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April 17, 2008

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APPLICATION NUMBER: 60/921,144
FILING DATE: March 30, 2007
RELATED PCT APPLICATION NUMBER: PCT/US08/04251

THE COUNTRY CODE AND NUMBER OF YOUR PRIORITY APPLICATION, TO BE USED FOR FILING ABROAD UNDER THE PARIS CONVENTION, IS US60/921,144

Certified by

Under Secretary of Commerce for Intellectual Property
and Director of the United States Patent and Trademark Office
**INVENTOR(S)**

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<th>Given Name (first and middle [if any])</th>
<th>Family Name or Surname</th>
<th>Residence (City and either State or Foreign Country)</th>
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<tr>
<td>Michel</td>
<td>Sadelain</td>
<td>New York, New York</td>
</tr>
<tr>
<td>Matthias</td>
<td>Stephan</td>
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**Additional inventors are being named on the separately numbered sheets attached hereto**

**TITLE OF THE INVENTION (500 characters max)**

ANTIGEN-SPECIFIC T CELLS AND METHODS OF USE

**APPLICATION DATA SHEET**

- **Application Data Sheet**: See 37 CFR 1.76
- **CD(s), Number of CDs**: [Blank]
- **Drawing(s) Number of Sheets**: 6
- **Other (specify)**: [Blank]
- **Specification Number of Pages (e.g. description of the invention)**: 50

**FEES DUE**

- **Filing Fee of $200 ($100 for small entity)**
- **Application Fee**: [Blank]
- **Total Fee Amount ($)**: 100.00

**METHOD OF PAYMENT**

- **A check or money order is enclosed to cover the filing fee and application size fee (if applicable)**: [Blank]
- **Payment by credit card**: Form PTO-2038 is attached.
- **The Director is hereby authorized to charge the filing fee and application size fee (if applicable) or credit any overpayment to Deposit Account Number**: 04-1105

A duplicative copy of this form is enclosed for fee processing.

**USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT**
**FEE TRANSMITTAL For FY 2006**

**Complete if Known**
- Application Number: Not Yet Assigned
- Filing Date: Concurrently Herewith
- First Named Inventor: Michel Sadelain
- Examiner Name: Not Yet Assigned
- Art Unit: N/A
- Attorney Docket No.: B6964P(51500)

**TOTAL AMOUNT OF PAYMENT**

| ($) | 100.00 |

**METHOD OF PAYMENT**

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For the above-identified deposit account, the Director is hereby authorized to: (check all that apply)

- [x] Charge fee(s) indicated below
- [X] Charge any additional fee(s) or underpayments of fee(s) under 37 CFR 1.16 and 1.17
- [X] Credit any overpayments

**FEE CALCULATION**

1. **BASIC FILING, SEARCH, AND EXAMINATION FEES**

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2. **EXCESS CLAIM FEES**

**Fee Description**
- Each claim over 20 (including Reissues): $50
- Each independent claim over 3 (including Reissues): $200
- Multiple dependent claims: $360

**Multiple Dependent Claims**

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3. **APPLICATION SIZE FEE**

If the specification and drawings exceed 100 sheets of paper (excluding electronically filed sequence or computer listings under 37 CFR 1.52(e)), the application size fee due is $250 ($125 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).

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4. **OTHER FEE(S)**

- Non-English Specification, $130 fee (no small entity discount)
- Other (e.g., late filing surcharge): 

**SUBMITTED BY**

- Signature: [Signature]
- Registration No./Attorney/Agent: 55,289
- Telephone: (617) 439-4444
- Date: March 30, 2007

**Name (Print/Type): Melissa Hunter-Ensor, Ph.D.**
The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

X No.

☐ Yes, the name of the U.S. Government agency and the Government contract number are:

________________________________________

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SIGNATURE

Melissa Hunter-Ensor, Ph.D.

TELEPHONE (617) 439-4444

Date March 30, 2007

REGISTRATION NO (if appropriate) 55,289

Docket Number 66964P(51590)
Certificate of Express Mailing Under 37 CFR 1.10

I hereby certify that this correspondence is being deposited with the United States Postal Service as Express Mail, Airbill No. EV 971741570 US in an envelope addressed to:

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Telephone Number (617) 239-0100

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Provisional Patent Application Transmittal (2 pages)
Fee Transmittal (1 page)
Application Data Sheet (2 pages)
Specification (50 pages)
Drawings (6 sheets)
Return Receipt Postcard
Charge $100.00 to deposit account 04-1105
ANTIGEN-SPECIFIC T CELLS AND METHODS OF USE

CROSS-REFERENCE TO RELATED APPLICATION

Each of the applications and patents cited in this text, as well as each document or reference cited in each of the applications and patents (including during the prosecution of each issued patent; "application cited documents"), and each of the PCT and foreign applications or patents corresponding to and/or claiming priority from any of these applications and patents, and each of the documents cited or referenced in each of the application cited documents, are hereby expressly incorporated herein by reference, and may be employed in the practice of the invention. More generally, documents or references are cited in this text, either in a Reference List before the claims, or in the text itself; and, each of these documents or references ("herein cited references"), as well as each document or reference cited in each of the herein cited references (including any manufacturer's specifications, instructions, etc.), is hereby expressly incorporated herein by reference.

STATEMENT OF RIGHTS TO INVENTIONS MADE UNDER FEDERALLY SPONSORED RESEARCH

The United States government may have certain rights in this invention by virtue of grant numbers PO1 CA59350.

BACKGROUND OF THE INVENTION

Prostate cancer is the most frequent cancer in males in the United States and the cause of nearly 31,000 deaths per year. When diagnosed early, cancer can be effectively treated by surgery or radiation. Postsurgical residual disease requires radiation and/or hormonal therapy, which may prevent tumor progression and metastasis. At present there is no curative treatment for hormone refractory, metastatic prostate cancer. Immunotherapy is a targeted therapy that in principle provides for the treatment of such cancers. Obstacles remain to induce tumor immunity, which requires the expansion of cytotoxic T lymphocytes to numbers sufficient to mediate tumor rejection. Among the mechanisms limiting efficient T cell priming and tumor rejection is the inherent absence of costimulatory ligands on many malignancies.
SUMMARY OF THE INVENTION

The present invention provides immunoresponsive cells, including T cells and Natural Killer (NK) cells, expressing at least one of an antigen-recognizing receptor and a co-stimulatory ligand and methods of use therefore for the treatment of neoplasia, infectious disease, and other pathologies.

In one aspect, the invention generally provides an immunoresponsive cell comprising a receptor that binds an antigen and an exogenous co-stimulatory ligand.

In another aspect, the invention provides a virus specific T cell expressing a vector encoding a polypeptide selected from any one or more of CD80, 4-1BBL, OX40L, CD70 and CD30L. In one embodiment, the virus specific T cell recognizes a virus selected from any one or more of Cytomegalovirus (CMV), Epstein Barr Virus (EBV), Human Immunodeficiency Virus (HIV), and influenza virus antigens.

In yet another aspect, the invention provides a tumor antigen-specific T cell expressing a vector encoding a polypeptide selected from the group consisting of CD80, 4-1BBL, OX40L, CD70 and CD30L. In one embodiment, the cell expresses CD80 and 4-1BBL. In another embodiment, the vector is a retroviral vector.

In yet another aspect, the invention provides a method of modulating an immune response in a subject, the method comprising administering an effective amount of an immunoresponsive cell of any previous aspect. In one embodiment, the method increases or reduces an immune response. In another embodiment, the method increases self-tolerance or increases tolerance to an organ transplant.

In yet another aspect, the invention provides a method of treating or preventing a neoplasia in a subject, the method comprising administering an effective amount of an immunoresponsive cell comprising a receptor that binds a tumor antigen and a vector encoding a co-stimulatory ligand. In one embodiment, the neoplasia is selected from the any one or more of prostate cancer, colon cancer, breast cancer, and glioblastoma. In another embodiment, the tumor antigen is prostate-specific membrane antigen, CD19, NY-ESO-1, WT-1 or hTERT.

In another aspect, the invention provides a method of enforcing tolerance in a subject, the method comprising administering an effective amount of an
immunoresponsive cell comprising a receptor that binds an antigen and a vector encoding a co-stimulatory ligand. In one embodiment, the method prevents or reduces an autoimmune disease or a disease associated with allogeneic transplantation.

In yet another aspect, the invention provides a method of treating or preventing a pathogen infection in a subject, the method comprising administering an effective amount of an immunoresponsive cell comprising a receptor that binds a viral antigen and a vector encoding a co-stimulatory ligand. In one embodiment, the pathogen is a virus, bacteria, fungus, protozoa or parasite. In another embodiment, the virus is selected from any one or more of Cytomegalovirus (CMV), Epstein Barr Virus (EBV), Human Immunodeficiency Virus (HIV), and influenza virus. In yet another embodiment, the cell is a T cell, a Natural Killer (NK) cell, or a cytotoxic T lymphocyte (CTL).

In still another aspect, the invention provides method for producing an antigen-specific immunoresponsive cell, the method comprising introducing into the immunoresponsive cell a nucleic acid sequence that encodes a chimeric antigen receptor, wherein the chimeric antigen receptor comprises an antigen-binding domain coupled to an intracellular signaling domain that activates an immunoresponsive cell. In one embodiment, the immunoresponsive cell is a T cell, CTL, or NK cell. In another embodiment, the antigen-binding domain is a tumor antigen-binding domain. In yet another embodiment, the tumor antigen is prostate specific membrane antigen (PSMA). In yet another embodiment, the intracellular signaling domain activates a T cell, CTL cell, or NK cell. In yet another embodiment, the intracellular signaling domain is the ζ-chain signaling domain.

In another aspect, the invention provides a method of treating a neoplasia in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of a T cell comprising a tumor antigen and an antigen presenting complex comprising at least two co-stimulatory ligands, wherein at least one of the two co-stimulatory ligands is selected from any one or more of a tumor necrosis factor (TNF) ligand and an immunoglobulin (Ig) superfamily ligand and combinations thereof, thereby treating cancer in the subject.

In another aspect, the invention provides a method of treating a neoplasia in a subject, the method comprising administering to the subject a therapeutically effective
amount of a Natural Killer (NK) cell comprising a tumor antigen and an antigen presenting complex comprising at least two co-stimulatory ligands, wherein at least one of the two co-stimulatory ligands is selected from any one or more of a tumor necrosis factor (TNF) ligand and an immunoglobulin (Ig) superfamily ligand and combinations thereof, thereby treating cancer in the subject. In one embodiment, the TNF ligand is selected from any one or more of 4-1BBL, OX40L, CD70, and CD30L, and LIGHT. In another embodiment, the Ig superfamily ligand is selected from CD80 and CD86. In yet another embodiment, the cell expresses at least two co-stimulatory ligands, where one is a TNF ligand (e.g., 4-1BBL) and the other is an Ig superfamily ligand (e.g., CD80).

In another aspect, the invention provides a method of treating an infectious disease in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of a T cell comprising a receptor specific for a viral antigen and an antigen presenting complex comprising at least two co-stimulatory ligands, wherein at least one of the two co-stimulatory ligands is selected from any one or more of a tumor necrosis factor (TNF) ligand and an immunoglobulin (Ig) superfamily ligand and combinations thereof, thereby treating the infectious disease in the subject.

In yet another aspect, the invention provides method of treating an infectious disease in a subject, comprising administering to the subject a therapeutically effective amount of a Natural Killer (NK) cell comprising a receptor specific for a viral antigen and an antigen presenting complex comprising at least two co-stimulatory ligands, wherein at least one of the two co-stimulatory ligands is selected from any one or more of a tumor necrosis factor (TNF) ligand and an immunoglobulin (Ig) superfamily ligand and combinations thereof, thereby treating the infectious disease in the subject. In one embodiment, the subject is an immunocompromised subject. In another embodiment, the TNF ligand is selected from any one or more of 4-1BBL, OX40L, CD70, LIGHT and CD30L. In yet another embodiment, the Ig superfamily ligand is selected from any one or more of CD80 and CD86. In yet another embodiment, the at least two co-stimulatory ligands are a TNF ligand and an Ig superfamily ligand. In another embodiment, the TNF ligand is 4-1BBL and the Ig superfamily ligand is CD80. In another embodiment, the antigen recognition complex is constitutively expressed on the surface of the cell. In another embodiment, the viral antigen is an antigen specific for Cytomegalovirus (CMV),
Epstein Barr Virus (EBV), Human Immunodeficiency Virus (HIV), or influenza virus. In another embodiment, the at least two co-stimulatory ligands are constitutively expressed on the surface of the cell.

In another aspect, the invention provides a pharmaceutical composition comprising an effective amount of an immunoresponsive cell of any previous aspect in a pharmaceutically acceptable excipient.

