effekt af lægemiddelkandidaten.

13. Fremgangsmåden ifølge patentkrav 12, hvori gnaveren er en rotte.

14. En nukleinsyremålrettet vektor, der omfatter:

30 et humant, genomisk DNA-fragment, der omfatter exon 2, 3 og 4 af et humant SIRPα-gen, flankeret af

en 5' homologigruppe, der omfatter et gnavergenomisk DNA-fragment opstrøms af exon 2 af et gnaver SIRPα-gen, og

35 en 3' homologigruppe, der omfatter et gnavergenomisk DNA-fragment nedstrøms af exon 4 af gnaver SIRPα-genet;

hvor integration af det humane, genomiske DNA-fragment i genomet af en celle fra en gnaver baseret på homolog rekombination resulterer i en udskiftning af exon 2, 3 og 4 af gnaver SIRPα-genet ved et endogent SIRPα-locus fra en gnaver med exon 2, 3 og 4 af det humane SIRPα-gen og danner et humaniseret

5 SIRPα-gen,

hvor det humaniserede SIRPα-gen er operativt forbundet med gnaverens SIRPα-promotor ved det endogene SIRPα-locus fra en gnaver og koder for et humaniseret SIRPα-protein, der omfatter en ekstracellulær del af det humane SIRPα-protein kodet af det humane SIRPα-gen og en intracellulær del af

10 gnaverens SIRPα-protein kodet af det pågældende SIRPα-gen, og

hvor gnaveren enten er en mus eller en rotte.

15. Den nukleinsyremålrettede vektor ifølge patentkrav 14, hvor det humaniserede SIRPα-gen omfatter exon 1, 5, 6, 7 og 8 af det pågældende SIRPα-gnavergen.

