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CHIMERIC TIM4 RECEPTORS AND USES THEREOF

Abstract

The present disclosure relates to chimeric Tim4 receptors, host cells modified to include chimeric Tim4 receptor molecules, and methods of making and using such receptor molecules and modified cells.

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Background/Summary

STATEMENT REGARDING SEQUENCE LISTING

[0001] This application contains a Sequence Listing, which has been submitted electronically in xml format and is hereby incorporated by reference in its entirety. Said xml copy, created on Apr. 16, 2025, is named SeqList-368779-40411.xml and is 94,977 bytes in size.

BACKGROUND

[0002] Upon exposure to antigen, naïve antigen-specific CD8+ T cells undergo differentiation that promotes their clonal expansion and development into functional, effector T cells that can kill cells expressing the cognate antigen (e.g., tumor cells). Following antigen clearance, the majority of effector T cells undergo apoptosis, and a subset of the surviving effector T cells differentiate into memory T cells that can confer long-term protection against antigen re-exposure. However, prolonged antigen exposure may result in T cell exhaustion, enabling the persistence of tumor cells. T cell exhaustion refers to a dysfunctional state acquired by T cells experiencing persistent TCR stimulation characterized by upregulated expression of immune checkpoint molecules (e.g., PD-1, CTLA-4, Tim-3), impaired effector function, poor proliferation, and metabolic defects. Engineered T cells expressing chimeric antigen receptors (CARs) can also develop exhaustion.

Description

DETAILED DESCRIPTION

[0003] In one aspect, the present disclosure provides chimeric T-cell immunoglobulin mucin protein 4 (Tim4) receptors. Chimeric Tim4 receptors of the present disclosure confer cytotoxic activity to chimeric Tim4 receptor-modified host cells, with the cytolytic activity being induced upon binding of the chimeric Tim4 receptor to its target antigen, phosphatidylserine. Embodiments of the chimeric Tim4 receptors described herein comprise a single chain chimeric protein, the single chain chimeric protein comprising: an extracellular domain comprising a Tim4 binding domain; an intracellular signaling domain comprising a first costimulatory signaling domain; and a transmembrane domain positioned between and connecting the extracellular domain and the intracellular signaling domain. In certain embodiments, the extracellular domain of the chimeric Tim4 receptors described herein optionally includes an extracellular spacer domain positioned between and connecting the binding domain and transmembrane domain.

[0004] In certain embodiments, cytotoxic chimeric Tim4 receptors may also be capable of costimulating T cells via a different signaling pathway than the “classical” T cell costimulation pathways (e.g., CD28). In addition to binding phosphatidylserine, Tim4 is also a ligand for Tim1, which is expressed on the surface of activated T cells. Tim4-induced Tim1 signaling has been found to costimulate T cell proliferation and survival (Hartt Meyers et al., 2005, Nat. Immunol. 6:455). Thus, in certain embodiments, cytotoxic chimeric Tim4 receptors may reduce or inhibit T cell exhaustion, or restore exhausted T cells by providing costimulatory signals via at least one signaling pathway. In certain embodiments, cytotoxic chimeric Tim4 receptors provide costimulatory signals via at least two distinct signaling pathways (e.g., via the selected costimulatory signaling domain in the cytotoxic chimeric Tim4 receptor and Tim1).

[0005] In certain embodiments, when expressed in a host cell, the chimeric Tim4 receptors of the present disclosure also confer engulfment activity to the host cell. For example, in certain such embodiments, binding of the chimeric Tim4 receptor expressed in a host cell to a

phosphatidylserine target may induce both cytolytic and engulfment responses by the host cell. In particular embodiments of modified host cells described herein, the host cell does not naturally exhibit an engulfment phenotype prior to modification with the chimeric Tim4 receptor.

[0006] In another aspect, host cells modified with chimeric Tim4 receptors of the present disclosure can be used in methods for eliminating target cells bearing surface exposed phosphatidylserine, e.g., for the treatment of cancer. In normal, healthy cells phosphatidylserine is located in the inner leaflet of the plasma membrane. However, certain cellular events, such as damage, apoptosis, necrosis, and stress, activates a "scramblase" that quickly exposes phosphatidylserine on the cell surface, where it can bind to receptors such as Tim4. Endogenous tumor-specific effector T cells can induce exposure of phosphatidylserine on the outer membrane of targeted tumor cells during cytolysis. Furthermore, certain cancer therapies (e.g., chemotherapy, radiotherapy, CAR-T cells, etc.) can induce exposure of phosphatidylserine on targeted tumor cells or cells in the tumor microenvironment by inducing apoptosis, cellular stress, cellular damage, etc. Host cells expressing the presently disclosed chimeric Tim4 receptors may clear damaged, stressed, apoptotic, or necrotic tumor cells bearing surface exposed phosphatidylserine by inducing apoptosis in the tumor cells bearing surface exposed phosphatidylserine. In certain embodiments, host cells expressing chimeric Tim4 receptors disclosed herein clear damaged, stressed, apoptotic, or necrotic tumor cells bearing surface exposed phosphatidylserine by inducing apoptosis and by engulfment. Host cells comprising chimeric Tim4 receptors according to the present description may be administered to a subject alone, or in combination with one or more additional therapeutic agents, including for example CAR-T cells, TCRs, antibodies, radiation therapy, chemotherapies, small molecules, oncolytic viruses, electropulse therapy, etc.

[0007] In another aspect, host cells modified with chimeric Tim4 receptors of the present disclosure can be used in methods for enhancing an effector response (e.g., a tumor specific immune response). Embodiments of the chimeric Tim4 receptors of the present disclosure are capable of costimulating T cells via at least one costimulatory signaling pathway upon binding phosphatidylserine. In certain embodiments, the chimeric Tim4 receptors described herein provide costimulatory signals via at least two distinct signaling pathways. In certain embodiments, the enhanced effector response is enhanced T cell proliferation, cytokine production, cytotoxic activity, persistence, or any combination thereof. Host cells expressing chimeric Tim4 receptors according to the present description may be administered to a subject alone, or in combination with one or more additional therapeutic agents, including for example CAR-T cells, TCRs, antibodies, radiation therapy, chemotherapies, small molecules, oncolytic viruses, electropulse therapy, etc.

[0008] In another aspect, host cells modified with chimeric Tim4 receptors of the present disclosure can be used in methods for inhibiting or reducing immune cell exhaustion. In certain embodiments, immune cell exhaustion refers to T cell exhaustion, NK cell exhaustion, or both. Tumor cells may provide continuous antigen stimulation to immune cells, often in the absence of costimulatory ligands, which may result in immune cell exhaustion (e.g., reduced proliferative capacity, reduced effector function, and upregulation of immunosuppressive molecules). Cancer therapies, such as chemotherapy, radiotherapy, CAR-T cell therapy, etc., can also provide prolonged antigen stimulation in the absence of costimulatory signals or when the strength or duration of costimulatory signals is limited. Chimeric Tim4 receptors of the present disclosure are capable of costimulating immune cells via at least one costimulatory signaling domain upon binding phosphatidylserine. In certain embodiments, chimeric Tim4 receptors provide costimulatory signals via at least two distinct signaling pathways. Host cells expressing chimeric Tim4 receptors may be administered to a subject alone, or in combination with one or more additional therapeutic agents, including for example CAR-T cells, TCRs, antibodies, radiation therapy, chemotherapies, small molecules, oncolytic viruses, electropulse therapy, etc.

[0009] In another aspect, host cells modified with chimeric Tim4 receptors of the present disclosure can be used to enhance the effect of a therapeutic agent that induces cellular stress, damage,

necrosis, or apoptosis. For example, certain therapeutic agents, such as chemotherapy, specific inhibitors of driver mutations associated with cancer (targeted therapy such as BRAF inhibitors, EGRF inhibitors, ALK/ROS1 kinase inhibitors), radiation therapy, UV light therapy, electropulse therapy, adoptive cellular immunotherapy (e.g., CAR-T cells, TCRs) and oncolytic viral therapy, can induce cell damage or death in tumor cells or diseased cells. Cells expressing a chimeric Tim4 receptor as presently described can bind to the phosphatidylserine moieties exposed on the outer leaflet of damaged or dying cells resulting from any one or more of such therapeutic agents and induce cytolysis or both cytolysis and engulfment of the targeted cells.