In another aspect, the invention provides a pharmaceutical composition for the treatment of a neoplasia comprising an effective amount of a tumor antigen-specific T cell of any previous aspect in a pharmaceutically acceptable excipient.

In another aspect, the invention provides a pharmaceutical composition for the treatment of a pathogen infection comprising an effective amount of a viral-specific T cell of the previous aspect in a pharmaceutically acceptable excipient. In one embodiment, the composition further comprising a cytokine selected from any one or more of IL-2, IL-3, IL-6, IL-11, IL-7, IL15, IL21, granulocyte macrophage colony stimulating factor, alpha, beta or gamma interferon and erythropoietin.

In another aspect, the invention provides a kit comprising an immunoresponsive cell comprising a receptor that binds an antigen and an exogenous co-stimulatory ligand. In one embodiment, the kit further comprises written directions for using said cell for the treatment of a subject having neoplasia, a pathogen infection, an autoimmune disorder, or an allogeneic transplant.

In various embodiments of any previous aspect, the method further comprises the step of obtaining the immunoresponsive cell or co-stimulatory ligand. In still other embodiments of the previous aspects, the co-stimulatory ligand is constitutively or inducibly expressed. In yet other embodiments of the previous aspects, at least two co-stimulatory ligands are constitutively expressed. In various embodiments of any previous aspect, the cell is selected from any one or more of a T cell, a Natural Killer (NK) cell, a cytotoxic T lymphocyte (CTL), and a regulatory T cell. In still other embodiments of the previous aspects, the antigen is a tumor or pathogen antigen, e.g., any one or more of prostate-specific membrane antigen (PSMA), Carcinoembryonic Antigen (CEA), IL13Ralpha, her-2, CD19, NY-ESO-1, HIV-1 Gag, Lewis Y, Mart-1, gp100, tyrosinase, WT-1, hTERT. In still other embodiments of the previous aspects, the cell expresses a
recombinant and/or an endogenous antigen receptor. In still other embodiments of the previous aspects, the co-stimulatory ligand is a tumor necrosis factor (TNF) ligand (e.g., 4-1BBL, OX40L, CD70, LIGHT, and CD30L) or an immunoglobulin (Ig) superfamily ligand (e.g., CD80 and CD86). In still other embodiments of the previous aspects, the immunoreponsive cell expresses at least one TNF ligand and at least one Ig superfamily ligand. In still other embodiments of the previous aspects, the cell expresses 4-1BBL and CD80. In still other embodiments of the previous aspects, an antigen presenting complex and/or co-stimulatory ligands are constitutively or inducibly expressed on the surface of the T cell. In still other embodiments of the previous aspects, the co-stimulatory ligand is expressed in a retroviral vector. In another embodiment, the tumor antigen is prostate-specific membrane antigen, CD19, NY-ESO-1, WT-1 or hTERT. In still other embodiments, the cell expresses a recombinant or an endogenous receptor for the antigen. In still other embodiments, the co-stimulatory ligand is a tumor necrosis factor (TNF) ligand or an immunoglobulin (Ig) superfamily ligand. In still other embodiments, the TNF ligand is selected from any one or more of 4-1BBL, OX40L, CD70, LIGHT, and CD30L. In still other embodiments, the Ig superfamily ligand is selected from the group consisting of CD80 and CD86.

Definitions

The term "ligand" as used herein refers to a molecule that binds to a receptor. In particular, the ligand binds a receptor on another cell, allowing for cell-to-cell recognition.

The term “constitutive expression” as used herein refers to expression under all physiological conditions.

The term “chimeric antigen receptor” (CAR) as used herein refers to a tumor antigen-binding domain that is fused to an intracellular signaling domain capable of activating T cells. Most commonly, the CAR’s extracellular binding domain is derived from a murine monoclonal antibody.

By “disease” is meant any condition or disorder that damages or interferes with the normal function of a cell, tissue, or organ. Examples of diseases include neoplasia or pathogen infection of cell.
By "effective amount" is meant an amount sufficient to arrest, ameliorate, or inhibit the continued proliferation, growth, or metastasis (e.g., invasion, or migration) of a neoplasia.

By "enforcing tolerance" is meant preventing the activity of self-reactive cells or immunoresponsive cells that target transplanted organs or tissues.

By "exogenous" is meant a nucleic acid molecule or polypeptide that is not endogenously present in the cell. The term "exogenous" would therefore encompass any recombinant nucleic acid molecule or polypeptide expressed in a cell, such as foreign, heterologous, and over-expressed nucleic acid molecules and polypeptides.

By a "heterologous nucleic acid molecule or polypeptide" is meant a nucleic acid molecule (e.g., a cDNA, DNA or RNA molecule) or polypeptide that is not normally present in a cell or sample obtained from a cell. This nucleic acid may be from another organism, or it may be, for example, an mRNA molecule that is not normally expressed in a cell or sample.

By "immunoresponsive cell" is meant a cell that functions in an immune response or a progenitor, or progeny thereof.

By "isolated cell" is meant a cell that is separated from the molecular and/or cellular components that naturally accompany the cell.

The term "tumor antigen-binding domain" as used herein refers to a domain capable of specifically binding a particular antigenic determinant or set of antigenic determinants present on a tumor.

The term "obtaining" as in "obtaining the agent" is intended to include purchasing, synthesizing or otherwise acquiring the agent (or indicated substance or material).

By "modulate" is meant positively or negatively alter. Exemplary modulations include a 1%, 2%, 5%, 10%, 25%, 50%, 75%, or 100% change.

By "neoplasia" is meant a disease characterized by the pathological proliferation of a cell or tissue and its subsequent migration to or invasion of other tissues or organs. Neoplasia growth is typically uncontrolled and progressive, and occurs under conditions that would not elicit, or would cause cessation of, multiplication of normal cells.

Neoplasias can affect a variety of cell types, tissues, or organs, including but not limited
to an organ selected from the group consisting of bladder, bone, brain, breast, cartilage, glia, esophagus, fallopian tube, gallbladder, heart, intestines, kidney, liver, lung, lymph node, nervous tissue, ovaries, pancreas, prostate, skeletal muscle, skin, spinal cord, spleen, stomach, testes, thymus, thyroid, trachea, urogenital tract, ureter, urethra, uterus, and vagina, or a tissue or cell type thereof. Neoplasias include cancers, such as sarcomas, carcinomas, or plasmacytomas (malignant tumor of the plasma cells).

By "receptor" is meant a polypeptide, or portion thereof, present on a cell membrane that selectively binds one or more ligand.

By "recognize" is meant selectively binds a target. A T cell that recognizes a virus typically expresses a receptor that binds an antigen expressed by the virus.

By "pathogen" is meant a virus, bacteria, fungi, parasite or protozoa capable of causing disease.

Exemplary viruses include, but are not limited to, Retroviridae (e.g. human immunodeficiency viruses, such as HIV-1 (also referred to as HDTV-III, LAVE or HTLV-III/LAV, or HIV-III; and other isolates, such as HIV-LP; Picornaviridae (e.g. polio viruses, hepatitis A virus; enteroviruses, human Coxsackie viruses, rhinoviruses, echoviruses); Calciviridae (e.g. strains that cause gastroenteritis); Togaviridae (e.g. equine encephalitis viruses, rubella viruses); Flaviridae (e.g. dengue viruses, encephalitis viruses, yellow fever viruses); Coronaviridae (e.g. coronaviruses); Rhabdoviridae (e.g. vesicular stomatitis viruses, rabies viruses); Filoviridae (e.g. ebola viruses); Paramyxoviridae (e.g. parainfluenza viruses, mumps virus, measles virus, respiratory syncytial virus); Orthomyxoviridae (e.g. influenza viruses); Bunyaviridae (e.g. Hantaan viruses, bunga viruses, phleboviruses and Nairo viruses); Arena viridae (hemorrhagic fever viruses); Reoviridae (e.g. reoviruses, orbiviruses and rotaviruses); Birnaviridae; Hepadnaviridae (Hepatitis B virus); Parovirida (paroviruses); Papaviridae (papilloma viruses, polyoma viruses); Adenoviridae (most adenoviruses); Herpesviridae (herpes simplex virus (HSV) 1 and 2, varicella zoster virus, cytomegalovirus (CMV), herpes virus; Poxviridae (variola viruses, vaccinia viruses, pox viruses); and Iridoviridae (e.g. African swine fever virus); and unclassified viruses (e.g. the agent of delta hepatitis (thought to be a defective satellite of hepatitis B virus), the agents of non-A, non-B
hepatitis (class 1 = internally transmitted; class 2 = parenterally transmitted (i.e. Hepatitis C); Norwalk and related viruses, and astroviruses).

Exemplary bacteria include, but are not limited to, Pasteurella, Staphyloccoci, Streptococcus, Escherichia coli, Pseudomonas species, and Salmonella species. Specific examples of infectious bacteria include but are not limited to, Helicobacter pyloris, Borelia burgdorferi, Legionella pneumophilia, Mycobacteria spp (e.g. M. tuberculosis, M. avium, M. intracellulare, M. kansaii, M. gordonae), Staphylococcus aureus, Neisseria gonorrhoeae, Neisseria meningitidis, Listeria monocytogenes, Streptococcus pyogenes (Group A Streptococcus), Streptococcus agalactiae (Group B Streptococcus), Streptococcus (virids group), Streptococcus faecalis, Streptococcus bovis, Streptococcus (anaerobic spp.), Streptococcus pneumoniae, pathogenic Campylobacter sp., Enterococcus sp., Haemophilus influenzae, Bacillus antracis, corynebacterium diphtheriae, corynebacterium sp., Erysipelothrix rhusiopathiae, Clostridium perfringens, Clostridium tetani, Enterobacter aerogenes, Klebsiella pneumoniae, Pasturella multocida, Bacteroides sp., Fusobacterium nucleatum, Streptobacillus moniliformis, Treponema pallidium, Treponema pertenue, Leptospira, Rickettsia. and Actinomyces israelii.

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

The term "tumor antigen" as used herein refers to any polypeptide expressed by a tumor that is capable of inducing an immune response.

By "virus antigen" is meant a polypeptide expressed by a virus that is capable of inducing an immune response.

The terms "comprises", "comprising", and are intended to have the broad meaning ascribed to them in U.S. Patent Law and can mean "includes", "including" and the like.

As used herein, "treatment" refers to clinical intervention in an attempt to alter the disease course of the individual or cell being treated, and can be performed either for prophylaxis or during the course of clinical pathology. Therapeutic effects of treatment
include, without limitation, preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, preventing metastases, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis. By preventing progression of a disease or disorder, a treatment can prevent deterioration due to a disorder in an affected or diagnosed subject or a subject suspected of having the disorder, but also a treatment may prevent the onset of the disorder or a symptom of the disorder in a subject at risk for the disorder or suspected of having the disorder.

The term "subject" as used herein refers to a vertebrate, preferably a mammal, more preferably a human.

The term "immunocompromised" as used herein refers to a subject who has an immunodeficiency. The subject is very vulnerable to opportunistic infections, infections caused by organisms that usually do not cause disease in a person with a healthy immune system, but can affect people with a poorly functioning or suppressed immune system.

Other aspects of the invention are described in the following disclosure and are within the ambit of the invention.

BRIEF DESCRIPTION OF THE FIGURES

The following Detailed Description, given by way of example, but not intended to limit the invention to specific embodiments described, may be understood in conjunction with the accompanying drawings.