DRAWINGS

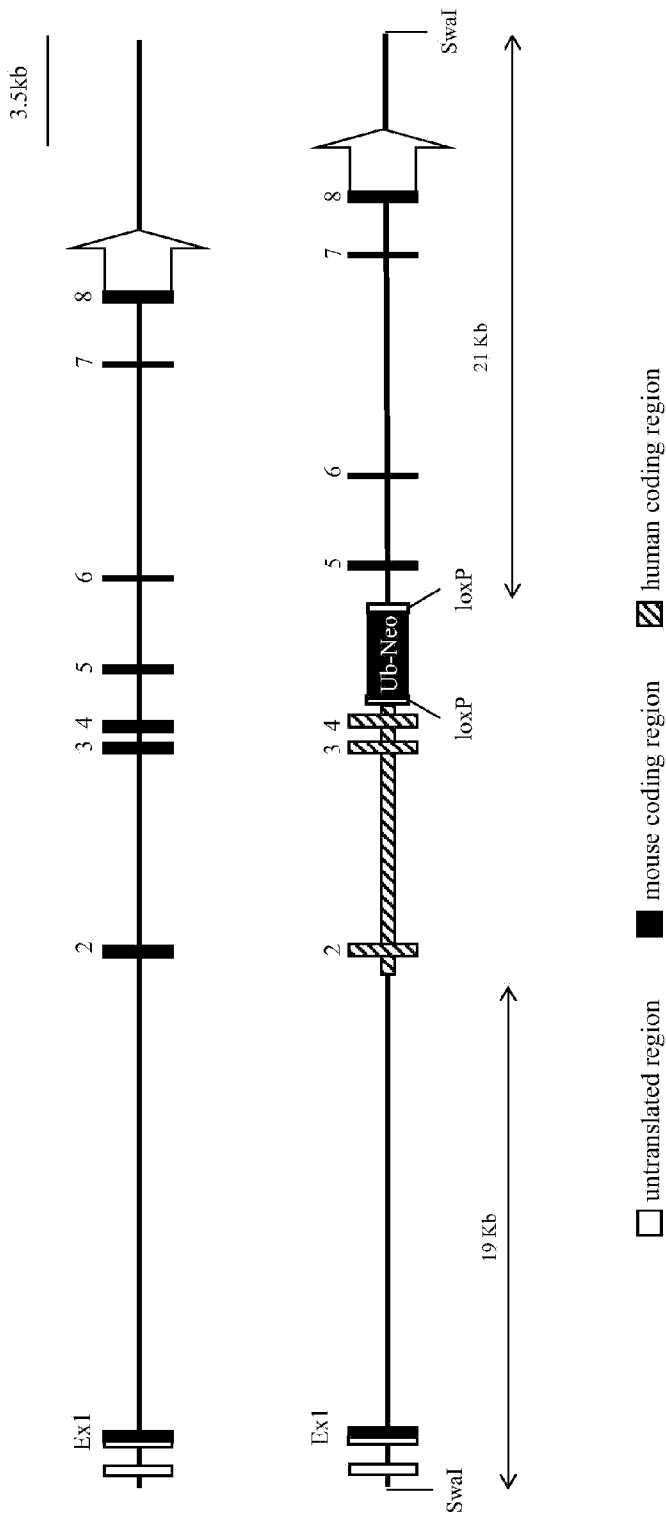


Figure 1

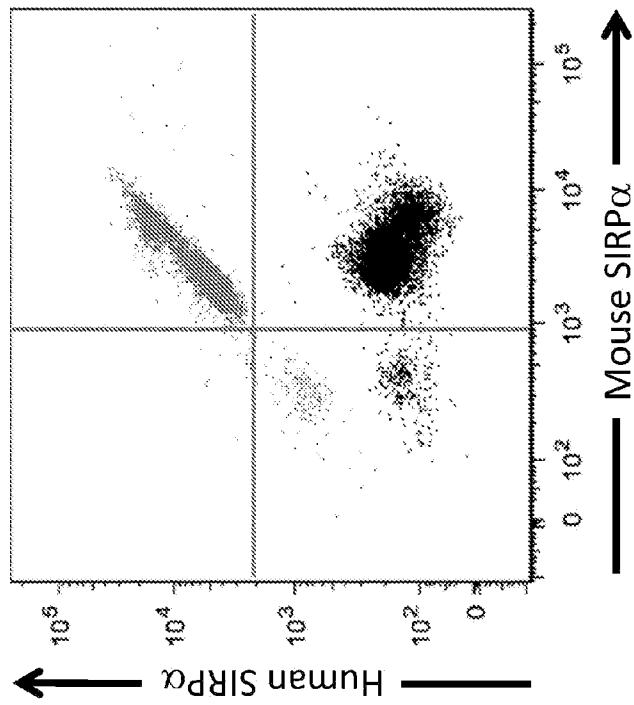


Figure 2

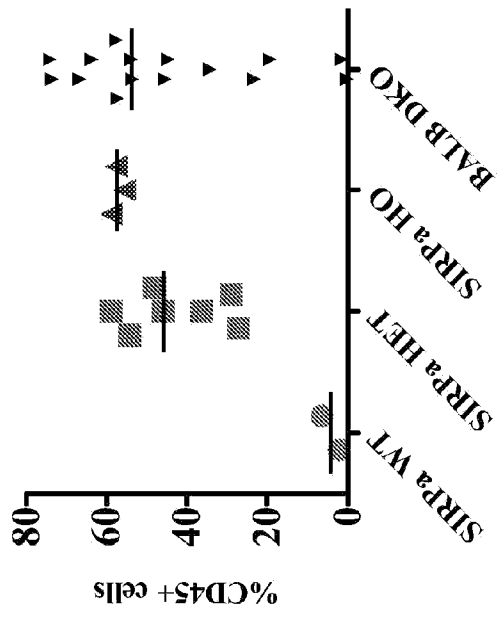


Figure 3

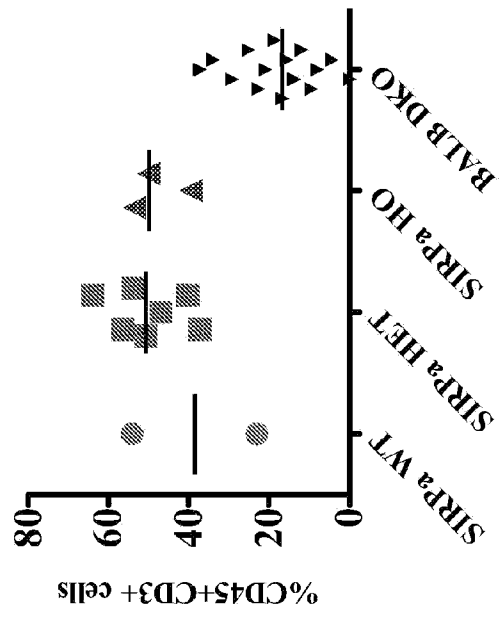