[0010] Prior to setting forth this disclosure in more detail, it may be helpful to an understanding thereof to provide definitions of certain terms to be used herein.

[0011] In the present description, any concentration range, percentage range, ratio range, or integer range is to be understood to include the value of any integer within the recited range and, when appropriate, fractions thereof (such as one tenth and one hundredth of an integer), unless otherwise indicated. Also, any number range recited herein relating to any physical feature, such as polymer subunits, size or thickness, are to be understood to include any integer within the recited range, unless otherwise indicated. As used herein, the term “about” means±20% of the indicated range, value, or structure, unless otherwise indicated. It should be understood that the terms “a” and “an” as used herein refer to “one or more” of the enumerated components. The use of the alternative (e.g., “or”) should be understood to mean either one, both, or any combination thereof of the alternatives. As used herein, the terms “include,” “have” and “comprise” are used synonymously, which terms and variants thereof are intended to be construed as non-limiting.

[0012] Terms understood by those in the art of antibody technology are each given the meaning acquired in the art, unless expressly defined differently herein. The term “antibody” is used in the broadest sense and includes polyclonal and monoclonal antibodies. An “antibody” may refer to an intact antibody comprising at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds, as well as an antigen-binding portion (or antigen-binding domain) of an intact antibody that has or retains the capacity to bind a target molecule. An antibody may be naturally occurring, recombinantly produced, genetically engineered, or modified forms of immunoglobulins, for example intrabodies, peptibodies, nanobodies, single domain antibodies, SMIPs, multispecific antibodies (e.g., bispecific antibodies, diabodies, triabodies, tetrabodies, tandem di-scFV, tandem tri-scFv, ADAPTIR). A monoclonal antibody or antigen-binding portion thereof may be non-human, chimeric, humanized, or human, preferably humanized or human. Immunoglobulin structure and function are reviewed, for example, in Harlow et al., Eds., Antibodies: A Laboratory Manual, Chapter 14 (Cold Spring Harbor Laboratory, Cold Spring Harbor, 1988). “Antigen-binding portion” or “antigen-binding domain” of an intact antibody is meant to encompass an “antibody fragment,” which indicates a portion of an intact antibody and refers to the antigenic determining variable regions or complementary determining regions of an intact antibody. Examples of antibody fragments include, but are not limited to, Fab, Fab′, F(ab′).sub.2, and Fv fragments, Fab′-SH, F(ab′).sub.2, diabodies, linear antibodies, scFv antibodies, VH, and multispecific antibodies formed from antibody fragments. A “Fab” (fragment antigen binding) is a portion of an antibody that binds to antigens and includes the variable region and CH1 of the heavy chain linked to the light chain via an inter-chain disulfide bond. An antibody may be of any class or subclass, including IgG and subclasses thereof (IgG.sub.1, IgG.sub.2, IgG.sub.3, IgG.sub.4), IgM, IgE, IgA, and IgD.

[0013] The term “variable region” or “variable domain” refers to the domain of an antibody heavy or light chain that is involved in binding of the antibody to antigen. The variable domains of the heavy chain and light chain (VH and VL, respectively) of a native antibody generally have similar structures, with each domain comprising four conserved framework regions (FRs) and three CDRs. (See, e.g., Kindt et al. Kuby Immunology, 6th ed., W.H. Freeman and Co., page 91 (2007)). A single VH or VL domain may be sufficient to confer antigen-binding specificity. Furthermore,

antibodies that bind a particular antigen may be isolated using a VH or VL domain from an antibody that binds the antigen to screen a library of complementary VL or VH domains, respectively. See, e.g., Portolano et al., J. Immunol. 150:880-887 (1993); Clarkson et al., Nature 352:624-628 (1991).

[0014] The terms “complementarity determining region” and “CDR,” which are synonymous with “hypervariable region” or “HVR,” are known in the art to refer to non-contiguous sequences of amino acids within antibody variable regions, which confer antigen specificity and/or binding affinity. In general, there are three CDRs in each heavy chain variable region (HCDR1, HCDR2, HCDR3) and three CDRs in each light chain variable region (LCDR1, LCDR2, LCDR3).

[0015] As used herein, the terms “binding domain”, “binding region”, and “binding moiety” refer to a molecule, such as a peptide, oligopeptide, polypeptide, or protein that possesses the ability to specifically and non-covalently bind, associate, unite, recognize, or combine with a target molecule (e.g., phosphatidylserine). A binding domain includes any naturally occurring, synthetic, semi-synthetic, or recombinantly produced binding partner for a biological molecule or other target of interest. In some embodiments, the binding domain is an antigen-binding domain, such as an antibody or functional binding domain or antigen-binding portion thereof. Exemplary binding domains include single chain antibody variable regions (e.g., domain antibodies, sFv, scFv, Fab), receptor ectodomains (e.g., Tim4), ligands (e.g., cytokines, chemokines), or synthetic polypeptides selected for the specific ability to bind to a biological molecule.

[0016] “T cell receptor” (TCR) refers to a molecule found on the surface of T cells (also referred to as T lymphocytes) that is generally responsible for recognizing antigens bound to major histocompatibility complex (MHC) molecules. The TCR is generally composed of a disulfide-linked heterodimer of the highly variable α and β chains (also known as TCRα and TCRβ, respectively) in most T cells. In a small subset of T cells, the TCR is made up of a heterodimer of γ and δ chains (also known as TCRγ and TCRδ, respectively). Each chain of the TCR is a member of the immunoglobulin superfamily and possesses one N-terminal immunoglobulin variable domain, one immunoglobulin constant domain, a transmembrane region, and a short cytoplasmic tail at the C-terminal end (see Janeway et al., *Immunobiology: The Immune System in Health and Disease,* 3.sup.rd Ed., Current Biology Publications, p. 4:33, 1997). TCRs of the present disclosure may be from various animal species, including human, mouse, rat, cat, dog, goat, horse, or other mammals. TCRs may be cell-bound (i.e., have a transmembrane region or domain) or in soluble form. TCRs include recombinantly produced, genetically engineered, fusion, or modified forms of TCRs, including for example, scTCRs, soluble TCRs, TCR fusion constructs (TRuC™, see, U.S. Patent Publication No. 2017/0166622).

[0017] The term “variable region” or “variable domain” of a TCR α-chain (Vα) and β-chain (Vβ), or Vγ and Vδ for γδ TCRs, are involved in binding of the TCR to antigen. The V.sub.α and V.sub.β of a native TCR generally have similar structures, with each variable domain comprising four conserved FRs and three CDRs. The V.sub.α domain is encoded by two separate DNA segments, the variable gene segment (V gene) and the joining gene segment (J gene); the V.sub.β domain is encoded by three separate DNA segments, the variable gene segment (V gene), the diversity gene segment (D gene), and the joining gene segment (J gene). A single V.sub.α or V.sub.β domain may be sufficient to confer antigen-binding specificity. “Major histocompatibility complex molecule” (MHC molecule) refers to a glycoprotein that delivers a peptide antigen to a cell surface. MHC class I molecules are heterodimers composed of a membrane spanning α chain (with three α domains) and a non-covalently associated β2 microglobulin. MHC class II molecules are composed of two transmembrane glycoproteins, α and β, both of which span the membrane. Each chain has two domains. MHC class I molecules deliver peptides originating in the cytosol to the cell surface, where peptide: MHC complex is recognized by CD8.sup.+ T cells. MHC class II molecules deliver peptides originating in the vesicular system to the cell surface, where they are recognized by CD4.sup.+ T cells. An MHC molecule may be from various animal species, including human,