Figures 1A-11 show that CD80 and 4-1BBL co-expression in human T-cells exceeds the in vitro costimulatory effect of CD80 and 4-1BBL on the antigen presenting cell (APC). 0.4 x 10^6 Pz1 transduced human T lymphocytes were cocultured on irradiated LnCap human prostatic tumor monolayers with costimulatory ligands expressed on the tumor (left side) or the T cell (right side). (O) CD80 only, (◆) CD80 plus 4-1BBL, (σ) CD80 plus OX40L, (υ) CD80 plus CD70, (ν) CD80 plus CD30L, (--) LnCap only. In each of Figures 1A, 1D, 1H, and 1I, arrows indicate the start of each cycle of stimulation with a constant starting T-cell population for each restimulation. No exogenous cytokines were added. Data are representative of three independent experiments. Each point or bar graph represents the mean ± standard error (SE) of three
randomly picked wells. Figure 1A is a schematic diagram showing the absolute count of CD8<sup>+</sup>Pz1<sup>+</sup> T-cells after repeated cycles LnCap stimulation. Panel picture insets illustrate *in vitro* co-culture condition. Figures 1B and 1C show the results of ELISA cytokine release assays for IL-2 (Figure 1B) at twenty-four hours and for IFN-γ (Figure 1C) at forty-eight hours after initial LnCap contact. To avoid cytokine levels above assay range, all samples were serial diluted with reagent diluent prior to analysis. Data represent the cytokine levels in three randomly chosen wells (symbols) and their mean (—). Figure 1D is a graph showing the results of the serial multicolor flow cytometry analysis of co-cultured T-cells between day 0 and day 7 for intracellular Granzyme B. Data are represented as the mean fluorescent intensity (MFI) gated on the CD8<sup>+</sup>Pz1<sup>+</sup> T-cell population. Figure 1E shows 5 hour <sup>51</sup>Cr-release assays of expanded T-cells three days after initial LnCap exposure targeting PSMA<sup>+</sup> RM1.PGLS tumor cells or RM1 tumor cells as control (small symbols). In the Figures, the term “E:T ratio” denotes effector-to-target ratio of CD8<sup>+</sup>Pz1<sup>+</sup> T-cells to tumor target. Figure 1F shows serial multicolor flow cytometry analysis as in Figure 1D for intracellular Bcl-xL. Figure 1G shows the percent viable negative CD8<sup>+</sup>Pz1<sup>+</sup> T-cells after serial LnCap cocultures as detected by multicolor flow cytometry. Data represent the percentage of Annexin-V/PI double-negative cells gated on the CD8<sup>+</sup>Pz1<sup>+</sup> population. Figure 1H shows results of a FACS analysis of the enrichment and subsequent retroviral gene transfer of CVM-specific cytotoxic T lymphocytes (CTLs) from peripheral blood of a seropositive HLA A2.1<sup>+</sup> donor after co-culture with irradiated pp65 presenting artificial antigen presenting cells (AAPCs). The frequency of CD8<sup>+</sup> pp65 tetramer<sup>+</sup> (TM) T-cells before (left panel) and after (middle panel) expansion was assessed by multicolor FACs seven days after AAPC co-culture in the absence of exogenous cytokines. T-cells were re-stimulated with 20 units (U) IL-2 and retrovirally transduced with CD80, 4-1BBL or a combination of both CD80 and 4-1BBL forty-eight hours later. The SFG-NIT bicistronic vector was used as the control vector. Fluorescence-Activated Cell-Sorting (FACS) analysis gated on the CD8<sup>+</sup>Pz1<sup>+</sup> T-cell population for CD80 and 4-1BBL expression was performed forty-eight hours after the last gene transfer (right panel). Figure 1I shows the serial co-culture of CMV-reactive cytotoxic T lymphocytes (CTLs) enriched and transduced as described in Figure 1H on HLA A2.1<sup>+</sup> pp65-presenting Caco-2 tumor cells under the same conditions as
described in Figures 1A-1G. Data are represented as the total number of CD8^+pp65-TM^+ T-cells.

Figures 2A-2C show the eradication of established - prostate-specific membrane antigen (PSMA) prostate carcinoma tumors in Scid/beige mice by Pz1^+ T-cells transduced with CD80 and 4-1BBL. Pz1 is a ζ chain-based fusion receptor specific for prostate-specific membrane antigen (PSMA) (Gade et al., Cancer Res. 65:9080-9088, 2005) Figure 2A shows in vivo bioluminescent imaging and corresponding coronal MRI scans of firefly luciferase^+ PC3-PSMA tumors in Scid/beige mice four weeks after systemic inoculation with 4 x 10^6 tumor cells (day 0 of T-cell treatment), and eighteen days after adoptive transfer of 4 x 10^6 CD8^+Pz1^+ or Pz1^+CD80^+4-1BBL^+ T lymphocytes. An equal number of T-cells bearing the human CD19-targeting chimeric antigen receptor 19z were injected in the control group. Pseudocolor images superimposed on conventional photographs are shown. The same animals imaged before and after treatment using bioluminescent imaging and MRI are shown. The two mice represent a total of n=10. Figure 1B shows three graphs. Bioluminescent tumor signal quantified per animal every two days over a time period of 28 days. Acquisitions with saturated pixels - although included in the figure to allow direct visual comparison between groups - were not used for photon quantification but repeated at a shorter acquisition time. The graph shows photons/second/cm^2/surface radius (sr) versus days after T cell injection. Every line corresponds to one animal with each dot representing the average photon count of the ventral and dorsal acquisition per animal at any given time point. Survival is illustrated in the Kaplan-Meier curve in Figure 2C.

Figures 3A and 3B show robust, yet tumor antigen dependent, in vivo proliferation of CD80^+4-1BBL^+ T lymphocytes. Figure 3A shows comparative in vivo bioluminescent imaging of adoptively transferred T cells in PC3-PSMA tumor bearing Scid/beige mice on days 0, 8 and 18 after the injection of 4 x 10^6 CD8^+ clickbeetle luciferase (click-luc)-expressing Pz1-transduced or Pz1^+CD80^+4-1BBL^+ transduced T-lymphocytes. As an antigen specificity control, an equal number of 19z^+CD80^+4-1BBL^+ T cells were infused. T-cell treatment started as in Figure 2 four weeks after the systemic injection of 4 X10^6 PC3-PSMA tumor cells. The five mice per group shown in each panel represent a total of n=8/group. Acquisitions with saturated pixels - although shown
in the figure to allow direct visual comparison were not used for photon quantification but repeated at a shorter acquisition time. Figure 3B shows three graphs. Clickbeetle luciferase signal intensities from sequential bioluminescence imaging were collected every 2 days after T-cell transfer over a sixteen day time period. Every line represents one animal with each dot showing the average photon count of the ventral and dorsal acquisition per animal at any given time point.

Figures 4A-4C show the mobilization of 4-1BBL and its receptors 4-1BB into the immunological synapse after induced T-cell/tumor cell cluster formation. As a functional outcome of this coalescence an enhanced accumulation of granzyme-B at the T-APC interphase could be detected Figure 4a is a set of four confocal micrographs exemplifying the polarization of 4-1BBL - expressed as a ds-Red fusion protein in combination with CD80 on CD8+ Pz1 transduced T cells - into the immunological synapse. T-cell/LnCap tumor clusters were incubated for 50 minutes before fixation, permeabilization and incubation with anti 4-1BB antiserum. To visualize lipid rafts in the forming synapse T lymphocytes were labeled with FITC conjugated cholera toxin (CT-FITC). Out of a total of 60 randomly chosen T-APC cell clusters with clear synapse formation a total of 56 clusters showed a concentration of 4-1BBL with its receptor 4-1BB at the contact zone. Figure 4B illustrates the augmented accumulation of granzyme-B at the immunological synapse. This accumulation is dependent on a functional engagement of 4-1BB by its ligand 4-1BBL, which is expressed on the same T cell surface. Primary human T lymphocytes were genetically modified as in Figure 4A. As indicated, retroviral vectors encoding Pz1 also express control shRNA or 4-1BB targeting shRNA under the control of the U6 promoter in their 3'LTR as described herein below. Cell conjugates in the top (Pz1+control shRNA) and middle (and Pz1+CD80+dsred 4-1BBL+control shRNA) row represent CD80+dsred 4-1BBL- untransduced and transduced T-lymphocytes, respectively, cultured in the same well and conjugated to LnCap on the same glass slide. Figure 4c shows the relative recruitment index (RRI) and the relative intensity, calculated as described herein below, of Granzyme-B-Alexa 647 at the T cell –antigen presenting cell interphase. Data points in each group show the calculated value of 35 analyzed conjugates (symbols) and their mean (−) of three independent experiments.
Figures 5A-5D show that CD80 and 4-1BBL-displaying T lymphocytes trans-costimulate unmodified, antigen specific bystander T-cells through physical contact. Peripheral T lymphocytes of a cytomegalovirus (CMV)-seropositive HLA A2.1\(^+\) donor were transduced with Pz1 or co-transduced with Pz1, CD80 and 4-1BBL. In parallel, CMV-specific, genetically unmodified cytotoxic T lymphocytes (CTLs) from the same donor were enriched by artificial antigen presenting cell (AAPC) co-culture as described herein below. These cells were labeled with carboxyfluorescein succinimidyl ester (CFSE). Figure 5 provides results with bead-sorted Pz1\(^+\) (Figure 5A) or Pz1\(^+\)CD80\(^+\)4-1BBL\(^+\) (Figure 5B) T lymphocytes were admixed to expanded CMV-reactive pp65\(^+\) CTLs at a 1:1 ratio and exposed to irradiated Caco-2 tumor cells retrovirally transduced to present surface pp65 in an HLA A2.1-dependent, as well as PSMA in an HLA-independent manner. Alternatively, a transwell membrane separated Pz1\(^+\)CD80\(^+\)4-1BBL\(^+\) T-cells from pp65\(^+\) T-cells, both engaging Caco-2 tumor (Figure C). Respective co-culture conditions are depicted in the left row (Figures 5A\(_1\), B\(_1\), C\(_1\)). Two days after tumor antigen contact intracellular Granzyme B levels gated on CD8\(^+\) T-cells were quantified by FACS analysis (Figures 5A\(_2\), B\(_2\), C\(_2\)). Granzyme B-antigen presenting cell (APC) mean fluorescent intensities (MFI) of the CFSE\(^-\) (Pz1\(^+\)) and CFSE\(^+\) (pp65\(^+\)) T cell sub-populations are summarized on top of each profile. On day 7 CFSE dilutions and Pz1\(^+\) compared to pp65\(^+\) T-cell fractions were analyzed by flow cytometry after pp65 tetramer and CD8 staining (Figure 5A\(_3\), B\(_3\), C\(_3\)). CFSE-MFI are indicated on top of each profile are based on pp65\(^+\)-populations after CD8\(^+\)-gating. Total cell counts of CD8\(^+\) pp65\(^+\) T-cells after the 7 day cultures are graphed in (Figure 5D). Each bar graph represents the mean ± SE of three randomly chosen wells. Data are representative of two independent experiments. No exogenous cytokines were added at any point of the co-culture.

Figures 6A and 6B show that adoptively transferred PSMA-redirected T lymphocytes can receive trans-costimulation from co-injected Pz1\(^+\)CD80\(^+\)4-1BBL\(^+\), but not from 19z\(^+\)CD80\(^+\)4-1BBL\(^+\) T-cells in Scid/beige mice. Figure 6A shows dual in vivo bioluminescence imaging of external Gaussia luciferase (x-gaus-luc) in RM1.PGLS and Raji tumors in addition to Clickbeetle luciferase (Click-luc) in tumor targeting T-cells. Two weeks after the systemic injection of 1 x 10\(^6\)CD19\(^+\)x-gaus-luc\(^+\)Raji tumors and two
days after the subsequent infusion of $5 \times 10^5$ PSMA$^+ \times$-gaus-luc$^+$ RM1.PG1S tumor cells into the same animals, these cells established tumors in the bone marrow (Raji) and lung (RM1). The animals were treated with a combination of three populations of T-cells transduced as indicated. Each animal received a total number of $12 \times 10^6$ CD8$^+$ chimeric antigen receptor$^+$ T-cells ($4 \times 10^6$ T-cells/transduction condition). The T-cell population listed third (Pz1$^+$, Pz1$^+$CD80$^+$4-1BBL$^+$, 19z$^+$CD80$^+$4-1BBL$^+$, left, middle, right row, respectively) were injected twelve hours after the combined injection of T-cells listed first and second to avoid T-cell/T-cell interaction in the lung due to crowding and not as a result of selective tumor antigen binding. At the indicated time points x-gaus-luc$^+$ tumor cells or click-luc$^+$ T-cells were monitored by bioluminescent imaging. On day 0 and day 4 a time period of at least 4 hours between tumor and T-cell imaging ensured the bioluminescent signal to return to background levels. A total number of $n=5$ Scid/beige mice were imaged per treatment group. Figure 5B shows a series of 6 graphs quantitating clickbeetle luciferase signal intensities from sequential bioluminescence imaging every day after T-cell transfer for a four day time period. Every line represents one animal with each dot showing the average photon count measured over the pulmonary area or both femurs, respectively at any given time point.

**DETAILED DESCRIPTION OF THE INVENTION**

The present invention provides cells, including genetically modified immunoresponsive cells (e.g., T cells, Natural Killer (NK) cells, cytotoxic T lymphocytes (CTL) cells) expressing at least one of an antigen-recognizing receptor and a co-stimulatory ligand and methods of use therefore for the treatment of neoplasia and other pathologies where an increase in an antigen-specific immune response is desired. The invention is based, at least in part, on the discovery that the constitutive retroviral expression of CD80 and 4-1BBL in co-transduced human T cells targeting prostate specific membrane antigen mounted a robust tumor-antigen-dependent T-cell proliferation coupled with a profound in vivo rejection of disseminated well-established prostate carcinoma tumors. Furthermore, CD80 and 4-1BBL expressing T cells provided costimulation of bystander T cells in trans in a contact dependent and antigen-specific manner at the tumor site. Taken together, the concept of genetically modified T cells as a
constitutive pool of costimulatory ligands to optimally costimulate themselves in addition to enhancing the immunogenicity within the tumor microenvironment represents a significant advance over conventional adoptive T cell therapy. Furthermore, as demonstrated ex vivo using enriched CMV-specific T lymphocytes, this approach is not limited to the treatment of neoplasias, but is amenable to a wide range of applications where an increase in an antigen-specific immune response is desired, such applications include not only the treatment of neoplasias, but also for the enhancement of an immune response against a pathogen infection or an infectious disease and to reinforce immune tolerance in regulatory T cells in the context of autoimmunity or allogeneic transplantation.

