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(71) Applicant: **MUSC FOUNDATION FOR RESEARCH DEVELOPMENT** [US/US]; 19 Hagood Avenue, Suite 909, Charleston, SC 29425 (US).

(72) Inventors: **RUBINSTEIN, Mark**; c/o Medical University of South Carolina, 86 Jonathan Lucas Street, Charleston, SC 29425 (US). **COLE, David**; c/o Medical University of South Carolina, President's Office, Colcock Hall, 176 Ashley Avenue, MSC 001, Charleston, SC 29425 (US).

(74) Agent: **BYRD, Marshall, P.**; Parker Highlander PLLC, 1120 S. Capital of Texas Highway, Building One, Suite 200, Austin, TX 78746 (US).

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(54) Title: CYTOKINE RECEPTOR GENES AND THE USE THEREOF TO ENHANCE THERAPY

(57) Abstract: Cells, such a T-cells, are provided that comprise cytokine receptors having increased activity in response to their ligand. For example, cell can comprise IL-2 and/or IL- 15 receptors having increased surface expression or signaling activity. Cells of the embodiments have a significant growth advantage in the presence of cytokines and can be used, e.g., for enhanced adoptive cell transfer therapies.

DESCRIPTION

CYTOKINE RECEPTOR GENES AND THE USE THEREOF TO ENHANCE THERAPY

[0001] This application claims the benefit of United States Provisional Patent Application No. 62/106,860, filed January 23, 2015 and 62/242,098, filed October 15, 2015, both of which are incorporated herein by reference.

GOVERNMENT SUPPORT CLAUSE

[0002] The invention was made with government support under Grant No. P01CA54778-01 awarded by the National Institutes of Health. The government has certain rights in the invention.

BACKGROUND OF THE INVENTION

1. Field of the Invention

[0003] The present invention relates generally to the fields of molecular biology, immunology and medicine. In particular, embodiments of the invention relate to transferring of cytokine receptor genes or modulation of cytokine receptor gene expression to enhance sensitivity to cytokine therapy.

2. Description of Related Art

[0004] Recently, there has been increasing interest in cell-based therapies to treat disease. For example, therapies using T-cells targeted to tumor antigens, such as chimeric antigen receptor T-cells, are being studied as potential new anti-cancer therapies. However, for such therapies to be effective, the therapeutic cells must be able to proliferate *in vivo*. Accordingly, many of these therapies rely upon administration of exogenous cytokines, such as IL-2, to the patients being treated. The administration of IL-2 and many other cytokines/protein therapeutics is often limited by dose limiting toxicity. In the case of IL-2, for example, therapeutic effector cells may respond inefficiently to the cytokine. Thus, there remains a need for therapeutic cells with enhanced proliferative capability or enhanced response to cytokines.

SUMMARY OF THE INVENTION

[0005] In a first embodiment there is provided an isolated cell comprising (i) an elevated surface expression level of at least one cytokine receptor or cytokine receptor co-stimulator (*e.g.*, relative to an activated or naïve T-cell); or (ii) a nucleic acid molecule encoding at least one cytokine receptor or cytokine receptor co-stimulator polypeptide comprising a mutation that increases the activity of the receptor. For example, the at least one cytokine receptor can be selected from IL-2R (a receptor for IL-2), IL-15R (a receptor for IL-15), IL-12R (a receptor for IL-12), IL-6R (a receptor for IL-6), GP130 (a receptor for IL-6, IL-35 or IL-27), IL-4R (a receptor for IL-4), IL-2Rγ (a receptor for IL-4 or IL-9), IL-27R (a receptor for IL-27), IL-2Rβ2 (a receptor for IL-35), IL-12Rβ1 (a receptor for IL-23), IL-23R (a receptor for IL-23), IL-9R (a receptor for IL-9), G-CSFR (a receptor for G-CSF and the neupogen or neulasta ligands) or GM-CSF (a receptor for GM-CSF). In a further aspects, the cytokine receptor co-stimulator is ICOS (which binds to ICOS ligand), 4-1BB (which binds to 4-1BB ligand) or CD28 (which binds to B7-1 or B7-2). In some aspects, the cell is a mammalian cell, such as a human cell. In further aspects, the cell is an immune cell, such as a T-cell, Natural Killer (NK) cell or NK T-cell. In specific aspects, the T-cell is a cytotoxic T-cell, an inflammatory T-cell, an effector T-cell or a memory T-cell. In further aspects, the T-cell is a $CD4^+$ or $CD8^+$ T-cell. In further aspects, the T cell is an alpha/beta T cell, a gamma/delta T cell, NK T cell, or other lymphocyte subpopulation. In still further aspects, the cell is comprised in a bone marrow graft cell population.

[0006] Thus, in a further embodiment there is provided an isolated transgenic cell comprising (i) an elevated surface expression level of a cytokine receptor; or (ii) a nucleic acid molecule encoding a cytokine receptor polypeptide comprising a mutation that increases the activity of the receptor (*e.g.*, when bound to the cytokine ligand). For example, in some aspects, the cytokine receptor is proliferative cytokine receptor, such as an IL-2 receptor (*e.g.*, IL-2Rα), GM-CSF receptor (*e.g.*, GM-CSFRα or GM-CSFRβc), G-CSF receptor, IL-12 receptor (*e.g.*, IL-12Rβ1 or IL-12Rβ2) or an IL-15 receptor (*e.g.*, IL-15Rα).

[0007] In further embodiment there is provided an isolated transgenic cell, such as a T-cell, comprising (i) an elevated surface expression level of an IL-2 receptor; or (ii) a nucleic acid molecule encoding an IL-2 receptor polypeptide comprising a mutation that increases the activity of the receptor. For example, the elevated surface expression level of an IL-2 receptor, can be elevated relative to the expression level exhibited by an activated T-

cell. In further aspects of the invention, expression will be elevated versus a naïve or unactivated T cells. In still further aspects, a cell of the embodiments may comprise IL-2R expression that is maintained in culture conditions that would normally down regulate IL-2R. In certain aspects, an IL-2 receptor polypeptide of the embodiments is an IL-2Rα, IL-2Rβ and/or IL-2Rγ polypeptide.

[0008] Thus, in specific aspects, an isolated cell of the embodiments comprises a nucleic acid molecule encoding an IL-2 receptor polypeptide comprising a mutation that increases the activity of the receptor. In particular aspects, the IL-2 receptor is IL-2Rα (also referred to as CD25). In other aspects, the IL-2 receptor is IL-2Rβ (also referred to as CD122) or IL-2Rγ (also referred to as CD132). In some aspects, the mutation increase surface expression, increases stability or increases ligand binding of the IL-2 receptor polypeptide. In certain aspects, the mutation disrupts one or more ribosylation sites on IL2-Rα (see, *e.g.*, Teege *et al.*, 2015, incorporated herein by reference). In further aspects, the signal sequence for recycling and/or endosomal sorting of the IL-2 receptor is modified to alter the natural distribution or re-expression of the receptor (see, *e.g.,* Amano *et al.,* 2013, incorporated herein by reference). In still further aspects, a nucleic acid molecule encoding the IL-2 receptor polypeptide is operably linked to a heterologous promoter. In certain aspects, the heterologous promoter is a ligand inducible or a ligand repressible promoter. In some aspects, the ligand inducible promoter is a tet-on promoter. In certain aspects, the nucleic acid molecule encoding the IL-2 receptor polypeptide is integrated into the genome of the cell or is encoded on an episomal vector. In further specific aspects, the nucleic acid molecule encoding the IL-2 receptor polypeptide is flanked by retroviral long terminal repeats or transposon repeats.

[0009] In a further embodiment there is provided an isolated transgenic cell, such as a T-cell, comprising (i) an elevated surface expression level of an IL-15 receptor; or (ii) a nucleic acid molecule encoding an IL-15 receptor polypeptide comprising a mutation that increases the activity of the receptor. For example, the elevated surface expression level of an IL-15 receptor, can be elevated relative to the expression level exhibited by an activated T-cell. In further aspects of the invention, expression will be elevated versus a naïve or unactivated T cells. In still further aspects, a cell of the embodiments may comprise IL-15R expression that is maintained in culture conditions that would normally down regulate IL-

15R. In certain aspects, an IL-15 receptor polypeptide of the embodiments is an IL-15Rα, IL-2Rβ and/or IL-2Rγ polypeptide.

[0010] Thus, in specific aspects, an isolated cell of the embodiments comprises a nucleic acid molecule encoding an IL-15 receptor polypeptide comprising a mutation that increases the activity of the receptor. In particular aspects, the IL-15 receptor is IL-15Rα. In other aspects, the IL-15 receptor is IL-2Rβ or IL-2Rγ. In some aspects, the mutation increase surface expression, increases stability or increases ligand binding of the IL-15 receptor polypeptide. In further aspects, the signal sequence for recycling and/or endosomal sorting of the IL-15 receptor is modified to alter the natural distribution or re-expression of the receptor (see, *e.g.,* Amano *et al.,* 2013, incorporated herein by reference). In still further aspects, a nucleic acid molecule encoding the IL-15 receptor polypeptide is operably linked to a heterologous promoter. In certain aspects, the heterologous promoter is a ligand inducible or a ligand repressible promoter. In some aspects, the ligand inducible promoter is a tet-on promoter. In certain aspects, the nucleic acid molecule encoding the IL-15 receptor polypeptide is integrated into the genome of the cell or is encoded on an episomal vector. In further specific aspects, the nucleic acid molecule encoding the IL-15 receptor polypeptide is flanked by retroviral long terminal repeats or transposon repeats.

[0011] In yet still a further aspect of the above embodiments, an isolated cell comprises a further transgene, such as a suicide gene, a chimeric antigen receptor (CAR) or a recombinant T-cell receptor (TCR). Such a further transgene may be encoded on the same nucleic acid molecule as a cytokine receptor of the embodiments or may be encoded on a separate molecule. In some aspects, the further transgene is a suicide gene that is operably linked to an inducible promoter. For example, the suicide gene can be a thymidine kinase gene. In further aspects a cell of the embodiments comprises a cell surface marker. For example, the cell can comprise a marker such as CD20 that can be depleted by antibody administration.

[0012] In some aspects, an isolated cell of the embodiments is a T-cell or an NK-cell targeted to an infectious disease or cancer cell antigen. For example, the cancer cell antigen can be an oncogene or a growth factor receptor. In particular aspects, the cancer cell antigen is CD19, CD20, GP240, 5T4, HER1, CD-33, CD-38, VEGFR-1, VEGFR-2, CEA, FGFR3, IGFBP2, IGF-1R, BAFF-R, TACI, APRIL, Fn14, EGFR, ERBB2, ERBB3 or mesothelin. In

further aspects, the isolated cell expresses a chimeric antigen receptor (CAR) or a recombinant T-cell receptor (TCR) targeted to an infectious disease or cancer cell antigen.

[0013] In a further embodiment there is provided a pharmaceutical composition comprising an isolated cell in accordance with any of the embodiments and aspects described above in a pharmaceutically acceptable carrier. In some aspects, the composition comprises between about $1x10^3$ and $1x10^4$, $1x10^5$, $1x10^6$, $1x10^7$, $1x10^8$, $1x10^9$, $1x10^{10}$, $1x10^{11}$ or $1x10^{12}$ cells in accordance with any of the embodiments and aspects described above.

[0014] In still a further embodiment, there is provided a method of providing a T-cell response in a human subject having a disease comprising administering an effective amount of T-cells having increased expression or activity of a cytokine receptor, as described above, to the subject. In certain aspects, the T-cell response is a regulatory T-cell response. In other aspects, the T-cell response is a cytotoxic T-cell response. In further aspects, the method further comprises administering a cytokine that stimulates T-cell proliferation to the subject. In particular aspects, the cytokine is IL-2 or IL-15. In some aspects, the cytokine administered to the subject (*e.g.*, IL-2 or IL-15) comprises a mutation that increases receptor binding. In some aspects, the cytokine has been modified to increase serum half-life. For example, the cytokine can be PEGylated or fused to an Fc polypeptide. In a further aspect, the cytokine may be bound to an antibody or soluble receptor. For example, IL-2 may be bound to anti-IL-2 mAb or IL-15 may be bound to soluble IL-15Rα with or without an Fc fusion (see, *e.g.,* Boyman *et al.*, 2006 and Rubinstein *et al.,* 2006, each of which are incorporated herein by reference).

[0015] In yet a further embodiment there is provided a method for controlling a T-cell response in a subject. In some aspects, such a method comprises identifying a subject who has been treated with a cell population of the embodiments (*e.g.*, cells comprising increased cytokine receptor activity, such as increased IL-2R activity) and administering a therapeutic the subject that selectively kills or inhibits proliferation of the cell population. For example, in the case of a cell population having increased IL-2R activity, a subject can be administered an IL-2-like molecule that is fused or conjugated to a toxin (such as ricin or gelonin). In some aspects, the IL-2-like molecule preferentially binds cells with elevated IL-2Rα expression and leads to their destruction or depletion. In further aspects, a subject can be administered an antibody to the cytokine receptor having increased activity. For example, a subject can be administered an antibody against IL-2Rα (or other receptor subunit protein) to

deplete or destroy cells that are no longer desirable. For instance, the antibody could be daclizumab (anti-IL-2Rα mAb).

[0016] In yet still a further embodiment, there is provided a method of producing therapeutic cells comprising: (i) selecting a population of cells having increased cytokine receptor activity (or cytokine co-receptor activity), for a proliferative cytokine receptor; and (ii) culturing the cells in the presence of a ligand for the proliferative cytokine receptor. In further aspects, the method may further comprise (i) selecting a population of cells having increased cytokine receptor activity (or cytokine co-receptor activity), for a proliferative cytokine receptor; (ii) culturing the cells in the presence of a ligand for the proliferative cytokine receptor, thereby producing an expanded call population; and (iii) selecting cells from the expanded population that do not have increased cytokine receptor activity. For example, an expanded cell population can be treated with and agent that reduces the activity of expression level of the cytokine receptor. In a further aspect, the method comprises (i) selecting a population of cells having increased IL-2 or IL-15 receptor activity; and (ii) culturing the cells in the presence of IL-2 or IL-15. In certain aspects, the cells having increased IL-2 or IL-15 receptor activity express or an elevated level of surface IL-2 or IL-15 receptor on their surface. In further aspects, selecting a population of cells having increased IL-2 or IL-15 receptor activity comprises contacting the cells a drug that increases IL-2 or IL-15 receptor expression. In certain aspects, selecting a population of cells having increased IL-2 or IL-15 receptor activity comprises sorting cells based on IL-2 or IL-15 receptor expression. In some particular aspects, the cells comprise a nucleic acid molecule encoding an IL-2 or IL-15 receptor polypeptide comprising a mutation that increases the activity of the receptor.

[0017] In a further embodiment, there is provided a method of producing cells with elevated receptor or receptor subunit expression (*e.g.*, using methods not requiring the transfer of genetic material). For example, therapeutic cells can be cultured with pharmacological agents before adoptive transfer and/or the pharmacological agents can be administered during or after adoptive transfer. For example, the pharmacological agents can include, without limitation, cytokines, agonists for co-stimulatory molecules, epigenetic drugs, or related compounds. Thus, therapeutic cells can be cultured in a cytokine such as IL-12 and/or IL-18 before adoptive transfer. For example, the therapeutic cells can be cultured in a cytokine such as IL-21 before adoptive transfer. In further aspects, the therapeutic cells

are cultured with an agonist against co-stimulatory molecules such as CD28, ICOS, or 4-1BB. In yet further aspects, the therapeutic cells are cultured with epigenetic drugs that target certain pathways, such as HDAC2 and G9a which repress expression of IL-2Ra (see, *e.g.*, Shin *et al.*, 2013, which is incorporated herein by reference).

[0018] In yet a further embodiment, there is provided a method of producing cells with elevated receptor or receptor subunit expression (*e.g.*, using methods not requiring the transfer of genetic material) comprising sorting or enriching cells for a receptor or a receptor subunit expression prior to culture, during culture, or immediately prior to adoptive transfer. For example, cell can be sorted or selected using an antibody against IL-2Rα (*e.g.*, by fluorescence assisted cell sorting (FACS), column purification or bead sorting of cells expressing elevated levels of IL-2Rα). Such sorted cells may be cultured for an additional period of time or immediately adoptively transferred. This method can optionally be used with cell and methods of the embodiments described herein above. In some aspects, this methodology has the added benefit of allowing the receptor to be used as selectable marker to enrich or isolate genetically modified T cells.

[0019] In still a further embodiment there is provided a method of treating a disease comprising transferring at least one receptor gene into at least one cell and treating said cell or cells with an agonist of the receptor transcribed by said receptor gene. In some aspects, the receptor gene is a cytokine receptor gene. In specific aspects, the cytokine receptor gene is Interleukin-2 receptor α (IL-2Rα). In particular aspects, the agonist is Interleukin-2 (IL-2). In certain aspects, the disease is cancer. In further aspects, the receptor gene is transferred into the at least one cell via adoptive cell therapy. In some aspects, the treatment does not require lymphodepletion. In other aspects, the at least one cell is a donor T cell. In certain aspects, a treatment method of the embodiments may require lower amounts of lymphodepletion relative to currently used clinical protocols for adoptive cell transfer. Furthermore, in some aspects, treatment methods of the embodiments can comprise low dose administration of a cytokine ligand (following adoptive transfer of cells). In particular, because cells of the embodiments have increased activity of a cytokine receptor (*e.g.*, IL-2R), lower doses of the receptor ligand (*e.g.*, IL-2) are effective to provide stimulation of the transferred cells.

[0020] In an additional embodiment, there is provided a method to expand specific populations of cells *in vivo*. In some aspects, patients may be directly injected with a vector

(*e.g.*, a retroviral vector) encoding a CAR linked to an IL-2Rα. Following injection, the patient is administered a IL-2-based therapy and the cells transduced with this vector will preferentially expand. Vectors for use according to the embodiments include, without limitation, a retroviral vector, a lentiviral vector, adenoviral vector, an adeno-associated viral vector or a plasmid vector (*e.g.*, delivered by a gene gun or liposome delivery system). In some cases, the responding cells would be only genetically modified for a short period of time and in other cases the cells would be permanently genetically modified. For example, the vector encoding the CAR and/or cytokine receptor can be an episomal vector or a mRNA vector.

[0021] In a further embodiment there is provided a method for providing an enhanced immune response in a subject. For example, an immunogenic composition can be administered to a subject in conjunction with a pharmacological agents to improve receptor or receptor subunit expression or activity (*e.g.,* to enhance IL-2R or IL-2Rα expression or activity). For instance, following immunization with an antigen the mammalian subject can be given pharmacological agent to improve receptor or receptor subunit expression. Examples of pharmacological agents according to this embodiment include, without limitation, epigenetic drugs targeting HDAC2 or G9a that improve the durability of IL-2Ra expression. In further aspects, a subject can be administered an IL-2-based therapy that will selectively expand those cells responding to vaccination and with elevated IL-2Ra.

[0022] Aspects of the invention provide that genetically transferring cytokine receptor genes, such as the high affinity IL-2Rα, into lymphocytes or other cells, will dramatically enhance sensitivity to cytokine therapy. There are multiple advantages to this approach: 1) It will not be necessary to give high amounts of IL-2, which is associated with life threatening toxicity, as adoptively transferred cells will respond to a much lower dose of IL-2; 2) It may be possible to make cells IL-2 responsive that are not IL-2 responsive; 3) Genetic modification of donor T cells with IL-2Rα allows for effective adoptive cellular therapy strategies in a lymphoreplete environment. Lymphodepletion, which, although highly toxic, is often required by patients undergoing adoptive cellular therapy in order to allow donor cells to efficiently engraft (FIG. 21c). This is thought to be due to the ability of lymphodepletion to increase the levels of endogenous cytokines. By eliminating lymphodepletion, patients may remain eligible for other types of therapies, such as checkpoint inhibition therapy.

[0023] Aspects of the embodiments refer to cytokine receptor activity. As used herein receptor activity refers to signaling from a receptor when bound to the receptor ligand. Thus, a cell having increased receptor activity can have, for example, increased receptor expression, increased receptor expression at the cell surface, increased affinity of the receptor for its ligand (*e.g.*, the ability of the receptor to bind to and/or release ligand), increased receptor stability or increased receptor half-life, all of which increase the signaling activity of the receptor in the presence of ligand.

[0024] Aspects, of the embodiments also refer to receptor genes. As used herein the term receptor gene encompasses genes of all subunits of a particular receptor (*e.g.*, the IL-2R α, β and γ subunits).

[0025] As used herein, "essentially free," in terms of a specified component, is used herein to mean that none of the specified component has been purposefully formulated into a composition and/or is present only as a contaminant or in trace amounts. The total amount of the specified component resulting from any unintended contamination of a composition is therefore well below 0.05%. Most preferred is a composition in which no amount of the specified component can be detected with standard analytical methods.

[0026] As used herein in the specification and claims, "a" or "an" may mean one or more. As used herein in the specification and claims, when used in conjunction with the word "comprising", the words "a" or "an" may mean one or more than one. As used herein, in the specification and claim, "another" or "a further" may mean at least a second or more.

[0027] As used herein in the specification and claims, the term "about" is used to indicate that a value includes the inherent variation of error for the device, the method being employed to determine the value, or the variation that exists among the study subjects.

[0028] Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating certain embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0029] The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

[0030] FIGS. 1a-1e - **IL-2/mAb but not IL-15/sIL-15Rα complexes induce potent effector T cell responses in tumor-bearing mice.** (a) Treatment scheme for B6 mice injected s.c. with B16 melanoma tumor cells 7 days prior to the adoptive transfer of $3x10^6$ pmel-1 Tc1 cells. Mice were then treated with hIL-2/mAb (clone 5355) or hIL-15/sIL-15Rα complexes. (b) Tumor volume from 'a' (n=9/group); each line represents one mouse. (*) Based on a log-rank test and time to sacrifice (at $400mm^2$) for analysis, mice treated with IL-2/mAb complexes had significantly improved outcomes versus each other condition ($p<0.001$ for each comparison). The average tumor areas when treatment was initiated ranged between 15-$20mm^2$ between the 4 groups. (c) The frequency of donor Tc1 cells in the blood of mice (n=4/group) treated as in 'a' but in the absence of tumor. Each point represents the average and bars indicate standard error. (d) The frequency of donor OT-I Tc1 cells in the blood of mice (n=5/group) treated with mIL-2/mAb_{CD122} (clone S4B6) or mIL-2/mAb_{CD25} (clone 1A12). Each point represents the average and bars indicate standard error. (e) The frequency of donor polyclonal T cells in the blood of mice (n=5/group) treated with hIL-2/mAb (clone 5355) complexes or vehicle alone. Each point represents the average and bars indicate standard error. For c-e, (**) indicates a significant difference ($p<0.001$) between indicated and other conditions. Random effects linear regression was used for modeling data and calculating p-values comparing conditions. All results are representative of at least 2 independent experiments.

[0031] FIGS. 2a-2h - **IL-2Rα mediates sustained signaling in effector $CD8^+$ T cells following withdrawal of IL-2.** (a) Diagram of the standard cytokine assay in which effector cells are assayed after incubation with titrated cytokine. (b,c) Levels of pSTAT5 in Tc1 and Tc0 cells that were cultured with increasing amounts of mIL-2 or mIL-15 for 1 hour. (d) As in 'b', except Tc1 cells were incubated as indicated for up to 2 hours with 200ng/ml of cytokine and assayed for pSTAT5. (e) Diagram of the cytokine pulse assay in which effector cells are incubated with saturating amounts of cytokine (200ng/ml). Cells are then washed

thoroughly, recultured at 37°C without additional cytokine, and assayed for pSTAT5. (f) Levels of pSTAT5 in Tc1 cells that were pulsed with mIL-2 with or without anti-IL-2Rα mAb (PC61 clone) for 1 hour, washed, and recultured at 37°C for the times indicated. (g,h) Levels of pSTAT5 in polyclonal effector T cells from wildtype (IL-2Rα$^{+/+}$) or IL-2Rα$^{+/-}$ mice that were pulsed for 1 hour with mIL-2 or mIL-15, and assayed as described in 'e'. Except for 'g' and 'h', all effector cells were generated from pmel-1 mice. All results are representative of at least 3 independent experiments.

[0032] FIGS. 3a-3g - **IL-2Rα facilitates sustained IL-2 signaling through creation of an extracellular reservoir and recycling.** (a) Presence of IL-2 on the surface of polyclonal T cells depends on IL-2Rα. Polyclonal effector CD8$^+$ T cells were pulsed for 2 hours with or without mIL-2. Prior to (and during) the pulse, T cells were incubated with anti-IL-2Rα mAb (PC61). Cells were then washed and stained for surface IL-2. (b) Time course of surface IL-2 on polyclonal T cells after reculture at 37°C. (c) Levels of pSTAT5 in Tc1 cells that were pulsed with IL-2, washed, and recultured at 37°C with or without anti-IL-2 mAb (clone S4B6 or 1A12). (d) Recycling of IL-2 on effector T cells. Pmel-1 Tc1 cells were incubated with hIL-2 or mIL-2 at 37°C for 2 hours. As indicated, cells were then acid washed and recultured at 37°C for 90 minutes in the presence of anti-hIL-2 mAb conjugated to Alexa647. Cells were then washed, fixed, and assayed by flow cytometry. (e) Recycling of IL-2 on pulsed cells while mixed with non-pulsed cells. Pmel-1 Tc1 cells were pulsed with hIL-2 at either 4°C or 37°C for 2 hours, and then acid washed. Cells were then mixed with non-pulsed CFSE-labeled Tc1 cells. The mixed cells were recultured at 37°C for 45 minutes in the presence of anti-hIL-2 mAb conjugated to Alexa647. Cells were then washed, fixed, and assayed by flow cytometry. (f) Internalized IL-2 leads to sustained pSTAT5 signaling. Pmel-1 Tc1 cells were pulsed with hIL-2 at either 4°C or 37°C for 2 hours, and then acid washed. Cells were then recultured at 37°C and assayed for pSTAT5. (g). Subcellular localization of hIL-2 and IL-2Rα (upper panel). Pmel-1 Tc1 cells were pulsed with hIL-2 (or media alone) for 1 hour at 37°C, and stained for hIL-2 and IL-2Rα. Cells were then imaged by confocal microscopy. Subcellular localization of hIL-2 and Rab5 (lower panel). As described for the upper panel, except cells were stained for Rab5. Results are representative of 3 independent experiments.

[0033] FIGS. 4a-4c - **IL-2Rα on donor T cells is critical for persistence in lymphoreplete but not lymphodepleted hosts.** (a) Wildtype and IL-2Rα$^{+/-}$ effector CD8$^+$ T

cells show similar persistence with or without IL-2 therapy. Effector T cells from wildtype and IL-2Rα$^{+/-}$ mice were activated, mixed, and injected into recipient mice (n=3-5/group). Mice received injections of hIL-2/mAb (clone 5355) complexes, hIL-15/sIL-15Rα complexes, or vehicle alone. The proportion of IL-2Rα$^{+/-}$ T cells among all donor T cells in the spleen was determined pre- and post- transfer. Each triangle represents one mouse and the bars indicate the mean. (b) The total number of donor T cells per spleen for the experiment shown in 'a'. The bars indicate the mean. The symbol (**) indicates a significant difference ($p<0.001$) between indicated conditions. (c) The total number of donor T cells in the spleen for the experiment shown in 'a'. Mice (n=5/group) were given total body irradiation (TBI, 600rad) one day prior to adoptive transfer of 10^7 Tc1 (pmel-1) cells, and then treated with hIL-2/mAb (clone 5355) or hIL-15/sIL-15Rα complexes. The frequency of donor T cells in the blood of mice was determined at the indicated time points. Each point represents the average and bars indicate standard error. The symbol (**) indicates a significant difference ($p<0.001$) between control and indicated conditions. All results are representative of 2 independent experiments.