mouse, rat, or other mammals.
[0018] “Chimeric antigen receptor” (CAR) refers to a chimeric protein comprising two or more distinct domains and can function as a receptor when expressed on the surface of a cell. CARs are generally composed of an extracellular domain comprising a binding domain that binds a target antigen, an optional extracellular spacer domain, a transmembrane domain, and an intracellular signaling domain (e.g., an immunoreceptor tyrosine-based activation motif (ITAM)-containing T cell activating motif, and optionally an intracellular costimulatory domain). In certain embodiments, an intracellular signaling domain of a CAR has an ITAM-containing T cell activating domain (e.g., CD3ζ) and an intracellular costimulatory domain (e.g., CD28). In certain embodiments, a CAR is synthesized as a single polypeptide chain or is encoded by a nucleic acid molecule as a single chain polypeptide.
[0019] A variety of assays are known for identifying binding domains of the present disclosure that specifically bind a particular target, as well as determining binding domain affinities, such as Western blot, ELISA, and BIACORE® analysis (see also, e.g., Scatchard et al., *Ann. N.Y. Acad. Sci.* 51:660, 1949; and U.S. Pat. Nos. 5,283,173, 5,468,614, or the equivalent). As used herein, “specifically binds” refers to an association or union of a binding domain, or a fusion protein thereof, to a target molecule with an affinity or K.sub.a (i.e., an equilibrium association constant of a particular binding interaction with units of 1/M) equal to or greater than 10.sup.5 M.sup.−1, while not significantly associating or uniting with any other molecules or components in a sample.
[0020] The terms “antigen” and “Ag” refer to a molecule that is capable of inducing an immune response. The immune response that is induced may involve antibody production, the activation of specific immunologically-competent cells, or both. Macromolecules, including proteins, glycoproteins, and glycolipids, can serve as an antigen. Antigens can be derived from recombinant or genomic DNA. As contemplated herein, an antigen need not be encoded (i) solely by a full length nucleotide sequence of a gene or (ii) by a “gene” at all. An antigen can be generated or synthesized, or an antigen can be derived from a biological sample. Such a biological sample can include, but is not limited, to a tissue sample, a tumor sample, a cell, or a biological fluid.
[0021] The term “epitope” or “antigenic epitope” includes any molecule, structure, amino acid sequence or protein determinant within an antigen that is specifically bound by a cognate immune binding molecule, such as an antibody or fragment thereof (e.g., scFv), T cell receptor (TCR), chimeric Tim4 receptor, or other binding molecule, domain or protein. Epitopic determinants generally contain chemically active surface groupings of molecules, such as amino acids or sugar side chains, and can have specific three dimensional structural characteristics, as well as specific charge characteristics. An epitope may be a linear epitope or a conformational epitope.
[0022] As used herein, the term “Tim4” (T-cell immunoglobulin and mucin domain containing protein 4), also known as “TimD4”, refers to a phosphatidylserine receptor that is typically expressed on antigen presenting cells, such as macrophages and dendritic cells. Tim4 mediates the phagocytosis of apoptotic, necrotic, damaged, injured, or stressed cells, which present phosphatidylserine (PtdSer) on the exofacial (outer) leaflet of the cell membrane. Tim4 is also capable of binding to Tim1 expressed on the surface of T cells and inducing proliferation and survival. In certain embodiments, Tim4 refers to human Tim4. An exemplary human Tim4 protein comprises an amino acid sequence of SEQ ID NO:1.
[0023] As used herein, the term “Tim4 binding domain” refers to the N-terminal immunoglobulin-fold domain of Tim4 that possesses a metal ion-dependent pocket that selectively binds PtdSer. An exemplary human Tim4 binding domain comprises an amino acid sequence of SEQ ID NO:2, and an exemplary mouse Tim4 binding domain comprises an amino acid sequence of SEQ ID NO:60. In certain embodiments, the Tim4 binding domain does not include a signal peptide.
[0024] As used herein, an “effector domain” is an intracellular portion of a fusion protein or receptor that can directly or indirectly promote a biological or physiological response in a cell expressing the effector domain when receiving the appropriate signal. In certain embodiments, an

effector domain is part of a protein or protein complex that receives a signal when bound, or it binds directly to a target molecule, which triggers a signal from the effector domain. An effector domain may directly promote a cellular response when it contains one or more signaling domains or motifs, such as an immunoreceptor tyrosine-based activation motif (ITAM). In other embodiments, an effector domain will indirectly promote a cellular response by associating with one or more other proteins that directly promote a cellular response.

[0025] As used herein, a “costimulatory signaling domain” refers to an intracellular signaling domain, or functional portion thereof, of a costimulatory molecule, which, when activated in conjunction with a primary or classic (e.g., ITAM-driven) activation signal (provided by, for example, a CD3ζ intracellular signaling domain), promotes or enhances a T cell response, such as T cell activation, cytokine production, proliferation, differentiation, survival, effector function, or combinations thereof. Costimulatory signaling domains include, for example, CD27, CD28, CD40L, GITR, NKG2C, CARD1, CD2, CD7, CD27, CD30, CD40, CD54 (ICAM), CD83, CD134 (OX-40), CD137 (4-1BB), CD150 (SLAMF1), CD152 (CTLA4), CD223 (LAG3), CD226, CD270 (HVEM), CD273 (PD-L2), CD274 (PD-L1), CD278 (ICOS), DAP10, LAT, LFA-1, LIGHT, NKG2C, SLP76, TRIM, ZAP70, or any combination thereof.

[0026] As used herein, an “immunoreceptor tyrosine-based activation motif (ITAM) activating domain” refers to an intracellular signaling domain or functional portion thereof which is naturally or endogenously present on an immune cell receptor or a cell surface marker and contains at least one immunoreceptor tyrosine-based activation motif (ITAM). ITAM refers to a conserved motif of YXXL/I-X.sub.6-8-YXXL/I. In certain embodiments an ITAM signaling domain contains one, two, three, four, or more ITAMs. An ITAM signaling domain may initiate T cell activation signaling following antigen binding or ligand engagement. ITAM-signaling domains include, for example, intracellular signaling domains of CD3γ, CD3δ, CD3ε, CD3ζ, CD79a, and CD66d.

[0027] “Junction amino acids” or “junction amino acid residues” refer to one or more (e.g., about 2-20) amino acid residues between two adjacent motifs, regions or domains of a polypeptide. Junction amino acids may result from the construct design of a chimeric protein (e.g., amino acid residues resulting from the use of a restriction enzyme site during the construction of a nucleic acid molecule encoding a chimeric protein).

[0028] “Nucleic acid molecule” and “polynucleotide” can be in the form of RNA or DNA, which includes cDNA, genomic DNA, and synthetic DNA. A nucleic acid molecule may be composed of naturally occurring nucleotides (such as deoxyribonucleotides and ribonucleotides), analogs of naturally occurring nucleotides (e.g., α-enantiomeric forms of naturally occurring nucleotides), or a combination of both. Modified nucleotides can have modifications in or replacement of sugar moieties, or pyrimidine or purine base moieties. Nucleic acid monomers can be linked by phosphodiester bonds or analogs of such linkages. Analogs of phosphodiester linkages include phosphorothioate, phosphorodithioate, phosphoroselenoate, phosphorodiselenoate, phosphoroanilothioate, phosphoranilidate, phosphoramidate, and the like. A nucleic acid molecule may be double stranded or single stranded, and if single stranded, may be the coding strand or non-coding (anti-sense strand). A coding molecule may have a coding sequence identical to a coding sequence known in the art or may have a different coding sequence, which, as the result of the redundancy or degeneracy of the genetic code, or by splicing, can encode the same polypeptide.