**Hematopoietic Cell Lineages**

Mammalian hematopoietic (blood) cells provide a diverse range of physiologic activities. Hematopoietic cells are divided into lymphoid, myeloid and erythroid lineages. The lymphoid lineage, comprising B, T and natural killer (NK) cells, provides for the production of antibodies, regulation of the cellular immune system, detection of foreign agents in the blood, detection of cells foreign to the host, and the like. The term “T cells” as used herein refers to lymphocytes that mature in the thymus and are chiefly responsible for cell-mediated immunity. T cells are involved in the adaptive immune system. The term “natural killer (NK) cells” as used herein refers to lymphocytes that are part of cell-mediated immunity and act during the innate immune response. They do not require prior activation in order to perform their cytotoxic effect on target cells. Cytotoxic T cells (CTL or killer T cells) are a subset of T lymphocytes capable of inducing the death of infected somatic or tumor cells.

**Cells for Use in the Methods of the Invention**

The present invention provides cells expressing at least one of an antigen-recognition receptor and a co-stimulatory ligand and methods of using such cells for the treatment of a disease that requires an enhanced immune response. In one approach, tumor antigen-specific T cells, NK cells, CTL cells or other immuno-responsive cells are used as shuttles for the selective enrichment of one or more co-stimulatory ligands for the
treatment or prevention of neoplasia. For example, a T cell expressing a co-stimulatory
ligands 4-1BBL and CD80 are constitutively co-expressed in a T cell that expresses a
chimeric antigen receptor PZ1 that recognizes and binds Prostate Specific Membrane
Antigen (PSMA)). Such cells are administered to a human subject in need thereof for the
treatment or prevention of prostate cancer. In another approach, viral antigen-specific T
cells, NK cells, CTL cells can be used for the treatment of viral diseases. For example,
CD80 and 4-1BBL are expressed in cy tromegalovirus (CMV)-specific cytotoxic T
lymphocytes for the treatment of CMV.

**Tumor antigen-specific T lymphocytes (and NK cells)**

Types of tumor antigen-specific human lymphocytes that can be used in the
methods of the invention include, without limitation, peripheral donor lymphocytes
genetically modified to express chimeric antigen receptors (CARs) (Sadelain, M., *et al.*
2003 *Nat Rev Cancer* 3:35-45), peripheral donor lymphocytes genetically modified to
express a full-length tumor antigen-recognizing T cell receptor complex comprising the α
derived from tumor infiltrating lymphocytes (TILs) in tumor biopsies (Panelli, M.C., *et al.*
and selectively *in vitro*-expanded antigen-specific peripheral blood leukocytes employing
artificial antigen-presenting cells (AAPCs) or pulsed dendritic cells (Dupont, J., *et al.*

Any suitable tumor antigen (antigenic peptide) is suitable for use in the tumor-
related embodiments described herein. Sources of antigen include, but are not limited to
cancer proteins. The antigen can be expressed as a peptide or as an intact protein or
portion thereof. The intact protein or a portion thereof can be native or mutagenized.

One suitable antigen is prostate specific membrane antigen (PSMA).

**Viral antigen-specific T lymphocytes (and NK cells)**

Suitable antigens for use in the treatment of pathogen infection or other infectious
disease, for example, in an immunocompromised subject include, without limitation, viral
antigens present in Cytomegalovirus (CMV), Epstein Barr Virus (EBV), Human
Immunodeficiency Virus (HIV), and influenza virus.
The unpurified source of CTLs may be any known in the art, such as the bone
marrow, fetal, neonate or adult or other hematopoietic cell source, e.g., fetal liver,
peripheral blood or umbilical cord blood. Various techniques can be employed to
separate the cells. For instance, negative selection methods can remove non-CTLs
initially. mAbs are particularly useful for identifying markers associated with particular
cell lineages and/or stages of differentiation for both positive and negative selections.

A large proportion of terminally differentiated cells can be initially removed by a
relatively crude separation. For example, magnetic bead separations can be used initially
to remove large numbers of irrelevant cells. Preferably, at least about 80%, usually at
least 70% of the total hematopoietic cells will be removed prior to cell isolation.

Procedures for separation include, but are not limited to, density gradient
centrifugation; resetting; coupling to particles that modify cell density; magnetic
separation with antibody-coated magnetic beads; affinity chromatography; cytotoxic
agents joined to or used in conjunction with a mAb, including, but not limited to,
complement and cytotoxins; and panning with antibody attached to a solid matrix, e.g.
plate, elutriation or any other convenient technique.

Techniques for separation and analysis include, but are not limited to, flow
cytometry, which can have varying degrees of sophistication, e.g., a plurality of color
channels, low angle and obtuse light scattering detecting channels, impedance channels.

The cells can be selected against dead cells, by employing dyes associated with
dead cells such as propidium iodide (PI). Preferably, the cells are collected in a medium
comprising 2% fetal calf serum (FCS) or 0.2% bovine serum albumin (BSA) or any other
suitable, preferably sterile, isotonic medium.

Accordingly, the invention generally provides an immunoresponsive cell, such as
a virus or tumor specific T cell comprising a receptor that binds an antigen and an
exogenous co-stimulatory ligand (e.g., CD80, 4-1BBL, OX40L, CD70 and CD30L).

**Vectors**

Genetic modification of immunoresponsive cells (e.g., T cells, CTL cells, NK
cells) can be accomplished by transducing a substantially homogeneous cell composition
with a recombinant DNA construct. Preferably, a retroviral vector is employed for the
introduction of the DNA construct into the cell. For example, a polynucleotide encoding a co-stimulatory ligand protein (e.g., tumor necrosis factor (TNF) ligand, such as 4-1BBL, OX40L, CD70, LIGHT, and CD30L, or an Ig superfamily ligand, such as CD80 and CD86), or a receptor that binds an antigen, or a variant, or a fragment thereof, can be cloned into a retroviral vector and expression can be driven from its endogenous promoter, from the retroviral long terminal repeat, or from a promoter specific for a target cell type of interest.

Co-stimulatory ligands

The interaction with at least one co-stimulatory ligand provides a non-antigen-specific signal required for full activation of a T cell. Co-stimulatory ligands include, without limitation, tumor necrosis factor (TNF) ligands, cytokines (such as IL-2, IL-15 or IL21), and immunoglobulin (Ig) superfamily ligands.

TNF ligands

Tumor necrosis factor (TNF) is a cytokine involved in systemic inflammation and stimulates the acute phase reaction. Its primary role is in the regulation of immune cells. Tumor necrosis factor (TNF) ligands share a number of common features. The majority of the ligands are synthesized as type II transmembrane proteins (extracellular C-terminus) containing a short cytoplasmic segment and a relatively long extracellular region. TNF ligands include, without limitation, nerve growth factor (NGF), CD40L (CD40L)/CD154, CD137L/4-1BBL, tumor necrosis factor alpha (TNFα), CD134L/OX40L/CD252, CD27L/CD70, Fas ligand (FasL), CD30L/CD153, tumor necrosis factor beta (TNFβ)/lymphotoxin-alpha (LTα), lymphotoxin-beta (LTβ), CD257/B cell-activating factor (BAFF)/Blys/THANK/Tall-1, glucocorticoid-induced TNF Receptor ligand (GITRL), and TNF-related apoptosis-inducing ligand (TRAIL), LIGHT (TNFSF14).

Ig Superfamily ligands

The immunoglobulin (Ig) superfamily is a large group of cell surface and soluble proteins that are involved in the recognition, binding, or adhesion processes of cells. These proteins share structural features with immunoglobulins -- they possess an immunoglobulin domain (fold). Immunoglobulin superfamily ligands include, without limitation, CD80 and CD86, both ligands for CD28.
For initial genetic modification of the cells to provide tumor or viral antigen-specific cells, a retroviral vector is generally employed for transduction, however any other suitable viral vector or delivery system can be used. For subsequent genetic modification of the cells to provide cells comprising an antigen presenting complex comprising at least two co-stimulatory ligands, retroviral gene transfer (transduction) likewise proves effective. Combinations of retroviruses and an appropriate packaging line are also suitable, where the capsid proteins will be functional for infecting human cells. Various amphotropic virus-producing cell lines are known, including, but not limited to, PA12 (Miller, et al. (1985) Mol. Cell. Biol. 5:431-437); PA317 (Miller, et al. (1986) Mol. Cell. Biol. 6:2895-2902); and CRIP (Danos, et al. (1988) Proc. Natl. Acad. Sci. USA 85:6460-6464). Non-amphotropic particles are suitable too, e.g., particles pseudotyped with VSVG, RD114 or GALV envelope and any other known in the art.


Other transducing viral vectors can be used to express a co-stimulatory ligand of the invention in an immunoresponsive cell. Preferably, the chosen vector exhibits high efficiency of infection and stable integration and expression (see, e.g., Cayouette et al., Human Gene Therapy 8:423-430, 1997; Kido et al., Current Eye Research 15:833-844, 1996; Bloomer et al., Journal of Virology 71:6641-6649, 1997; Naldini et al., Science 272:263-267, 1996; and Miyoshi et al., Proc. Natl. Acad. Sci. U.S.A. 94:10319, 1997). Other viral vectors that can be used include, for example, adenoviral, lentiviral, and adeno-associated viral vectors, vaccinia virus, a bovine papilloma virus, or a herpes virus, such as Epstein-Barr Virus (also see, for example, the vectors of Miller, Human Gene Therapy 15-14, 1990; Friedman, Science 244:1275-1281, 1989; Eglitis et al., BioTechniques 6:608-614, 1988; Tolstoshev et al., Current Opinion in Biotechnology 1:55-61, 1990; Sharp, The Lancet 337:1277-1278, 1991; Cornetta et al., Nucleic Acid Research and Molecular Biology 36:311-322, 1987; Anderson, Science 226:401-409, 1984; Moen, Blood Cells 17:407-416, 1991; Miller et al., Biotechnology 7:980-990,
1989; Le Gal La Salle et al., Science 259:988-990, 1993; and Johnson, Chest 107:77S-83S, 1995). Retroviral vectors are particularly well developed and have been used in clinical settings (Rosenberg et al., N. Engl. J. Med 323:370, 1990; Anderson et al., U.S. Pat. No. 5,399,346).

Non-viral approaches can also be employed for the expression of a protein in cell. For example, a nucleic acid molecule can be introduced into a cell by administering the nucleic acid in the presence of lipofection (Feigner et al., Proc. Natl. Acad. Sci. U.S.A. 84:7413, 1987; Ono et al., Neuroscience Letters 17:259, 1990; Brigham et al., Am. J. Med. Sci. 298:278, 1989; Staubinger et al., Methods in Enzymology 101:512, 1983), asialoorosomucoid-polysyne conjugation (Wu et al., Journal of Biological Chemistry 263:14621, 1988; Wu et al., Journal of Biological Chemistry 264:16985, 1989), or by micro-injection under surgical conditions (Wolff et al., Science 247:1465, 1990). Other non-viral means for gene transfer include transfection in vitro using calcium phosphate, DEAE dextran, electroporation, and protoplast fusion. Liposomes can also be potentially beneficial for delivery of DNA into a cell. Transplantation of normal genes into the affected tissues of a subject can also be accomplished by transferring a normal nucleic acid into a cultivatable cell type ex vivo (e.g., an autologous or heterologous primary cell or progeny thereof), after which the cell (or its descendants) are injected into a targeted tissue or are injected systemically.

cDNA expression for use in polynucleotide therapy methods can be directed from any suitable promoter (e.g., the human cytomegalovirus (CMV), simian virus 40 (SV40), or metallothionein promoters), and regulated by any appropriate mammalian regulatory element. For example, if desired, enhancers known to preferentially direct gene expression in specific cell types can be used to direct the expression of a nucleic acid. The enhancers used can include, without limitation, those that are characterized as tissue- or cell-specific enhancers. Alternatively, if a genomic clone is used as a therapeutic construct, regulation can be mediated by the cognate regulatory sequences or, if desired, by regulatory sequences derived from a heterologous source, including any of the promoters or regulatory elements described above.
The resulting cells can then be grown under conditions similar to those for unmodified cells, whereby the modified cells can be expanded and used for a variety of purposes.