[0034] FIGS. 5a-5b - **IL-2/mAb complexes selectively enhance the persistence of donor T cells.** B6 mice (n=6-7/group) were injected intravenously with $8x10^6$ Tc1 pmel-1 CD8+ T cells. On days 0, 2, 4, 6, as indicated, mice received (i.p.) either hIL-2/mAb (clone 5355) or hIL-15/sIL-15Rα complexes. (a) The frequency of donor CD8+ T cells in the spleens, lymph nodes and liver were determined on day 8. Each triangle represents one mouse and the bar indicates the mean. The symbol (**) indicates a significant difference ($p<0.001$) between indicated conditions. (b) Splenocytes from mice treated as in 'A' were stimulated with or without $hgp100_{25-33}$ peptide for 5 hours. The frequency of donor T cells positive for both IFNγ and TNFα was determined by flow cytometry. Results are representative of 2 independent experiments.

[0035] FIG. 6 - **In the absence of donor T cells, hIL-2/mAb and IL-15/sIL-15Rα complexes mediate comparable anti-tumor immunity.** B6 mice (n=8/group) were injected with B16 tumor cells. The next day, mice were given i.p. injections as indicated of either hIL-2/mAb (clone 5355) or hIL-15/sIL-15Rα complexes for 7 days. (For hIL-2/mAb we used 1 μg of cytokine and 5 μg of antibody, and for hIL-15/sIL-15Rα we used 0.5 μg of cytokine and 2.3 μg of soluble receptor per injection.) Tumors were measured in a blinded fashion twice a week. Each line is representative of one mouse. IL-2/mAb and IL-15/sIL-15Rα

complexes significantly increased the time to sacrifice versus the control condition (log-rank test, <0.05). Results are representative of 2 independent experiments.

[0036] FIG. 7 - **Treatment with IL-2/mAb, IL-2/mAb$_{CD25}$, and IL-15/sIL-15Rα complexes induces differential expansion of CD8$_+$ memory-phenotype T cells, NK cells, and T regulatory cells.** B6 mice (n=5/group) were injected with hIL-2/mAb (clone 5355), hIL-2/mAb$_{CD25}$ (clone 5344.111), or hIL-15/IL15Rα complexes on days 0, 2, 4, and 6. Spleens were harvested on day 8 and stained for T regulatory cells (CD4$_+$CD25$_+$FOXP3$_+$), memory-phenotype (MP) CD8 T cells (CD8$_+$CD44^{hi}), and NK cells (NK1.1$_+$TCRβ-B220-). Mice also received adoptive transfer of Tc1 cells (data not shown). (**, p<0.001 or *, p=0.008) indicates a significant difference between indicated conditions and control. Data is representative of two independent experiments.

[0037] FIG. 8 - **Tc1 but not Tc0 effector CD8$_+$ T cells show preferential responsiveness to IL-2/mAb complexes.** B6 mice (n=6-7/group) were injected intravenously with 8x10$_6$ Tc1 or Tc0 pmel-1 CD8$_+$ T cells. On days 0, 2, 4, 6, as indicated, mice received (i.p.) either hIL-2/mAb (clone 5355) or hIL-15/sIL-15Rα complexes. The top graph shows the frequency of donor CD8$_+$ T cells in the spleens on day 8. The bottom graph shows the absolute number of donor T cells on day 8. Each triangle represents one mouse and the bar indicates the mean. These data are from the same experiment shown in supplemental figure 1. Values were log-transformed prior to comparison of means by two-sample t-tests.

[0038] FIGS. 9a-9b - **Blockade of IL-2Rα has minimal impact on Tc1 cells in response to titrated IL-2(a) or IL-15(b).** Using a standard cytokine responsiveness assay, Tc1 cells from pmel-1 mice were incubated with titrated amounts of mIL-2 or mIL-15 for 30 minutes and assayed for pSTAT5. As indicated, anti-IL-2Rα mAb (PC61) was added at 5 μg/ml. Results are representative of 3 similar experiments.

[0039] FIGS. 10a-10b - **Tc1 effector CD8$_+$ T cells exhibit comparable functional sensitivity to IL-2 and IL-15 *in vitro*.** Tc1 CD8$_+$ T cells generated from pmel-1 TCR transgenic mice were plated with either IL-2 or IL-15. After 48 hours, the frequency of proliferating (a) and viable (b) cells was assayed by Ki67 staining and propidium iodide (PI) exclusion, respectively. Cells were then analyzed by flow cytometry.

[0040] FIGS. 11a-11c - **Tc1 effector CD8$_+$ T cells pulsed with IL-2 mediate sustained cytokine signaling.** (a) In the cytokine pulse assay, Tc1 or Tc0 effector CD8$_+$ T

cells were incubated overnight at 37°C with mIL-2 (200ng/ml), mIL-15 (200ng/ml), or without cytokine. Cells were then washed thoroughly, recultured at 37°C without additional cytokine, and assayed for phosphorylation of STAT5. The frequency of cells staining positive for pSTAT5 are shown for (b) Tc0 and (c) Tc1 cells. Results are representative of 3 independent experiments.

[0041] FIG. 12 - **IL-2 mediated sustained cytokine signaling is IL-2Rα-dependent in 11 independent experiments.** Tc1 cells from pmel-1 mice were pulsed with mIL-2 with or without anti-IL-2Rα mAb (PC61 clone) for 90 minutes, then washed and recultured at 37°C. Cells were harvested at the times indicated and stained for pSTAT5. Each symbol represents one of 11 independent experiments.

[0042] FIGS. 13a-13b - **Tc1 effector CD8+ T cells pulsed with IL-2 exhibit IL-2Rα-dependent proliferation after cytokine withdrawal.** (a) Tc1 cells from pmel-1 TCR transgenic mice were pre-incubated as indicated with anti-IL-2Rα mAb (PC61) or isotype control antibody for 15 minutes. Then, mIL-2 or mIL-15 was added for 2 hours at 37°C. Cells were then washed three times and resuspended in culture media without cytokine for 18 hours. During the last hour of culture, BrdU was added. Cells were then stained for BrdU and CD8, and analyzed by flow cytometry. (b) The frequency of CD8+ T cells positive for BrdU staining in cytokine-treated cultures is indicated by the black line and the number in the upper right quadrant. Control cultures without cytokine are indicated by the shaded histogram. Results are representative of 3 independent experiments.

[0043] FIG. 14 - **Human IL-2 mediates sustained cytokine signaling on mouse Tc1 effector CD8+ T cells.** Tc1 cells from pmel-1 mice were pulsed with hIL-2 with or without anti-IL-2Rα mAb (PC61 clone) for 90 minutes, then washed and recultured at 37°C. Cells were harvested at the times indicated and stained for pSTAT5. Results are representative of 5 independent experiments.

[0044] FIGS. 15a-15b - **Human effector CD8+ T cells pulsed with IL-2 mediate sustained IL-2Rα-dependent signaling.** (a) Human PBMCs activated with plate-bound anti-CD3 mAb for 3 days were pulsed with either hIL-2 or hIL-15 at 37°C for one hour. Effector cells were then washed to remove unbound cytokine and recultured in media without cytokine at 37°C. At the indicated times, cells were fixed and stained for CD8 and pSTAT5. The percentage indicates the frequency of CD8+ T cells staining positive for pSTAT5. (b)

Human PBMCs from two healthy adult donors were activated for 2 days with plate-bound anti-CD3 mAb. Effector cells were then pulsed with hIL-2 in the absence or presence of an anti-IL-2Rα pAb (R&D systems, AB-223-NA) for two hours. pSTAT5 was assessed in these cells at the indicated times in a manner similar to 'a'. For 'a & b', similar results were obtained with $CD8^+$ T cells derived from 4 healthy adult donors.

[0045] FIGS. 16a-16b - **Human IL-2/mAb (clone 5355), but not mouse IL-2/mAb$_{CD122}$ (clone S4B6) complexes, are permissive to IL-2Rα-dependent sustained signaling *in vitro*.** (a) Tc1 cells from pmel-1 TCR transgenic mice were incubated with hIL-2 with or without excess anti-hIL-2 mAb (clone 5355, 10 μg/ml) to generate hIL-2/mAb *in vitro*. In replicate wells, anti-IL-2Rα mAb (clone PC61) was added during the incubation step to block IL-2Rα-dependent signaling. Cells were then washed and recultured at 37°C for the time indicated. Phosphorylation of STAT5 was assessed at the indicated time points by flow cytometry. (b) As in 'a', except mouse IL-2 and anti-mIL-2 mAb (clone S4B6, 10 μg/ml) were used to generate mL-2/mAb$_{CD122}$ *in vitro*. Results are representative of two independent experiments.

[0046] FIG. 17 - **Antibodies for mouse and human IL-2 are species-specific.** Tc1 cells from pmel-1 TCR transgenic mice were pulsed with mIL-2 (200ng/ml), hIL-2 (200ng/ml), or mIFNγ (200ng/ml) for 45 minutes. Cells were then stained with either anti-mIL-2 mAb or anti-hIL-2 mAb directly conjugated to Alexa647 and analyzed by flow cytometry. Data are representative of 3 independent experiments.

[0047] FIGS. 18a-18b - **Detection of hIL-2 by confocal microscopy is species-specific and dependent on pulsing cells with cytokine at 37°C.** (a) Tc1 cells generated from pmel-1 TCR transgenic mice were pulsed with hIL-2 (200ng/ml), mIL-2 (200ng/ml), or media alone (control) for 90 minutes at 37°C. Cells were then fixed, permeabilized, and stained with anti-hIL-2 mAb prior to being mounted onto slides. IL-2 staining in confocal images is represented as a red pseudocolor. (b) As in 'a', except cells were pulsed with hIL-2 at 4°C or 37°C. All results are representative of at least two independent experiments.

[0048] FIG. 19 - **Detection of mIL-2 by confocal microscopy.** (a) Tc1 cells generated from pmel-1 TCR transgenic mice were pulsed with mIL-2 (200ng/ml) or media alone (control) for 90 minutes at 37°C. Cells were then fixed, permeabilized, and stained with

anti-mIL-2 mAb prior to being mounted onto slides. Results are representative of two independent experiments.

[0049] FIGS. 20a-20c - **Colocalization of hIL-2 with EEA-1 and LAMP-1 by confocal microscopy.** Tc1 cells from pmel-1 TCR transgenic mice were pulsed with hIL-2 for 90 minutes at 37°C, and stained for hIL-2, EEA1, or LAMP-1. Cells were then imaged by confocal microscopy to determine the subcellular localization of hIL-2 relative to EEA-1 and LAMP-1. Nine representative images for EEA1/IL-2 and LAMP-1/IL-2 were taken, and scored blindly by three independent observers. The percent colocalization was determined by counting the sum of IL-2 directly colocalizing (yellow) versus IL-2 colocalizing (yellow) plus IL-2 alone (green). Each solid line below denotes readings by one rater of % colocalization of EEA-1 and LAMP-1. "X" values and dashed line indicate estimated colocalization from regression model, adjusting for rater variability. Mean difference in LAMP-1 and EEA-1 colocalization is statistically significant (p=0.010).

[0050] FIGS. 21a-21c - **In lymphodepleted mice, IL-15/sIL-15Rα and hIL-2/mAb mediate comparable engraftment of Tc1 effector CD8+ T cells.** (a) Diagram depicting the ability of IL-2 to preferentially engage IL-2Rα^{hi} donor T cells, while IL-15 requires removal of host cells for equivalent activity on donor T cells. (b) Mice (n=5/group) were treated without (top) or with (bottom) total body irradiation (TBI, 600rad) one day prior to adoptive transfer of 10_7 pmel-1 Tc1 cells. Then on days 0, 2, 4, and 6, mice were treated with hIL-2/mAb (clone 5355) or hIL-15/sIL-15Rα complexes. Spleens were harvested on day 8. Each triangle represents one mouse and the bar indicates the mean. (**) indicates a significant difference ($p<0.001$) between control and indicated conditions. Results are representative of 2 independent experiments. (c) A schematic showing the effect of IL-2 of adoptive cell transfer (ACT) therapy with ACT only, ACT plus lymphodepletion and ACT using cells having increased IL-2R activity (IL-2Rα^{hi}).

[0051] FIG. 22 – An example nucleic acid vector of the embodiments. Shown is a retroviral vector encoding T-cell receptor (TCR) genes (alpha and beta) and an IL-2Rα (CD25) molecule. The TCR could be specific for, as an example, a tumor antigen such as MART-1 or tyrosinase. In this case, the vector could be used to genetically modify T cells, which will then be transferred into a cancer patient. A patient having cells comprising such a vector will be able to respond much more efficiently to IL-2-based therapies.

[0052] FIG. 23 – Low-dose IL-2 leads to preferential expansion of adoptively transferred donor tumor-reactive T cells by engagement of IL-2Rα. B6 mice were injected with 250,000 B16-F1 tumor cells (s.c.). Eight days later, mice were adoptively transferred with $3x10^6$ tumor-reactive activated T cells (pmel-1) conditioned with IL-12 to induce high levels of IL-2Rα. On the day of adoptive T cell transfer, 2 days later, and 4 days later, mice were treated with hIL-2 (1.5ug), hIL-2/mAb complexes (1.5ug hIL-2 and 7.5ug anti-hIL-2 mAb (MAB602)), or hIL-15/sIL-15Rα-Fc complexes (0.5ug hIL-15 + 2.3ug sIL-15Rα-Fc). On day 6 after adoptive transfer, mice were bled and the frequency of donor T cells ($CD8^+Thy1.1^+$) in the peripheral blood was determined. Each triangle represents an individual mouse and the bar indicates the mean. The number in parenthesis and in red indicates the frequency of donor T cells in the blood.

DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

I. The Present Embodiments

[0053] In some aspects, methods detailed herein concern adoptively transferring lymphocytes that have been modified to express elevated levels of cytokine receptor genes or cytokine receptor subunit genes. For example, tumor-reactive T cells can be modified to express IL-2Rα. Upon adoptive transfer, these cells will have enhanced ability to respond to the exogenous ligand. Thus, in in this example, tumor-reactive T cells will have enhance ability to respond to exogenously administered IL-2 or a similar IL-2-based reagent. Cells responding to cytokine have significant advantage for growing and mediating effector functions such as killing tumor cells. Thus, this approach may allow clinicians to administer adoptive cellular therapy without having to precondition patients with chemotherapy or radiation to deplete host lymphocytes which normally compete for cytokine. Moreover, the instant methods provide the ability to genetically modify lymphocytes *in vivo* and provide them cytokine receptor genes or cytokine receptor subunit genes. For example, a subject may be injected with a retroviral vector containing IL-2Ra and a CD19-reactive CAR. Cells modified with such a vector would be very responsive to IL-2-based therapy, and therefore, this method would provide an effective means for expanding such cells. Methods, such those described above, have application for cancer therapy as well as for the treatment of other disease. For example, T regulatory cells might be genetically modified with IL-2Ra, and exhibit improved responsiveness to IL-2 therapy, and thus, this approach could have application for the treatment of autoimmune disease.

[0054] As noted above, in some specific examples, methods of the embodiments can be used to produce cells having enhanced responsiveness to IL-2. Administration of IL-2 is a critical component of many T cell-based strategies for cancer therapy. However, IL-2 has a short half-life and dose limiting toxicity. Furthermore, as IL-2 selectively expands T regulatory cells, it has been proposed that IL-15-based therapies may more effectively support adoptively transferred effector T cells. The findings here show that genetically transferring cytokine receptor genes, such as IL-2Rα, into lymphocytes or other cells dramatically enhances sensitivity to cytokine therapy. This approach is easily adopted for other cytokines or injectable protein therapeutics dependent on receptor expression. For example, the technique could be used to genetically transfer IL-15Rα to modulate lymphocyte responsiveness to IL-15. It is also possible to create chimeric or novel receptors combing different ligand binding and cell signaling properties. It is also possible to genetically add receptors *in vivo* through novel gene transfer techniques. Alternatively, in some instances, receptor genes may be introduced in a transient method (such as RNA electroporation), so that the impact is not long lasting.

[0055] In some embodiments, the treatment entails genetically modifying lymphocytes with other proteins that enhance cytokine receptor gene expression. This could include the transfer of transcription factors that lead to up-regulation of cytokine receptors or enhance the cellular machinery necessary for cytokine responsiveness.

[0056] In another embodiment, receptor expression is modulated in other ways than outlined above. For example, modulation of the levels of IL-2Rβ and IL-2Rγ, either individually, together, or with or without modulation of IL-2Rα, may be done. As part of this, modulation may be done by increasing the expression of these receptors or by inhibiting the expression of the endogenous receptor(s). For example, genetic modification of IL-2Rα and Il-2Rβ may be necessary for optimal responsiveness to IL-2.

[0057] As an additional aspect of the embodiments, mutant or altered versions of IL-2 may be used. For example, a mutant recombinant IL-2 molecule may be used to enhance binding to IL-2Rα. In some cases a mutant IL-2 may also have altered affinity for IL-2Rα dependent on pH (and thus, may undergo differential intracellular trafficking). The treatment may use an IL-2 molecule fused to another protein such as IgG. These altered IL-2 molecules may provide for improved IL-2 responsiveness and act in an additive or synergistic manner to the genetic modification of T cells as proposed above.

[0058] In another embodiment, altered receptor molecules are designed. For example, a version of IL-2Rα with improved sensitivity to IL-2 may be more effective upon genetic modification of lymphocytes.

[0059] In another embodiment, genetic constructs including long terminal repeats (LTR) linking T-cell receptor (TCR) or chimeric antigen receptor (CAR) genes to cytokine receptor subunits are created. For example, TCRα or TCRβ is linked to IL-2Ra, where the TCR genes are reactive against a melanoma tumor antigen. The genetic construct used could be a retroviral vector, lentiviral vector, or any other means of genetically modifying T cells using DNA or RNA. In addition to these genetic elements, other genes may be linked to this construct such as a selectable marker (CD34 or GFP) or a suicide gene to allow killing of the adoptively transferred cell population.

[0060] In some embodiments, this approach is used to modify other cells, such as specific lympohcyte subusets (such as T regulatory cells, Tc1 cells, or Th17 cells), or completely different classes of lympohcytes such as natural killer cells.

[0061] As detailed above, in some embodiments a method of treating a patient is provided. For example, in the case of a patient with metastatic melanoma, who seeks treatment with adoptive cellular therapy, tumor infiltrating lympohcytes (TIL) can be isolated from this patient and expanded to later numbers for adoptive transfer. During this process, the TIL can be genetically modified with a retroviral vector encoding an IL-2R gene. While normally, such a patient might be given lymphodepleting non-myeloablative chemotherapy with cyclophosphamide and fludarabine, with IL-2Rα-modified TIL, this patient may not require such chemotherapy to enhance TIL efficacy or may require a lower dose of chemotherapy. In this situation, the patient may be given low dose IL-2 therapy. Alternatively, the patient could be given another IL-2-based molecule such as an IL-2 fusion protein.

[0062] In a further example, a patient with B cell-derived malignancy, who seeks treatment with adoptive cellular therapy, can have peripheral blood genetically modified with a CAR vector also containing an IL-2Rα gene. In some cases, there may also be a suicide gene in this vector. The patient can be treated with the CAR-IL-2Rα-modified T cells and low dose IL-2. In this case, the patient may not require chemotherapy to suppress the host immune cells.

[0063] In another example, a patient with metastatic melanoma who seeks treatment with adoptive cellular therapy, can have tumor infiltrating lympohcytes (TIL) isolated and expanded to sufficient numbers for adoptive transfer. During this process, the TIL can be genetically modified with a retroviral vector encoding IL-12 receptor (IL-12Rβ1 and/or IL-12Rβ2). In this situation, very low doses of IL-12 may augment ability of TIL to mediate anti-tumor efficacy. In this case, IL-12 can be given at lower doses and may not be toxic to the patient. This example could be applied to any cytokine, ligand, or protein therapy where efficacy is impacted by dose limiting toxicity.

[0064] In still another example, a patient may require a bone marrow transplant. In this case, the bone marrow cells may be genetically modified with a vector encoding IL-2Rα, GM-CSF receptor (GM-CSF receptor α and βc), or G-CSF receptor (GCSF-receptor). In this case, the patient may be given GM-CSF or G-CSF ligand, to induce improved reconstitution of the bone marrow graft or IL-2 to selectively reconstitute T cells within the graft. This technology could be used with any cytokine or ligand receptor system.

[0065] Still another example of method of treatment in accordance with the embodiments concerns a patient with metastatic melanoma who seeks treatment with adoptive cellular therapy. Tumor infiltrating lympohcytes (TIL) can be isolated from this patient and expanded to sufficient numbers for adoptive transfer. During this process, the TIL can be genetically modified with a retroviral vector encoding a mutated IL-2Rα gene. The mutation may eliminate potential ribosylation sites, and therefore make the IL-2Rα more responsive to IL-2 therapy. Alternatively, the IL-2Rα molecule may be mutated so that the intracellular signaling domain from another receptor subunit or costimulatory molecule is engineered into the intracellular portion of IL-2Rα. In this case, the IL-2Rα may improve T cell function in novel ways. For this patient, while normally, he or she may be given lymphodepleting non-myeloablative chemotherapy with cyclophosphamide and fludarabine, with IL-2Rα-modified TIL, this patient may not require such chemotherapy to enhance TIL efficacy. In this situation, the patient may be given low dose IL-2 therapy. Alternatively, the patient could be given another IL-2-based molecule such as an IL-2 fusion protein.

II. Examples

[0066] The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques

disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

Example 1 – Materials and Methods

[0067] *Study Design.* This was a preclinical study to assess the efficacy of cytokine therapy to augment anti-tumor T cell immune responses. The inventors found that IL-2-based therapies were more efficacious than IL-15-based therapies in the tumor model, and thereafter, focused on understanding the mechanism of this differential response. For *in vivo* experiments, the numbers of mice are outlined in the figure legends. For all experiments, the number of independent replicates is outlined in each figure legend. Randomization and blinding for tumor experiments was done as described in the tumor methods below. Additional study design details are also included in the statistical analysis section below.

[0068] *Recombinant proteins and antibodies.* Human (h) IL-15, hIL-2, and anti-hCD3 mAb (clone OKT3) were kindly provided by the NCI Biological Resources Branch Preclinical Repository. Mouse (m) IL-2, mIL-12, and mIL-15 were purchased from Shenandoah Biotechnology. Recombinant sIL-15Ra-Fc (551-MR-100) and anti-hIL-2 mAb (clone 5355) were purchased from R&D systems. Anti-hIL-2 mAb_{CD25} (clone 5344.111) was obtained from BD Bioscience. Anti-mIL-2 mAb_{CD122} (clone S4B6) and anti-IL-2Rα (clone PC61) were obtained from Bioxcell. Anti-mIL-2 mAb_{CD25} (clone JES6-1A12), anti-mCD3 mAb (clone 145-2C11), and anti-mCD28 mAb (clone 37.51) were obtained from the UCSF monoclonal antibody core. Antibodies used for flow cytometric and confocal analysis are described below.

[0069] *Mice and tumor cells.* C57BL/6 (B6), B6.PL (Thy1.1), B6(CD45.1), pmel-1 TCR transgenic, and OT-I TCR transgenic mice were purchased from Jackson Laboratory. All animals were housed under specific pathogen-free conditions in accordance with institutional and federal guidelines. For tumor experiments, B16-F1 cells were obtained from ATCC.

[0070] *T cell cultures.* Mouse Tc1 and Tc0 cells were generated from pmel-1 and OT-I TCR transgenic mice as previously described (Rubinstein *et al.*, 2012). Briefly, splenocytes were cultured for three days with relevant peptide (for pmel-1, $hgp100_{25-33}$ peptide (KVPRNQDWL) and for OT-I, $OVA_{257-264}$ peptide (SIINFEKL)) and cultured with (Tc1) or without (Tc0) mIL-12 (10ng/ml). Polyclonal mouse T cells were generated by culturing B6 splenocytes for three days with plate-bound anti-CD3 mAb (clone 145-2C11, 1ug/ml) unless otherwise stated. Activated human T cells were generated by culturing de-identified human PBMCs (Research Blood Components) from healthy adult donors for two or three days with plate-bound anti-CD3 mAb (clone OKT3, 1ug/ml).

[0071] *Tumor and persistence studies in mice.* For tumor experiments, B6 mice were challenged subcutaneously with $2.5x10^5$ B16-F1 tumor cells. Prior to randomizing mice to treatment groups, some mice were excluded due to abnormal tumor growth. As indicated, mice were treated by adoptive transfer of activated T cells (Tc1 or Tc0) by intravenous tail vein injection. Cytokine complexes were administered by intraperitoneal injection on days 0, 2, 4, and 6 after adoptive transfer unless otherwise indicated. Cytokine complexes used include: hIL-15/sIL-15Rα, hIL-15 (0.5ug)/sIL-15Ra-Fc (2.3ug); hIL-2/mAb, hIL-2 (1.5ug)/anti-IL-2 mAb (7.5ug, clone 5355); hIL-2/mAb_{CD25}, hIL-2 (1.5ug)/anti-IL-2 mAb (7.5ug, clone 5344.111); mIL-2/mAb_{CD122}, mIL-2 (1.5ug)/anti-IL-2 mAb (7.5ug, clone S4B6); and mIL-2/mAb_{CD25}, mIL-2 (1.5ug)/anti-IL-2 mAb (7.5ug, clone JES6-1A12). Tumor growth was measured by caliper every 2-4 days by personnel blinded to the treatment regimen. Tumor surface area (mm^2) was calculated as length x width. Mice were sacrificed when tumors reached $400mm^2$. For persistence studies, mice received adoptive transfer of activated T cells (Tc1 or Tc0). Peripheral blood lymphocytes or indicated organs were stained for CD8 and either Thy1.1 or CD45.1 to identify donor T cells. In experiments with a mixed transfer, the inventors used effector T cells from wildtype (Thy1.1) and IL-2Rα$^{+/-}$ (Thy1.2) mice that were activated with plate-bound anti-CD3/anti-CD28 mAb, mixed, and transferred into B6(CD45.1) mice.

[0072] Where indicated, mice also received total body irradiation (600rad) one day prior to adoptive T cell transfer. In all adoptive transfer experiments, donor and recipient mice were gender-matched and were 6-12 weeks of age. All animals were housed under specific pathogen-free conditions in accordance with institutional and federal guidelines.

[0073] *Flow cytometry.* Flow cytometry analysis was performed as previously described (*17*). The antibodies used in this study include CD8 (53-6.7), CD25 (PC61), CD45.1 (A20), IFNγ (XMG1.2), STAT5 pY694 (47/Stat5(pY694)), Thy1.1 (A20), and TNFα (TN3-19.12).These were purchased from BD Bioscience, Biolegend (San Diego, CA), and eBioscience (San Diego, CA). For analysis of phosphorylation of STAT5, the inventors followed the manufacturer's protocol using Lyse/Fix and PermIII buffer (BD Bioscience). To examine cellular proliferation, cells were fixed and permeabilized according to the manufacturer's protocol for Cytofix/Cytoperm (BD Bioscience) and stained with anti-Ki67 mAb (SolA15, eBioscience). Alternatively, BrdU (10μm) was added one hour prior to harvest, and cells were analyzed for BrdU incorporation as previously described (Rubinstein *et al.*, 2008). For Foxp3 staining, the inventors followed the protocol outlined in the Foxp3 kit (eBioscience). Flow cytometry was performed on BD LSRII and BD FACSAccuri. Data were analyzed using FlowJo software (TreeStar). In all experiments, initial gating of live cells was performed using forward scatter and side scatter parameters, and cells were then gated on live lymphocytes. Isotype and fluorescence minus one (FMO) controls were performed as required. For experiments assessing IL-2, the inventors always included control conditions without IL-2 pulsing.