[0029] “Encoding” refers to the inherent property of specific polynucleotide sequences, such as DNA, cDNA, and mRNA sequences, to serve as templates for synthesis of other polymers and macromolecules in biological processes having either a defined sequence of nucleotides (i.e., rRNA, tRNA and mRNA) or a defined sequence of amino acids and the biological properties resulting therefrom. Thus, a polynucleotide encodes a protein if transcription and translation of mRNA corresponding to that polynucleotide produces the protein in a cell or other biological system. Both a coding strand and a non-coding strand can be referred to as encoding a protein or other product of the polynucleotide. Unless otherwise specified, a “nucleotide sequence encoding

an amino acid sequence" includes all nucleotide sequences that are degenerate versions of each other and that encode the same amino acid sequence.
[0030] As used herein, the terms "peptide," "polypeptide," and "protein" are used interchangeably, and refer to a compound comprised of amino acid residues covalently linked by peptide bonds. A protein or peptide must contain at least two amino acids, and no limitation is placed on the maximum number of amino acids that can comprise a protein's or peptide's sequence. Polypeptides include any peptide or protein comprising two or more amino acids joined to each other by peptide bonds. As used herein, the term refers to both short chains, which also commonly are referred to in the art as peptides, oligopeptides and oligomers, for example, and to longer chains, which generally are referred to in the art as proteins, of which there are many types. "Polypeptides" include, for example, biologically active fragments, substantially homologous polypeptides, oligopeptides, homodimers, heterodimers, variants of polypeptides, modified polypeptides, derivatives, analogs, fusion proteins, among others. The polypeptides include natural peptides, recombinant peptides, synthetic peptides, or a combination thereof.
[0031] As used herein, the term "mature polypeptide" or "mature protein" refers to a protein or polypeptide that is secreted or localized in the cell membrane or inside certain cell organelles (e.g., the endoplasmic reticulum, golgi, or endosome) and does not include an N-terminal signal peptide.
[0032] A "signal peptide", also referred to as "signal sequence", "leader sequence", "leader peptide", "localization signal" or "localization sequence", is a short peptide (usually 15-30 amino acids in length) present at the N-terminus of newly synthesized proteins that are destined for the secretory pathway. A signal peptide typically comprises a short stretch of hydrophilic, positively charged amino acids at the N-terminus, a central hydrophobic domain of 5-15 residues, and a C-terminal region with a cleavage site for a signal peptidase. In eukaryotes, a signal peptide prompts translocation of the newly synthesized protein to the endoplasmic reticulum where it is cleaved by the signal peptidase, creating a mature protein that then proceeds to its appropriate destination.
[0033] The term "chimeric" refers to any nucleic acid molecule or protein that is not endogenous and comprises sequences joined or linked together that are not normally found joined or linked together in nature. For example, a chimeric nucleic acid molecule may comprise regulatory sequences and coding sequences that are derived from different sources, or regulatory sequences and coding sequences that are derived from the same source but arranged in a manner different than that found in nature.
[0034] As used herein, the term "endogenous" or "native" refers to a gene, protein, compound, molecule or activity that is normally present in a host or host cell, including naturally occurring variants of the gene, protein, compound, molecule, or activity.
[0035] As used herein, "homologous" or "homolog" refers to a molecule or activity from a host cell that is related by ancestry to a second gene or activity, e.g., from the same host cell, from a different host cell, from a different organism, from a different strain, from a different species. For example, a heterologous molecule or heterologous gene encoding the molecule may be homologous to a native host cell molecule or gene that encodes the molecule, respectively, and may optionally have an altered structure, sequence, expression level or any combination thereof.
[0036] As used herein, "heterologous" nucleic acid molecule, construct or sequence refers to a nucleic acid molecule or portion of a nucleic acid molecule that is not native to a host cell, but can be homologous to a nucleic acid molecule or portion of a nucleic acid molecule from the host cell. The source of the heterologous nucleic acid molecule, construct or sequence can be from a different genus or species. In some embodiments, the heterologous nucleic acid molecules are not naturally occurring. In certain embodiments, a heterologous nucleic acid molecule is added (i.e., not endogenous or native) into a host cell or host genome by, for example, conjugation, transformation, transfection, transduction, electroporation, or the like, wherein the added molecule can integrate into the host cell genome or exist as extra-chromosomal genetic material (e.g., as a plasmid or other form of self-replicating vector), and can be present in multiple copies. In addition, "heterologous"

refers to a non-native enzyme, protein or other activity encoded by a non-endogenous nucleic acid molecule introduced into the host cell, even if the host cell encodes a homologous protein or activity.

[0037] As used herein, the term “engineered,” “recombinant,” “modified” or “non-natural” refers to an organism, microorganism, cell, nucleic acid molecule, or vector that has been modified by introduction of a heterologous nucleic acid molecule, or refers to a cell or microorganism that has been genetically engineered by human intervention—that is, modified by introduction of a heterologous nucleic acid molecule, or refers to a cell or microorganism that has been altered such that expression of an endogenous nucleic acid molecule or gene is controlled, deregulated or constitutive, where such alterations or modifications can be introduced by genetic engineering. Human-generated genetic alterations can include, for example, modifications introducing nucleic acid molecules (which may include an expression control element, such as a promoter) encoding one or more proteins, chimeric receptors, or enzymes, or other nucleic acid molecule additions, deletions, substitutions, or other functional disruption of or addition to a cell's genetic material. Exemplary modifications include those in coding regions or functional fragments thereof heterologous or homologous polypeptides from a reference or parent molecule. Additional exemplary modifications include, for example, modifications in non-coding regulatory regions in which the modifications alter expression of a gene or operon.

[0038] As used herein, the term “transgene” refers to a gene or polynucleotide encoding a protein of interest (e.g., chimeric Tim4 receptor) whose expression is desired in a host cell and that has been transferred by genetic engineering techniques into a cell. A transgene may encode proteins of therapeutic interest as well as proteins that are reporters, tags, markers, suicide proteins, etc. A transgene may be from a natural source, modification of a natural gene, or a recombinant or synthetic molecule. In certain embodiments, a transgene is a component of a vector.

[0039] The term “overexpressed” or “overexpression” of an antigen refers to an abnormally high level of antigen expression in a cell. Overexpressed antigen or overexpression of antigen is often associated with a disease state, such as in hematological malignancies and cells forming a solid tumor within a specific tissue or organ of a subject. Solid tumors or hematological malignancies characterized by overexpression of a tumor antigen can be determined by standard assays known in the art.

[0040] The “percent identity” between two or more nucleic acid or amino acid sequences is a function of the number of identical positions shared by the sequences (i.e., % identity=number of identical positions/total number of positions×100), taking into account the number of gaps, and the length of each gap that needs to be introduced to optimize alignment of two or more sequences. The comparison of sequences and determination of percent identity between two or more sequences can be accomplished using a mathematical algorithm, such as BLAST and Gapped BLAST programs at their default parameters (e.g., Altschul et al., *J. Mol. Biol.* 215:403, 1990; see also BLASTN at www.ncbi.nlm.nih.gov/BLAST).

[0041] A “conservative substitution” is recognized in the art as a substitution of one amino acid for another amino acid that has similar properties. Exemplary conservative substitutions are well known in the art (see, e.g., WO 97/09433, page 10, published Mar. 13, 1997; Lehninger, Biochemistry, Second Edition; Worth Publishers, Inc. NY: NY (1975), pp. 71-77; Lewin, Genes IV, Oxford University Press, NY and Cell Press, Cambridge, MA (1990), p. 8).

[0042] The term “promoter” as used herein is defined as a DNA sequence recognized by the synthetic machinery of the cell, or introduced synthetic machinery, required to initiate the specific transcription of a polynucleotide sequence.