**Administration**

Compositions comprising genetically modified immunoresponsive cells of the invention (e.g., T cells, NK cells, CTL cells, or their progenitors) can be provided systemically or directly to a subject for the treatment of a neoplasia, pathogen infection, or infectious disease. In one embodiment, cells of the invention are directly injected into an organ of interest (e.g., an organ affected by a neoplasia). Alternatively, compositions comprising genetically modified immunoresponsive cells are provided indirectly to the organ of interest, for example, by administration into the circulatory system (e.g., the tumor vasculature). Expansion and differentiation agents can be provided prior to, during or after administration of the cells to increase production of T cells, NK cells, or CTL cells *in vitro* or *in vivo*.

The modified cells can be administered in any physiologically acceptable vehicle, normally intravascularly, although they may also be introduced into bone or other convenient site where the cells may find an appropriate site for regeneration and differentiation (e.g., thymus). Usually, at least $1 \times 10^5$ cells will be administered, preferably $1 \times 10^6$, eventually reaching $1 \times 10^{10}$, or more. Genetically modified immunoresponsive cells of the invention can comprise a purified population of cells. Those skilled in the art can readily determine the percentage of genetically modified immunoresponsive cells in a population using various well-known methods, such as fluorescence activated cell sorting (FACS). Preferable ranges of purity in populations comprising genetically modified immunoresponsive cells are about 50 to about 55%, about 55 to about 60%, and about 65 to about 70%. More preferably the purity is about 70 to about 75%, about 75 to about 80%, about 80 to about 85%; and still more preferably the purity is about 85 to about 90%, about 90 to about 95%, and about 95 to about 100%. Dosages can be readily adjusted by those skilled in the art (e.g., a decrease in purity may require an increase in dosage). The cells can be introduced by injection, catheter, or the like. If desired, factors can also be included, including, but not limited to, interleukins, e.g. IL-2, IL-3, IL-6, and IL-11, as well as the other interleukins, the colony
stimulating factors, such as G-, M- and GM-CSF, interferons, e.g. -gamma-interferon and erythropoietin.

Compositions of the invention include pharmaceutical compositions comprising genetically modified immunoresponsive cells or their progenitors and a pharmaceutically acceptable carrier. Administration can be autologous or heterologous. For example, immunoresponsive cells, or progenitors can be obtained from one subject, and administered to the same subject or a different, compatible subject. Peripheral blood derived immunoresponsive cells of the invention or their progeny (e.g., in vivo, ex vivo or in vitro derived) can be administered via localized injection, including catheter administration, systemic injection, localized injection, intravenous injection, or parenteral administration. When administering a therapeutic composition of the present invention (e.g., a pharmaceutical composition containing a genetically modified immunoresponsive cell), it will generally be formulated in a unit dosage injectable form (solution, suspension, emulsion).

**Formulations**

Compositions of the invention comprising genetically modified immunoresponsive cells can be conveniently provided as sterile liquid preparations, e.g., isotonic aqueous solutions, suspensions, emulsions, dispersions, or viscous compositions, which may be buffered to a selected pH. Liquid preparations are normally easier to prepare than gels, other viscous compositions, and solid compositions. Additionally, liquid compositions are somewhat more convenient to administer, especially by injection. Viscous compositions, on the other hand, can be formulated within the appropriate viscosity range to provide longer contact periods with specific tissues. Liquid or viscous compositions can comprise carriers, which can be a solvent or dispersing medium containing, for example, water, saline, phosphate buffered saline, polyol (for example, glycerol, propylene glycol, liquid polyethylene glycol, and the like) and suitable mixtures thereof.

Sterile injectable solutions can be prepared by incorporating the genetically modified immunoresponsive cells utilized in practicing the present invention in the required amount of the appropriate solvent with various amounts of the other ingredients,
as desired. Such compositions may be in admixture with a suitable carrier, diluent, or excipient such as sterile water, physiological saline, glucose, dextrose, or the like. The compositions can also be lyophilized. The compositions can contain auxiliary substances such as wetting, dispersing, or emulsifying agents (e.g., methylcellulose), pH buffering agents, gelling or viscosity enhancing additives, preservatives, flavoring agents, colors, and the like, depending upon the route of administration and the preparation desired. Standard texts, such as "REMINGTON'S PHARMACEUTICAL SCIENCE", 17th edition, 1985, incorporated herein by reference, may be consulted to prepare suitable preparations, without undue experimentation.

Various additives which enhance the stability and sterility of the compositions, including antimicrobial preservatives, antioxidants, chelating agents, and buffers, can be added. Prevention of the action of microorganisms can be ensured by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, and the like. Prolonged absorption of the injectable pharmaceutical form can be brought about by the use of agents delaying absorption, for example, aluminum monostearate and gelatin. According to the present invention, however, any vehicle, diluent, or additive used would have to be compatible with the genetically modified immunoresponsive cells or their progenitors.

The compositions can be isotonic, i.e., they can have the same osmotic pressure as blood and lacrimal fluid. The desired isotonicity of the compositions of this invention may be accomplished using sodium chloride, or other pharmaceutically acceptable agents such as dextrose, boric acid, sodium tartrate, propylene glycol or other inorganic or organic solutes. Sodium chloride is preferred particularly for buffers containing sodium ions.

Viscosity of the compositions, if desired, can be maintained at the selected level using a pharmaceutically acceptable thickening agent. Methylcellulose is preferred because it is readily and economically available and is easy to work with. Other suitable thickening agents include, for example, xanthan gum, carboxymethyl cellulose, hydroxypropyl cellulose, carbomer, and the like. The preferred concentration of the thickener will depend upon the agent selected. The important point is to use an amount that will achieve the selected viscosity. Obviously, the choice of suitable carriers and

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other additives will depend on the exact route of administration and the nature of the particular dosage form, e.g., liquid dosage form (e.g., whether the composition is to be formulated into a solution, a suspension, gel or another liquid form, such as a time release form or liquid-filled form).

Those skilled in the art will recognize that the components of the compositions should be selected to be chemically inert and will not affect the viability or efficacy of the genetically modified immunoresponsive cells as described in the present invention. This will present no problem to those skilled in chemical and pharmaceutical principles, or problems can be readily avoided by reference to standard texts or by simple experiments (not involving undue experimentation), from this disclosure and the documents cited herein.

One consideration concerning the therapeutic use of genetically modified immunoresponsive cells of the invention is the quantity of cells necessary to achieve an optimal effect. The quantity of cells to be administered will vary for the subject being treated. In a one embodiment, between $10^4$ to $10^8$, between $10^5$ to $10^7$, or between $10^6$ and $10^7$ genetically modified immunoresponsive cells of the invention are administered to a human subject. In preferred embodiments, at least about $1 \times 10^7$, $2 \times 10^7$, $3 \times 10^7$, $4 \times 10^7$, and $5 \times 10^7$ genetically modified immunoresponsive cells of the invention are administered to a human subject. The precise determination of what would be considered an effective dose may be based on factors individual to each subject, including their size, age, sex, weight, and condition of the particular subject. Dosages can be readily ascertained by those skilled in the art from this disclosure and the knowledge in the art.

The skilled artisan can readily determine the amount of cells and optional additives, vehicles, and/or carrier in compositions and to be administered in methods of the invention. Typically, any additives (in addition to the active stem cell(s) and/or agent(s)) are present in an amount of 0.001 to 50 % (weight) solution in phosphate buffered saline, and the active ingredient is present in the order of micrograms to milligrams, such as about 0.0001 to about 5 wt %, preferably about 0.0001 to about 1 wt %, still more preferably about 0.0001 to about 0.05 wt % or about 0.001 to about 20 wt %, preferably about 0.01 to about 10 wt %, and still more preferably about 0.05 to about 5 wt %. Of course, for any composition to be administered to an animal or human, and
for any particular method of administration, it is preferred to determine therefore: toxicity, such as by determining the lethal dose (LD) and LD50 in a suitable animal model e.g., rodent such as mouse; and, the dosage of the composition(s), concentration of components therein and timing of administering the composition(s), which elicit a suitable response. Such determinations do not require undue experimentation from the knowledge of the skilled artisan, this disclosure and the documents cited herein. And, the time for sequential administrations can be ascertained without undue experimentation.

Methods of Treatment

Provided herein are methods for treating neoplasia in a subject. Also contemplated herein are methods for treating a pathogen infection or other infectious disease in a subject, such as an immunocompromised human subject. The methods comprise administering a T cell, NK cell, or CTL cell of the invention in an amount effective to achieve the desired effect, be it palliation of an existing condition or prevention of recurrence. For treatment, the amount administered is an amount effective in producing the desired effect. An effective amount can be provided in one or a series of administrations. An effective amount can be provided in a bolus or by continuous perfusion.

An "effective amount" (or, "therapeutically effective amount") is an amount sufficient to effect a beneficial or desired clinical result upon treatment. An effective amount can be administered to a subject in one or more doses. In terms of treatment, an effective amount is an amount that is sufficient to palliate, ameliorate, stabilize, reverse or slow the progression of the disease, or otherwise reduce the pathological consequences of the disease. The effective amount is generally determined by the physician on a case-by-case basis and is within the skill of one in the art. Several factors are typically taken into account when determining an appropriate dosage to achieve an effective amount. These factors include age, sex and weight of the subject, the condition being treated, the severity of the condition and the form and effective concentration of the antigen-binding fragment administered.

For adoptive immunotherapy using antigen-specific T cells, cell doses in the range of $10^9$ are typically infused. Upon administration of the genetically modified cells
into the host and subsequent differentiation, T cells are induced that are specifically directed against the specific antigen. "Induction" of T cells can include inactivation of antigen-specific T cells such as by deletion or anergy. Inactivation is particularly useful to establish or reestablish tolerance such as in autoimmune disorders. The modified cells can be administered by any method known in the art including, but not limited to, intravenous, subcutaneous, intranodal, intratumoral, intrathecal, intrapleural, intraperitoneal and directly to the thymus.

**Therapeutic Methods**

The invention provides methods for increasing an immune response in a subject in need thereof. In one embodiment, the invention provides methods for treating or preventing a neoplasia in a subject. The invention provides therapies that are particularly useful for the treatment of subjects having prostate cancer, or metastatic prostate cancer that is not amenable to conventional therapeutic interventions. Suitable human subjects for therapy typically comprise two treatment groups that can be distinguished by clinical criteria. Subjects with "advanced disease" or "high tumor burden" are those who bear a clinically measurable tumor. A clinically measurable tumor is one that can be detected on the basis of tumor mass (e.g., by palpation, CAT scan, sonogram, mammogram or X-ray; positive biochemical or histopathologic markers on their own are insufficient to identify this population). A pharmaceutical composition embodied in this invention is administered to these subjects to elicit an anti-tumor response, with the objective of palliating their condition. Ideally, reduction in tumor mass occurs as a result, but any clinical improvement constitutes a benefit. Clinical improvement includes decreased risk or rate of progression or reduction in pathological consequences of the tumor.

A second group of suitable subjects is known in the art as the "adjuvant group." These are individuals who have had a history of neoplasia, but have been responsive to another mode of therapy. The prior therapy can have included (but is not restricted to, surgical resection, radiotherapy, and traditional chemotherapy. As a result, these individuals have no clinically measurable tumor. However, they are suspected of being at risk for progression of the disease, either near the original tumor site, or by metastases. This group can be further subdivided into high-risk and low-risk individuals. The
subdivision is made on the basis of features observed before or after the initial treatment. These features are known in the clinical arts, and are suitably defined for each different neoplasia. Features typical of high-risk subgroups are those in which the tumor has invaded neighboring tissues, or who show involvement of lymph nodes.

Another group have a genetic predisposition to neoplasia but have not yet evidenced clinical signs of neoplasia. For instance, women testing positive for a genetic mutation associated with breast cancer, but still of childbearing age, can wish to receive one or more of the antigen-binding fragments described herein in treatment prophylactically to prevent the occurrence of neoplasia until it is suitable to perform preventive surgery.