[0074] *In vitro experiments.* For functional assays, Tc1 or Tc0 cells were incubated with cytokines and assayed for pSTAT5, Ki67, BrdU, or propidium iodide exclusion. For pulse assays, cells were incubated with or without cytokine at 200ng/ml at either 4°C or 37°C for 90 minutes unless otherwise indicated. Cells were then washed at least three times, replated without cytokine, and assayed for pSTAT5. When added during the pulse step, anti-IL-2Rα mAb was added 15 minutes prior to cytokine addition. Acid wash was performed by washing cells twice for 2 minutes at 4°C with an acid wash buffer consisting of complete media adjusted to pH3.5 or pH3.75 with 1N HCl. For analysis of recycling of IL-2 to the cell surface, acid washed cells were replated in media at 37°C for the indicated amount of time with anti-IL-2 mAb conjugated to Alexa647. To assess IFNγ and TNFα production, the inventors added $hgp100_{25-33}$ (1ug/ml) or PMA (50ng/mL) and ionomycin (1μM) to splenocytes for 6 hours in the presence of brefeldin A (GolgiStop, BD Bioscience).

[0075] *Confocal Microscopy.* Tc1 cells were incubated with hIL-2 (200ng/ml), mIL-2 (200ng/ml), or no cytokine, for 1 hour at either 4°C or 37°C unless otherwise stated. Cells were washed, fixed, and permeabilized using the Cytofix/Cytoperm protocol. To determine

the subcellular localization of internalized IL-2 by confocal microscopy, cells were stained with anti-hIL-2 mAb and either anti-IL-2Rα pAb (R&D systems), anti-Rab5 mAb (C8B1, Cell Signaling), anti-LAMP1 mAb (1D4B, company), or anti-EEA1 mAb (C45B10, Cell Signaling). To detect anti-IL-2Rα, the inventors used an anti-goat IgG conjugated to Alexa488 (R&D systems). To detect EEA-1 and Rab5, the inventors used an anti-rabbit IgG conjugated to Alexa488 ($F(ab')_2$ fragment, Cell Signaling). After washing, cells were transferred to SuperFrost microscope slides via cytospin. Immunofluorescence staining was visualized with a confocal microscope (Olympus Fluoview FV10i laser scanning confocal microscope system, Olympus) using a 60x water immersion objective (1.2 NA). Image analysis was performed using the FV10-ASW 1.7 software. In all images, IL-2 staining is presented as a red pseudocolor. In all experiments, cells pulsed without IL-2 were used as the primary control.

[0076] *Statistical Analyses.* Before analysis, graphical displays were made of all data vs. conditions to identify the need for transformations to adhere to model assumptions. For experiments comparing outcomes at a fixed point in time, log transforms were taken and comparisons of means performed using two-sample t-tests or linear regression (depending on the number of conditions). Where appropriate, t-tests assumed unequal variance across conditions. Comparisons of conditions where mice were followed over time were made at individual timepoints based on random effects linear regression models (with random effects to account for correlation of data from the same mouse over repeated measures) with the outcome (*e.g.* % T-cells) log-transformed. Graphical displays were used to assess appropriateness of transformation. Residual plots were inspected to assess assumptions of linear regression models. Time to sacrifice was compared across groups using log-rank tests. Time to sacrifice was compared across groups using log-rank tests. Percent colocalization was compared with log(percent) as the outcome (due to skewness) and main effects of LAMP-1 (vs. EEA-1) and rater. The LAMP-1 effect was evaluated based on the Wald test of the regression coefficient. Model results were exponentiated to provide point estimates for LAMP-1 and EEA-1 colocalization. In the interest of addressing the hypotheses and not over-testing, the inventors did not perform hypothesis tests for every possible comparison in each figure. Where comparisons were insignificant ($p>0.05$) it is stated in the text; where tests were significant, it is stated and/or indicated with asterisks in figures. P-values are reported to two significant digits, except when the p-value is less than 0.001; for p-values smaller than 0.001, it is reported as '$p<0.001$'. P-values are not corrected for multiple comparisons. For all

analyses, statistical significance was based on a two-sided α level of 0.05. Statistical analyses were performed using Stata/IC (version 12.1) and R statistical software.

Example 2 – Results

[0077] IL-*2- but not IL-15- therapy mediates anti-tumor immunity after adoptive transfer of activated CD8$^+$ T cells.* To assess the impact of cytokine therapy on adoptively transferred effector CD8$^+$ T cells, the inventors used IL-2/anti-IL-2 mAb (IL-2/mAb) and IL-15/sIL-15Rα-Fc (IL-15/sIL-15Rα) complexes, in which the antibody or receptor acts as a carrier molecule to improve the half-life and biological activity of free cytokine *in vivo* (Rubinstein *et al.*, 2006; Stoklasek *et al.*, 2006; Boyman *et al.*, 2006). To test effector T cell responsiveness to cytokines in a clinically relevant model, B6 mice were injected (s.c.) with B16 melanoma tumor cells (**FIG. 1A**). After the establishment of palpable tumors, unirradiated mice received activated IL-12-conditioned T cells (Tc1) from pmel-1 TCR transgenic mice, from which CD8$^+$ T cells recognize an endogenous B16 tumor antigen (H-2D^b-restricted gp100$_{25\text{-}33}$ peptide). The inventors have shown these Tc1 effector cells are highly efficacious against tumor in lymphodepleted mice (Rubinstein *et al.*, 2012). For the first week after adoptive transfer, IL-15/sIL-15Rα or IL-2/mAb (clone 5355) complexes were administered every 48 hours. While 6 of 9 mice that received IL-2/mAb complexes were cured of established tumor, mice that received either IL-15/sIL-15Rα complexes or no cytokine therapy showed no tumor regression (**FIG. 1b**). To better understand this differential response, the inventors assessed the persistence of donor Tc1 cells in recipients that received treatment with IL-2/mAb complexes or IL-15/sIL-15Rα complexes. Independent of the presence of tumor, only IL-2/mAb complexes enhanced the persistence of effector CD8$^+$ T cells in a systemic fashion across multiple organs (**FIGS. 1c** and **5a**). Notably, without lymphodepletion or vaccination, the inventors routinely achieved sustained donor T cell frequencies of 20% or higher in the peripheral blood. Furthermore, donor Tc1 cells were equally functional across treatment groups as indicated by the ability to produce IFNγ and TNFα (**FIG. 5b**). Finally, as a control, the inventors found that the transfer of tumor-reactive effector CD8$^+$ T cells was necessary for curative therapy. Thus, tumor-bearing mice treated with only IL-2/mAb or IL-15/sIL-15Rα complexes exhibited minimally delayed tumor growth, albeit comparable between cytokine conditions (**FIG. 6**).

[0078] *Donor T cell expression of IL-2Rα is critical for preferential IL-2-mediated responses.* The preferential response of effector CD8$^+$ T cells to IL-2/mAb but not IL-15/sIL-

15Rα complexes was contrary to the expectation. This response was not dose related as IL-2/mAb and IL-15/sIL-15Rα complexes expanded IL-2Rβγ^{hi} cells such as memory-phenotype CD8$^+$ T cells and NK cells to a similar extent *in vivo* (**FIG. 7**) (Rubinstein *et al.*, 2008). However, only IL-2/mAb complexes expanded T regulatory cells (**FIG. 7**), which are characterized by their expression of IL-2Rα. As IL-12-conditioned (Tc1) effector CD8$^+$ T cells express very high levels of IL-2Rα (Rubinstein *et al.*, 2012), the results suggested an unappreciated role for cell surface IL-2Rα on effector T cells in dictating responsiveness to IL-2 therapy. To formally test this, the inventors made use of two anti-IL-2 mAbs with the ability to differentially redirect IL-2 based on lymphocyte cell surface IL-2Rα expression. IL-2/mAb$_{CD25}$ complexes (clone 1A12) preferentially expand IL-2Rα^{hi} lymphocytes, while IL-2/mAb$_{CD122}$ complexes (clone S4B6) act in an IL-2Rα-independent manner (Boyman *et al.*, 2006; Spangler *et al.*, 2015). The inventors tested these two complexes in lymphoreplete mice injected with Tc1 cells. For only this experiment, the inventors generated Tc1 cells from another TCR transgenic mouse, OT-I, to confirm the results with a different TCR. While IL-2/mAb$_{CD122}$ complexes mediated a minimal increase in persistence, IL-2/mAb$_{CD25}$ complexes induced donor T cell levels of greater than 60% of total lymphocytes (**FIG. 1d**). To further confirm that this effect was dependent on IL-2Rα and not on IL-12 conditioning or selective TCR engagement, the inventors stimulated polyclonal T cells from wildtype mice with plate-bound anti-CD3 mAb, a method that generates IL-2Rα^{hi} effector CD8$^+$ T cells. Upon adoptive transfer into lymphoreplete mice, IL-2/mAb complexes (clone 5355) greatly enhanced the persistence of polyclonal T cells (**FIG. 1e**). Finally, as an additional control, Tc0 cells, which have lower levels of surface IL-2Rα (Rubinstein *et al.*, 2012), showed limited IL-2/mAb-driven persistence (**FIG. 8**).

[0079] *IL-2Rα induces sustained IL-2 signaling in effector CD8$^+$ T cells after cytokine withdrawal.* To uncover the mechanism behind the remarkable IL-2Rα-dependent responsiveness of effector Tc1 cells *in vivo*, the inventors assayed IL-2 and IL-15 activity downstream of IL-2Rβγ using standard *in vitro* assays quantifying phosphorylation of STAT5 (a proximal signaling event), viability, and proliferation (**FIG. 2a**). In the context of STAT5 phosphorylation in response to titrated cytokine, the inventors found that Tc1 (IL-2Rα^{hi}) cells exhibited marginally increased sensitivity to IL-2 versus IL-15 when compared to Tc0 effector cells (IL-2Rα^{med}) (**FIGS. 2b-2c**), which is consistent with previous findings (Lisiero *et al.*, 2011). The addition of a blocking antibody (anti-IL-2Rα mAb, PC61 clone) also

showed a minimal benefit of IL-2Rα engagement on Tc1 cells in comparison between titrated IL-2 and IL-15 (**FIGS. 9a-9b**). Notably, Tc1 cells responded comparably to IL-2 and IL-15 in standard assays of proliferation and viability (**FIGS. 10a-10b**). Importantly, there was no difference in the kinetics of STAT5 phosphorylation between cells cultured in IL-2 or IL-15 (**FIG. 2d**). The mildly enhanced sensitivity of Tc1 cells to IL-2 versus IL-15 seemed unlikely to account for the dramatic difference in activity observed *in vivo*. Therefore, the inventors hypothesized that IL-2Rα does not simply improve cellular affinity for IL-2, but allows for sustained IL-2 signaling after a T cell transitions from a cytokine-rich to a cytokine-free environment. To test this idea, the inventors used a cytokine pulse assay. Tc1 and Tc0 cells were cultured overnight with a saturating dose of IL-2 or IL-15, washed, and replated without cytokine as shown in **FIG. 2e**. Consistent with the hypothesis, only pre-culture of Tc1 cells with IL-2 led to sustained STAT5 phosphorylation in the absence of additional cytokine (**FIGS. 11a-11c**). To directly test the role of IL-2Rα in promoting sustained signaling on effector $CD8^+$ T cells, the inventors cultured Tc1 cells for 90 minutes with IL-2 in the absence or presence of blocking anti-IL-2Rα antibody (PC61 clone). This shorter pulse was equally sufficient for inducing sustained signaling as indicated by STAT5 phosphorylation (**FIG. 2f**). Importantly, blockade of IL-2Rα completely abolished the sustained IL-2 signaling as indicated by STAT5 phosphorylation and proliferation (**FIGS. 2f, 12, and 13a-13b**). Polyclonal effector $CD8^+$ T cells activated in the absence of IL-12 also showed sustained IL-2 signaling, and importantly, effector cells generated from IL-$2R\alpha^{+/-}$ mice showed roughly half the sustained IL-2 signaling (**FIG. 2g**). To ensure that these cells had similar IL-2Rβγ signaling potential, the inventors pulsed wildtype and IL-$2R\alpha^{+/-}$ effector $CD8^+$ T cells with IL-15 and found no differences in their response (**FIG. 2h**). Notably, the ability to induce sustained IL-2 signaling on mouse effector cells was observed with human and mouse IL-2 (**FIG. 14**). Furthermore, culture of human effector T cells with hIL-2 but not hIL-15 led IL-2Rα-dependent sustained STAT5 phosphorylation (**FIGS. 15a-15b**). Finally, to verify that IL-2/mAb complexes (clone 5355) used in the *in vivo* experiments were permissive to engagement of IL-2Rα, the inventors repeated the pulse assay with hIL-2 and excess anti-IL-2 mAb. *In vitro* generated IL-2/mAb complexes induced sustained IL-2 signaling that was dependent on IL-2Rα (**FIG. 16a**). In contrast, IL-2/mAb_{CD122} complexes (clone S4B6), which do not engage IL-2Rα (Boyman *et al.*, 2006; Spangler *et al.*, 2015), failed to induce sustained signaling *in vitro* (**FIG. 16b**).

[0080] *IL-2Rα facilitates sustained IL-2 signaling through creation of an extracellular reservoir and recycling.* To understand how IL-2Rα promotes sustained IL-2 signaling, the inventors hypothesized two non-mutually exclusive possibilities. First, IL-2Rα may bind IL-2 and create a cell-surface cytokine reservoir due to the high ratio of surface IL-2Rα to IL-2Rβγ, as IL-2/IL-2Rα internalization can only occur in the presence of both IL-2Rβ and γ (Robb and Greene, 1987; Takeshita *et al.*, 1992). Such a reservoir of IL-2 bound to IL-2Rα would mediate gradual signaling by continually feeding the rate-limiting, endocytosed IL-2Rβγ. In support of this possibility, the inventors detected high surface levels of IL-2 on effector $CD8^+$ T cells that gradually waned after extended culture, and this cell-surface IL-2 was dependent on available IL-2Rα (**FIGS. 3a-3b**). Furthermore, antibodies against IL-2 added after the removal of free cytokine from IL-2 pulsed cells were able to dampen sustained signaling (**FIG. 3c**). A second possible way in which IL-2Rα might sustain signaling is by promoting recycling of IL-2 from within the cell to the surface, thus allowing for repetitive signaling. To test this hypothesis, Tc1 cells were pulsed with IL-2 at 37°C to allow for cytokine internalization. Cells were then stripped of surface IL-2 using an acid wash. Upon reculture at 37°C, the inventors were able to detect re-appearance of either mIL-2 or hIL-2 on the cell surface (**FIG. 3d**). Minimal surface IL-2 was observed when cells were pulsed at 4°C or on the surface of mixed bystander Tc1 cells (**FIG. 3e**). Importantly, the species-specificity of the reagents precluded autocrine production as the source of cell surface IL-2 after acid wash (**FIG. 17**). In additional support of IL-2Rα-mediated recycling, the inventors observed sustained pSTAT5 signaling after acid washing of cells pulsed with hIL-2 at 37°C but not 4°C (**FIG. 3f**). Because internalization of IL-2Rαβγ does not occur at 4°C, these data provide further support that sustained signaling occurs in part through an IL-2Rα bound pool of internalized IL-2. It is notable that the inventors could not block sustained STAT5 signaling in cells pulsed with mIL-2 at 4°C by acid washing, possibly reflecting a higher affinity of mIL-2 for mIL-2Rα compared with that of hIL-2 for mIL-2Rα (Spangler *et al.*, 2015; Liu *et al.*, 1996). Finally, confocal microscopy showed discrete punctate structures of either mIL-2 or hIL-2 when cells were incubated with cytokine at 37°C but not 4°C (**FIGS. 18a, 18b, and 19**). These punctate structures colocalized with IL-2Rα, Rab5, and EEA1, but less frequently with LAMP-1, consistent with intracellular IL-2 being accessible to the recycling pathway (**FIGS. 3g, 3h and 20a-20c**) (Grant and Donaldson, 2009; Mu *et al.*, 1995). Taken together, these results suggest that IL-2Rα both promotes an extracellular reservoir for IL-2 and mediates recycling of IL-2.

[0081] *IL-2Rα expression on donor CD8$^+$ T cells provides a competitive advantage to IL-2 therapy in a lymphoreplete but not lymphopenic host environment.* The results thus far suggest that the differential responsiveness of Tc1 cells to IL-2- and IL-15 therapy *in vivo* is a consequence of IL-2Rα on donor T cells providing a competitive advantage to accessing cytokine. To formally test this hypothesis, the inventors initially attempted to activate T cells from wildtype and IL-2Ra$^{-/-}$ mice. However, this proved technically not feasible for us as T cells isolated from IL-2Ra$^{-/-}$ mice were resistant to normal activation, likely due to the immune alterations in the absence of IL-2 responsiveness (Willerford *et al.*, 1995). Therefore, the inventors used polyclonal IL-2Rα$^{+/-}$ T cells, as these cells activated comparably to wildtype T cells and had approximately half the expression of IL-2Rα (**FIG. 4a**). Using the Thy1.1 congenic marker to distinguish between genotypes, these two cell populations were mixed and adoptively transferred into non-irradiated B6(CD45.1) recipient mice. Mice were treated with IL-2/mAb or IL-15/sIL-15Rα for 1 week. The inventors hypothesized that IL-2Rα$^{+/-}$ donor CD8$^+$ T cells would not persist as well as their wildtype counterparts due to loss of one allele. In contrast to the expectations, wildtype and IL-2Rα$^{+/-}$ donor T cells did not show differential responsiveness to treatment with IL-2/mAb or IL-15/sIL-15Rα complexes (**FIG. 4a-4b**). These results suggest a threshold of IL-2Rα *in vivo*, both in terms of level and durability of expression, that when reached is sufficient for providing donor cells a competitive advantage to IL-2 therapy in a lymphoreplete environment.

[0082] As an alternative means of assessing the role of IL-2Rα on donor T cells *in vivo*, the inventors compared the responsiveness of IL-2Rα^{hi} donor T cells to IL-2- and IL-15 therapy with the addition of lymphodepletion to destroy host cells. The inventors predicted that the advantage of IL-2Rα-competent cytokine therapy would be lost in the absence of host IL-2Rβγ$^+$ lymphocytes competing for cytokine (**FIG. 21a**). Thus, mice were given total body irradiation (600rad) prior to adoptive transfer of effector Tc1 CD8$^+$ T cells, and then treated for one week with IL-2/mAb and IL-15/sIL-15Rα complexes. Consistent with the prediction, both IL-2 and IL-15 therapy effectively augmented the persistence of donor cells both in the blood and in the spleen, and only in lymphodepleted mice (**FIGS. 4c and 21b-c**). These results demonstrate a critical role for IL-2Rα on donor T cells in promoting IL-2 responsiveness in a lymphoreplete host environment.

[0083] It was also shown that low-dose IL-2 leads to preferential expansion of adoptively transferred donor tumor-reactive T cells by engagement of IL-2Rα (**FIG. 23**). B6 mice were injected with 250,000 B16-F1 tumor cells (s.c.). Eight days later, mice were adoptively transferred with $3x10^6$ tumor-reactive activated T cells (pmel-1) conditioned with IL-12 to induce high levels of IL-2Rα. On the day of adoptive T cell transfer, 2 days later, and 4 days later, mice were treated with hIL-2 (1.5ug), hIL-2/mAb complexes (1.5ug hIL-2 and 7.5ug anti-hIL-2 mAb (MAB602)), or hIL-15/sIL-15Rα-Fc complexes (0.5ug hIL-15 + 2.3ug sIL-15Rα-Fc). On day 6 after adoptive transfer, mice were bled and the frequency of donor T cells ($CD8^+Thy1.1^+$) in the peripheral blood was determined.

* * *

[0084] All of the methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

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WHAT IS CLAIMED IS:

1. An isolated transgenic cell comprising:

(i) an elevated surface expression level of at least one cytokine receptor or cytokine receptor co-stimulator, relative to an activated T-cell; or

(ii) a nucleic acid molecule encoding at least one cytokine receptor or cytokine receptor co-stimulator polypeptide comprising a mutation that increases the activity of the receptor.

2. The isolated transgenic cell of claim 1, wherein the at least one cytokine receptor is the IL-2 or IL-15 receptor.

3. The isolated cell of claim 1, wherein the at least one cytokine receptor is IL-12Rβ1 and/or IL-12Rβ2.

4. The isolated cell of claim 1, wherein the at least one cytokine receptor is IL-6Rα1 and/or GP130.

5. The isolated cell of claim 1, wherein the at least one cytokine receptor is IL-4Rα and/or IL-2Rγ.

6. The isolated cell of claim 1, wherein the at least one cytokine receptor is IL-21Rα and/or IL-2Rγ.

7. The isolated cell of claim 1, wherein the at least one cytokine receptor is IL-27R and/or GP130. In this case the ligand is IL-27 or an IL-27-derived molecule.

8. The isolated cell of claim 1, wherein the at least one cytokine receptor is IL-2Rβ2 and/or GP130.

9. The isolated cell of claim 1, wherein the at least one cytokine receptor is IL-12Rβ1 and/or IL-23R.

10. The isolated cell of claim 1, wherein the at least one cytokine receptor is IL-9Rα and/or IL-2Rγ.

11. The isolated cell of claim 1, wherein the at least one cytokine receptor co-stimulator is ICOS.

12. The isolated cell of claim 1, wherein the at least one cytokine receptor co-stimulator is 4-1BB.

13. The isolated cell of claim 1, wherein the at least one cytokine receptor co-stimulator is CD28.

14. The isolated cell of claim 1, wherein the at least one cytokine receptor is G-CSFR.

15. The isolated cell of claim 1, wherein the at least one cytokine receptor is GM-CSFRα or GM-CSFRβc.

16. The isolated cell of claim 1, wherein the cell is a human cell.

17. The isolated cell of claim 1, wherein the cell is a T-cell.

18. The isolated cell of claim 17, wherein the T-cell is a T-cell targeted to cancer cell antigen.

19. The isolated cell of claim 17, wherein the T-cell is a $CD4^+$ or $CD8^+$ T-cell.

20. The isolated cell of claim 17, wherein the T-cell is an effector T-cell or a memory T-cell.

21. The isolated cell of claim 1, wherein the cell is a Natural Killer (NK) cell or a NK T-cell.

22. The isolated cell of claim 1, wherein the cell is comprised in a bone marrow graft cell population.

23. The isolated cell of claim 18, wherein the cancer cell antigen is a growth factor receptor.

24. The isolated cell of claim 18, wherein the cancer cell antigen is GP240, 5T4, HER1, CD-33, CD-38, VEGFR-1, VEGFR-2, CEA, FGFR3, IGFBP2, IGF-1R, BAFF-R, TACI, APRIL, Fn14, EGFR, ERBB2, ERBB3 CD19, CD20 or mesothelin.

25. The isolated cell of claim 1, wherein the cell expresses a chimeric antigen receptor (CAR) or a recombinant T-cell receptor (TCR).

26. The isolated cell of claim 1, comprising a nucleic acid molecule encoding a cytokine receptor polypeptide comprising a mutation that increases the activity of the receptor upon ligand binding.

27. The isolated cell of claim 26, comprising a nucleic acid molecule encoding a IL-2 receptor or IL-15 receptor polypeptide comprising a mutation that increases the activity of the receptor upon ligand binding.

28. The isolated cell of claim 27, comprising a nucleic acid molecule encoding the IL-2 receptor polypeptide comprising a mutation that increases the activity of the receptor.

29. The isolated cell of claim 28, wherein the IL-2 receptor is IL-2Rα.

30. The isolated cell of claim 28, wherein the IL-2 receptor is IL-2Rβ or IL-2Rγ.

31. The isolated cell of claim 28, wherein the mutation increase surface expression, increases stability or increases ligand binding of the IL-2 receptor polypeptide.

32. The isolated cell of claim 28, wherein the mutation disrupts one or more ribosylation sites in IL2-Rα.

33. The isolated cell of claim 28, wherein the mutation alters intracellular trafficking of the IL-2 receptor.

34. The isolated cell of claim 27, comprising a nucleic acid molecule encoding the IL-15 receptor polypeptide comprising a mutation that increases the activity of the receptor.

35. The isolated cell of claim 34, wherein the mutation increase surface expression, increases stability or increases ligand binding of the IL-15 receptor polypeptide.

36. The isolated cell of claim 34, wherein the IL-15 receptor is IL-15Rα.

37. The isolated cell of claim 34, wherein the IL-15 receptor is IL-2Rβ or IL-2Rγ.

38. The isolated cell of claim 1, comprising the nucleic acid molecule encoding cytokine receptor polypeptide operably linked to a heterologous promoter.

39. The isolated cell of claim 38, comprising the nucleic acid molecule encoding an IL-2 receptor or IL-15 receptor polypeptide, is operably linked to a heterologous promoter.

40. The isolated cell of claim 39, wherein the heterologous promoter is a ligand inducible or a ligand repressible promoter.

41. The isolated cell of claim 39, wherein the heterologous promoter is a ligand inducible promoter.

42. The isolated cell of claim 41, wherein the ligand inducible promoter is a tet-on promoter.

43. The isolated cell of claim 1, further comprising a suicide gene is operably linked to an inducible promoter.

44. The isolated cell of claim 26, wherein the nucleic acid molecule encoding the cytokine receptor polypeptide is integrated into the genome of the cell.

45. The isolated cell of claim 44, wherein the nucleic acid molecule encoding the cytokine receptor polypeptide is flanked by retroviral long terminal repeats or transposon repeats.

46. A pharmaceutical composition comprising an isolated cell in accordance with anyone of claims 1-45 in a pharmaceutically acceptable carrier.

47. The pharmaceutical composition of claim 46, comprising between about $1x10^3$ and $1x10^{11}$ cells in accordance with any one of claims 1-45.

48. A method of providing a T-cell response in a human subject having a disease comprising administering an effective amount of T-cells in accordance with claim 17 to the subject.

49. The method of claim 48, wherein the T-cell response is a regulatory T-cell response.

50. The method of claim 48, wherein the T-cell response is a cytotoxic T-cell response.

51. The method of claim 48, wherein the T-cell response is a $CD4^+$ T-cell response.

52. The method of claim 48, further comprising administering a cytokine that stimulates T-cell proliferation to the subject.

53. The method of claim 52, wherein the cytokine is IL-2 or IL-15.

54. The method of claim 52, wherein the cytokine comprises a mutation that increases receptor binding or reduces ligand release from the receptor.

55. The method of claim 52, wherein the cytokine has been modified to increase serum half-life.

56. The method of claim 55, wherein the cytokine has been PEGylated or fused to an Fc polypeptide.

57. The method of claim 55, wherein the cytokine has been modified to redirect target cell specificity.

58. The method of claim 55, wherein the cytokine is associated with an antibody or soluble receptor.

59. The method of claim 48, further comprising administering an agent that increased IL-2 or IL-15 receptor expression to the subject.