[0043] As used herein, the term “promoter/regulatory sequence” means a nucleic acid sequence which is required for expression of a gene product operably linked to the promoter/regulatory sequence. In some instances, this sequence may be the core promoter sequence and in other instances, this sequence may also include an enhancer sequence and other regulatory elements

which are required for expression of the gene product. The promoter/regulatory sequence may, for example, be one which expresses the gene product in a tissue specific manner.
[0044] A “constitutive” promoter is a nucleotide sequence which, when operably linked with a polynucleotide which encodes or specifies a gene product, causes the gene product to be produced in a cell under most or all physiological conditions of the cell.
[0045] An “inducible” promoter is a nucleotide sequence which, when operably linked with a polynucleotide which encodes or specifies a gene product, causes the gene product to be produced in a cell substantially only when an inducer which corresponds to the promoter is present in the cell.
[0046] A “tissue-specific” promoter is a nucleotide sequence which, when operably linked with a polynucleotide encodes or specified by a gene, causes the gene product to be produced in a cell substantially only if the cell is a cell of the tissue type corresponding to the promoter.
[0047] The phrase “under transcriptional control” or “operatively linked” as used herein means that a promoter is in the correct location and orientation in relation to a polynucleotide to control the initiation of transcription by RNA polymerase and expression of the polynucleotide.
[0048] A “vector” is a nucleic acid molecule that is capable of transporting another nucleic acid. Vectors may be, for example, plasmids, cosmids, viruses, or phage. The term should also be construed to include non-plasmid and non-viral compounds which facilitate transfer of nucleic acid into cells. An “expression vector” is a vector that is capable of directing the expression of a protein encoded by one or more genes carried by the vector when it is present in the appropriate environment.
[0049] In certain embodiments, the vector is a viral vector. Examples of viral vectors include, but are not limited to, adenovirus vectors, adeno-associated virus vectors, retrovirus vectors, gamma retrovirus vectors, and lentivirus vectors. “Retroviruses” are viruses having an RNA genome. “Gamma retrovirus” refers to a genus of the retroviridae family. Examples of gamma retroviruses include mouse stem cell virus, murine leukemia virus, feline leukemia virus, feline sarcoma virus, and avian reticuloendotheliosis viruses. “Lentivirus” refers to a genus of retroviruses that are capable of infecting dividing and non-dividing cells. Examples of lentiviruses include, but are not limited to HIV (human immunodeficiency virus, including HIV type 1 and HIV type 2, equine infectious anemia virus, feline immunodeficiency virus (FIV), bovine immune deficiency virus (BIV), and simian immunodeficiency virus (SIV).
[0050] In other embodiments, the vector is a non-viral vector. Examples of non-viral vectors include lipid-based DNA vectors, modified mRNA (modRNA), self-amplifying mRNA, closed-ended linear duplex (CELiD) DNA, and transposon-mediated gene transfer (PiggyBac, Sleeping Beauty). Where a non-viral delivery system is used, the delivery vehicle can be a liposome. Lipid formulations can be used to introduce nucleic acids into a host cell in vitro, ex vivo, or in vivo. The nucleic acid may be encapsulated in the interior of a liposome, interspersed within the lipid bilayer of a liposome, attached to a liposome via a linking molecule that is associated with both the liposome and the nucleic acid, contained or complexed with a micelle, or otherwise associated with a lipid.
[0051] As used herein, the term “engulfment” refers to a receptor-mediated process wherein endogenous or exogenous cells or particles greater than 100 nm in diameter are internalized by a phagocyte or host cell of the present disclosure. Engulfment is typically composed of multiple steps: (1) tethering of the target cell or particle via binding of an engulfment receptor to a pro-engulfment marker or antigenic marker directly or indirectly (via a bridging molecule) on a target cell or particle; and (2) internalization or engulfment of the whole target cell or particle, or a portion thereof. In certain embodiments, internalization may occur via cytoskeletal rearrangement of a phagocyte or host cell to form a phagosome, a membrane-bound compartment containing the internalized target. Engulfment may further include maturation of the phagosome, wherein the phagosome becomes increasingly acidic and fuses with lysosomes (to form a phagolysosome),

whereupon the engulfed target is degraded (e.g., “phagocytosis”). Alternatively, phagosome-lysosome fusion may not be observed in engulfment. In yet another embodiment, a phagosome may regurgitate or discharge its contents to the extracellular environment before complete degradation. In some embodiments, engulfment refers to phagocytosis. In some embodiments, engulfment includes tethering of the target cell or particle by the phagocyte of host cell of the present disclosure, but not internalization. In some embodiments, engulfment includes tethering of the target cell or particle by the phagocyte of host cell of the present disclosure and internalization of part of the target cell or particle.

[0052] As used herein, the term “phagocytosis” refers to an engulfment process of cells or large particles (≥0.5 μm) wherein tethering of a target cell or particle, engulfment of the target cell or particle, and degradation of the internalized target cell or particle occurs. In certain embodiments, phagocytosis comprises formation of a phagosome that encompasses the internalized target cell or particle and phagosome fusion with a lysosome to form a phagolysosome, wherein the contents therein are degraded. In certain embodiments, during phagocytosis, following binding of a chimeric Tim4 receptor expressed on a host cell of the present disclosure to a phosphatidylserine expressed by a target cell or particle, a phagocytic synapse is formed; an actin-rich phagocytic cup is generated at the phagocytic synapse; phagocytic arms are extended around the target cell or particle through cytoskeletal rearrangements; and ultimately, the target cell or particle is pulled into the phagocyte or host cell through force generated by motor proteins. As used herein, “phagocytosis” includes the process of “efferocytosis”, which specifically refers to the phagocytosis of apoptotic or necrotic cells in a non-inflammatory manner.

[0053] The term “immune system cell” or “immune cell” means any cell of the immune system that originates from a hematopoietic stem cell in the bone marrow. Hematopoietic stem cells give rise to two major lineages, a myeloid progenitor cell (which give rise to myeloid cells such as monocytes, macrophages, dendritic cells, megakaryocytes and granulocytes) and a lymphoid progenitor cell (which give rise to lymphoid cells such as T cells, B cells and natural killer (NK) cells). Exemplary immune system cells include a CD4+ T cell, a CD8+ T cell, a CD4− CD8− double negative T cell, a γδ T cell, a regulatory T cell, a natural killer cell, and a dendritic cell. Macrophages and dendritic cells may also be referred to as “antigen presenting cells” or “APCs,” which are specialized cells that can activate T cells when a major histocompatibility complex (MHC) receptor on the surface of the APC complexed with a peptide interacts with a TCR on the surface of a T cell.

[0054] The term “T cells” refers to cells of T cell lineage. “Cells of T cell lineage” refer to cells that show at least one phenotypic characteristic of a T cell or a precursor or progenitor thereof that distinguishes the cells from other lymphoid cells, and cells of the erythroid or myeloid lineages. Such phenotypic characteristics can include expression of one or more proteins specific for T cells (e.g., CD3.sup.+, CD4.sup.+, CD8.sup.+), or a physiological, morphological, functional, or immunological feature specific for a T cell. For example, cells of the T cell lineage may be progenitor or precursor cells committed to the T cell lineage; CD25.sup.+ immature and inactivated T cells; cells that have undergone CD4 or CD8 linage commitment; thymocyte progenitor cells that are CD4.sup.+CD8.sup.+ double positive; single positive CD4.sup.+ or CD8.sup.+; TCRαβ or TCR γδ; or mature and functional or activated T cells. The term “T cells” encompasses naïve T cells (CD45 RA+, CCR7+, CD62L+, CD27+, CD45RO−), central memory T cells (CD45RO.sup.+, CD62L.sup.+, CD8.sup.+), effector memory T cells (CD45RA+, CD45RO−, CCR7−, CD62L−, CD27−), mucosal-associated invariant T (MAIT) cells, Tregs, natural killer T cells, and tissue resident T cells.

[0055] The term “B cells” refers to cells of the B cell lineage. “Cells of B cell lineage” refer to cells that show at least one phenotypic characteristic of a B cell or a precursor or progenitor thereof that distinguishes the cells from other lymphoid cells, and cells of the erythroid or myeloid lineages. Such phenotypic characteristics can include expression of one or more proteins specific for B cells (e.g., CD19.sup.+, CD72+, CD24+, CD20.sup.+), or a physiological, morphological, functional, or

immunological feature specific for a B cell. For example, cells of the B cell lineage may be progenitor or precursor cells committed to the B cell lineage (e.g., pre-pro-B cells, pro-B cells, and pre-B cells); immature and inactivated B cells or mature and functional or activated B cells. Thus, "B cells" encompass naïve B cells, plasma cells, regulatory B cells, marginal zone B cells, follicular B cells, lymphoplasmacytoid cells, plasmablast cells, and memory B cells (e.g., CD27.sup.+, IgD.sup.−).