Human neoplasia subjects having any of the following neoplasias: glioblastoma, melanoma, neuroblastoma, adenocarcinoma, glioma, soft tissue sarcoma, and various carcinomas (including prostate and small cell lung cancer) are especially appropriate subjects. Suitable carcinomas further include any known in the field of oncology, including, but not limited to, astrocytoma, fibrosarcoma, myxosarcoma, liposarcoma, oligodendrogloma, ependymoma, medulloblastoma, primitive neural ectodermal tumor (PNET), chondrosarcoma, osteogenic sarcoma, pancreatic ductal adenocarcinoma, small and large cell lung adenocarcinomas, chordoma, angiosarcoma, endotheliosarcoma, squamous cell carcinoma, bronchoalveolar carcinoma, epithelial adenocarcinoma, and liver metastases thereof, lymphangiosarcoma, lymphangioendotheliosarcoma, hepatoma, cholangiocarcinoma, synovioma, mesothelioma, Ewing’s tumor, rhabdomyosarcoma, colon carcinoma, basal cell carcinoma, sweat gland carcinoma, papillary carcinoma, sebaceous gland carcinoma, papillary adenocarcinoma, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms’ tumor, testicular tumor, medulloblastoma, craniohypophyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, neuroblastoma, retinoblastoma, leukemia, multiple myeloma, Waldenstrom’s macroglobulinemia, and heavy chain disease, breast tumors such as ductal and lobular adenocarcinoma, squamous and adenocarcinomas of the uterine cervix, uterine and ovarian epithelial carcinomas, prostatic adenocarcinomas, transitional squamous cell carcinoma of the bladder, B and T
cell lymphomas (nodular and diffuse) plasmacytoma, acute and chronic leukemias, malignant melanoma, soft tissue sarcomas and leiomyosarcomas.

The subjects can have an advanced form of disease, in which case the treatment objective can include mitigation or reversal of disease progression, and/or amelioration of side effects. The subjects can have a history of the condition, for which they have already been treated, in which case the therapeutic objective will typically include a decrease or delay in the risk of recurrence.

Accordingly, the invention provides a method of treating or preventing a neoplasia in a subject, the method comprising administering an effective amount of an immunoresponsive cell comprising a receptor that binds a tumor antigen and a vector encoding a co-stimulatory ligand. In one embodiment, the neoplasia is selected from the group consisting of prostate cancer, colon cancer, breast cancer, and glioblastoma. In another embodiment, the tumor antigen is prostate-specific membrane antigen, CD19, NY-ESO-1, WT-1 or hTERT.

In another approach, the invention provides a method of enforcing tolerance in a subject, the method comprising administering an effective amount of an immunoresponsive cell comprising a receptor that binds an antigen and a vector encoding a co-stimulatory ligand. In one embodiment, the method prevents or reduces an autoimmune disease or a disease associated with allogeneic transplantation.

As a consequence of constitutive surface expression of co-stimulatory ligands, adoptively transferred human T or NK cells are endowed with augmented proliferative, cytolytic, and survival capacities in an intrinsically poorly immunogenic tumor or immunodeficient environment devoid of co-stimulatory ligands. Furthermore, subsequent to their localization to tumor or viral infection and their proliferation, co-stimulatory ligand-expressing T cells turn the tumor or viral infection site into a highly conductive environment for a wide range of immune cells involved in the physiological anti-tumor or antiviral response (tumor infiltrating lymphocytes, NK-, NKT- cells, dendritic cells, and macrophages).

In other embodiments, the invention provides methods for treating subjects with a pathogen infection (e.g., viral infection, bacterial infection, fungal infection, parasite infection, or protozoal infection. The invention is particularly useful for enhancing an
immune response in an immunocompromised subject. Exemplary viral infections susceptible to treatment using a method of the invention include, but are not limited to, Cytomegalovirus (CMV), Epstein Barr Virus (EBV), Human Immunodeficiency Virus (HIV), and influenza virus infections.

Accordingly, the invention provides a method of treating or preventing a pathogen infection in a subject, the method comprising administering an effective amount of an immunoresponsive cell as described herein.

**Kits**

The invention provides kits for the treatment or prevention of a neoplasia, pathogen infection, immune disorder or allogeneic transplant. In one embodiment, the kit includes a therapeutic or prophylactic composition containing an effective amount of an immunoresponsive cell comprising one or more co-stimulatory ligands in unit dosage form. In some embodiments, the kit comprises a sterile container which contains a therapeutic or prophylactic vaccine; such containers can be boxes, ampules, bottles, vials, tubes, bags, pouches, blister-packs, or other suitable container forms known in the art. Such containers can be made of plastic, glass, laminated paper, metal foil, or other materials suitable for holding medicaments.

If desired the immunoresponsive cell is provided together with instructions for administering the cell to a subject having or at risk of developing a neoplasia, pathogen infection, immune disorder or allogeneic transplant. The instructions will generally include information about the use of the composition for the treatment or prevention of neoplasia, pathogen infection, immune disorder or allogeneic transplant. In other embodiments, the instructions include at least one of the following: description of the therapeutic agent; dosage schedule and administration for treatment or prevention of a neoplasia, pathogen infection, immune disorder or allogeneic transplant or symptoms thereof; precautions; warnings; indications; counter-indications; overdosage information; adverse reactions; animal pharmacology; clinical studies; and/or references. The instructions may be printed directly on the container (when present), or as a label applied to the container, or as a separate sheet, pamphlet, card, or folder supplied in or with the container.
Recombinant methods are well known in the art. The practice of the invention employs, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology, biochemistry and immunology, which are within the skill of the art. Such techniques are explained fully in the literature, such as, "Molecular Cloning: A Laboratory Manual", second edition (Sambrook et al., 1989); "Oligonucleotide Synthesis" (Gait, ed., 1984); "Animal Cell Culture" (Freshney, ed., 1987); "Methods in Enzymology" (Academic Press, Inc.); "Handbook of Experimental Immunology" (Wei & Blackwell, eds.); "Gene Transfer Vectors for Mammalian Cells" (Miller & Calos, eds., 1987); "Current Protocols in Molecular Biology" (Ausubel et al., eds., 1987); "PCR: The Polymerase Chain Reaction", (Mullis et al., eds., 1994); and "Current Protocols in Immunology" (Coligan et al., eds., 1991). These techniques are applicable to the production of the polynucleotides and polypeptides, and, as such, can be considered in making and practicing the invention. Particularly useful techniques for are discussed in the sections that follow.

The following examples are provided as a further description of the invention, and to illustrate but not limit the invention.

EXAMPLES

Example 1. T cells co-expressing CD80 and 4-1BBL strongly respond to antigen in vitro.

In order to determine whether constitutive, high-level expression of selected costimulatory ligands could compensate for the absence of these ligands on antigen presenting cells (APCs), including tumor cells, the antigen-specific response of human T cells with costimulatory ligands expressed in antigen presenting cells vs. T cells was compared. Peripheral blood T lymphocytes were either specific for PSMA, following transduction of the Pz1 receptor, and activated by PSMA+ LNCaP cells (Gade et al., Cancer Res. 65:9080-9088, 2005) or specific for the cytomegalovirus pp65 antigen, and exposed to pp65-presenting Caco-2 tumor cells. Pz1-transduced T cells were co-cultured with LNCaP cells expressing either CD80 (Gade et al., Cancer Res. 65:9080-9088, 2005) or CD80 combined with 4-1BBL, OX40L, CD70 or CD30L. Alternatively, the same
Pz1+ T cells were transduced with the costimulatory ligands and co-cultured with unmodified LNCaP cells.

Pz1-transduced T cells failed to expand after 3 weekly stimulations with unmodified LNCaP, as previously described (Gade et al., Cancer Res. 65:9080-9088, 2005), but expanded 47-fold on CD80+ LNCaP cells (Figure 1A, left panel). When co-expressing CD80 with 4-1BBL or CD70, LNCaP cells induced a median 172- and 201-fold increase in PSMA-specific CD8+ T cells, respectively (Figure 1A, left panel). LNCaP cells co-expressing CD80 with OX40L or CD30L only supported a modest expansion (median 70- and 58-fold increase, respectively). In the absence of costimulatory ligands on the APC, constitutive expression of CD80 in human T lymphocytes did not markedly enhance T cell expansion (median 15-fold increase over 21 days) (Fig 1a, right). The addition of OX40L or CD70L mildly improved T-cell expansion to a median 35- and 41-fold, respectively. In sharp contrast, T lymphocytes modified to co-express CD80 and 4-1BBL not only exhibited a vigorous and sustained proliferation kinetics when challenged with the unmodified LNCaP cells (median 1042-fold expansion), but also mounted a median 6-fold higher T cell response than obtained with Pz1+ T cells challenged with CD80+4-1BBL+ tumor cells (median 172-fold expansion).

Pz1+ T cells constitutively expressing CD80 and 4-1BBL secreted 2.6-fold higher levels of IFN-γ 24 hrs and 48 hrs after initial antigen encounter, respectively (Figure 1B-c, right panel), than T cells stimulated with CD80+4-1BBL+ LNCaP (Figure 1b-c, left), whereas levels of IL-2 secretion were comparable. Intracellular granzyme-B and Bcl-xL staining were performed daily for a 7-day period after initial exposure to LNCaP cells (Figure 1d,f). Granzyme-B levels peaked after 24 hrs (median 6.6-fold above day 0), irrespective of what co-stimulatory ligands were expressed by the APC. However, when expressed by the Pz1-transduced T lymphocytes, 4-1BBL cooperated with CD80 to further amplify the peak amount of granzyme B by 2.5 fold, which was followed by a less rapid decline (Figure 1D). The high content of cytolytic granules in these T cells correlated with a substantially improved cytotoxic T-cell response in $^{51}$chromium release assays (Figure 1E). The induction of Bcl-XL showed a similar expression pattern, with CD80- and 4-1BBL- doubly transduced T cells showing the strongest induction (Figure
which was associated with lesser T cell apoptosis, as reflected by the increase in Annexin-V/PI-double negative T lymphocytes 7 days after antigenic stimulation (Figure 1G).

Despite their differentiated phenotype, CMV-specific T lymphocytes were highly susceptible to retroviral gene transfer of CD80 and 4-1BBL cDNAs subsequent to a brief co-culture with HLA A2.1+ artificial antigen presenting cells. When exposed to consecutive monolayers of the colonic tumor cell line Caco-2, genetically modified to present the CMV protein pp65 by HLA A2.1, only T cells equipped with the co-stimulatory ligand pair CD80 and 4-1BBL exhibited a sustained proliferative response with a median 209-fold expansion (Figure 1I).

In aggregate, these in vitro studies demonstrated that constitutive expression of co-stimulatory ligands in primary human T cells strongly potentiates antigen-induced T cell activation and, furthermore, substitutes for the lack of co-stimulation provided by antigen-presenting cells such as tumor cells.

Example 2. T cells co-expressing CD80 and 4-1BBL eradicate established, systemic tumors.

To investigate the potency of the T cells described herein in vivo, a model of multifocal prostate cancer was established utilizing PSMA+ PC-3 tumor cells (Gong et al., Neoplasia 1:123-7, 1999, which is hereby incorporated by reference in its entirety) which were also transduced with firefly luciferase to quantitatively monitor systemic tumor progression. Using dual-modality bioluminescence and magnetic resonance (MRI) imaging, established tumors could be visualized 4 weeks after intravenous tumor inoculation, showing lungs, cervical lymph nodes, bone marrow, and kidneys as the main sites of disease (Figure 2A). Animals were screened for comparable tumor burden and randomly assigned to a T cell treatment group, consisting of a single intravenous infusion of 4 x 10^6 Pz1+ T cells expressing either CD80 or 4-1BBL alone, or both.

The baseline anti-tumor response obtained with Pz1+ T cells showed a short-term reduction of tumor burden, followed by delayed tumor progression and a modest improvement of survival (Figure 2C, median survival of 59 and 47 days with Pz1+ vs 19z1+ T cells, respectively, P = 0.0001), compared to treatment with an equal dose of
19Z-transduced T cells targeted to the tumor irrelevant antigen CD19. Constitutive expression of either CD80 or 4-1BBL alone in PSMA-targeted T cells only marginally augmented this therapeutic response, extending median survival to 63 and 66 days, respectively (P = 0.0077, P = 0.056, respectively). T cells co-expressing CD80 and 4-1BBL induced major responses, with 7/10 treated animals becoming tumor-free and remaining so 200 days after T cell infusion (Figure 3C). Three other treated mice showed marked initial tumor regression (Figure 2B) but only partial responses resulting in 100-day survival only. These mice ultimately succumbed to tumor relapsing in lymph nodes of the neck and shoulder area (confirmed by histopathology).

**Example 3. In vivo T cell expansion is robust, yet limited, and antigen-specific.**

To closely monitor T-cell migration and accumulation in relation to tumor localization and tumor burden, the adoptively transferred T-cells were marked with clickbeetle luciferase (Ponomarev et al., Eur J Nucl Med Mol Imaging, 2004 May;31(5):740-51) Serial imaging of Pz1+ T cells showed a progressive increase in signal reaching a peak 4 days after T-cell injection (Figures 3A and 3B). A low-level signal remained detectable up to day 18. A direct correlation was observed between bioluminescent signal intensity and *ex vivo* T cell count in pulmonary cell suspensions in 3 representative animals per treatment group.

Pz1+ T cells also expressing CD80 and 4-1BBL showed a greater and more sustained increase reaching a nadir by day 9, after which the T cell signal gradually declined, indicating lymphocyte contraction. Some signal could still be detected up to day 100 (Figure 3).