Figure 4

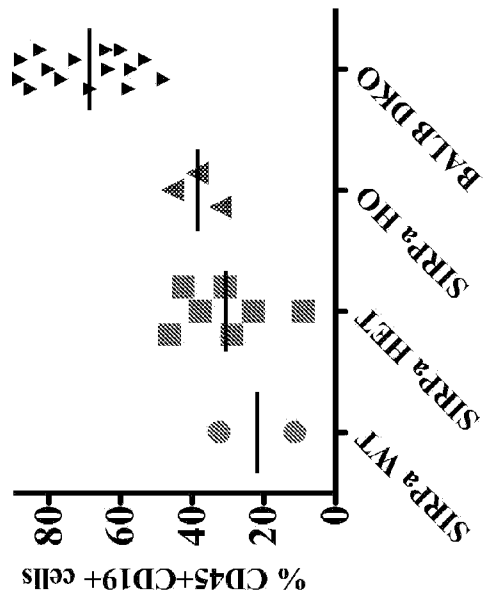


Figure 5

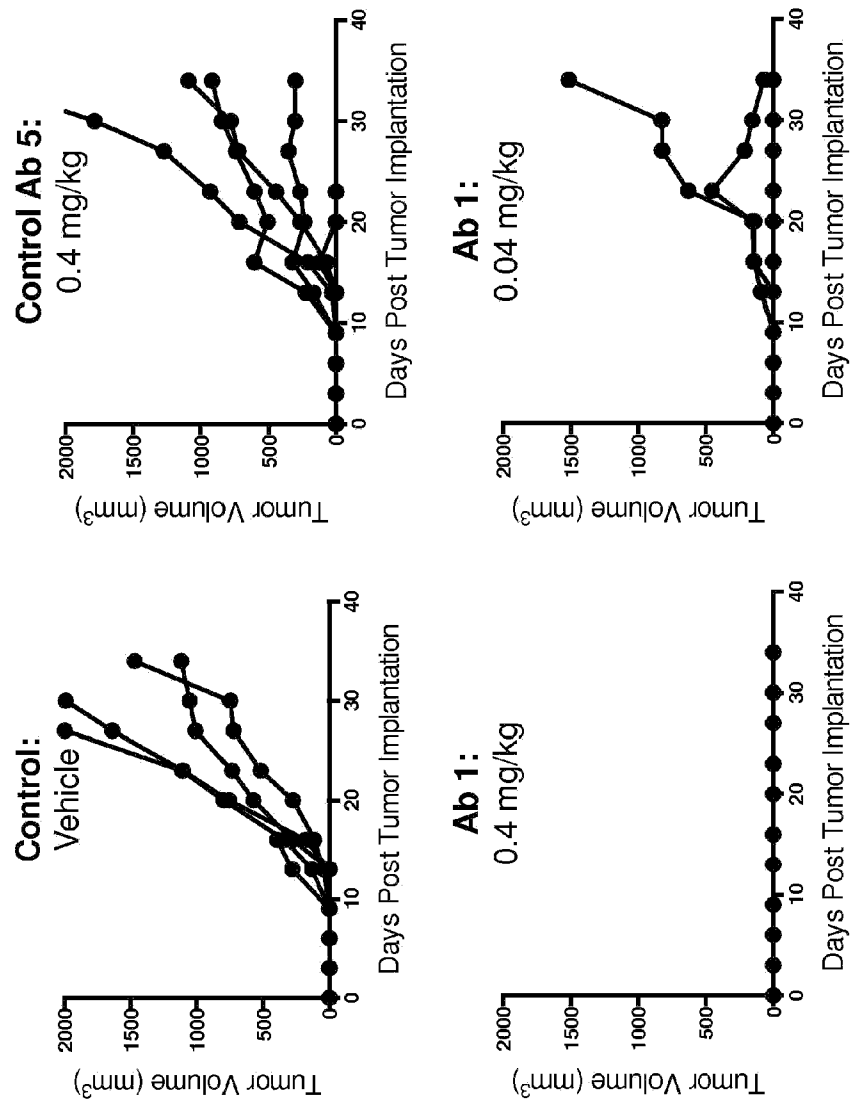


Figure 6

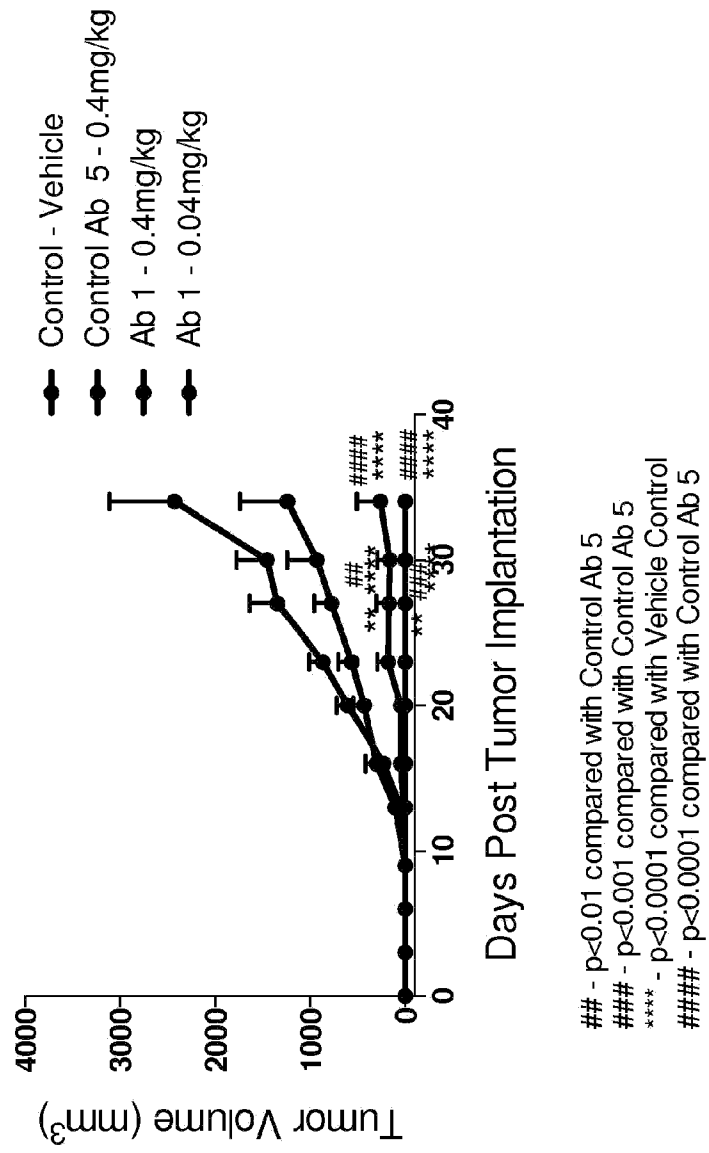


Figure 7