60. The method of claim 59, the agent is a cytokine, an agonist of a costimulatory molecule or an epigenetic drug.

61. The method of claim 60, wherein the agent is a drug targeting HDAC2 or G9a.

62. A method of producing therapeutic cells comprising:

(i) selecting a population of cells having an increased activity of at least a one cytokine receptor; and

(ii) culturing the cells in the presence of a ligand for said at one cytokine receptor.

63. The method of claim 62, wherein the at least one cytokine receptor is a IL-2R.

64. The method of claim 62, wherein the cells having increased activity of at least a one cytokine receptor express an elevated level the at least one cytokine receptor on their surface.

65. The method of claim 64, wherein selecting a population of cells having increased activity of at least a one cytokine receptor comprises contacting the cells with an agent that increases expression of said at least one cytokine receptor.

66. The method of claim 65, wherein the agent is a cytokine, an agonist of a costimulatory molecule or an epigenetic drug.

67. The method of claim 65, wherein the agent is a drug targeting HDAC2 of G9a.

68. The method of claim 64, wherein selecting a population of cells having increased activity of at least a one cytokine receptor comprises sorting cells based on expression of the at least one cytokine receptor.

69. The method of claim 63, wherein the cells comprise a nucleic acid molecule encoding the at least one cytokine receptor comprising a mutation that increases the activity of the receptor.

70. The method of claim 63, further comprising introducing a nucleic acid molecule encoding the at least one cytokine receptor into the population of cells.

71. The method of claim 70, wherein the nucleic acid molecule encoding the at least one cytokine receptor is comprised in a retroviral, lentiviral, adenoviral or adenoassociated viral vector.

72. The method of claim 63, wherein said culturing is *in vivo*.

73. A method of treating a disease comprising:

transferring at least one receptor gene into at least one cell, and

treating said cell or cells with an agonist of the receptor transcribed by said receptor gene.

74. The method of claim 73, wherein the receptor gene is a cytokine receptor gene.

75. The method of claim 74, wherein the cytokine receptor gene is Interleukin-2 receptor alpha (IL-2Rα).

76. The method of claim 73, wherein the agonist is Interleukin-2 (IL-2).

77. The method of claim 73, wherein the disease is cancer.

78. The method of claim 73, wherein the receptor gene is transferred into the at least one cell via adoptive cell therapy.

79. The method of claim 73, wherein the treatment does not require lymphodepletion.

80. The method of claim 73, wherein the at least one cell is a donor T cell.

81. The method of claim 73, wherein the at least one cell further comprises a CAR.

82. The method of claim 81, wherein the at least one cell comprises a transgene encoding a CAR and the at least one receptor gene.

83. The method of claim 73, wherein the at least one cell further comprises a TCR.

84. The method of claim 81, wherein the at least one cell comprises a transgene encoding a TCR and the at least one receptor gene.

85. The method of claim 81, wherein the at least one receptor gene is linked to a suicide gene.

86. The method of claim 81, wherein the at least one receptor gene is comprised in viral vector.

87. The method of claims 86, wherein the viral vector is a retroviral or lentiviral vector.

88. The method of claim 73, wherein the at least one receptor gene is linked to a regulatory element to allow regulated expression of the receptor.

89. The method of claim 73, further comprising contacting the at least one cell with an antibody against the receptor encoded by the at least one receptor gene.

90. The method of claim 89, wherein the antibody is daclizumab.

91. The method of claim 73, wherein the at least one receptor gene is linked to a gene encoding a cell surface molecule that is recognized by an antibody.

92. The method of claim 91, wherein the cell surface molecule is CD20.

93. The method of claim 91, further comprising contacting the at least one cell with an antibody against the cell surface molecule.

94. The method of claim 93, wherein the antibody is rituximab.

95. A method of treating a disease comprising:

injecting a mammalian subject with a vector encoding a receptor gene, and

treating the mammalian subject with an agonist of the receptor encoded by the receptor gene.

96. A method comprising:

administering an antigenic composition to a subject to induce an antigen-specific lymphocyte response, and

administering a pharmacological agent to the subject to induce expression of a cytokine receptor; and

administering a ligand for the cytokine receptor to the subject.

97. The method of claim 96, wherein the pharmacological agent is a drug targeting HDAC2 or G9a.

98. The method of claim 96, wherein the a pharmacological agent is vector encoding the cytokine receptor.

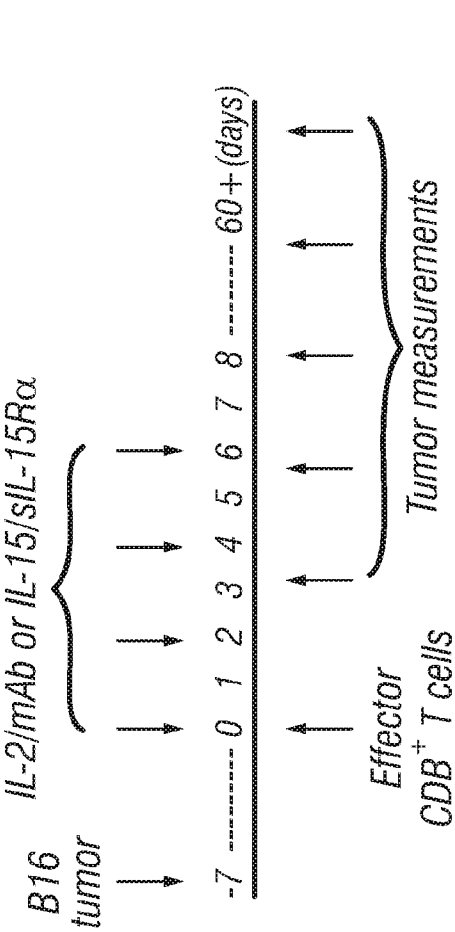


FIG. 1A

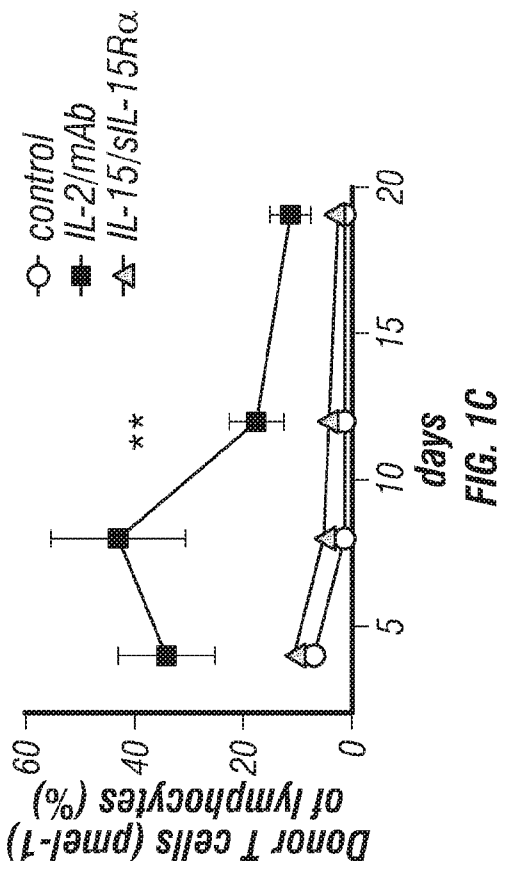


FIG. 1C

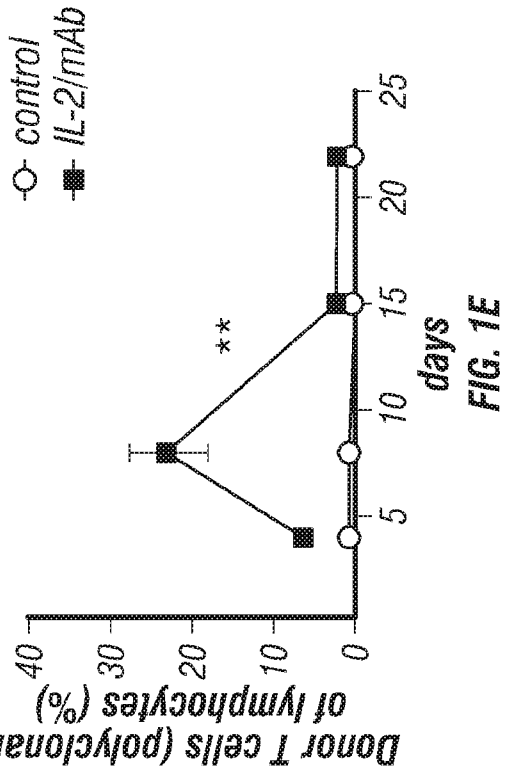


FIG. 1E

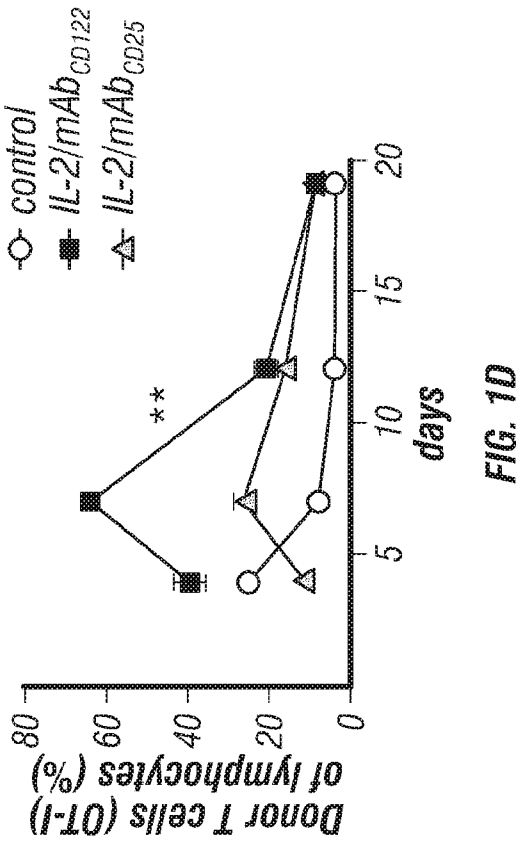


FIG. 1D

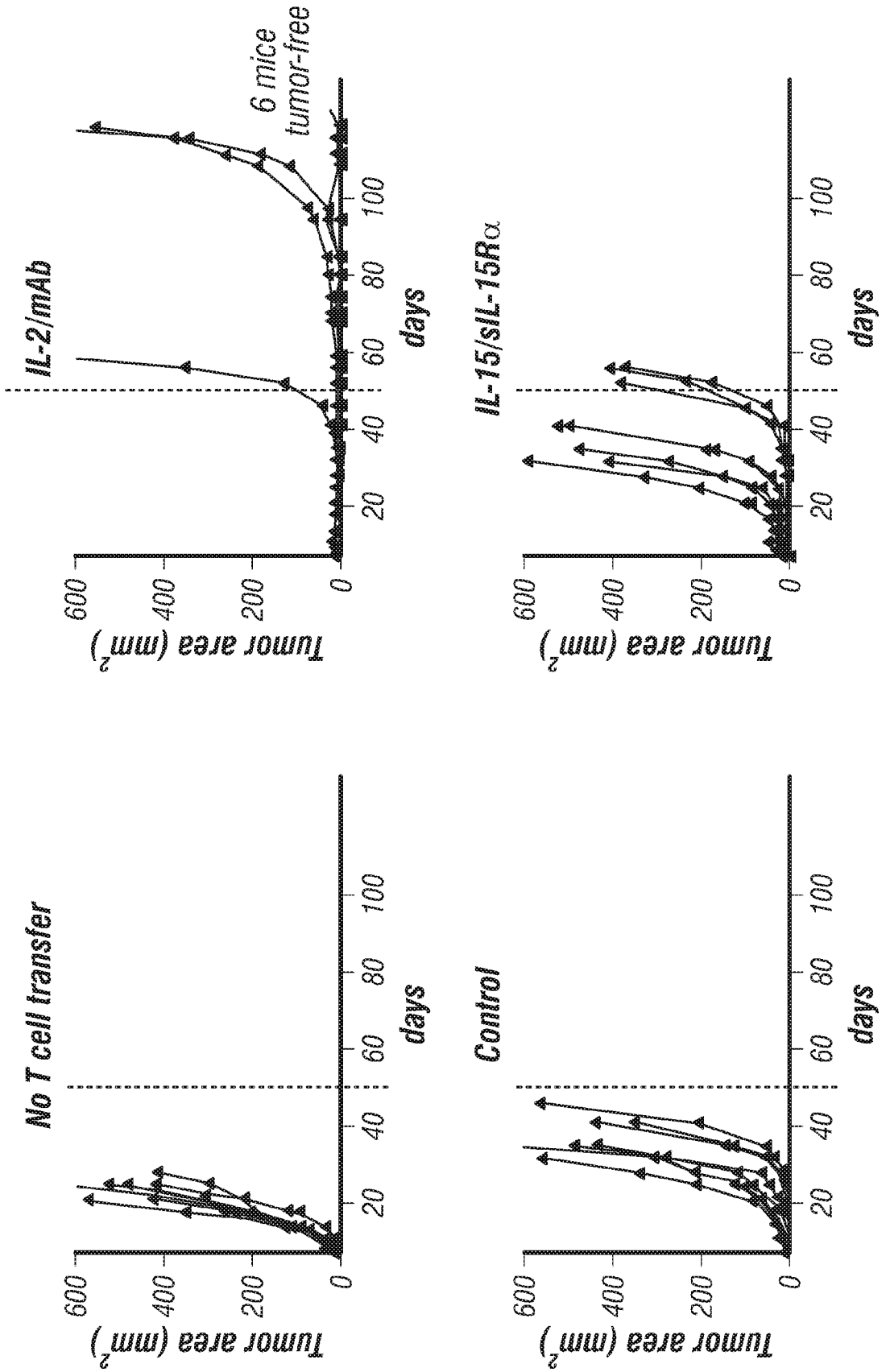

IL-2/mAb
6 mice tumor-free
IL-15/sIL-15Rα
No T cell transfer
Control
Tumor area (mm²)
days
0
200
400
600
20
40
60
80
100