Histopathological evaluation of treated animals after a 100-day time period excluded lymphoproliferative disease. The potentiating effect of CD80 and 4-1BBL transduction on T cell expansion was completely abrogated in 19z1+ T-lymphocytes. It is, thus, demonstrated herein that T cells co-expressing CD80 and 4-1BBL expand in antigen-dependent fashion before eventually entering a contraction phase resulting in major, if not complete, T cell disappearance.
Example 4. CD80, 4-1BBL and their receptors colocalize in the immunological synapse

To address whether constitutively expressed co-stimulatory ligands may activate T cells in cis, it was first examined whether these ligands co-localize with their cognate receptors during T cell activation. Both CD28 and 4-1BB have been shown to amplify TCR signaling after recruitment into central membrane compartments of the immunological synapse. To test whether colocalization of CD80 and 4-1BBL to the T-cell:tumor-cell contact area would be a prerequisite for enhanced T cell activation, a dsRed 4-1BBL fusion protein was co-expressed with CD80 in Pz1-transduced CD8+ T lymphocytes. Immediately prior to admixing with unmodified LNCaP cells, T-cells were labeled with FITC-conjugated cholera toxin β subunit to visualize lipid raft clusters after synapse formation.

As summarized in Figure 4A, following LnCap tumor engagement, 4-1BBL mobilized into cholera-toxin-FITC-positive T-cell/tumor-cell contact areas, as did its receptor 4-1BB (55/60 randomly selected cell clusters chosen from 3 independent experiments). To investigate the functional consequence of this interaction, the impact on the co-localization of granzyme B, a downstream effector molecule which is redirected to the T-cell/tumor-cell interface after reorientation of the microtubule organization center in functional T cells was examined. Granzyme-B aggregates that formed in CD8+ T cells 90 minutes after conjugate formation with LNCaP cells were quantified in the presence or absence of 4-1BBL. To ensure that the measured differences solely reflected effects of co-stimulatory ligand polarization into the synapse at a single cell level, and not random bystander activation during the process of gene transfer, granzyme B aggregation in PZ1+ T-lymphocytes that were co-transduced with 4-1BBLdsRed and CD80 was compared directly with T-cells from the same well carrying only the PZ1 gene.

As illustrated in Figure 4B, a defined, albeit sparse, granzyme B condensation was localized near the contact zone in cell clusters of Pz1-expressing T-cells and their cognate tumor target. In 4-1BBL+CD80+ T cells, greater granzyme B+ accumulation was detected, with an increase of quantifiable granzyme B (Figure 4C). To further confirm that these observed differences were, indeed, a result of 4-1BB engagement with
4-1BBL within the same cell, 4-1BB expression was knocked down by stably co-expressing 4-1BBL and a 4-1BB-specific shRNA. 4-1BB knock-down significantly diminished granzyme B density, despite the obvious presence of 4-1BBL in the T-cell junction in all acquired cell clusters. These findings strongly indicate that the co-stimulatory ligand can functionally engage its receptor on the same T-cell surface after antigen-induced coalescence in the immunological synapse.

Example 5. **Trans-costimulation of antigen-specific bystander T cells**

The constitutive expression of co-stimulatory ligands may also enable genetically modified T cells to co-stimulate T cells in *trans*. Indeed, the presence of some triangular three-cell clusters comprising CD80+4-1BBL+ and CD80−4-1BBL− T cells was noted in confocal studies suggesting that bystander co-stimulation may occur through cell contact. To provide functional evidence for *trans*-co-stimulation, a co-culture system was devised in which PSMA-specific T cells expressing CD80 and 4-1BBL were admixed with CFSE-labeled CD80− and 4-1BBL-untransduced T lymphocytes. As shown in Figure 5A,B, unmodified CMV pp65-specific effector memory T-cells lacking constitutive CD80 and 4-1BBL expression were effectively co-stimulated by autologous, bystander CD80+4-1BBL+ T-lymphocytes. Physical T-cell-T-cell contact was a prerequisite for CFSE-labeled CMV-reactive responder CTLs to receive activation signals from co-cultured PSMA-redirected T-cells expressing co-stimulatory ligands. Separation of these two lymphocyte pools by a transwell membrane abrogated the strong induction of granzyme-B, as well as the robust T-cell expansion mounted in pp65-responder cells, consistent with a cell contact-dependent response (Figure 5C).

These observations prompted investigation of whether T cell-mediated trans-co-stimulation is operative *in vivo* in the context of tumor rejection. To this end, two previously described animal models were combined: the RM1.PGLS tumor, in which tumor is essentially confined to the lung (PSMA+ RM1.PGLS, Gade et al., Cancer Res. 65:9080-9088, 2005), and the Raji tumor model with selective bone marrow tropism (CD19+ Raji, (Brentjens et al., Nat Med. 2003 Mar;9(3):279-86). All mice were treated with Pz1-transduced T cells, which also expressed clickbeetle luciferase, as shown in
Figure 3. Luciferase-negative Pz1+ T cells, either expressing or lacking CD80 and 4-1BBL, were subsequently infused.

To further examine whether CD80+4-1BBL+ T cells would have to be locally activated to support Pz1+ T cell function, clickbeetle-positive 19z1-transduced T cells, which have been shown to eliminate medullary Raji tumors (Brentjens et al., Nat Med. 2003 Mar;9(3):279-86), were also administered. By expressing Gaussia luciferase (Brentjens et al., Nat Med. 2003 Mar;9(3):279-86) in RM1.PGLS and Raji tumor cells, and clickbeetle luciferase in T-cells, dual bioluminescence in vivo imaging could be employed to monitor tumor progression, as well as spatial and temporal T cell accumulation. As shown in Figure 6a, all treatment groups showed comparable initial trafficking of luciferase-positive lymphocytes to RM1 tumors in the pulmonary area.

Three days after adoptive transfer, the bystander effect of Pz1+CD80+4-1BBL+ T cells on the clickbeetle-positive Pz1+ T cells was still modest (1.2-fold). The subsequent decay of the signal emanating from Pz1+ T cells stands in sharp contrast to that recorded in animals also given Pz1+CD80+4-1BBL+, which showed a median 5-fold amplified T-cell signal over the following 5-day period (Figure 6A,B). Importantly, this effect was selective for the PSMA-targeted T cells, since 19z1-transduced T-lymphocytes, which infiltrated the established Raji tumors in both femurs, did not show an enhanced response.

Collectively, these in vitro and in vivo studies indicate that, in addition to their auto-co-stimulatory effect, CD80+4-1BBL+ T cells may locally enhance T cell activation by providing co-stimulation in trans.

As reported herein above, genetically modified T cells can efficiently provide their own costimulation by constitutively expressing different costimulatory ligands in viral or tumor antigen-specific T cells. These genetically modified T cells could mount strong responses to antigen-presenting cells lacking costimulatory ligands. In fact, coexpression of retrovirally transduced CD80 and 4-1BBL into human primary, PSMA-specific T cells not only compensates for the lack of these ligands on the APC during serial antigen stimulation in vitro, but this stimulation surpasses the proliferative response induced by APCs expressing these same costimulatory ligands (Fig 1). Having assessed several costimulatory ligands and combinations thereof, CD80 and 4-1BBL were found...
to provide the strongest T cell activation in response to antigen (Fig 1). Adoptively transferred PSMA-targeted T lymphocytes co-expressing CD80 plus 4-1BBL effectively rejected established PC-3 tumors in the majority of treated mice (70% long-term tumor-free survival), in contrast to 0% with the same dose of non-CD80/4-1BBL–transduced T cells (Fig. 2). The CD80+4-1BBL+ T cells exhibited superior proliferation, cytokine secretion and survival in vitro (Fig 1) and superior proliferation and persistence in vivo, resulting in a 100-fold greater T cell biomass 1 week after infusion in tumor-bearing mice (Fig 3). These findings underscore the biological activity and remarkable potency of constitutive, high-level expression of costimulatory ligands in T cells.

To investigate the mechanism of action constitutive ligand expression, the spatial distribution of CD80 and 4-1BBL and their respective receptors on T cells was examined before and after tumor engagement. Studies over the past years have revealed the relocalization of CD28 and 4-1BB together with the T-cell receptor into central components of the forming immunological synapse during lymphocyte activation. Likewise, the costimulatory ligands CD80 and CD40L have been reported to aggregate from the APC membrane into the site of T cell activation supporting the idea of the immunological synapse as the ordered contact area facilitating and propagating costimulatory ligand-receptor interactions. As reported herein above, CD80 and 4-1BBL accumulated in the contact zone between T cells and LNCaP tumor cells, colocalizing with CD28 and 4-1BB. Furthermore, functional ligand-receptor interactions in these condensations on a single cell level are indicated by the strong accumulation of granzyme-B in synapses containing concentrated 4-1BBL foci. On antigen contact with the target cell the microtubule organizing center localizes towards the immunological synapse and mediates directed movement of lytic granules towards the T APC interphase. Notably, this translocation has been shown to be severely impaired in sub optimally stimulated or anergized tumor infiltrating lymphocytes. The immunological synapse seems to be highly suited to orchestrate this auto-costimulation. After maturation, it displays a highly compartmentalized pattern with condensed domains of receptors propagating costimulation in vicinity to T-cell receptors and proteins involved in early downstream signaling events. Without wishing to be bound by theory, it is possible that the physical interaction of 4-1BBL with its receptor could occur either during early raft
reorganization or between adjoining plasma membrane folds displaying 4-1BBL and 4-1BB in the center of the synapse. Conceivably, auto-costimulation as a genetic strategy equips T lymphocytes with costimulatory capacity irrespective of the immunogenicity of the antigen presenting cell. Furthermore, the genetically induced constitutive presence of 4-1BBL on T cells ensures costimulation "on demand" by the prompt engagement of the transiently upregulated 4-1BB in response to TCR activation. This effect is self regulated as CD80+4-1BBL+ T-cell expansion was markedly attenuated in response to the third LnCap co-culture (median 8.6 fold) compared to initial tumor challenge (median 13.2 fold, stat, Fig 1a, right panel). Similarly, following their robust T cell response in PC3-tumor bearing mice clickbeetle signal of CD80 and 4-1BBL co-transduced T lymphocytes gradually declined to 0.17 % of its peak value over the following 80 days (Fig. 3b). Interestingly, serial FACS and gene expression profiles of in vitro expanded CD80 and 4-1BBL transduced T lymphocytes revealed a stepwise downregulation of CD28. The reduced availability of CD28 may serve to put a break on excessive auto-costimulation in the absence of tumor antigen.

Costimulation in trans is a second mechanism likely to be involved in the enhancement effect of CD80 and 4-1BBL expression on T lymphocytes. Proliferative as well as effector functions of T-cells engaging antigen in the absence of CD80 or 4-1BBL could be restored by in-vitro co-culture or in-vivo co-injection of antigen specific CD80 and 4-1BBL displaying T cells (Fig 5, 6). Thus, the immediate activation effect of constitutive CD80 and 4-1BBL expression on genetically enhanced T-cells would extend to unmodified bystander cells. Whereas soluble factors, such as IL-2 or other cytokines may account for some effects in trans, the absence of trans-activation in a transwell co-culture system underscores the importance of physical contact between the responder- and the CD80 and 4-1BBL displaying T cell population. The ability of a T cell to integrate antigen receptor and costimulatory signals when the agonistic MHC-peptide complex and CD80 are presented on different cells has been confirmed in several in vitro co-culture settings. Choosing tumor reactive human T lymphocytes as a platform of the combined CD80 and 4-1BBL expression takes advantage of their active tumor infiltration coupled with a robust proliferation exclusively in the tumor environment – two key features absent in conventional monoclonal antibody or cytokine tumor therapies. This
would broaden the anti tumor immune response initially limited to PSMA to a heterogeneous population of endogenous tumor infiltrating lymphocytes, thus helping to prevent tumor escapes variants.

The constitutive expression of CD80 and 4-1BBL taps into the activation potential of endogenously expressed costimulation receptors and engage them according to their physiological expression pattern. This approach is not restricted to the chimeric antigen receptor as shown in the expression of CD80 and 4-1BBL in CMV-specific CTLs (Fig. 1H,1). Collectively, the constitutive expression of the costimulatory ligand pair CD80 and 4-1BBL has established human T lymphocytes as a viable and therapeutically successful means to obviate the need for APC mediated costimulation. In particular the dual costimulatory effect in auto and trans qualifies CD80 and 4-1BBL cotransduced T cells as a substantially improved treatment modality in the arsenal of adoptive T cell therapies. Moreover, this discovery can be used for the expression of virtually any antigen-recognizing receptor in combination with a co-stimulatory ligand in an immunoresponsive cell. Such cells are useful in a variety of applications where an increase in an immune response is desirable, including but not limited to the prevention or treatment of neoplasias, infectious diseases, or to enforce immune tolerance in the context of autoimmune disease or allogeneic transplantation.