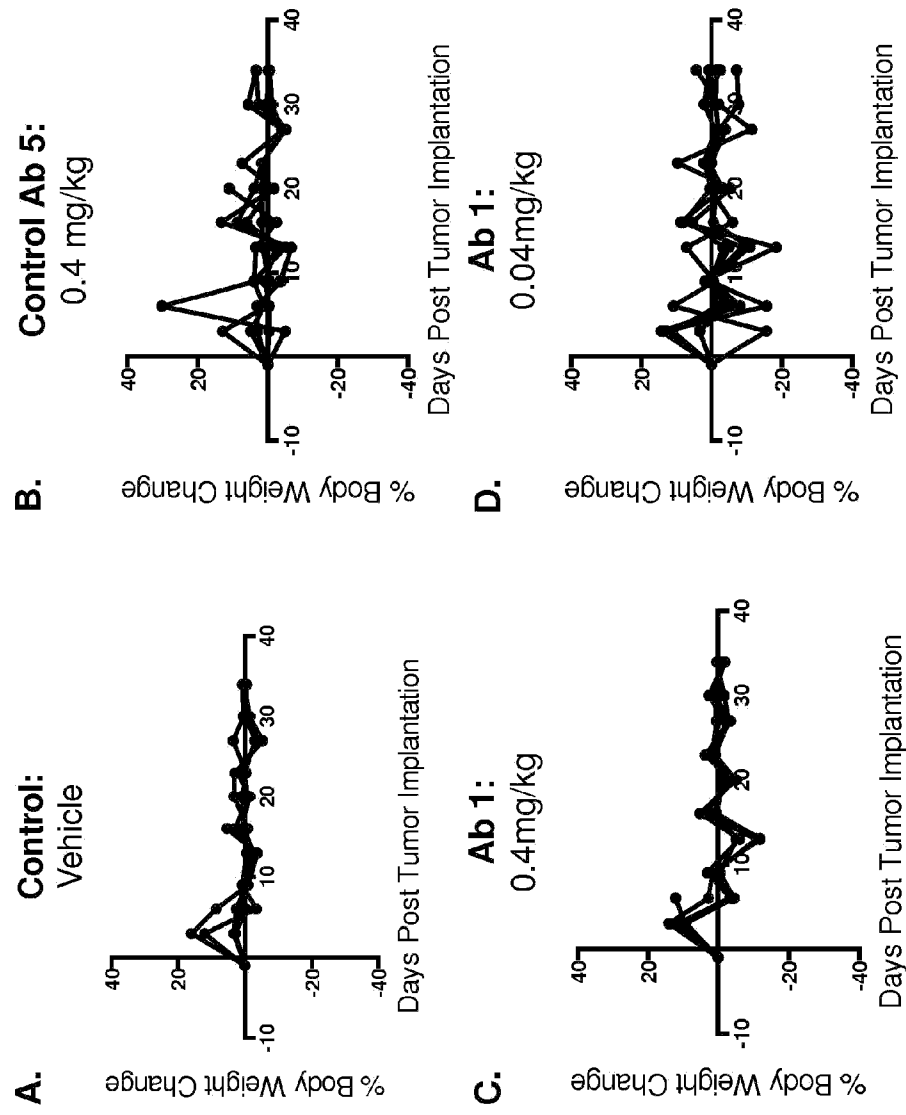


Figure 8

SEKVENSLISTE

Sekvenslisten er udeladt af skriftet og kan hentes fra det Europæiske Patent Register.

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