[0056] The term "cytotoxic activity," also referred to as "cytolytic activity," with respect to a cell (e.g., a T cell or NK cell) expressing an immune receptor (e.g., a TCR) or a chimeric Tim4 receptor according to the present disclosure on its surface, means that upon antigen-specific signaling (e.g., via the TCR, chimeric Tim4 receptor), the cell induces a target cell to undergo apoptosis. In some embodiments, a cytotoxic cell may induce apoptosis in a target cell via the release of cytotoxins, such as perforin, granzyme, and granulysin, from granules. Perforins insert into the target cell membrane and form pores that allow water and salts to rapidly enter the target cell. Granzymes are serine proteases that induce apoptosis in the target cell. Granulysin is also capable of forming pores in the target cell membrane and is a proinflammatory molecule. In some embodiments, a cytotoxic cell may induce apoptosis in a target cell via interaction of Fas ligand, which is upregulated on T cell following antigen-specific signaling, with Fas molecules expressed on the target cell. Fas is an apoptosis-signaling receptor molecule on the surface of a number of different cells.

[0057] The term "exhaustion" with respect to immune cells refers to a state of immune cell dysfunction defined by poor effector function (e.g., reduced cytokine production, reduced cytotoxic activity), reduced proliferative capacity, increased expression of immune checkpoint molecules, and a transcriptional state distinct from that of functional effector or memory cells. In certain embodiments, an exhausted immune cell becomes unresponsive to the presence of its target antigen. Immune cell exhaustion may result from chronic exposure to a target antigen (e.g., as may result from chronic infection) or when it enters an immunosuppressive environment (e.g., a tumor microenvironment). In certain embodiments, immune cell exhaustion refers to T cell exhaustion, NK cell exhaustion, or both. In certain embodiments, exhausted T cells exhibit; (a) increased expression of PD-1, TIGIT, LAG3, TIM3, or any combination thereof; (b) decreased production of IFN-γ, IL-2, TNF-α, or any combination thereof; or both (a) and (b). In certain embodiments, exhausted NK cells exhibit; (a) increased expression of PD-1, NKG2A, TIM3, or any combination thereof; (b) decreased production of IFN-γ, TNF-α, or both; or both (a) and (b).

[0058] A "disease" is a state of health of a subject wherein the subject cannot maintain homeostasis, and wherein, if the disease is not ameliorated, then the subject's health continues to deteriorate. In contrast, a "disorder" or "undesirable condition" in a subject is a state of health in which the subject is able to maintain homeostasis, but in which the subject's state of health is less favorable than it would be in the absence of the disorder or undesirable condition. Left untreated, a disorder or undesirable condition does not necessarily result in a further decrease in the subject's state of health.

[0059] The term "cancer" as used herein is defined as disease characterized by the rapid and uncontrolled growth of aberrant cells. The aberrant cells may form solid tumors or constitute a hematological malignancy. Cancer cells can spread locally or through the bloodstream and lymphatic system to other parts of the body. Examples of various cancers include, but are not limited to, breast cancer, prostate cancer, ovarian cancer, cervical cancer, skin cancer, pancreatic cancer, colorectal cancer, renal cancer, liver cancer, brain cancer, lymphoma, leukemia, lung cancer and the like.

[0060] The term "subject," "patient" and "individual" are used interchangeably herein and are intended to include living organisms in which an immune response can be elicited (e.g., mammals). Examples of subjects include humans, primates, cows, horses, sheep, dogs, cats, mice, rats, rabbits, guinea pigs, pigs, and transgenic species thereof

[0061] "Adoptive cellular immunotherapy" or "adoptive immunotherapy" refers to the

administration of naturally occurring or genetically engineered disease antigen-specific immune cells (e.g., T cells). Adoptive cellular immunotherapy may be autologous (immune cells are from the recipient), allogeneic (immune cells are from a donor of the same species) or syngeneic (immune cells are from a donor genetically identical to the recipient).

[0062] "Autologous" refers to any material (e.g., a graft of organ, tissue, cells) derived from the same subject to which it is later to be re-introduced.

[0063] "Allogeneic" refers to a graft derived from a different subject of the same species.

[0064] A "therapeutically effective amount" or "effective amount" of a chimeric protein or cell expressing a chimeric protein of this disclosure (e.g., a chimeric Tim4 receptor or a cell expressing a chimeric Tim4 receptor) refers to that amount of protein or cells sufficient to result in amelioration of one or more symptoms of the disease, disorder, or undesired condition being treated. When referring to an individual active ingredient or a cell expressing a single active ingredient, administered alone, a therapeutically effective dose refers to the effects of that ingredient or cell expressing that ingredient alone. When referring to a combination, a therapeutically effective dose refers to the combined amounts of active ingredients or combined adjunctive active ingredient with a cell expressing an active ingredient that results in a therapeutic effect, whether administered serially or simultaneously.

[0065] "Treat" or "treatment" or "ameliorate" refers to medical management of a disease, disorder, or undesired condition of a subject. In general, an appropriate dose or treatment regimen comprising a host cell expressing a chimeric protein of this disclosure is administered in an amount sufficient to elicit a therapeutic or prophylactic benefit. Therapeutic or prophylactic/preventive benefit includes improved clinical outcome; lessening or alleviation of symptoms associated with a disease, disorder, or undesired condition; decreased occurrence of symptoms; improved quality of life; longer disease-free status; diminishment of extent of disease, disorder, or undesired condition; stabilization of disease state; delay of disease progression; remission; survival; prolonged survival; or any combination thereof.

[0066] The term "anti-tumor effect" refers to a biological effect which can be manifested by a decrease in tumor volume, a decrease in the number of tumor cells, a decrease in the number of metastases, an increase in life expectancy, or amelioration of various physiological symptoms associated with a cancerous condition. An "anti-tumor effect" can also be manifested by prevention of a hematological malignancy or tumor formation.

[0067] "Autoimmune disease" refers to a disorder that results from an autoimmune response. An autoimmune disease is the result of an inappropriately excessive response to a self-antigen. An autoimmune response may involve self-reactive B-cells that produce autoantibodies, self-reactive T-cells, or both. An "autoantibody" as used herein is an antibody produced by a subject that binds to a self-antigen also produced by the subject.

[0068] Additional definitions are provided throughout the present disclosure.

Chimeric Tim4 Receptors

[0069] In one aspect, the present disclosure provides a chimeric Tim4 receptor comprising a single chain chimeric protein, the single chain chimeric protein comprising: an extracellular domain comprising a Tim4 binding domain; an intracellular signaling domain comprising a first costimulatory signaling domain; and a transmembrane domain positioned between and connecting the extracellular domain and intracellular signaling domain. In certain embodiments, the extracellular domain of the chimeric Tim4 receptors described herein optionally includes an extracellular spacer domain positioned between and connecting the binding domain and transmembrane domain. When expressed in a host cell, chimeric Tim4 receptors of the present disclosure can confer a phosphatidylserine-specific, cytotoxic phenotype to the modified host cell (e.g., the host cell becomes cytotoxic to a stressed, damaged, injured, apoptotic, or necrotic cell expressing phosphatidylserine on its surface). In certain embodiments, the chimeric Tim4 receptors induce apoptosis in targeted cells via release of granzymes, perforin, granulysin, or any

combination thereof. In further embodiments, cells expressing a chimeric Tim4 receptor according to the present description exhibit an engulfment phenotype specific to phosphatidylserine presenting cells.

[0070] The intracellular signaling domain can include one or more effector (also referred to as "costimulatory signaling") domains that costimulate the modified host cell. Signaling by the costimulatory signaling domain(s) is triggered by binding of the extracellular domain to phosphatidylserine. In certain embodiments, the intracellular signaling domain comprises a first costimulatory signaling domain. In further embodiments, the intracellular signaling domain comprises a first costimulatory signaling domain and a second costimulatory signaling domain. Chimeric Tim4 receptors according to the present disclosure can be used in a variety of therapeutic methods where clearance of apoptotic, necrotic, damaged, or stressed cells is beneficial, while providing costimulation that enhances cellular immune response, reduces immune cell exhaustion, or both.

[0071] Component parts of the fusion proteins of the present disclosure are further described in detail herein.