**Embodiments of the Invention**

From the foregoing description, it will be apparent that variations and modifications may be made to the invention described herein to adopt it to various usages and conditions. Such embodiments are also within the scope of the following claims.

The recitation of a listing of elements in any definition of a variable herein includes definitions of that variable as any single element or combination (or subcombination) of listed elements. The recitation of an embodiment herein includes that embodiment as any single embodiment or in combination with any other embodiments or portions thereof.

All patents and publications mentioned in this specification are herein incorporated by reference to the same extent as if each independent patent and publication was specifically and individually indicated to be incorporated by reference.
What is claimed is:

1. An immunoresponsive cell comprising a receptor that binds an antigen and an exogenous co-stimulatory ligand.

2. The method of claim 1, wherein the co-stimulatory ligand is constitutively or inducibly expressed.

3. The method of claim 1, wherein at least two co-stimulatory ligands are constitutively expressed.

4. The immunoresponsive cell of claim 1, wherein the cell is selected from the group consisting of a T cell, a Natural Killer (NK) cell, a cytotoxic T lymphocyte (CTL), and a regulatory T cell.

5. The immunoresponsive cell of claim 1, wherein the antigen is a tumor or pathogen antigen.

6. The immunoresponsive cell of claim 3, wherein the antigen is selected from the group consisting of prostate-specific membrane antigen (PSMA), Carcinoe embryonic Antigen (CEA), IL13Ralpha, her-2, CD19, NY-ESO-1, HIV-1 Gag, Lewis Y, Mart-1, gp100, tyrosinase, WT-1, hTERT.

7. The immunoresponsive cell of claim 1, wherein the cell expresses a recombinant and/or an endogenous antigen receptor.

8. The immunoresponsive cell of claim 1, wherein the co-stimulatory ligand is a tumor necrosis factor (TNF) ligand or an immunoglobulin (Ig) superfamily ligand.

9. The immunoresponsive cell of claim 8, wherein the TNF ligand is selected from the group consisting of 4-1BBL, OX40L, CD70, LIGHT, and CD30L.
10. The immunoresponsive cell of claim 8, wherein the Ig superfamily ligand is selected from the group consisting of CD80 and CD86.

11. The immunoresponsive cell of any one of claims 1-10, wherein the immunoresponsive cell expresses a TNF ligand and an Ig superfamily ligand.

12. The immunoresponsive cell of claim 11, wherein the TNF ligand is 4-1BBL and the Ig superfamily ligand is CD80.

13. The immunoresponsive cell of any one of claims 1-12, wherein an antigen presenting complex is constitutively expressed on the surface of the T cell.

14. The cell of any one of claims 1-12, wherein the co-stimulatory ligands are constitutively expressed on the surface of the T cell.

15. The immunoresponsive cell of any one of claims 1-12, wherein the co-stimulatory ligand is expressed in a retroviral vector.

16. A virus specific T cell expressing a vector encoding a polypeptide selected from the group consisting of CD80, 4-1BBL, OX40L, CD70 and CD30L.

17. The virus specific T cell of claim 16, wherein the T cell recognizes a virus selected from the group consisting of Cytomegalovirus (CMV), Epstein Barr Virus (EBV), Human Immunodeficiency Virus (HIV), and influenza virus antigens.

18. A tumor antigen-specific T cell expressing a vector encoding a polypeptide selected from the group consisting of CD80, 4-1BBL, OX40L, CD70 and CD30L.

19. The tumor antigen-specific T cell of claim 15, wherein the cell expresses CD80 and 4-1BBL.
20. The tumor antigen-specific T cell of claim 15, wherein the vector is a retroviral vector.

21. A method of modulating an immune response in a subject, the method comprising administering an effective amount of an immunoresponsive cell of any one of claims 1-17.

22. The method of claim 18, wherein the method increases or reduces an immune response.

23. The method of claim 18, wherein the method increases self-tolerance or increases tolerance to an organ transplant.

24. A method of treating or preventing a neoplasia in a subject, the method comprising administering an effective amount of an immunoresponsive cell comprising a receptor that binds a tumor antigen and a vector encoding a co-stimulatory ligand.

25. The method of claim 18, wherein the neoplasia is selected from the group consisting of prostate cancer, colon cancer, breast cancer, and glioblastoma.

26. The method of claim 18, wherein the tumor antigen is prostate-specific membrane antigen, CD19, NY-ESO-1, WT-1 or hTERT.

27. A method of enforcing tolerance in a subject, the method comprising administering an effective amount of an immunoresponsive cell comprising a receptor that binds an antigen and a vector encoding a co-stimulatory ligand.

28. The method of claim 27, wherein the method prevents or reduces an autoimmune disease or a disease associated with allogeneic transplantation.
29. A method of treating or preventing a pathogen infection in a subject, the method comprising administering an effective amount of an immunoresponsive cell comprising a receptor that binds a viral antigen and a vector encoding a co-stimulatory ligand.

30. The method of claim 29, wherein the pathogen is a virus, bacteria, fungus, protozoa or parasite.

31. The method of claim 29, wherein the virus is selected from the group consisting of Cytomegalovirus (CMV), Epstein Barr Virus (EBV), Human Immunodeficiency Virus (HIV), and influenza virus.

32. The method of any one of claims 21-30, wherein the cell is a T cell, a Natural Killer (NK) cell, or a cytotoxic T lymphocyte (CTL).

33. The method of any one of claims 21-30, wherein the cell expresses a recombinant or an endogenous receptor for the antigen.

34. The method of any one of claims 21-30, wherein the co-stimulatory ligand is a tumor necrosis factor (TNF) ligand or an immunoglobulin (Ig) superfamily ligand.

35. The method of any one of claims 21-31, wherein the TNF ligand is selected from the group consisting of 4-1BBL, OX40L, CD70, LIGHT, and CD30L.

36. The method of any one of claims 21-31, wherein the Ig superfamily ligand is selected from the group consisting of CD80 and CD86.

37. The method of any one of claims 21-31, wherein the immunoresponsive cell express a TNF ligand and an Ig superfamily ligand.

38. The method of any one of claims 21-31, wherein the TNF ligand is 4-1BBL and the Ig superfamily ligand is CD80.
39. The method of any one of claims 21-31, wherein an antigen presenting complex is constitutively expressed on the surface of the T cell.

40. The method of any one of claims 21-31, wherein the co-stimulatory ligands are constitutively expressed on the surface of the T cell.

41. The method of any one of claims 21-31, wherein the vector is a retroviral vector.

42. A method for producing an antigen-specific immunoresponsive cell, the method comprising introducing into the immunoresponsive cell a nucleic acid sequence that encodes a chimeric antigen receptor, wherein the chimeric antigen receptor comprises an antigen-binding domain coupled to an intracellular signaling domain that activates an immunoresponsive cell.

43. The method of claim 42, wherein the immunoresponsive cells is a T cell, CTL, or NK cell.

44. The method of claim 42, wherein the antigen-binding domain is a tumor antigen-binding domain.

45. The method of claim 42, wherein the tumor antigen is prostate specific membrane antigen (PSMA).

46. The method of claim 42, wherein the intracellular signaling domain activates a T cell, CTL cell, or NK cell.

47. The method of claim 42, wherein the intracellular signaling domain is the ζ-chain signaling domain.
48. A method of treating a neoplasia in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of a T cell comprising a tumor antigen and an antigen presenting complex comprising at least two co-stimulatory ligands, wherein at least one of the two co-stimulatory ligands is selected from the group consisting of: a tumor necrosis factor (TNF) ligand and an immunoglobulin (Ig) superfamily ligand and combinations thereof, thereby treating cancer in the subject.

49. A method of treating a neoplasia in a subject, the method comprising administering to the subject a therapeutically effective amount of a Natural Killer (NK) cell comprising a tumor antigen and an antigen presenting complex comprising at least two co-stimulatory ligands, wherein at least one of the two co-stimulatory ligands is selected from the group consisting of: a tumor necrosis factor (TNF) ligand and an immunoglobulin (Ig) superfamily ligand and combinations thereof, thereby treating cancer in the subject.

50. The method of claim 48 or 49, wherein the TNF ligand is selected from the group consisting of: 4-1BBL, OX40L, CD70, and CD30L, and LIGHT.

51. The method of claim 48 or 49, wherein the Ig superfamily ligand is selected from the group consisting of: CD80 and CD86.

52. The method of claim 48 or 49, wherein the at least two co-stimulatory ligands are a TNF ligand and an Ig superfamily ligand.

53. The method of claim 52, wherein the TNF ligand is 4-1BBL and the Ig superfamily ligand is CD80.

54. The method of claim 48 or 49, wherein the antigen recognition complex is constitutively expressed on the surface of the cell.
55. The method of claim 48 or 49, wherein the tumor antigen is prostate specific membrane antigen (PSMA).

56. The method of claim 48 or 49, wherein the at least two co-stimulatory ligands are constitutively expressed on the surface of the cell.

57. A method of treating an infectious disease in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of a T cell comprising a receptor specific for a viral antigen and an antigen presenting complex comprising at least two co-stimulatory ligands, wherein at least one of the two co-stimulatory ligands is selected from the group consisting of: a tumor necrosis factor (TNF) ligand and an immunoglobulin (Ig) superfamily ligand and combinations thereof, thereby treating the infectious disease in the subject.

58. A method of treating an infectious disease in a subject, comprising administering to the subject a therapeutically effective amount of a Natural Killer (NK) cell comprising a receptor specific for a viral antigen and an antigen presenting complex comprising at least two co-stimulatory ligands, wherein at least one of the two co-stimulatory ligands is selected from the group consisting of: a tumor necrosis factor (TNF) ligand and an immunoglobulin (Ig) superfamily ligand and combinations thereof, thereby treating the infectious disease in the subject.

59. The method of claim 57 or 58, wherein the subject is an immunocompromised subject.

60. The method of claim 57 or 58, wherein the TNF ligand is selected from the group consisting of: 4-1BBL, OX40L, CD70, LIGHT and CD30L.

61. The method of claim 57 or 58, wherein the Ig superfamily ligand is selected from the group consisting of: CD80 and CD86.
62. The method of claim 57 or 58, wherein the at least two co-stimulatory ligands are a TNF ligand and an Ig superfamily ligand.

63. The method of claim 57 or 58, wherein the TNF ligand is 4-1BBL and the Ig superfamily ligand is CD80.

64. The method of claim 57 or 58, wherein the antigen recognition complex is constitutively expressed on the surface of the cell.

65. The method of claim 57 or 58, wherein the viral antigen is an antigen specific for Cytomegalovirus (CMV), Epstein Barr Virus (EBV), Human Immunodeficiency Virus (HIV), or influenza virus.

66. The method of claim 57 or 58, wherein the at least two co-stimulatory ligands are constitutively expressed on the surface of the cell.

67. A pharmaceutical composition comprising an effective amount of an immunoresponsive cell of any one of claims 1-20 in a pharmaceutically acceptable excipient.

68. A pharmaceutical composition for the treatment of a neoplasia comprising an effective amount of a tumor antigen-specific T cell of any one of claim 15-17 in a pharmaceutically acceptable excipient.

69. A pharmaceutical composition for the treatment of a pathogen infection comprising an effective amount of a viral-specific T cell of claim 14 in a pharmaceutically acceptable excipient.

70. The pharmaceutical composition of any one of claims 67-69, further comprising a cytokine selected from the group consisting of IL-2, IL-3, IL-6, IL-11, IL7, IL15, IL21,
granulocyte macrophage colony stimulating factor, alpha, beta or gamma interferon and erythropoietin.

71. A kit for treatment of a neoplasia, pathogen infection, an autoimmune disorder, or an allogeneic transplant, the kit comprising an imuno-responsive cell comprising a receptor that binds an antigen and an exogenous co-stimulatory ligand.

72. The kit of claim 71, wherein the kit further comprises written instructions for using said cell for the treatment of a subject having a neoplasia, a pathogen infection, an autoimmune disorder, or an allogeneic transplant.

73. The method of any one of claims 21-67, further comprising the step of an immunoresponsive obtaining the immunoresponsive cell or co-stimulatory ligand.
ANTIGEN-SPECIFIC T CELLS AND METHODS OF USE

Abstract of the Disclosure

The present invention provides immunoresponsive cells, including T cells, cytotoxic T cells, regulatory T cells, and Natural Killer (NK) cells, expressing at least one of an antigen-recognizing receptor and a co-stimulatory ligand and methods of use therefore for the treatment of neoplasia and other pathologies where an increase in an antigen-specific immune response is desired.

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Figure 1
Figure 2
Figure 3
Figure 5
Application Data Sheet

Application Information

Application Type:: Provisional
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