FIG. 1B

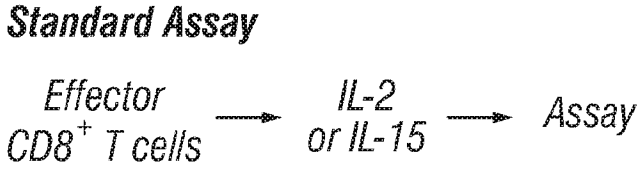


FIG. 2A

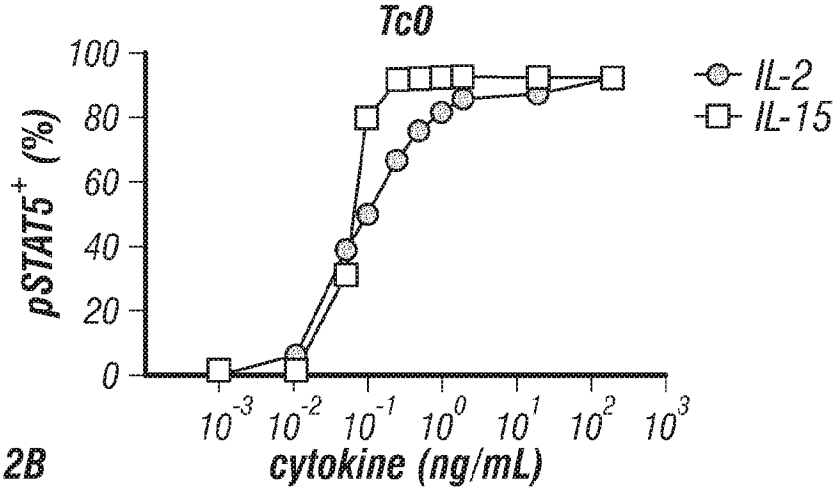


FIG. 2B

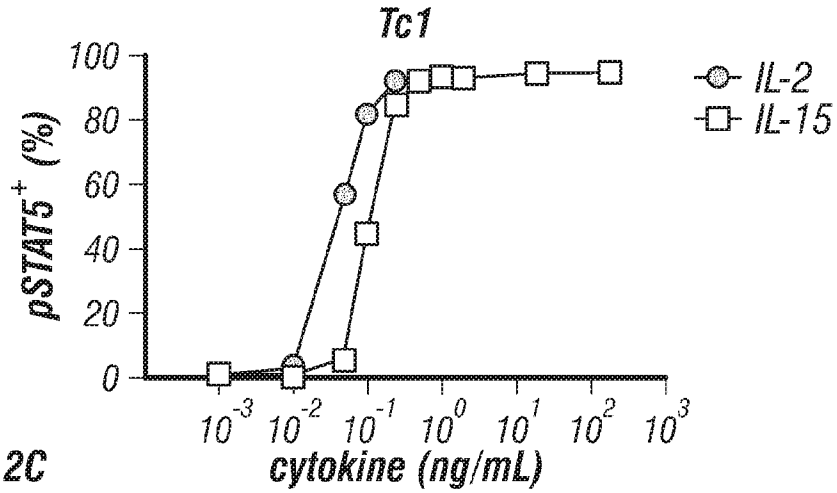


FIG. 2C

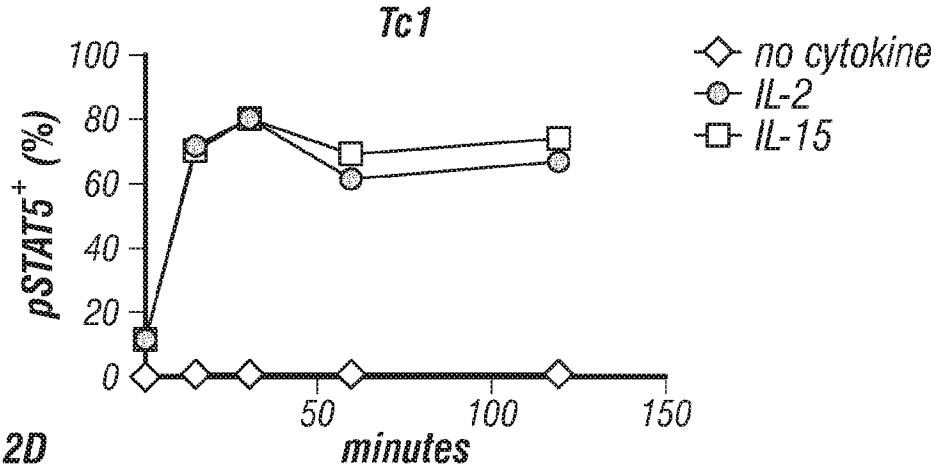


FIG. 2D

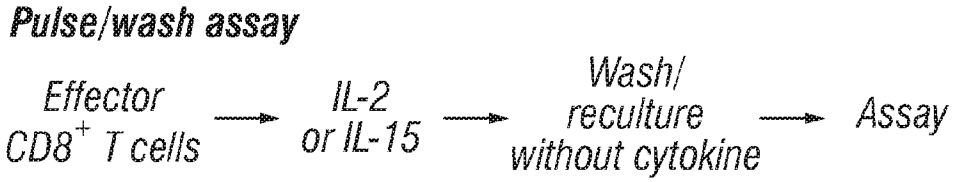


FIG. 2E

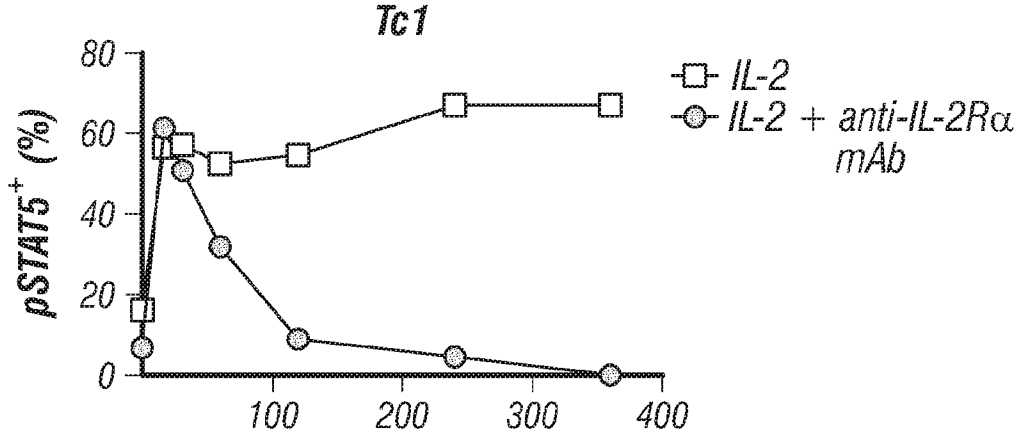


FIG. 2F

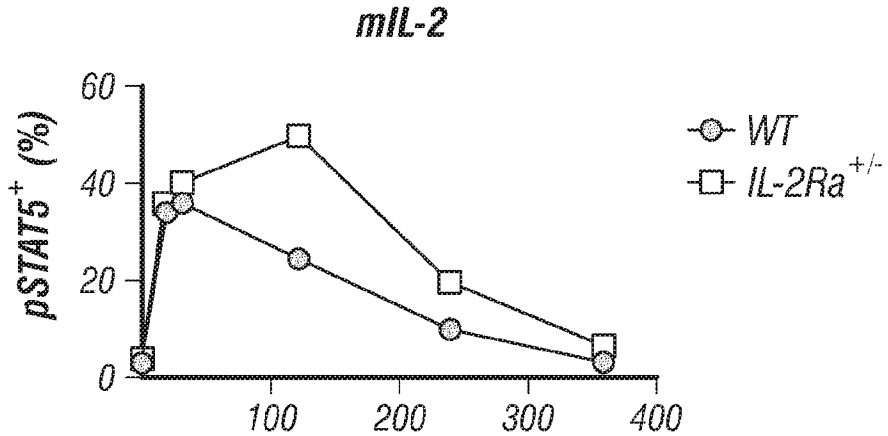


FIG. 2G

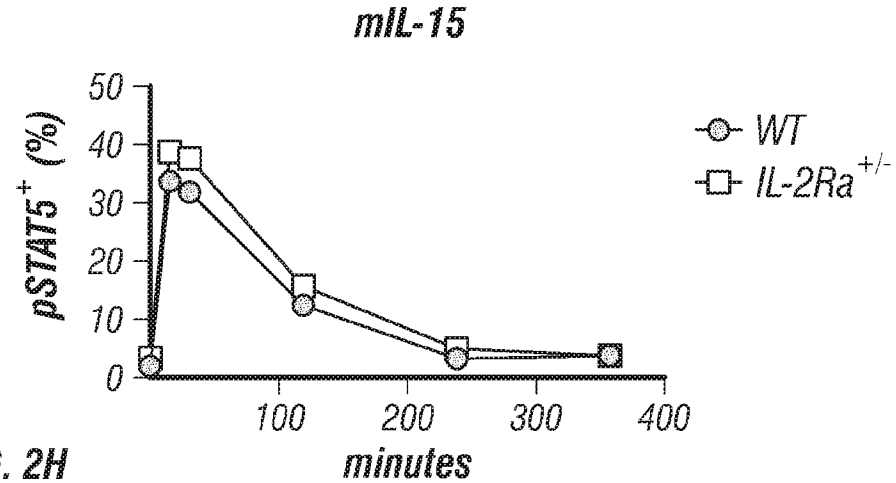


FIG. 2H

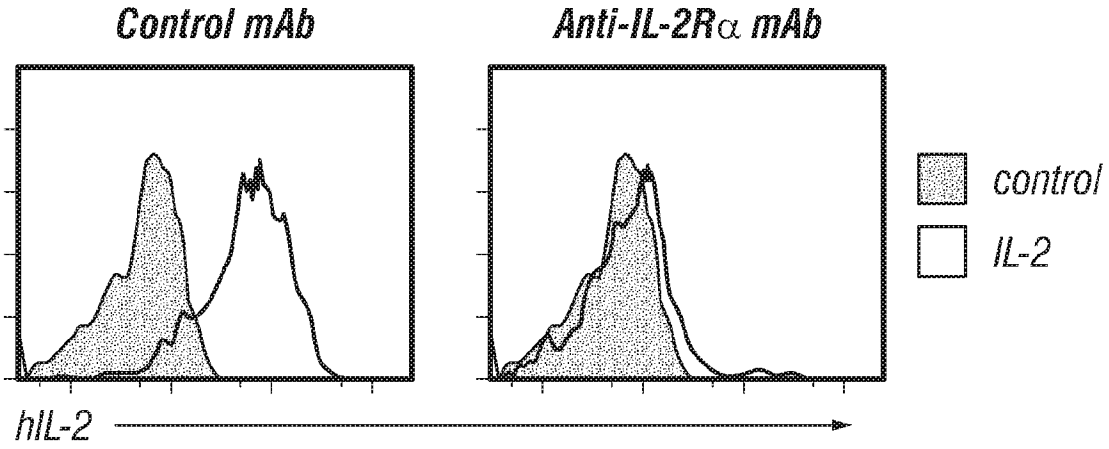


FIG. 3A

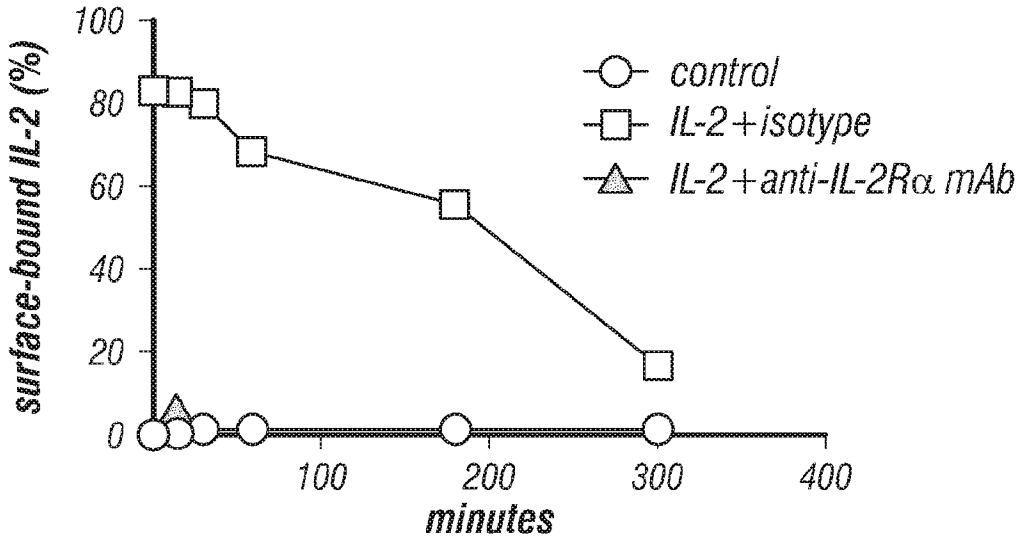


FIG. 3B

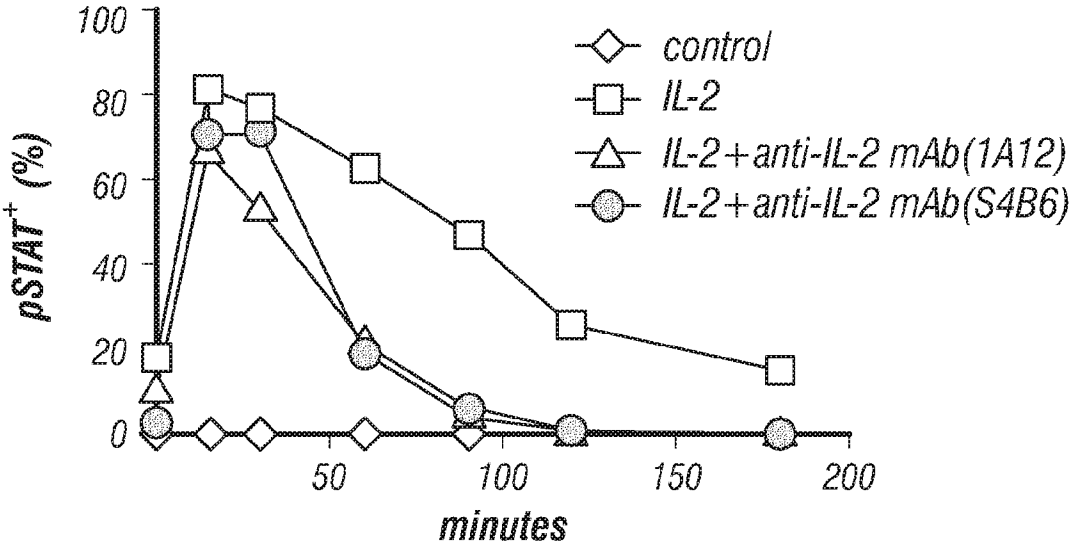


FIG. 3C

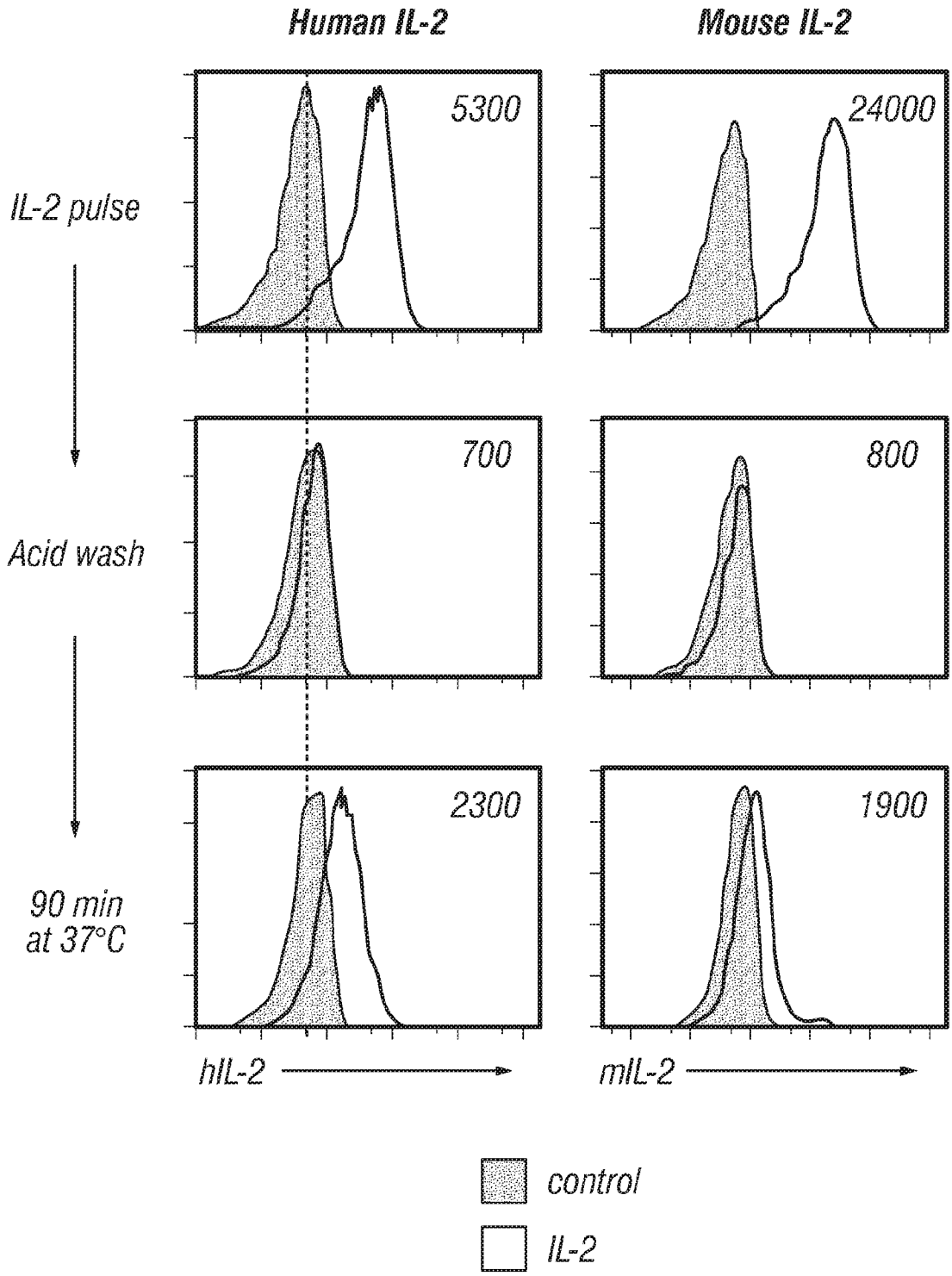

Human IL-2
Mouse IL-2
IL-2 pulse
Acid wash
90 min
at 37°C
5300
24000
700
800
2300
1900
hIL-2
mIL-2
control
IL-2

FIG. 3D

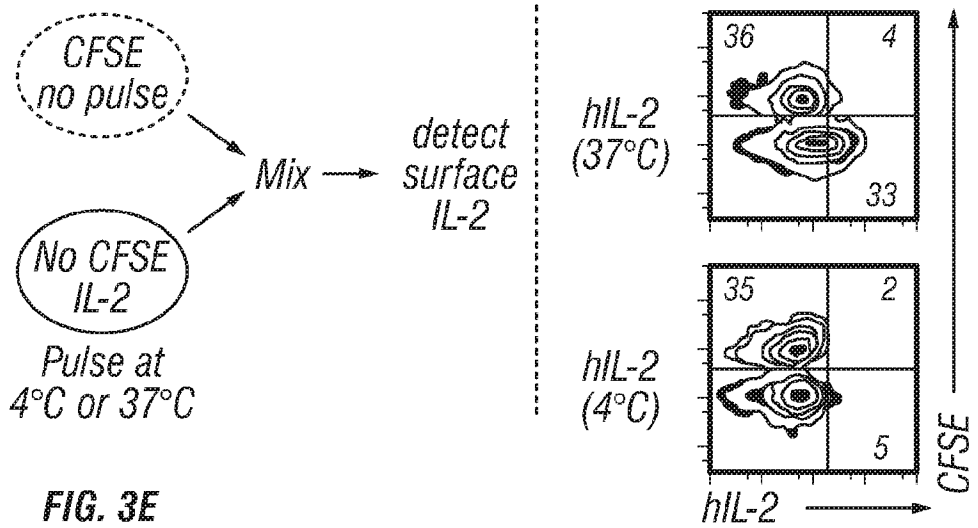

CFSE
no pulse
Mix
detect
surface
IL-2
No CFSE
IL-2
Pulse at
4°C or 37°C
hIL-2
(37°C)
hIL-2
(4°C)
36
4
33
35
2
5
CFSE
hIL-2

FIG. 3E

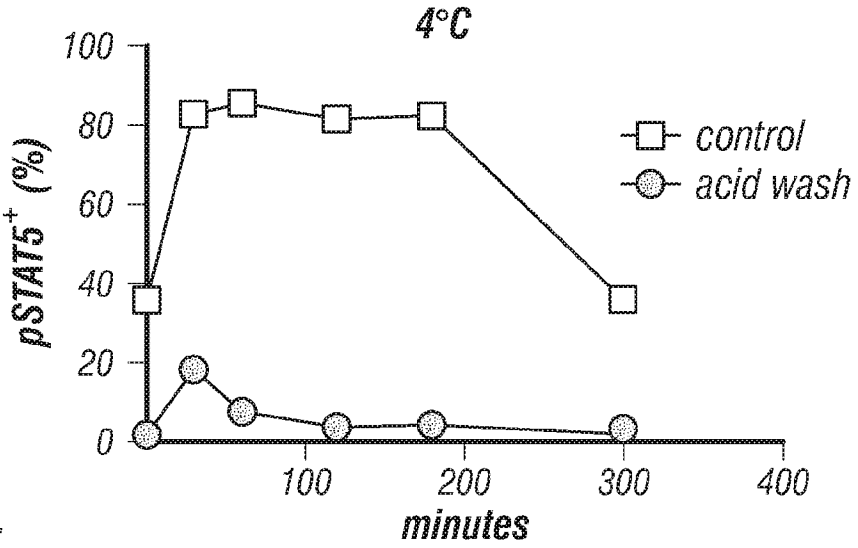
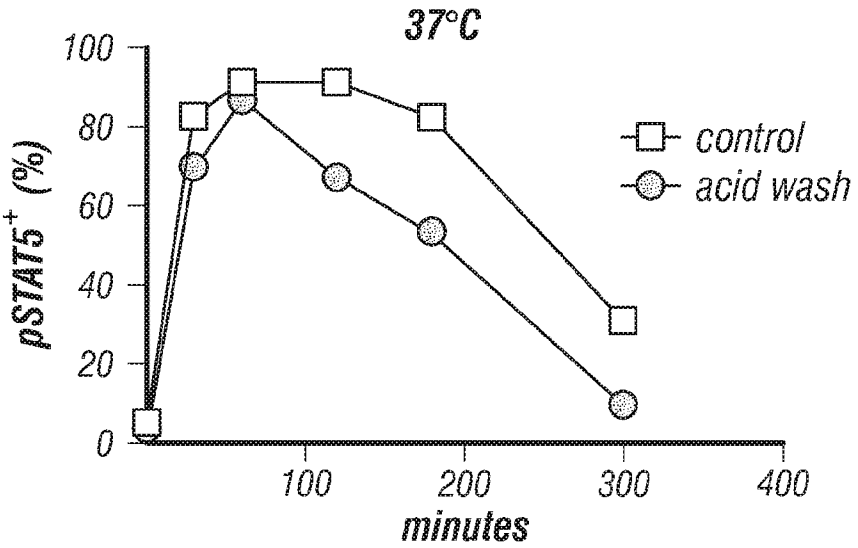

4°C
pSTAT5+ (%)
100
80
60
40
20
0
100
200
300
400
minutes
control
acid wash

FIG. 3F


37°C
pSTAT5+ (%)
100
80
60
40
20
0
100
200
300
400
minutes
control
acid wash

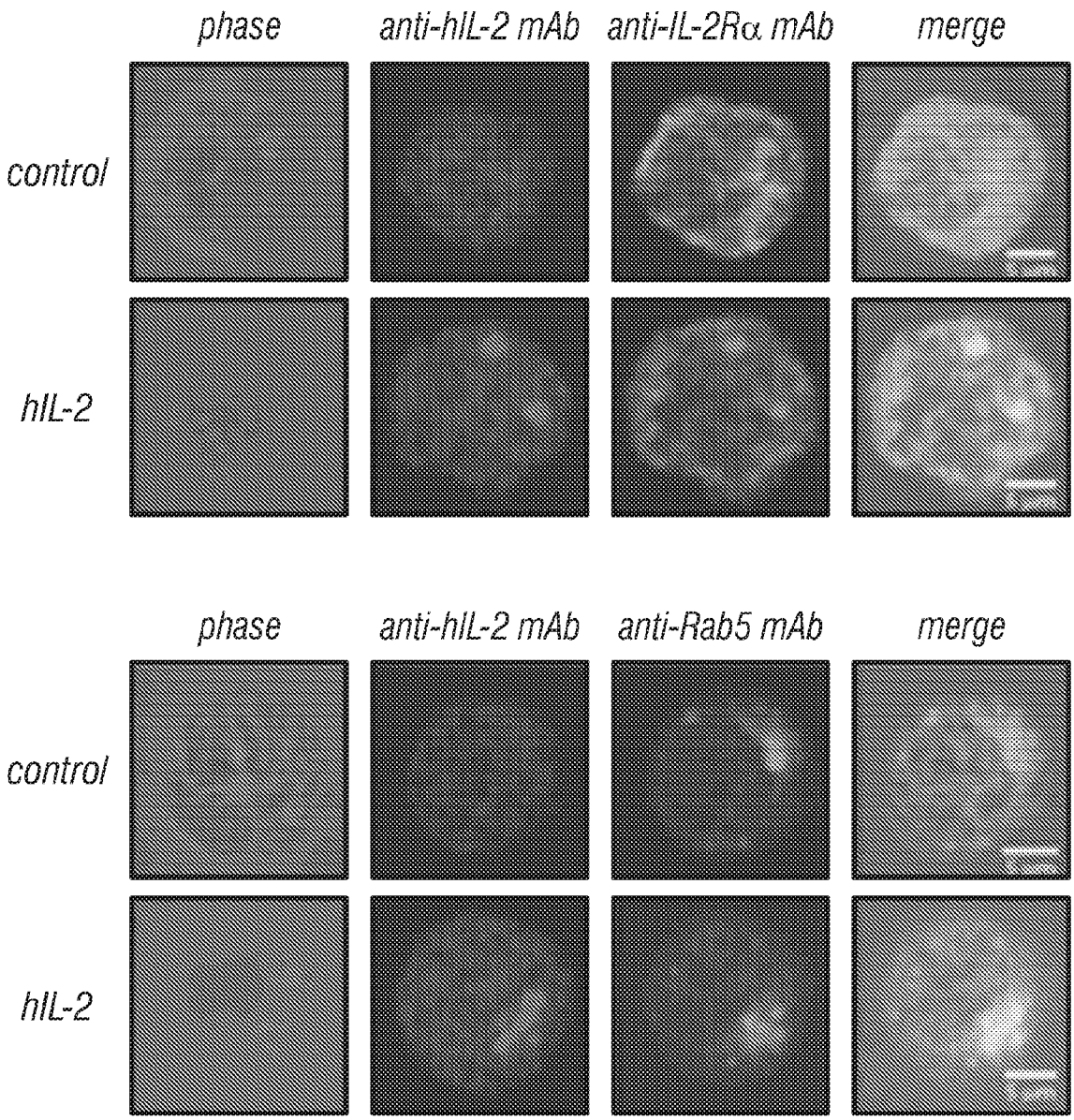

phase
anti-hIL-2 mAb
anti-IL-2Rα mAb
merge
control
hIL-2
phase
anti-hIL-2 mAb
anti-Rab5 mAb
merge
control
hIL-2

FIG. 3G

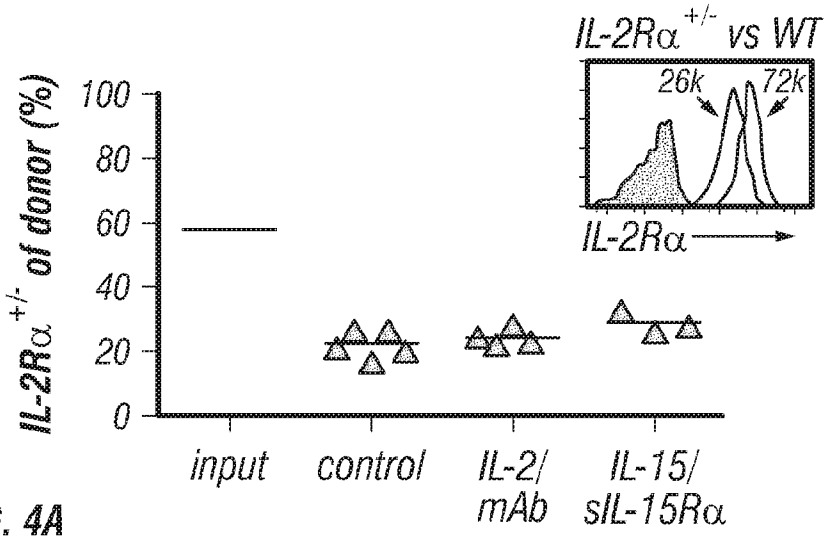


FIG. 4A

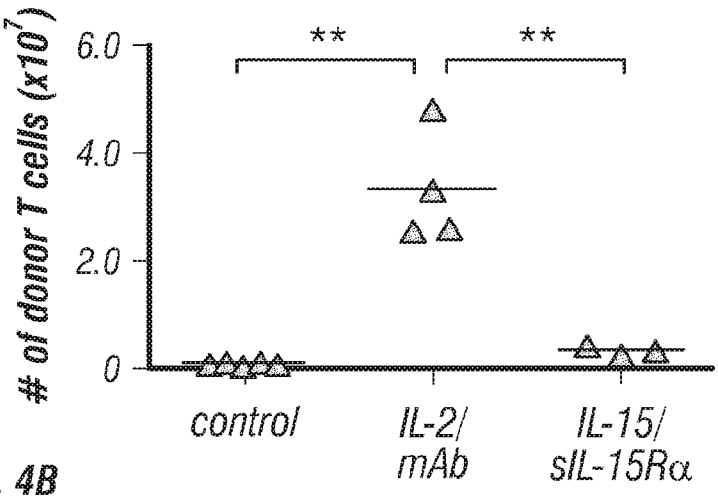


FIG. 4B

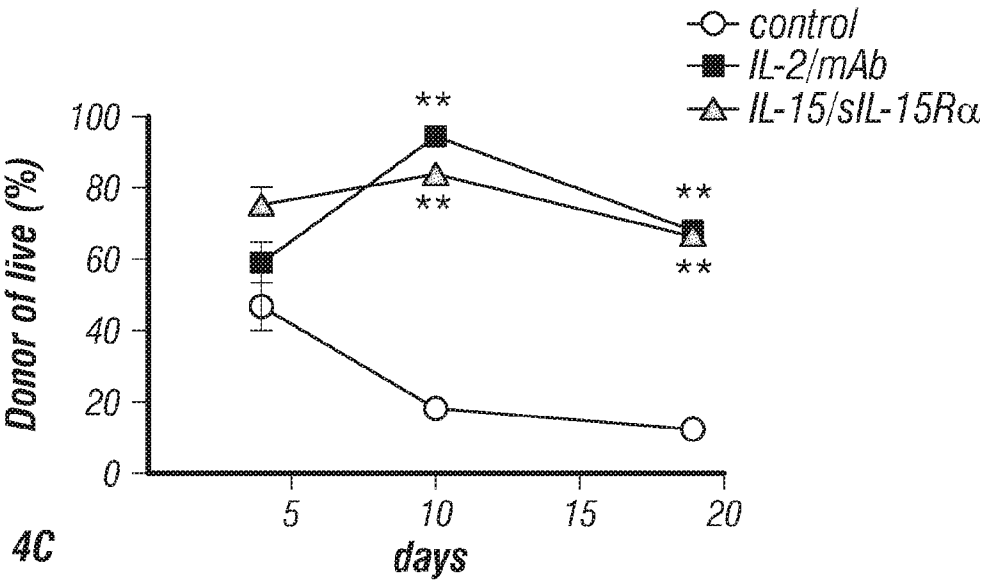


FIG. 4C

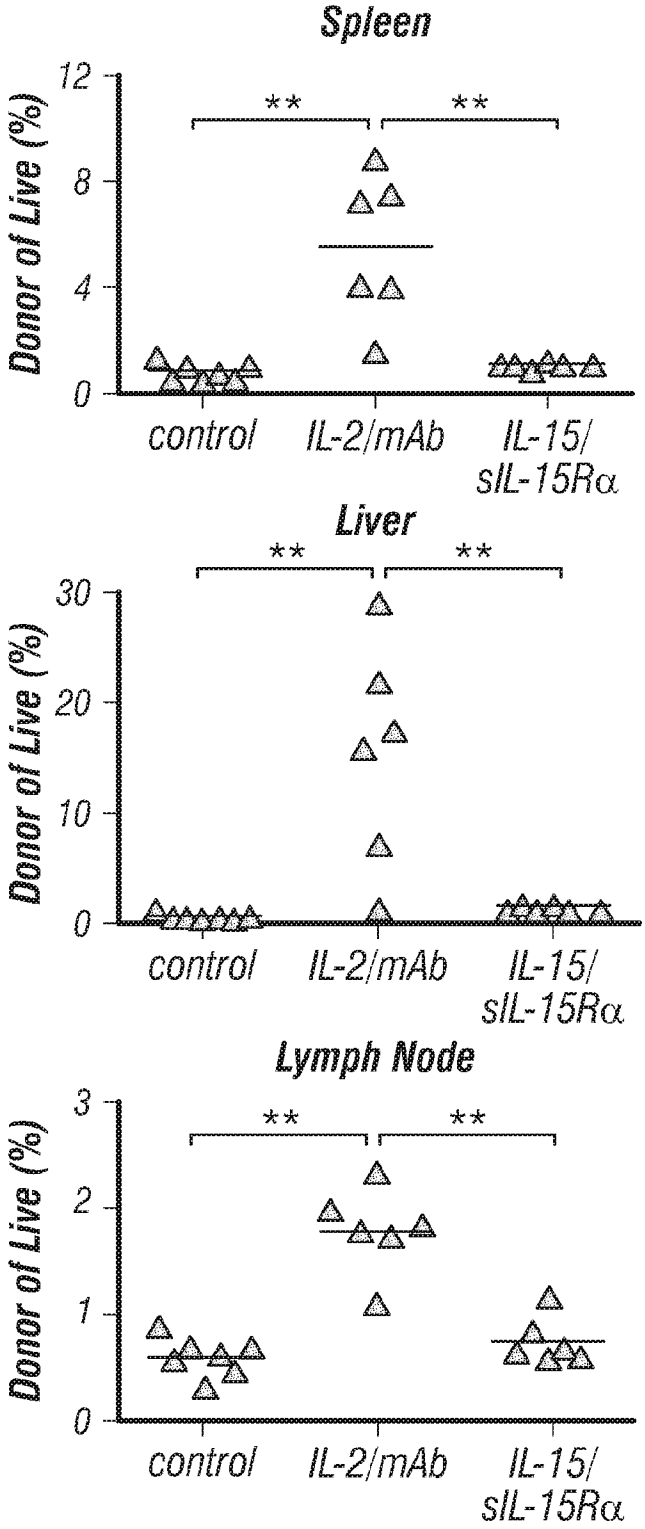

Spleen
Donor of Live (%)
12
8
4
0
**
**
control
IL-2/mAb
IL-15/
sIL-15Rα
Liver
Donor of Live (%)
30
20
10
0
**
**
control
IL-2/mAb
IL-15/
sIL-15Rα
Lymph Node
Donor of Live (%)
3
2
1
0
**
**
control
IL-2/mAb
IL-15/
sIL-15Rα

FIG. 5A

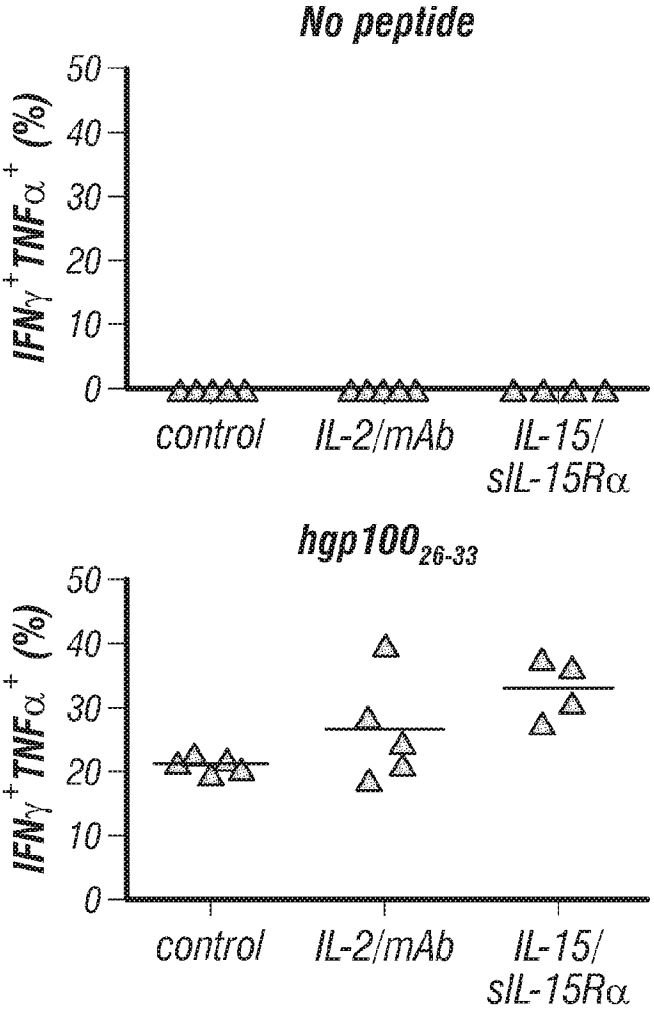

No peptide
IFNγ+TNFα+ (%)
50
40
30
20
10
0
control
IL-2/mAb
IL-15/
sIL-15Rα
hgp100 26-33
IFNγ+TNFα+ (%)
50
40
30
20
10
0
control
IL-2/mAb
IL-15/
sIL-15Rα

FIG. 5B

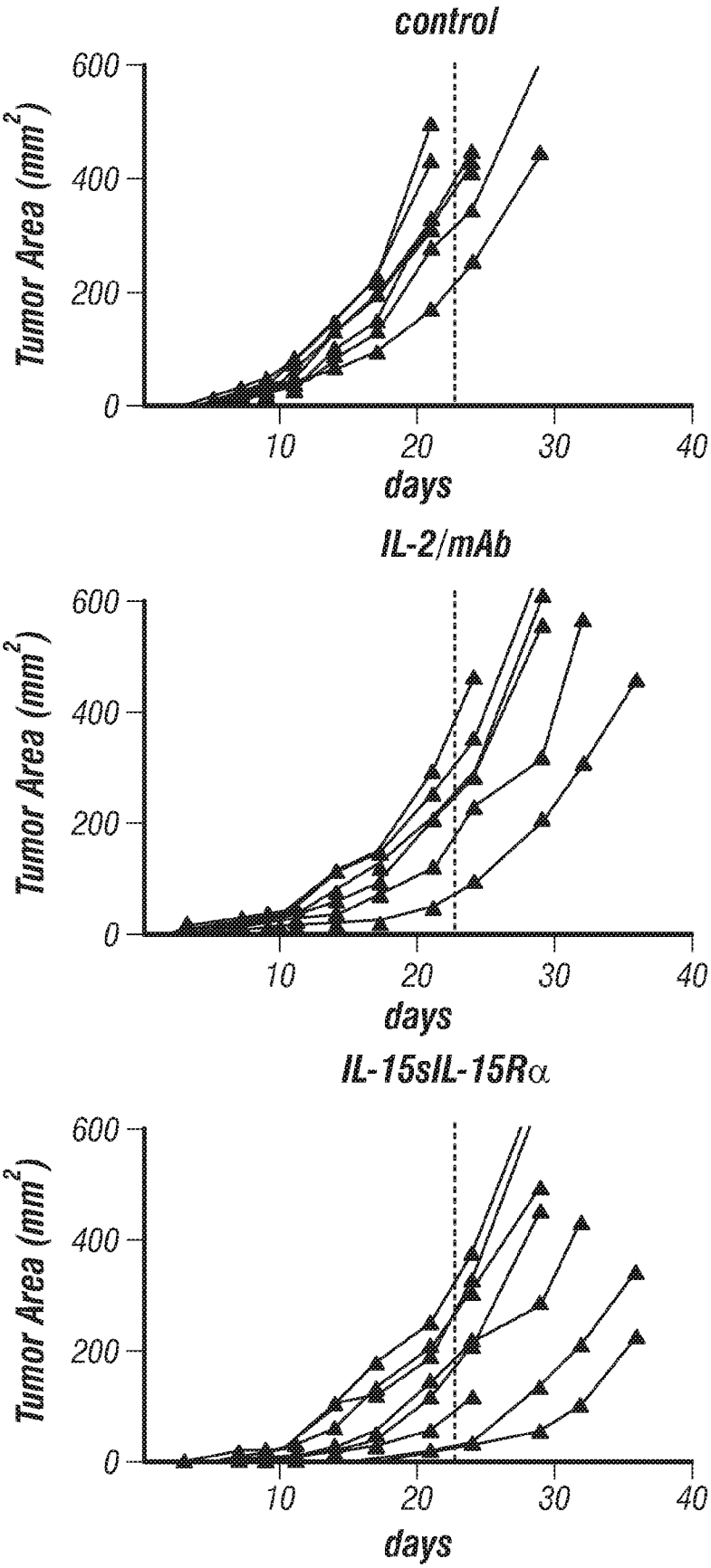

control
Tumor Area (mm2)
600
400
200
0
10
20
30
40
days
IL-2/mAb
Tumor Area (mm2)
600
400
200
0
10
20
30
40
days
IL-15sIL-15Rα
Tumor Area (mm2)
600
400
200
0
10
20
30
40
days