Extracellular Domain

[0072] As described herein, a chimeric Tim4 receptor comprises an extracellular domain comprising a Tim4 binding domain. The Tim4 binding domain confers specificity to phosphatidylserine (PtdSer), which is a phospholipid with a negatively charged head-group and a component of the cell membrane. In healthy cells, phosphatidylserine is preferentially found in the inner leaflet of the cell membrane. However, when cells are stressed, damaged or undergo apoptosis or necrosis, phosphatidylserine is exposed on the outer leaflet of the cell membrane. Thus, phosphatidylserine may be used as a marker to distinguish stressed, damaged, apoptotic, necrotic, pyroptotic, or oncotic cells. Binding of phosphatidylserine by the Tim4 binding domain may block the interaction between the phosphatidylserine and another molecule and, for example, interfere with, reduce or eliminate certain functions of the phosphatidylserine (e.g., signal transduction). In some embodiments, the binding of a phosphatidylserine may induce certain biological pathways or identify the phosphatidylserine molecule or a cell expressing phosphatidylserine for elimination.

[0073] A Tim4 binding domain suitable for use in a chimeric Tim4 receptor of the present disclosure may be any polypeptide or peptide derived from a Tim4 molecule that specifically binds phosphatidylserine. In certain embodiments, the Tim4 binding domain is derived from human Tim4. An exemplary human Tim4 molecule is provided in Uniprot. Ref. Q96H15 (SEQ ID NO:1). An exemplary human Tim4 binding domain comprises or consists of an amino acid sequence of SEQ ID NO:2 or amino acids 25-314 of SEQ ID NO:2. An exemplary mouse Tim4 binding domain comprises or consists of an amino acid sequence of SEQ ID NO:60 or amino acids 23-279 of SEQ ID NO: 60. In certain embodiments, the Tim4 binding domain comprises or consists of an amino acid sequence having at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to SEQ ID NO:2 or amino acids 25-314 of SEQ ID NO:2, or SEQ ID NO:60 or amino acids 23-279 of SEQ ID NO: 60. In certain embodiments, the Tim4 binding domain comprises an amino acid sequence having at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acid modifications (e.g., deletions, additions, substitutions) to an amino acid sequence of SEQ ID NO:2 or amino acids 25-314 of SEQ ID NO:2, or SEQ ID NO: 60 or amino acids 23-279 of SEQ ID NO:60.

[0074] In certain embodiments, the extracellular domain optionally comprises an extracellular, non-signaling spacer or linker domain. Where included, such a spacer or linker domain may position the binding domain away from the host cell surface to further enable proper cell/cell contact, binding, and activation. When included in a chimeric receptor as described herein, an extracellular spacer domain is generally located between the extracellular binding domain and the transmembrane domain of the chimeric Tim4 receptor. The length of the extracellular spacer may be varied to

optimize target molecule binding based on the selected target molecule, selected binding epitope, binding domain size and affinity (see, e.g., Guest et al., *J. Immunother.* 28:203-11, 2005; PCT Publication No. WO 2014/031687). In certain embodiments, an extracellular spacer domain is an immunoglobulin hinge region (e.g., IgG1, IgG2, IgG3, IgG4, IgA, IgD). An immunoglobulin hinge region may be a wild type immunoglobulin hinge region or an altered wild type immunoglobulin hinge region. An altered IgG.sub.4 hinge region is described in PCT Publication No. WO 2014/031687, which hinge region is incorporated herein by reference in its entirety. In a particular embodiment, an extracellular spacer domain comprises a modified IgG.sub.4 hinge region having an amino acid sequence of ESKYGPPCPPCP (SEQ ID NO:3). Other examples of hinge regions that may be used in the chimeric Tim4 receptors described herein include the hinge region from the extracellular regions of type 1 membrane proteins, such as CD8a, CD4, CD28 and CD7, which may be wild-type or variants thereof. In further embodiments, an extracellular spacer domain comprises all or a portion of an immunoglobulin Fc domain selected from: a CH1 domain, a CH2 domain, a CH3 domain, or combinations thereof (see, e.g., PCT Publication WO2014/031687, which spacers are incorporated herein by reference in their entirety). In yet further embodiments, an extracellular spacer domain may comprise a stalk region of a type II C-lectin (the extracellular domain located between the C-type lectin domain and the transmembrane domain). Type II C-lectins include CD23, CD69, CD72, CD94, NKG2A, and NKG2D.

[0075] In certain embodiments, an extracellular domain comprises polynucleotide sequences derived from any mammalian species, including humans, primates, cows, horses, goats, sheep, dogs, cats, mice, rats, rabbits, guinea pigs, pigs, transgenic species thereof, or any combination thereof. In certain embodiments, an extracellular domain is murine, human, or chimeric.

Intracellular Signaling Domain

[0076] The intracellular signaling domain of a chimeric Tim4 receptor as described herein is an intracellular effector domain and is capable of transmitting functional signals to a cell in response to binding of the extracellular domain of the chimeric Tim4 receptor and phosphatidylserine. The signals transduced by the intracellular signaling domain promote effector function of the chimeric Tim4 receptor containing cell. Examples of effector function include cytotoxic activity, secretion of cytokines, proliferation, anti-apoptotic signaling, persistence, expansion, engulfment of a target cell or particle expressing phosphatidylserine on its surface, or any combination thereof.

[0077] In certain embodiments, an intracellular signaling domain comprises a costimulatory signaling domain. The costimulatory signaling domain may be any portion of a costimulatory signaling molecule that retains sufficient signaling activity. In some embodiments, a full length or full length intracellular component of a costimulatory signaling molecule is used. In some embodiments, a truncated portion of a costimulatory signaling molecule or intracellular component of a costimulatory signaling molecule is used, provided that the truncated portion retains sufficient signal transduction activity. In further embodiments, a costimulatory signaling domain is a variant of a whole or truncated portion of a costimulatory signaling molecule, provided that the variant retains sufficient signal transduction activity (i.e., is a functional variant).

[0078] In certain embodiments, the costimulatory signaling domain comprises a CD27, CD28, CD40L, GITR, NKG2C, CARD1, CD2, CD7, CD27, CD30, CD40, CD54 (ICAM), CD83, CD134 (OX-40), CD137 (4-1BB), CD150 (SLAMF1), CD152 (CTLA4), CD223 (LAG3), CD226, CD270 (HVEM), PD-1, CD273 (PD-L2), CD274 (PD-L1), B7-H3 (CD276), ICOS (CD278), DAP10, LAT, LFA-1  custom-character, LIGHT, NKG2C, SLP76, TRIM, or ZAP70 signaling domain. In particular embodiments, the costimulatory signaling domain comprises an T] 951HI 71HI7<1 HI7=  custom-characterFR  custom-character1QKF  custom-character  custom-characterI 66f4HI6=  custom-characterTX  custom-characterI7<=.1tw9  custom-characterGG  custom-characterI 68<.xnlsf  custom-characteritr f  custom-character3 Fs j} jr u  custom-characterHI7=ht  custom-character  custom-character  custom-characteritr  custom-character htr  custom-charactertw ht  custom-charactertkfs fr  custom-character xjvzjshj tkXJV