FIG. 6

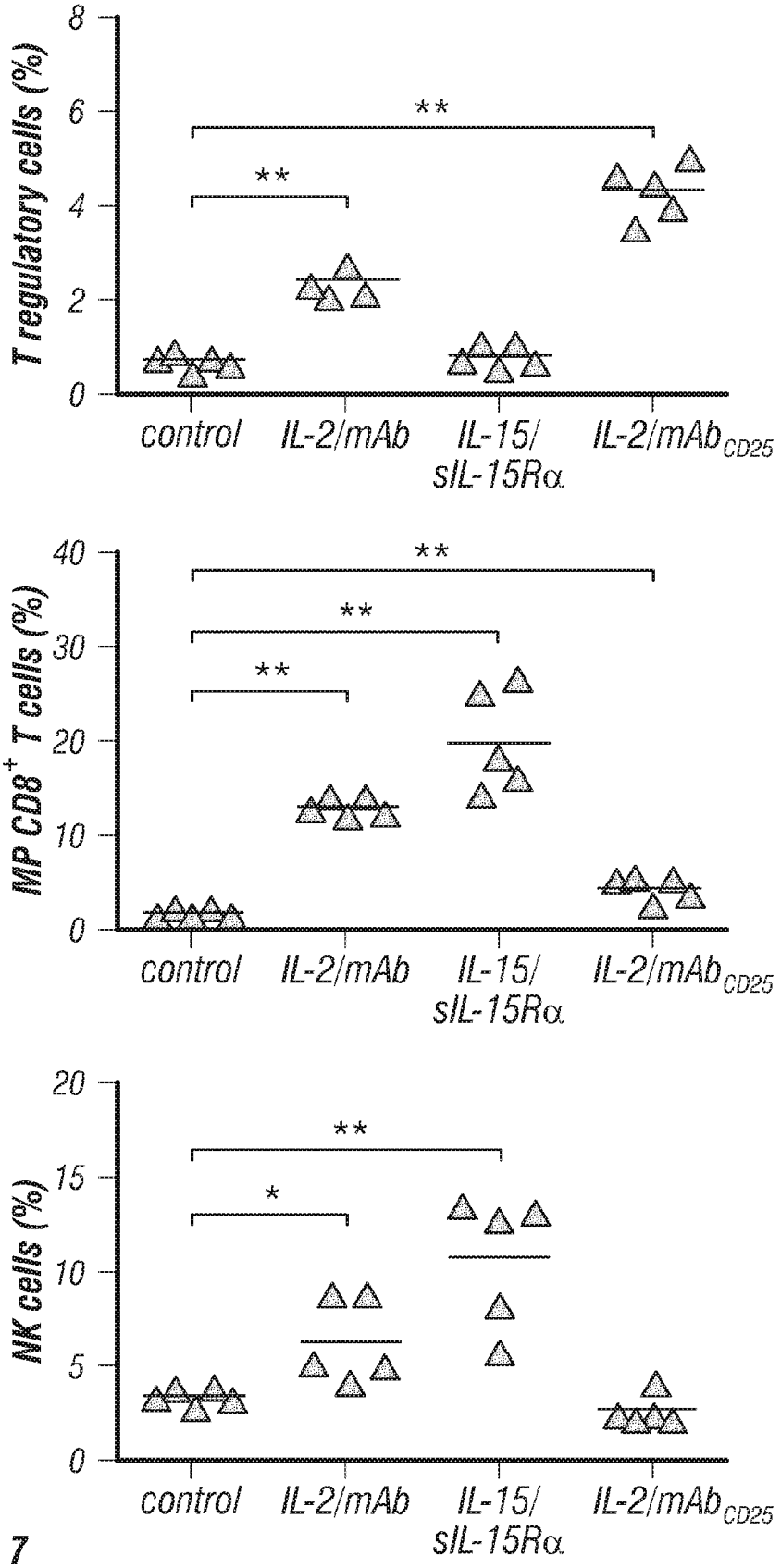

T regulatory cells (%)
8
6
4
2
0
**
**
control
IL-2/mAb
IL-15/
sIL-15Rα
IL-2/mAb CD25
MP CD8+ T cells (%)
40
30
20
10
0
**
**
**
control
IL-2/mAb
IL-15/
sIL-15Rα
IL-2/mAb CD25
NK cells (%)
20
15
10
5
0
**
*
control
IL-2/mAb
IL-15/
sIL-15Rα
IL-2/mAb CD25

FIG. 7

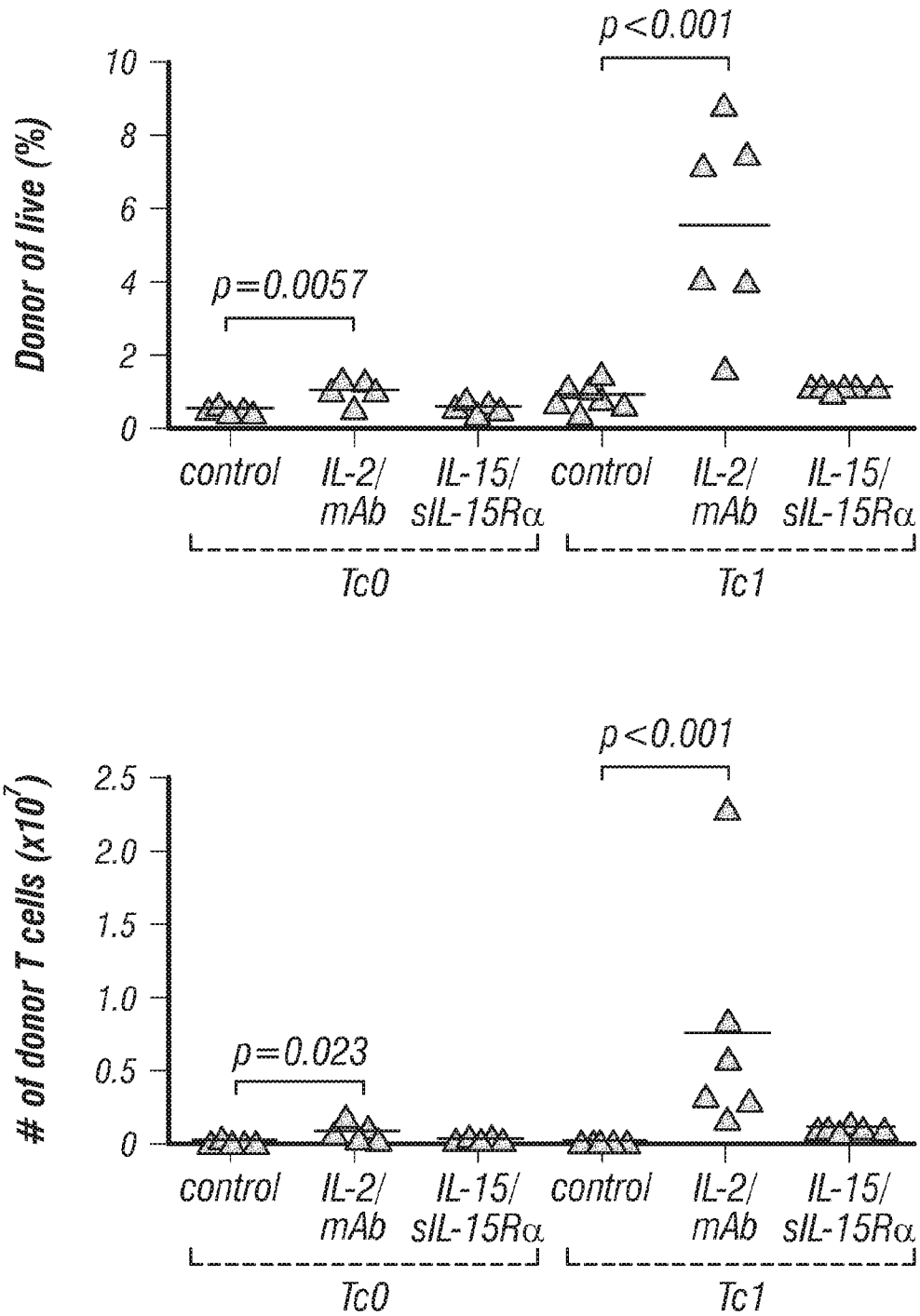

p<0.001
p=0.0057
Donor of live (%)
10
8
6
4
2
0
control
IL-2/ mAb
IL-15/ sIL-15Rα
control
IL-2/ mAb
IL-15/ sIL-15Rα
Tc0
Tc1
p<0.001
p=0.023
# of donor T cells (x10^7)
2.5
2.0
1.5
1.0
0.5
0
control
IL-2/ mAb
IL-15/ sIL-15Rα
control
IL-2/ mAb
IL-15/ sIL-15Rα
Tc0
Tc1

FIG. 8

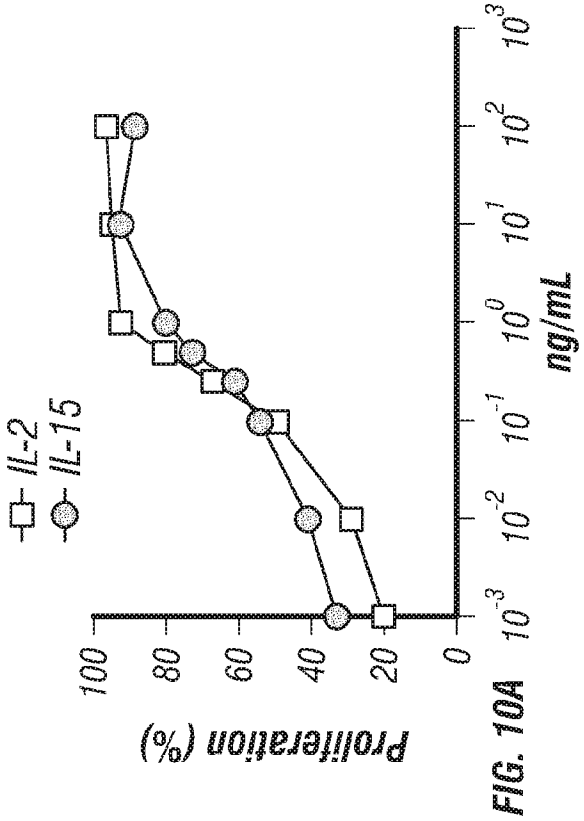


FIG. 10A

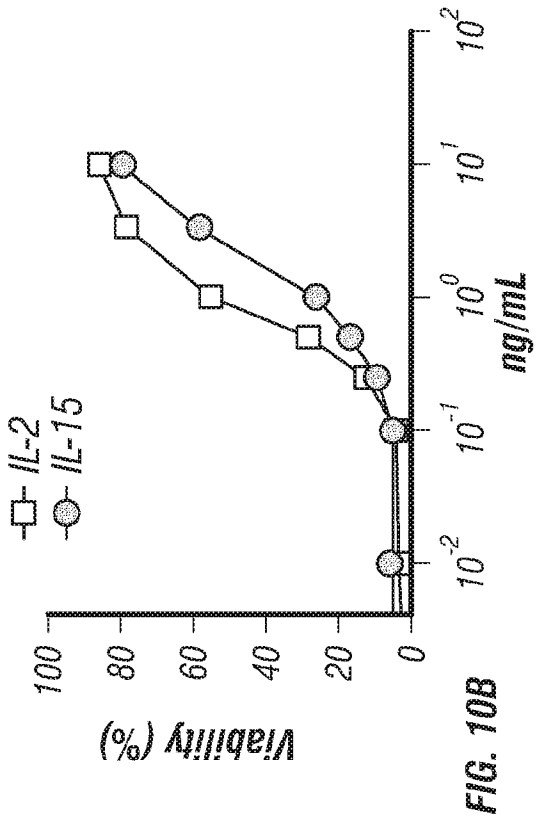


FIG. 10B

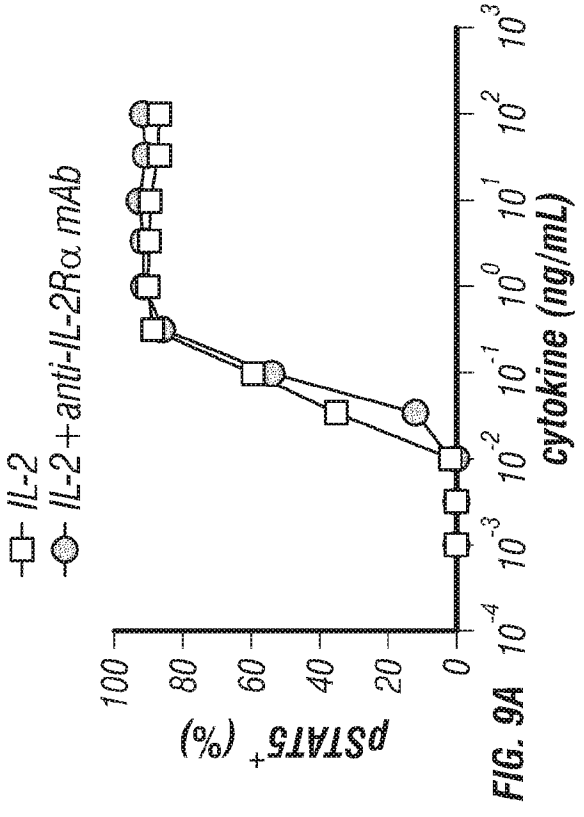


FIG. 9A

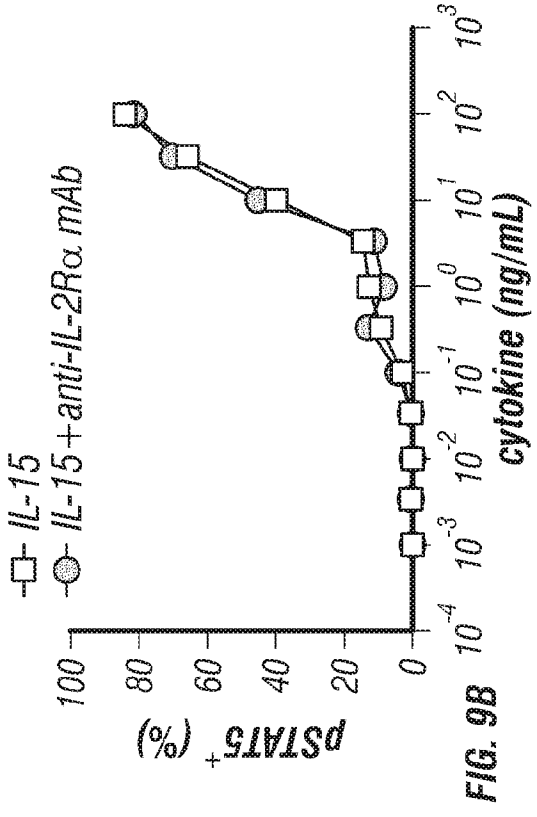


FIG. 9B

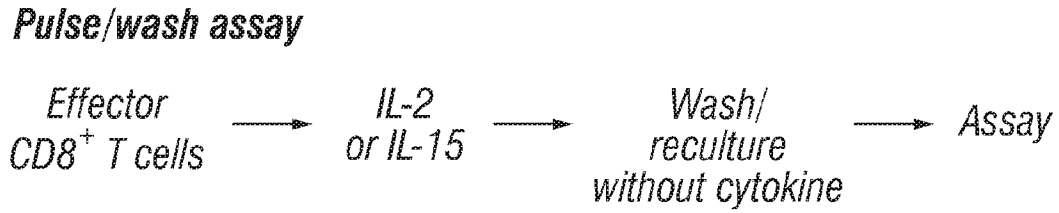


FIG. 11A

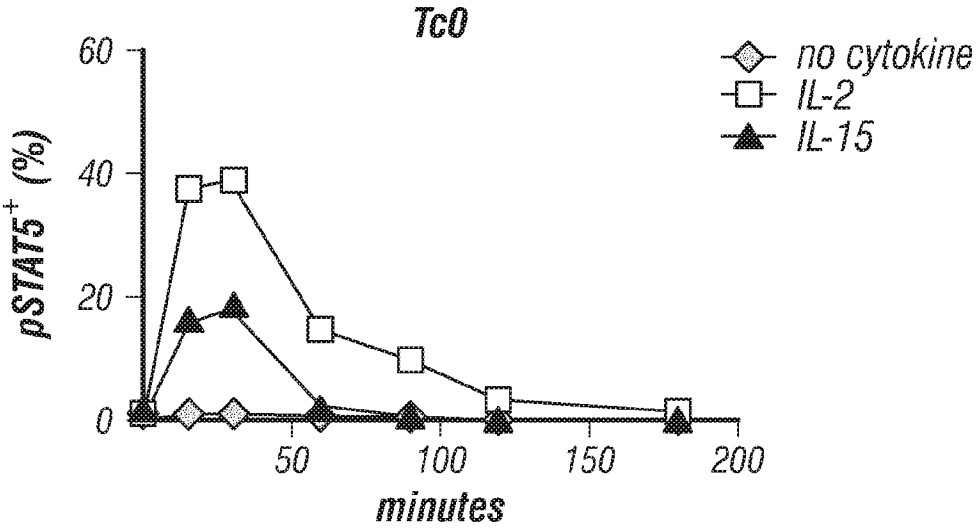


FIG. 11B

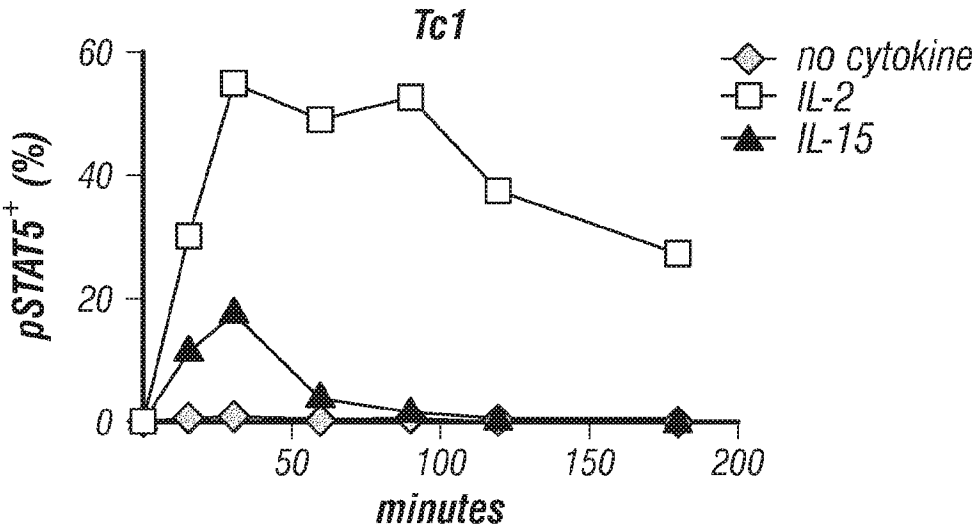


FIG. 11C

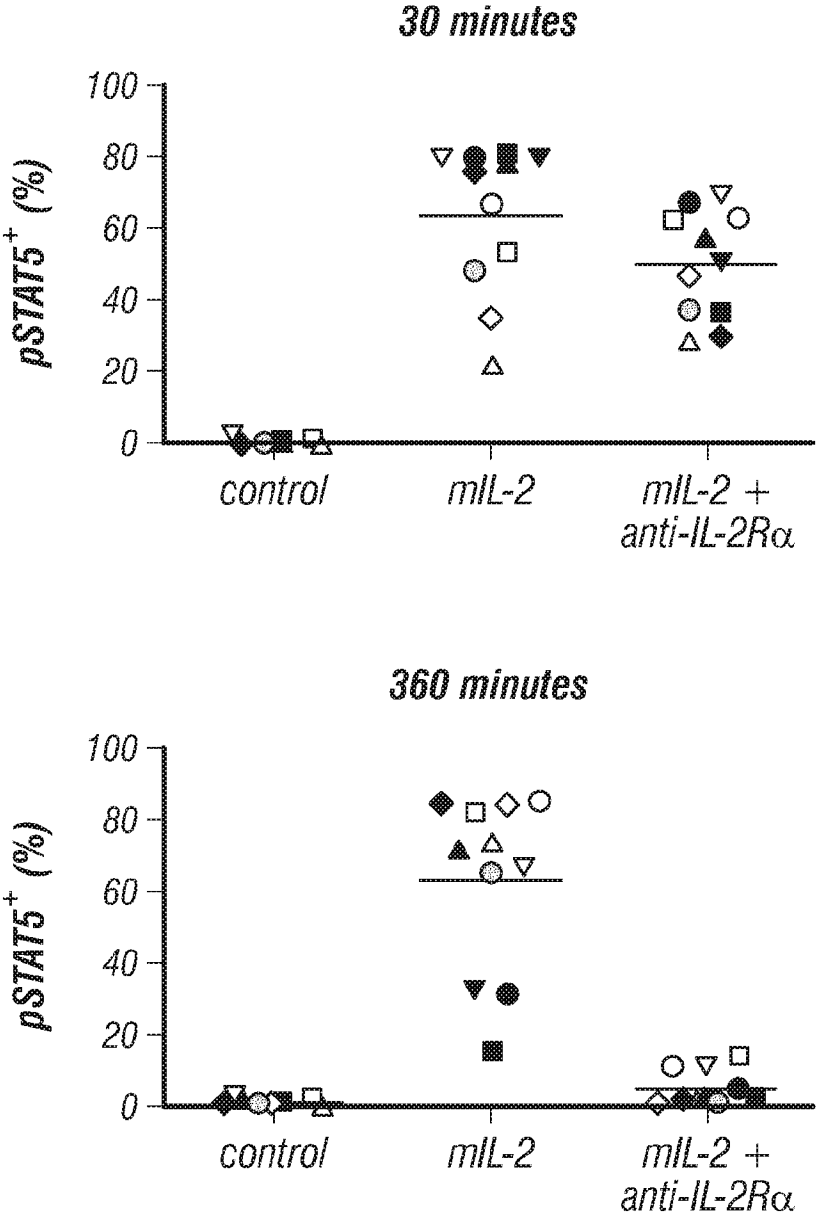

30 minutes
pSTAT5+ (%)
100
80
60
40
20
0
control
mIL-2
mIL-2 + anti-IL-2Rα
360 minutes
pSTAT5+ (%)
100
80
60
40
20
0
control
mIL-2
mIL-2 + anti-IL-2Rα

FIG. 12

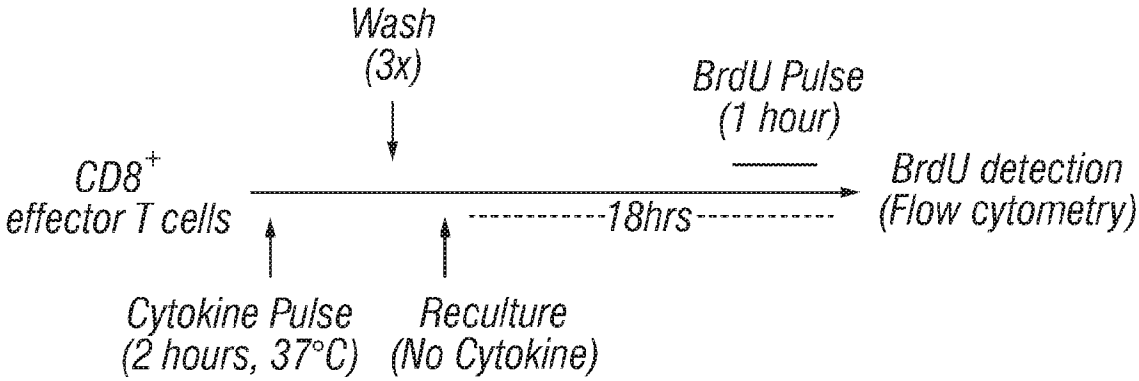


FIG. 13A

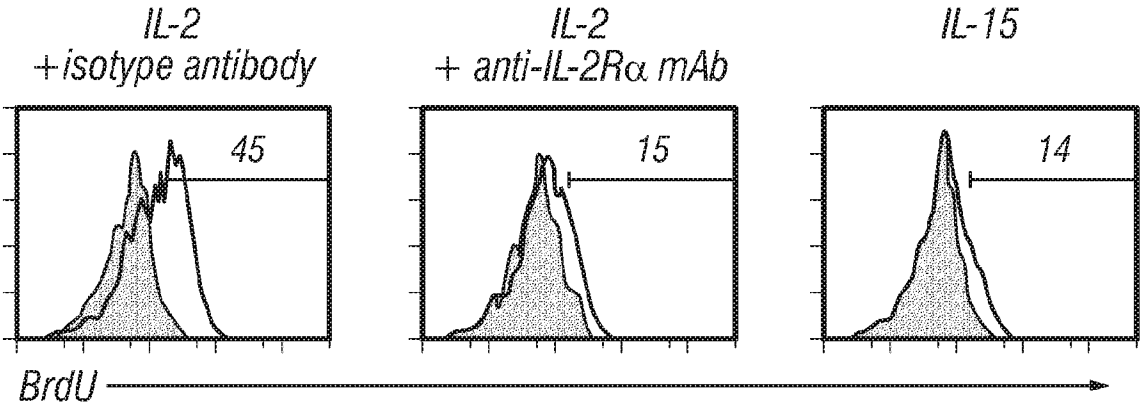


FIG. 13B

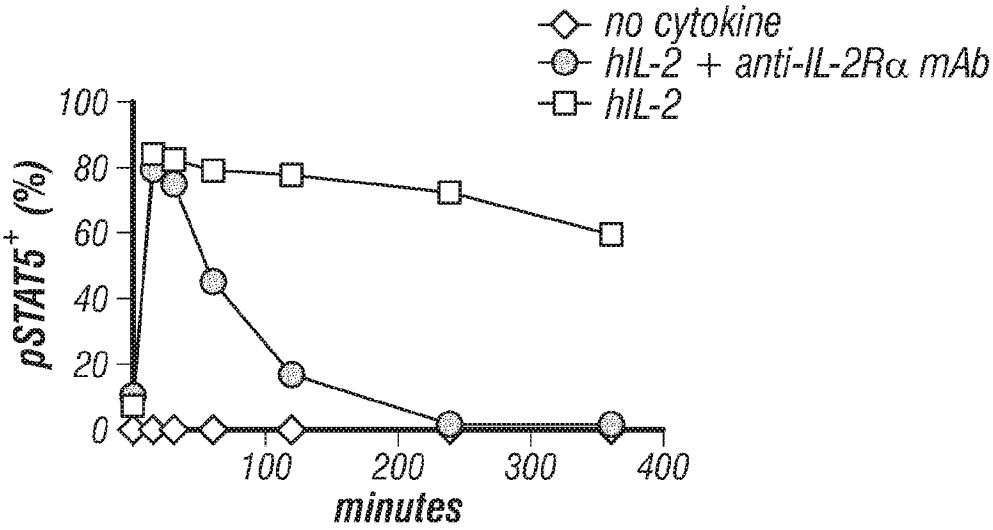


FIG. 14

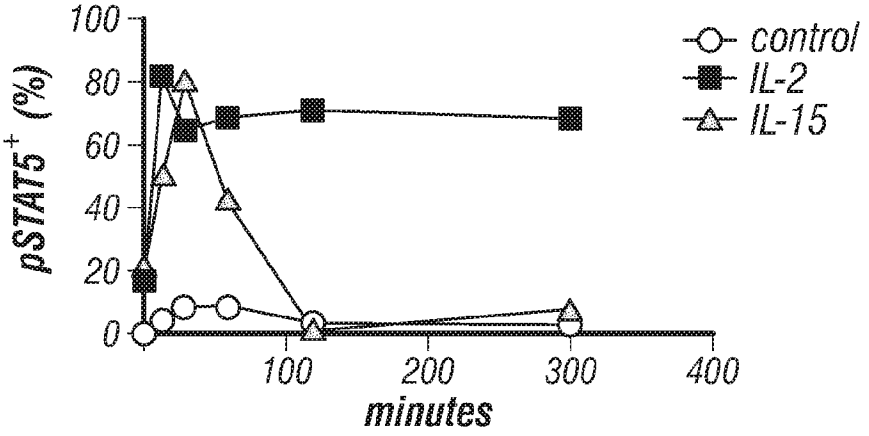


FIG. 15A

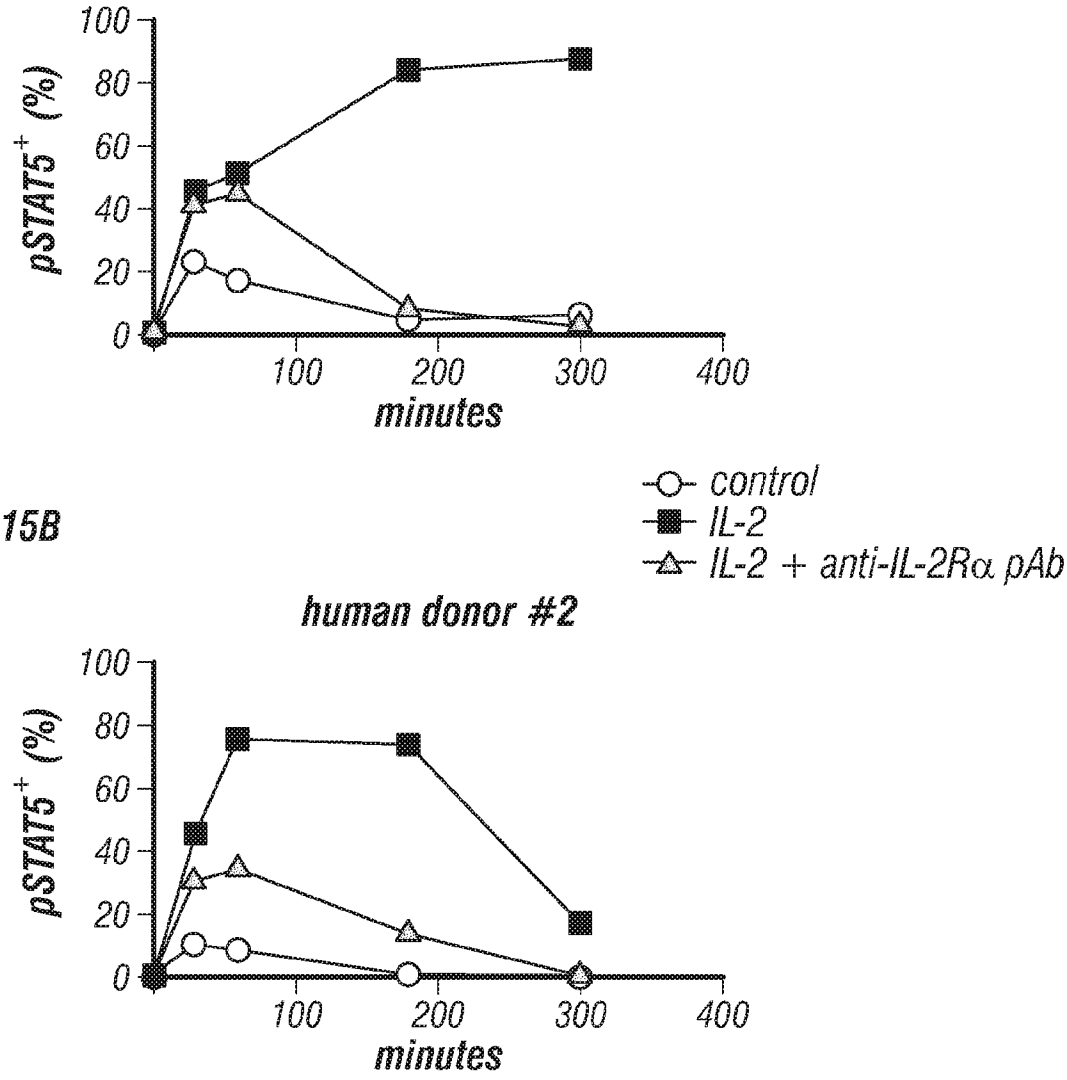


FIG. 15B

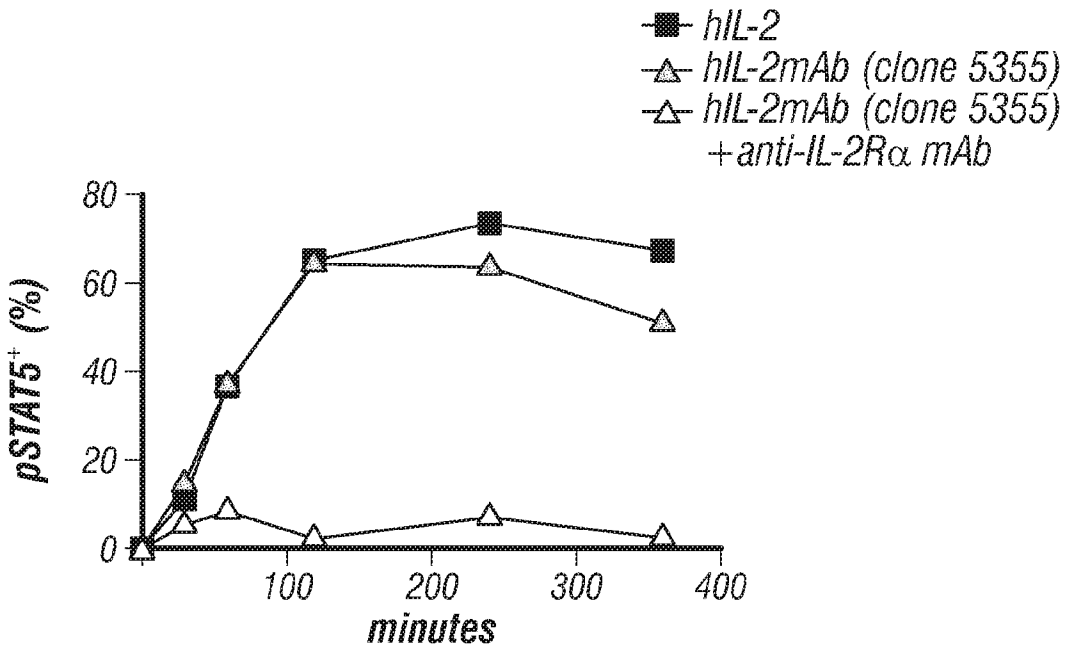


FIG. 16A

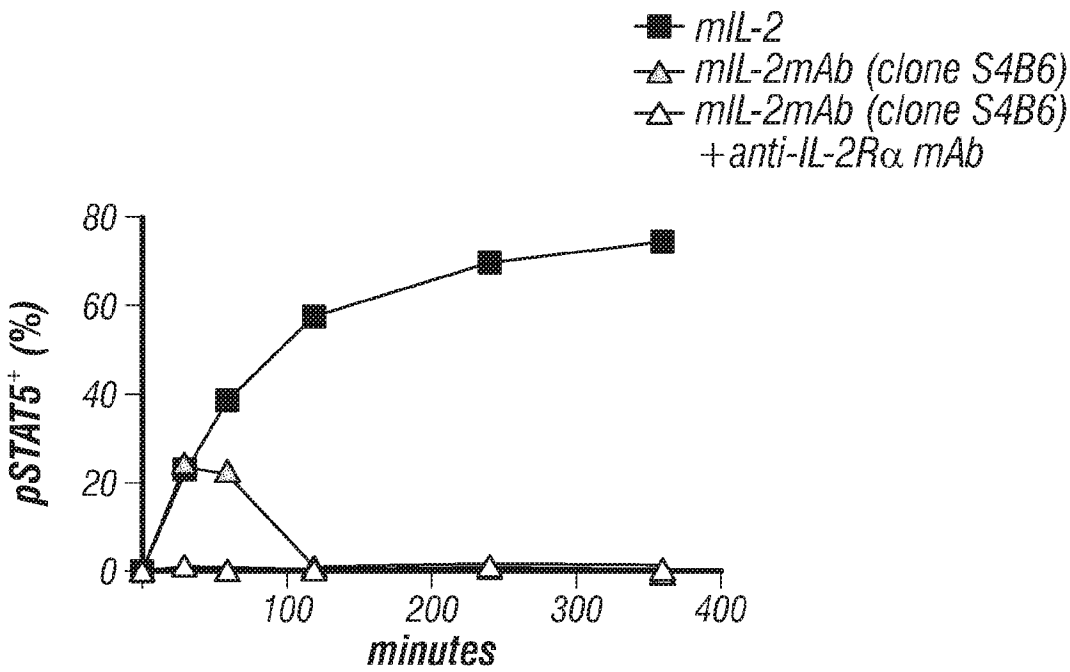


FIG. 16B

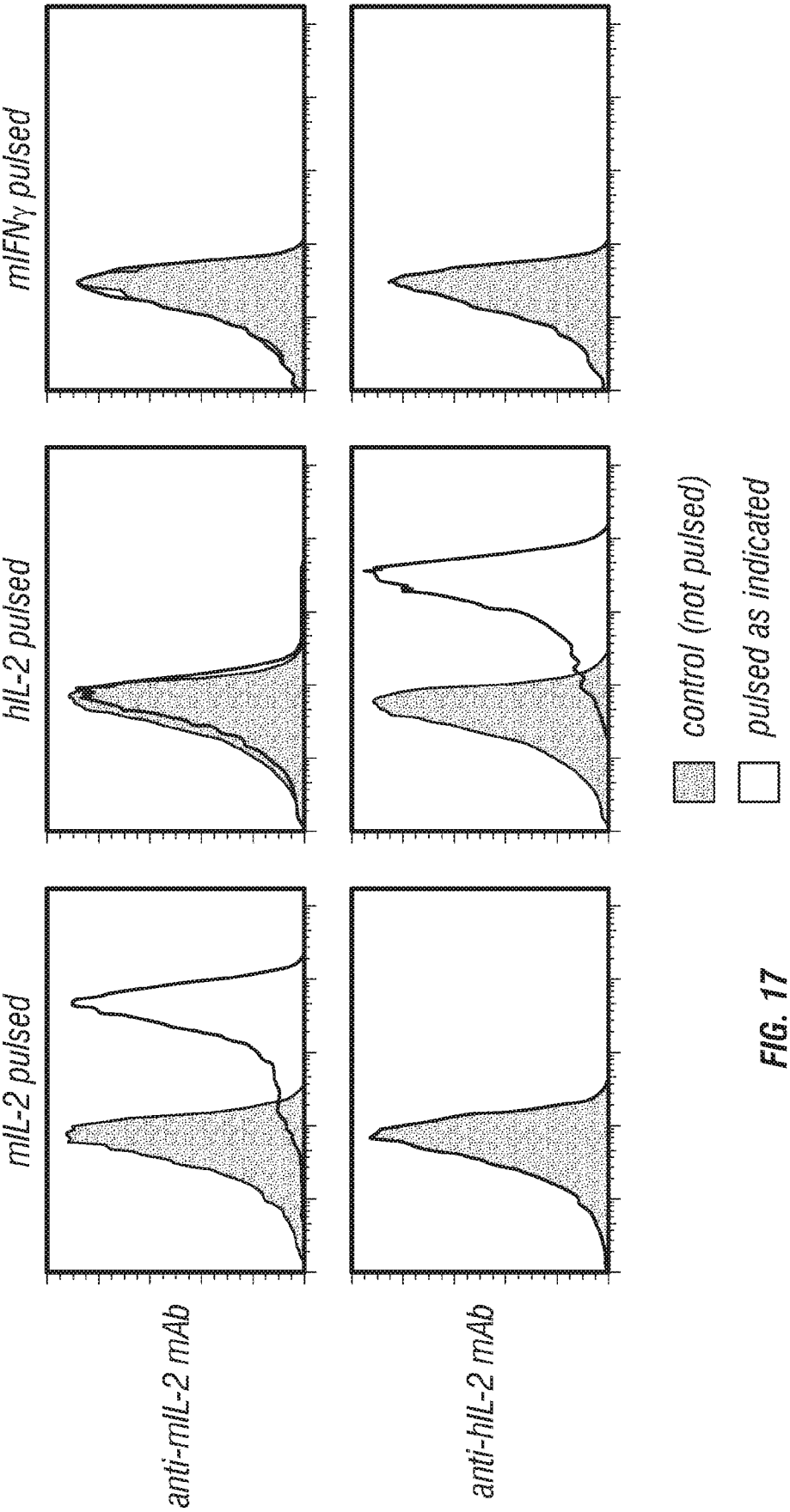

mIFNγ pulsed
hIL-2 pulsed
mIL-2 pulsed
anti-mIL-2 mAb
anti-hIL-2 mAb
control (not pulsed)
pulsed as indicated

FIG. 17

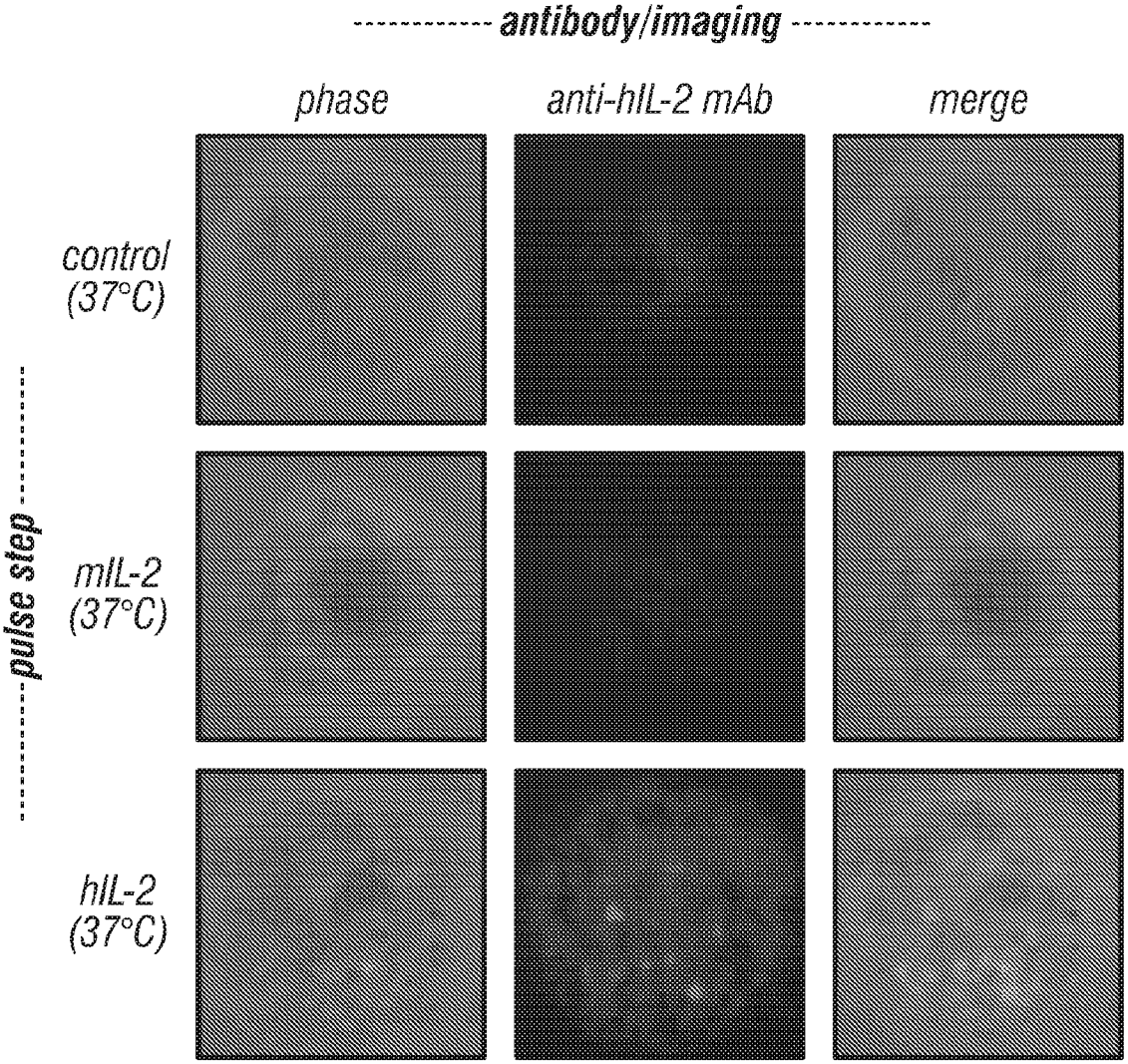

antibody/imaging
phase
anti-hIL-2 mAb
merge
pulse step
control (37°C)
mIL-2 (37°C)
hIL-2 (37°C)

FIG. 18A

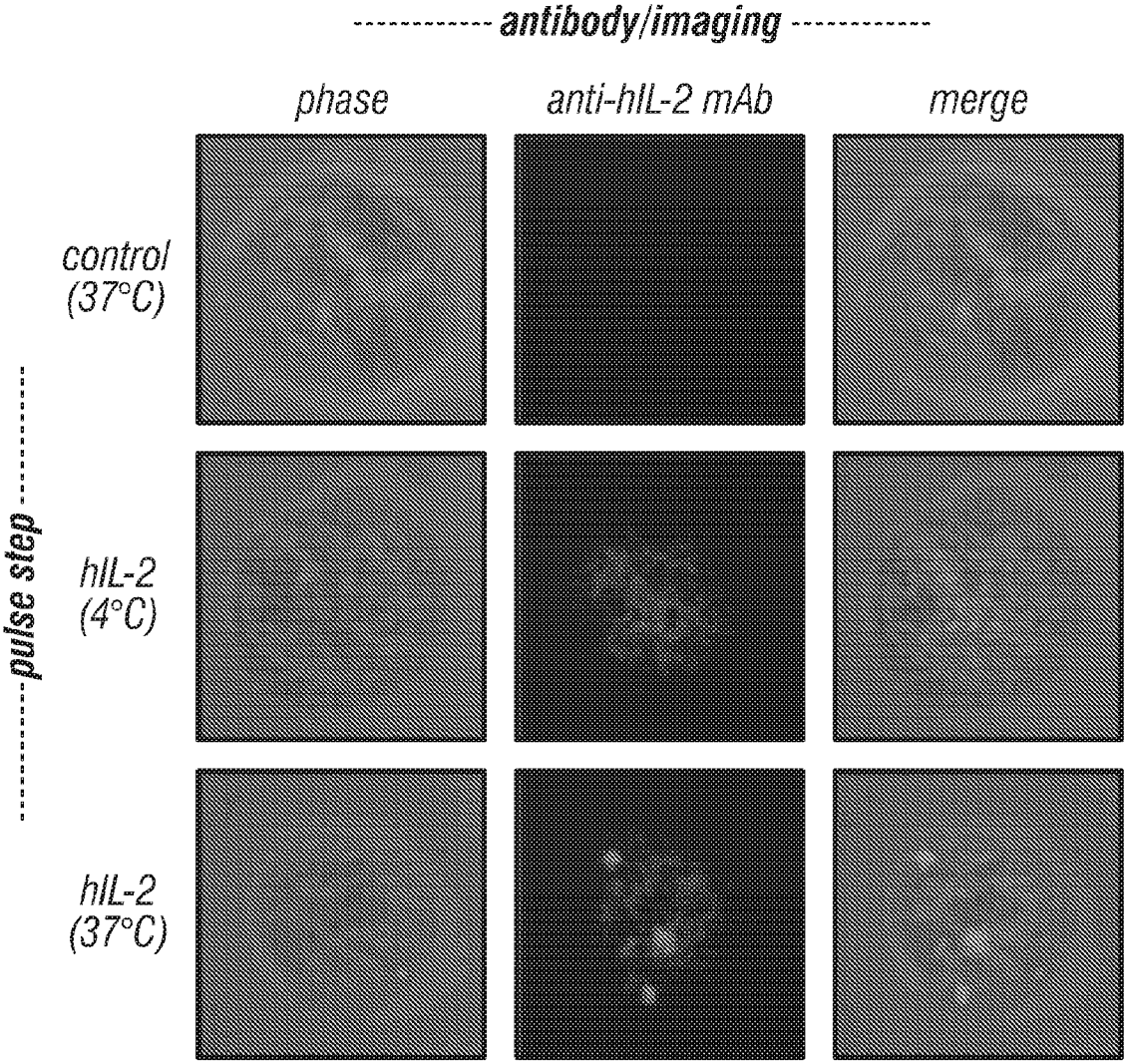

antibody/imaging
phase
anti-hIL-2 mAb
merge
pulse step
control (37°C)
hIL-2 (4°C)
hIL-2 (37°C)

FIG. 18B

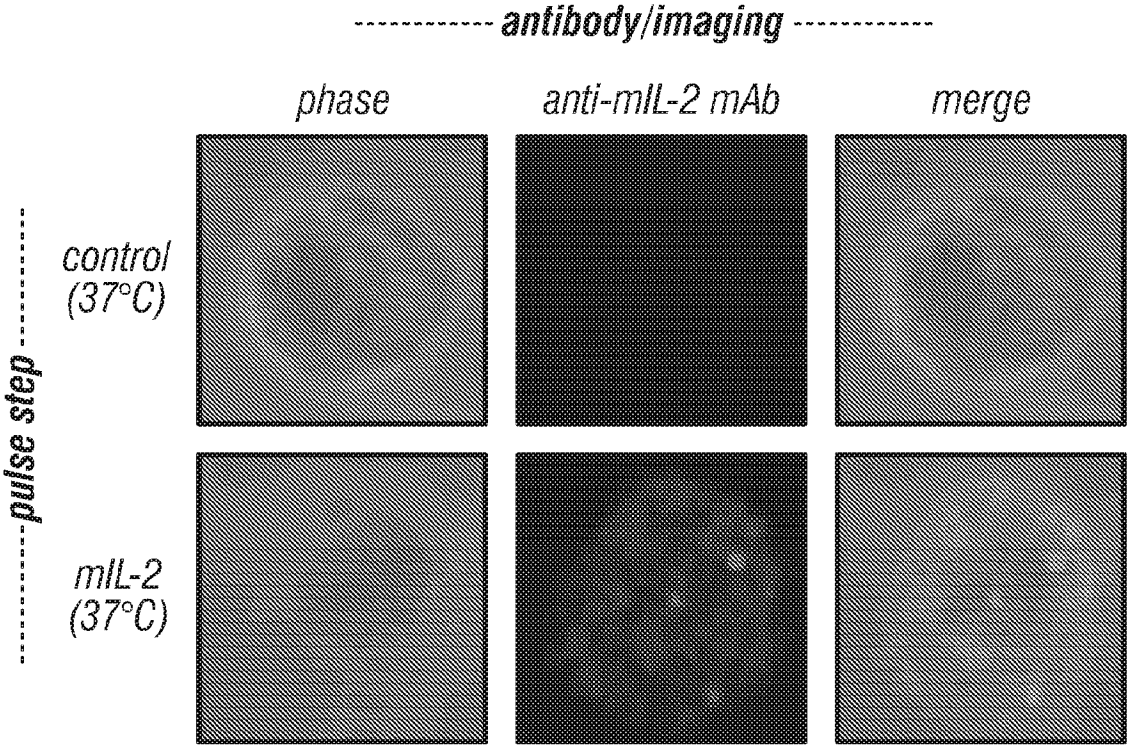

antibody/imaging
phase
anti-mIL-2 mAb
merge
pulse step
control (37°C)
mIL-2 (37°C)

FIG. 19

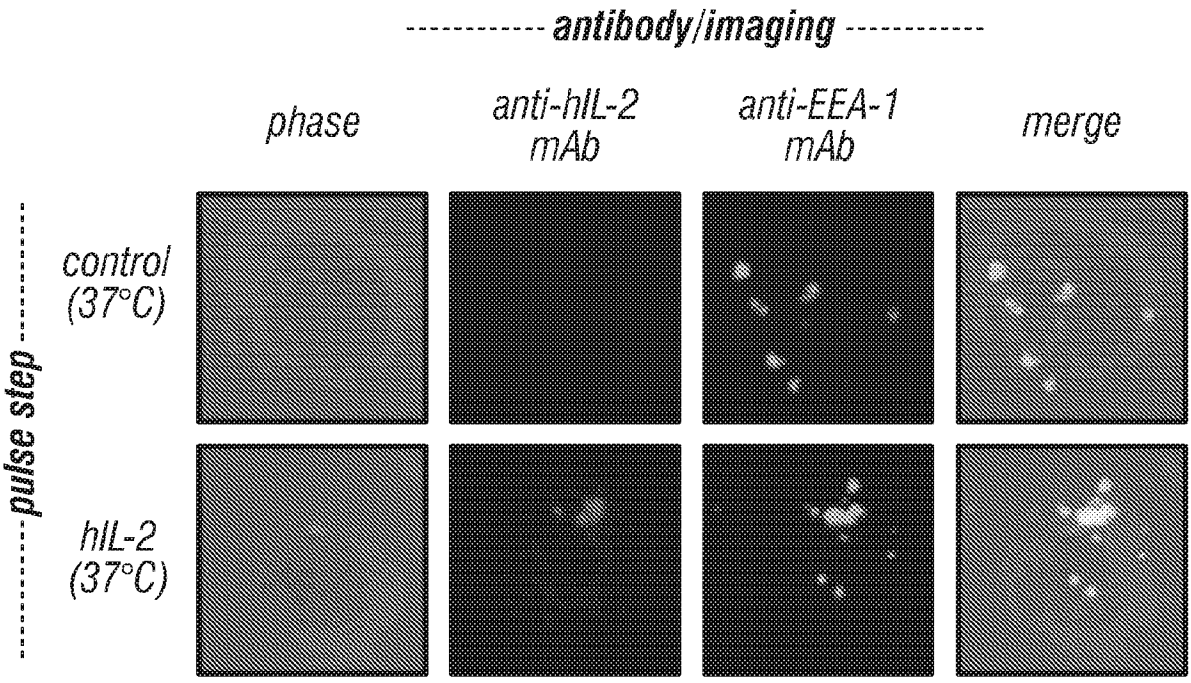


FIG. 20A

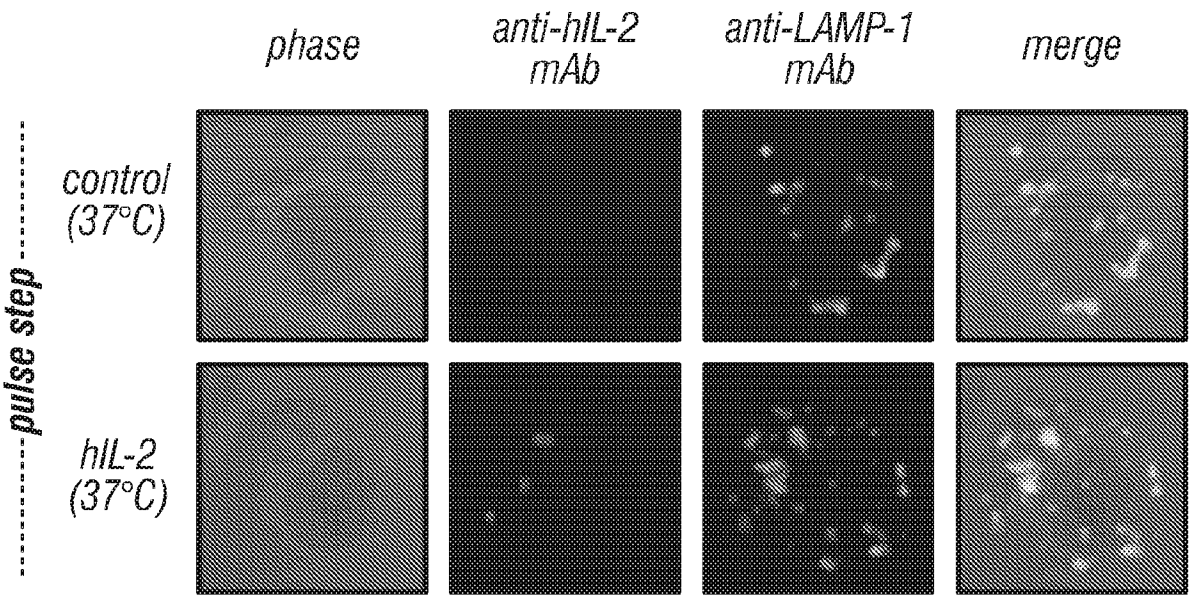


FIG. 20B

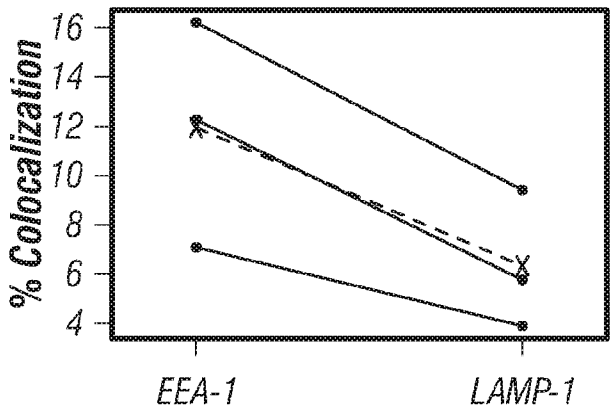
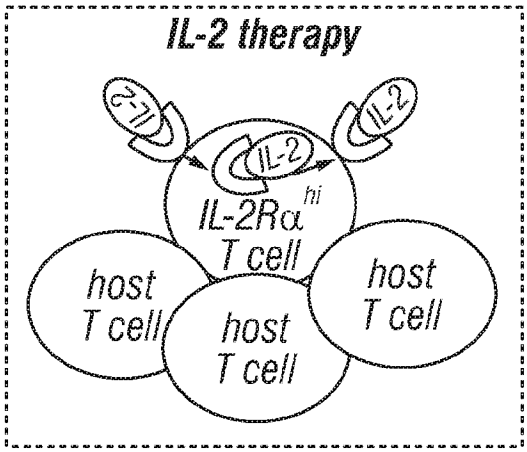
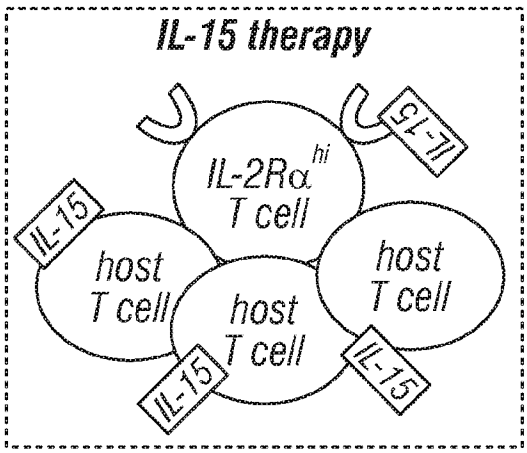


FIG. 20C





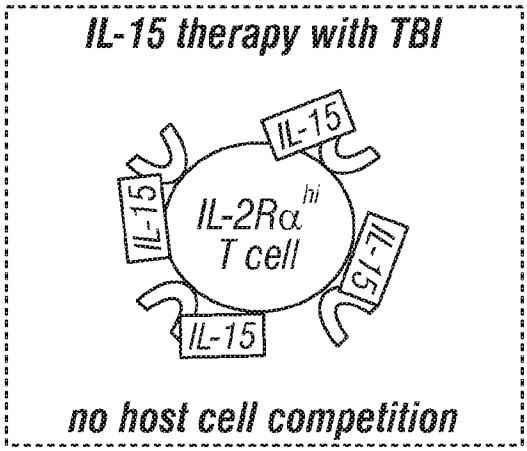


FIG. 21A

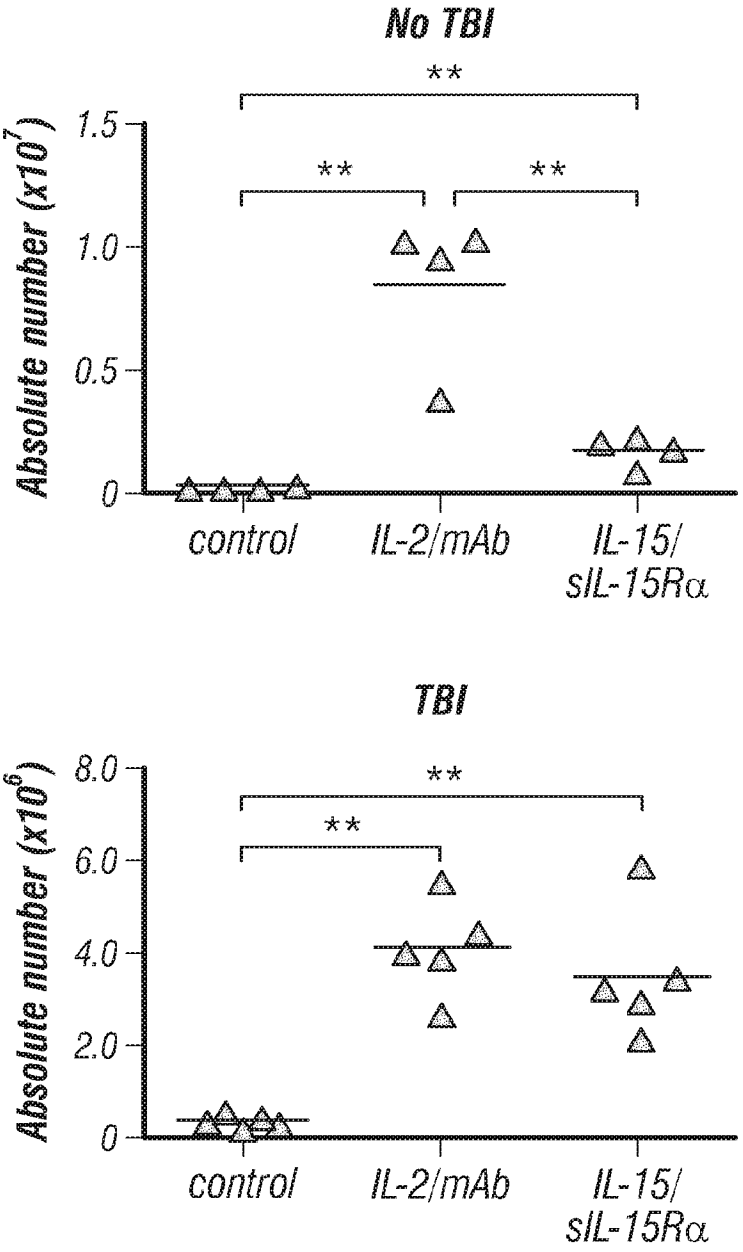

No TBI
**
**
**
Absolute number (x10^7)
1.5
1.0
0.5
0
control
IL-2/mAb
IL-15/
sIL-15Rα
TBI
**
**
Absolute number (x10^6)
8.0
6.0
4.0
2.0
0
control
IL-2/mAb
IL-15/
sIL-15Rα

FIG. 21B

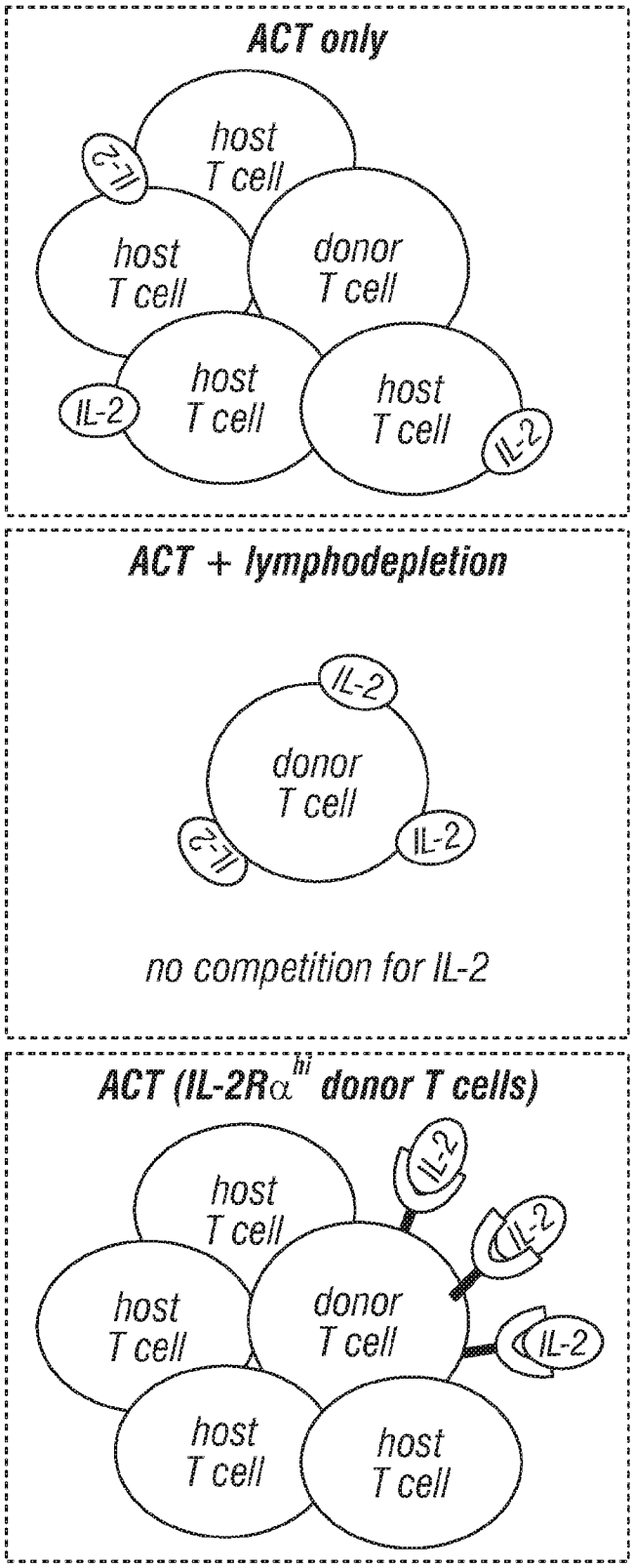

ACT only
host T cell
IL-2
host T cell
donor T cell
host T cell
IL-2
host T cell
IL-2
ACT + lymphodepletion
IL-2
donor T cell
IL-2
IL-2
no competition for IL-2
ACT (IL-2Rα hi donor T cells)
host T cell
IL-2
IL-2
host T cell
donor T cell
IL-2
host T cell
host T cell

FIG. 21C

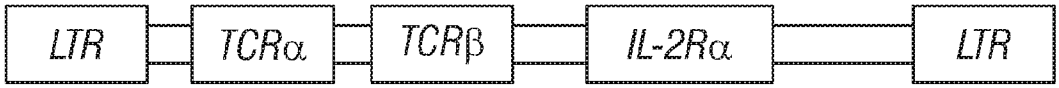

LTR
TCRα
TCRβ
IL-2Rα
LTR

FIG. 22

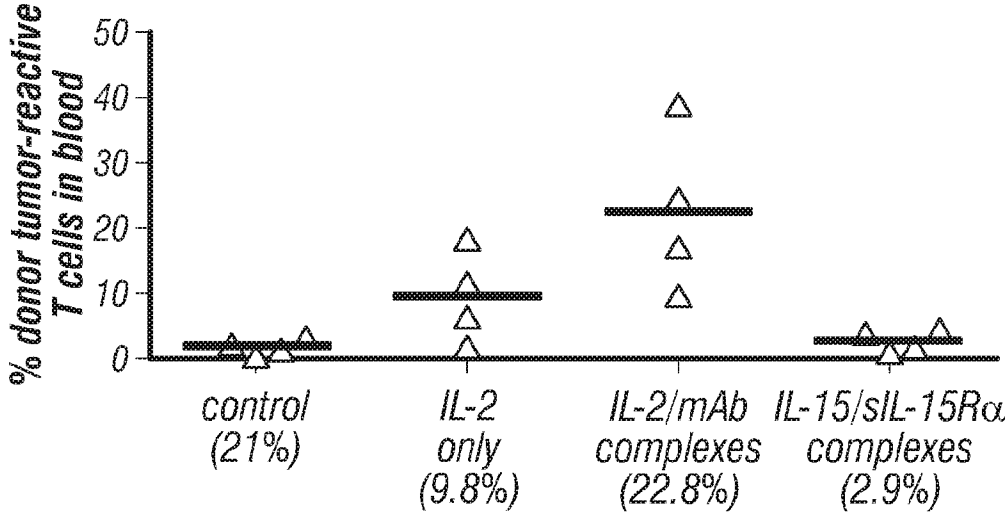

% donor tumor-reactive T cells in blood
50
40
30
20
10
0
control (21%)
IL-2 only (9.8%)
IL-2/mAb complexes (22.8%)
IL-15/sIL-15Rα complexes (2.9%)

FIG. 23

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 16/14516

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 35/17, C07K 14/705, C07K 14/725 (2016.01)
CPC - A61K 35/17, C07K 14/7051, C07K 14/7155
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - A61K 35/17, C07K 14/705, C07K 14/725 (2016.01)
CPC - A61K 35/17, C07K 14/7051, C07K 14/7155

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
CPC - A61K 48/00, C12N 15/63
(keyword limited: terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatBase, Google Scholar, Google Patents
Search terms: T cell, T-cell, T lymphocyte, NK cell, natural killer, cell, transgenic, engineered, modified, vector, plasmid, IL-2, IL2, IL-15, IL15, receptor, IL-2R, IL2R, IL-15R, IL15R, co-stimulatory, costimulatory, ICOS, CD278, 41BB, 4-1BB, CD28, G-CSFR, inducible, promot

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2012/0093842 A1 (ESHHAR et al.) 19 April 2012 (19.04.2012) para [0014], [0021], [0022], [0023], [0024], [0025], [0054], [0058], [0060], [0062], [0063], [0064], [0068], [0075], [0091]; claims 42, 45	1-2, 8, 10, 16-21, 23-31, 33, 38-39, 44-45, (46-47)/(1-2,8,10,16-21,23-31,33,38-39,44-45) --- 11, 22, 32, 40-43, (46-47)/(11,22,32,40-43)
Y	US 2014/0286987 A1 (BELLICUM PHARMACEUTICALS, INC.) 25 September 2014 (25.09.2014) para [0002], [0011], [0015], [0098] [0117], [0157], [0159], [0225]	11, 22, 40-43, (46-47)/(11,22,40-43)
Y	BACHA et al., Interleukin 2 receptor-targeted cytotoxicity. Interleukin 2 receptor- mediated action of a diphtheria toxin-related interleukin 2 fusion protein. J Exp Med. 1 February 1988, Vol 167, No 2, pp 612-22. Especially p 619, para 5	32, (46-47)/32
Y, P	WO 2015/123527 A1 (BELLICUM PHARMACEUTICALS INC.) 20 August 2015 (20.08.2015) entire document	11, 40-43

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

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
07 June 2016	01 JUL 2016
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 16/14516

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

-------please see extra sheet-------

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-2, 8, 10, 11, 16-33, 38-45, (46-47)(in part), limited to IL-2 receptor and ICOS

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

---Continuation of: Box No. III Observations where unity of invention is lacking----

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I+: Claims 1-47, drawn to an isolated transgenic cell and pharmaceutical composition comprising said isolated cell comprising: (i) an elevated surface expression level of at least one cytokine receptor or cytokine receptor co-stimulator, relative to an activated T-cell; or (ii) a nucleic acid molecule encoding at least one cytokine receptor or cytokine receptor costimulator polypeptide comprising a mutation that increases the activity of the receptor. The isolated transgenic cell will be searched to the extent that the transgenic cell encompasses:
- the at least one cytokine receptor is the IL-2 receptor (claim 2),
- the at least one cytokine receptor co-stimulator is ICOS (CD278) (claim 11).

It is believed that claims claims 1-2, 8, 10, 11, 16-33, 38-45, (46-47)(in part) encompass this first named invention, and thus these claims will be searched without fee to the extent that they encompass a transgenic cell that comprises the IL-2 cytokine receptor and the ICOS (CD278) cytokine receptor co-stimulator. Additional cytokine receptor(s) and cytokine receptor costimulator(s) will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected cytokine receptor(s) and cytokine receptor costimulator(s). Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. An exemplary election would be a transgenic cell that comprises the IL-15 cytokine receptor [claim 2] and the 4-1BB cytokine receptor co-stimulator [claim 12], i.e. claims 1-2, 12, 16-27, 34-45, (46-47)(in part),
limited to IL-15 receptor and 4-1BB.

Group II: Claims 48-61 and 96-98, drawn to a method of providing a T-cell response in a human subject having a disease comprising administering an effective amount of T-cells; and to a method of administering an antigenic composition to a subject to induce an antigen -specific lymphocyte response, and administering a pharmacological agent to the subject to induce expression of a cytokine receptor, and administering a ligand for the cytokine receptor to the subject

Group III: Claims 62-72, drawn to a method of producing therapeutic cells comprising: (i) selecting a population of cells having an increased activity of at least a one cytokine receptor; and (ii) culturing the cells in the presence of a ligand for said at one cytokine receptor.

Group IV: Claims 73-95, drawn to a method of treating a disease comprising: transferring at least one receptor gene into at least one cell or by injecting a mammal with a vector encoding said receptor gene; and treating said cell or mammalian subject with an agonist of the receptor transcribed by said receptor gene.

The inventions listed as Groups I+, II, III, and IV do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features

Group I+ requires a composition comprising an isolated transgenic cell, not required by Groups II, III, and IV. Further, the technical feature of each of the inventions listed as Group I+ is the specific cytokine receptor and cytokine receptor costimulator recited therein. Each invention requires a cytokine receptor and cytokine receptor costimulator not required by any of the other inventions, and not required by Groups II, III, IV.

Group II requires method steps of administering T cells for providing a T cell response to a disease; as well as method steps of an antigenic composition to a subject to induce an antigen-specific lymphocyte response, and administering a pharmacological agent to the subject to induce expression of a cytokine receptor, and administering a ligand for the cytokine receptor to the subject, not required by Groups I+, III, IV.

Group III requires method steps for producing therapeutic cells by screening cells for increased cytokine receptor activity and culturing said cells, not required by Groups I+, II, IV.

Group IV requires method steps for treating a disease by transferring at least one receptor gene into at least one cell or mammalian subject, and treating said cell or subject with an agonist of the receptor transcribed by said receptor gene, not required by Groups I+, II, III.

-------please see continuation on next extra sheet-------

----Continuation of: Box No. III Observations where unity of invention is lacking----

Common Technical Features

The feature shared by Groups I+, II, III, IV is a receptor, more specifically a cytokine receptor.

The feature shared by Groups I+ and IV is a nucleic acid encoding a cytokine receptor, and a cell comprising said nucleic acid (claims 1, 73).

The feature shared by Groups II and IV is treatment of a disease using cells that express a receptor, more specifically a cytokine receptor (claims 48, 73).

The feature shared by Groups II and III is a ligand for a cytokine receptor (claims 62, 96).

The feature shared by Groups I+ and III is a cell having an increased activity of at least a one cytokine receptor (claims 1, 26, 27, 62).

The feature shared by the inventions listed as Group I+ is the isolated transgenic cell of claims 1 and the pharmaceutical composition of claim 46.

The feature shared by Group I+ and II is the T cell of claim 17.

However, these shared technical features do not represent a contribution over prior art, because the shared technical features are taught by US 2012/0093842 A1 to Eshhar et al. (hereinafter 'Eshhar').

Eshhar discloses a cytokine receptor (para [0012] "Another type of immune cell receptor is the IL-2 receptor.").

Eshhar discloses a nucleic acid encoding a cytokine receptor, and a cell comprising said nucleic acid (para [0019] "The chimeric gene comprises a first gene segment encoding the scFv of a specific antibody, ... linked by a flexible linker, and a second gene segment which comprises a DNA sequence encoding partially or entirely the transmembrane and cytoplasmic, and optionally the extracellular, domains of a lymphocyte-triggering molecule corresponding to a lymphocyte receptor or part thereof"; para [0058] "examples of immune cell [lymphocyte] trigger molecules are any one of the IL-2 receptor (IL-2R)", note that IL2R is a cytokine receptor; para [0020] "The present invention further relates to suitable vectors for transfecting cells of the type defined above with the chimeric gene").

Eshhar discloses treatment of a disease using cells that express a receptor, more specifically a cytokine receptor (para [0023] "The genetically engineered lymphocyte cells of the present invention may be used in new therapeutic treatment processes"; claim 42 "An adoptive immunotherapeutic method of treating a patient having a condition, comprising administering to the patient cells that have been transformed with a chimeric gene, the chimeric gene containing a) a first DNA segment encoding a single-chain Fv (scFv) domain of an antibody and b) a second DNA segment encoding a lymphocyte-activating molecule ... wherein the second DNA segment of the chimeric gene encodes: ... iii) a receptor molecule which transduces a costimulatory signal for lymphocyte activation; or iv) a subunit of an IL-2 receptor"; claim 45 "The method of claim 42, wherein the second DNA segment of the chimeric gene encodes a subunit of an IL-2 receptor and the subunit is a a or B subunit of an IL-2 receptor", note that IL2R is a cytokine receptor).

Eshhar discloses a ligand for a cytokine receptor (para [0012] "Another type of immune cell receptor is the IL-2 receptor [cytokine receptor]. ...When stimulated by IL-2 [ligand], lymphocytes undergo proliferation and activation.")

Eshhar disclose [claims 26, 27] a cell comprising a nucleic acid molecule encoding a IL-2 receptor comprising a mutation that increases the activity of the receptor upon ligand binding (para [0060] "Most recently prepared chimeras between these polypeptides and the ... IL-2 receptor chain (28) ... proved to be active in signalling T cell stimulation even in the absence of other TCR/CD3 components"; para [0063] "In vivo, cells expressing these genetically engineered chimeric receptors will home to their target, will be stimulated by it to attract other effector cells, or, by itself, will mediate specific destruction of the target cells. ... It is expected that such anti-tumor cytolysis can also be independent of exogenous supply of IL-2, thus providing a specific and safer means for adoptive immunotherapy").

Eshhar discloses [claim 1] An isolated transgenic cell (para [0021] "The present invention further relates to cells of the type defined above into which such chimeric gene has been introduced so as to obtain its expression, and also to pharmaceutical prophylactic and curative compositions containing an effective quantity of such cells"; para [0023] "T cells or NK cells isolated from a patient may be transfected with DNA encoding a chimeric gene including the variable region of an antibody directed toward a specific antigen, and then returned to the patient") comprising:
(i) an elevated surface expression level of at least one cytokine receptor or cytokine receptor co-stimulator, relative to an activated T-cell (claim 42 "cells that have been transformed with a chimeric gene, the chimeric gene containing a) a first DNA segment encoding a single -chain Fv (scFv) domain of an antibody and b) a second DNA segment encoding a lymphocyte-activating molecule to express the scFv domain and the lymphocyte-activating molecule on the surface of the lymphocytes and to provide antibody specificity, wherein the second DNA segment of the chimeric gene encodes: ... iii) a receptor molecule which transduces a costimulatory signal for lymphocyte activation; or iv) a subunit of an IL-2 receptor"; para [0062] "the resultant chimera is expressed on the surface of an immune cell in to which the corresponding gene was genetically introduced", expression of the chimeric cytokine receptor in the transformed cell would inherently result in elevated cytokine receptor expression level as compared to an activated T-cell that has not been transformed with the chimeric gene); or
(ii) a nucleic acid molecule encoding at least one cytokine receptor or cytokine receptor costimulator polypeptide comprising a mutation that increases the activity of the receptor (para [0060] "Most recently prepared chimeras between these polypeptides and the ... IL-2 receptor chain (28) ... proved to be active in signalling T cell stimulation even in the absence of other TCR/CD3 components"; para [0063] "In vivo, cells expressing these genetically engineered chimeric receptors will home to their target, will be stimulated by it to attract other effector cells, or, by itself, will mediate specific destruction of the target cells. ... It is expected that such anti-tumor cytolysis can also be independent of exogenous supply of IL-2, thus providing a specific and safer means for adoptive immunotherapy").

-------please see continuation on next extra sheet-------

----Continuation of: Box No. III Observations where unity of invention is lacking----

Eshhar discloses [claim 17] wherein the cell is a T-cell (para [0064] "In preferred embodiments, the immune cells are T-cells or NK-cells").

Eshhar discloses [claim 46] a pharmaceutical composition comprising an isolated cell in a pharmaceutically acceptable carrier (para [0021] "The present invention further relates to cells of the type defined above into which such chimeric gene has been introduced so as to obtain its expression, and also to pharmaceutical prophylactic and curative compositions containing an effective quantity of such cells."; para [0068] "The transformed cells of the present invention may be administered in the form of a pharmaceutical composition with suitable pharmaceutically acceptable excipients").

As the technical features were known in the art at the time of the invention, they cannot be considered special technical features that would otherwise unify the groups.

Groups I+, II, III, and IV therefore lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.

NOTE: Claim 7 is objected to for consisting of two sentences. As drafted, claim 7 states:
"7. The isolated cell of claim 1, wherein the at least one cytokine receptor is IL-27R and/or GP130. In this case the ligand is IL-27 or an IL-27-derived molecule."
For the purposes of this ISA Search and Written Opinion, claim 7 is construed as follows:
"7. The isolated cell of claim 1, wherein the at least one cytokine receptor is IL-27R and/or GP130."