custom-charactertw; 73 Fs j} jr custom-characterT] 95 htcustom-character custom-charactersfcustom-characterl itr custom-character htr custom-characterjx twht custom-character tkfs fr custom-character xjvzjshj tkXJV custom-character ST?: 3 Fs j} jr custom-characterHI 7 htcustom-charactercustom-charactercustom-character itr custom-character htr uwxjx twhtcustom-character tkfs fr custom-character custom-character xjvzjshj tkXJV custom-character ST?; 3 Fs j} jr custom-character9 custom-characterGG custom-charactercustom-charactercustom-character itr custom-character comprises twht custom-character tkan amino acid sequence of SEQ ID NO:7. An exemplary CD27 costimulatory signaling domain comprises twhtcustom-character tkan amino acid sequence of SEQ ID NO:8. An exemplary ICAM-1 costimulatory signaling domain comprises twhtcustom-character tkan amino acid sequence of SEQ ID NO:9. An exemplary LFA-1 costimulatory signaling domain comprises twhtcustom-character tkan amino acid sequence of SEQ ID NO: 10. An exemplary ICOS costimulatory signaling domain comprises twht custom-character tkan amino acid sequence of SEQ ID NO:11. An exemplary CD30 costimulatory signaling domain comprises twhtcustom-character custom-character tkan amino acid sequence of SEQ ID NO:12. An exemplary CD40 costimulatory signaling domain comprises twhtcustom-character tkan amino acid sequence of SEQ ID NO:13. An exemplary PD-1 costimulatory signaling domain comprises twhtcustom-character tkan amino acid sequence of SEQ ID NO:14. An exemplary CD7 costimulatory signaling domain comprises twht custom-character tkan amino acid sequence of SEQ ID NO:15. An exemplary LIGHT costimulatory signaling domain comprises tw htcustom-character tkan amino acid sequence of SEQ ID NO: 16. An exemplary NKG2C costimulatory signaling domain comprises twht custom-character tkan amino acid sequence of SEQ ID NO:17. An exemplary B7-H3 costimulatory signaling domain comprises tw htcustom-character tkan amino acid sequence of SEQ ID NO:18. In certain embodiments, the costimulatory signaling domain comprises twht custom-character tkan amino acid sequence having at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to any one of SEQ ID NOS: 4-18 and 62. In certain embodiments, the costimulatory signaling domain comprises an amino acid sequence having at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acid modifications (e.g., deletions, additions, substitutions) to an amino acid sequence of any one of SEQ ID NOS: 4-18 and 62. In certain embodiments, the intracellular signaling comprises a second costimulatory signaling domain. In preferred embodiments, the first costimulatory signaling domain and second costimulatory signaling domain are different.

[0079] In certain embodiments, the intracellular signaling domain further comprises an ITAM-containing activating domain. The ITAM-containing activating domain may recapitulate TCR signaling independently of endogenous TCR complexes. In certain embodiments, signaling via the ITAM-containing activating domain leads to mediation of a T cell response, including, but not limited to, proliferation, activation, differentiation, and the like. The ITAM-containing activating domain may be any portion of an ITAM-containing activating domain molecule that retains sufficient signaling activity. In some embodiments, a full length or full length intracellular component of an ITAM-containing activating domain molecule is used. In some embodiments, a truncated portion of an ITAM-containing activating domain molecule or intracellular component of an ITAM-containing activating domain molecule is used, provided that the truncated portion retains sufficient signal transduction activity. In further embodiments, an ITAM-containing activating domain is a variant of a whole or truncated portion of an ITAM-containing activating domain molecule, provided that the variant retains sufficient signal transduction activity (i.e., is a functional variant).

[0080] Examples of ITAM-containing activating domains that may be used in the chimeric Tim4 receptors of the present disclosure include those derived from CD3ζ, CD3γ, CD38, CD38, CD5, CD22, CD79a, CD278 (ICOS), DAP10, and CD66d. In specific embodiments, the ITAM-

containing activating domain is a CD3ζ signaling domain. An exemplary CD3ζ signaling domain comprises twht custom-character tkan amino acid sequence of SEQ ID NO:63 or 19. An exemplary CD3γ signaling domain comprises twht custom-character tkan amino acid sequence of SEQ ID NO:20. An exemplary CD3δ signaling domain comprises twht custom-character tkan amino acid sequence of SEQ ID NO: 21. An exemplary CD3ε signaling domain comprises twht custom-character tkan amino acid sequence of SEQ ID NO:22. An exemplary CD5 signaling domain comprises tw ht custom-character tkan amino acid sequence of SEQ ID NO:23. An exemplary CD22 signaling domain comprises twht custom-character tkan amino acid sequence of SEQ ID NO:24. An exemplary CD79a signaling domain comprises twht custom-character tkan amino acid sequence of SEQ ID NO:25. An exemplary DAP10 signaling domain comprises twht custom-character tk an amino acid sequence of SEQ ID NO:26. An exemplary CD66d signaling domain comprises twht custom-character tkan amino acid sequence of SEQ ID NO:27. In certain embodiments, the ITAM-containing activating domain comprises twht custom-character tkan amino acid sequence having at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to any one of SEQ ID NOS: 63 and 19-27. In certain embodiments, the CD3ζ signaling domain comprises an amino acid sequence having at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acid modifications (e.g., deletions, additions, substitutions) of an amino acid sequence to any one of SEQ ID NOS: 19-27 and 63.

[0081] In another embodiment, an intracellular signaling domain comprises a CD28 costimulatory signaling domain and a CD3ζ signaling domain. In another embodiment, an intracellular signaling domain comprises a 4-1BB costimulatory signaling domain and a CD3ζ signaling domain. In yet another embodiment, an intracellular signaling domain comprises a CD27 costimulatory signaling domain and a CD3ζ signaling domain. In another embodiment, an intracellular signaling domain comprises a ICOS costimulatory signaling domain and a CD3ζ signaling domain. In another embodiment, an intracellular signaling domain comprises a LFA-1 costimulatory signaling domain and a CD3ζ signaling domain. In another embodiment, an intracellular signaling domain comprises an OX40 costimulatory signaling domain and a CD3ζ signaling domain. In yet another embodiment, an intracellular signaling domain comprises a CD2 costimulatory signaling domain and a CD3ζ signaling domain. In still another embodiment, an intracellular signaling domain comprises an ICAM-1 costimulatory signaling domain and a CD3ζ signaling domain.

[0082] Intracellular signaling domains may be derived from a mammalian species, including humans, primates, cows, horses, goats, sheep, dogs, cats, mice, rats, rabbits, guinea pigs, pigs, and transgenic species thereof.

Transmembrane Domain

[0083] The transmembrane domain of a chimeric Tim4 receptor connects and is positioned between the extracellular domain and the intracellular signaling domain. The transmembrane domain is a hydrophobic alpha helix that transverses the host cell membrane. The transmembrane domain may be directly fused to the binding domain or to the extracellular spacer domain if present. In certain embodiments, the transmembrane domain is derived from an integral membrane protein (e.g., receptor, cluster of differentiation (CD) molecule, enzyme, transporter, cell adhesion molecule, or the like). In one embodiment, the transmembrane domain is selected from the same molecule as the molecule from which the extracellular domain is derived. In another embodiment, the transmembrane domain is selected from the same molecule as the molecule from which the intracellular signaling domain is derived. For example, a chimeric Tim4 receptor may comprise a Tim4 binding domain and a Tim4 transmembrane domain. In another example, a chimeric Tim4 receptor may comprise a CD28 transmembrane domain and a CD28 costimulatory signaling domain. In certain embodiments, the transmembrane domain and the extracellular domain are derived from different molecules; the transmembrane domain and the intracellular signaling domain are derived from different molecules; or the transmembrane domain, extracellular domain,

and intracellular signaling domain are all derived from different molecules. Examples of transmembrane domains that may be used in chimeric Tim4 receptors of the present disclosure include transmembrane domains from Tim4, CD3ζ, CD3γ, CD3δ, CD3ε, CD28, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, CD154, CD27, CD28, 4-1BB, OX40, CD30, CD40, PD-1, ICOS, LFA-1, CD2, CD7, LIGHT, NKG2C, and B7-H3. An exemplary Tim4 transmembrane domain comprises or consists of an amino acid sequence of SEQ ID NO: 28 or 59. An exemplary CD28 transmembrane domain comprises or consists of an amino acid sequence of SEQ ID NO:29. An exemplary 4-1BB transmembrane domain comprises or consists of an amino acid sequence of SEQ ID NO:30. An exemplary OX40 transmembrane domain comprises or consists of an amino acid sequence of SEQ ID NO:31. An exemplary CD27 transmembrane domain comprises or consists of an amino acid sequence of SEQ ID NO:32. An exemplary ICOS transmembrane domain comprises or consists of an amino acid sequence of SEQ ID NO: 33. An exemplary CD2 transmembrane domain comprises or consists of an amino acid sequence of SEQ ID NO:34. An exemplary LFA-1 transmembrane domain comprises or consists of an amino acid sequence of SEQ ID NO:35. An exemplary CD30 transmembrane domain comprises or consists of an amino acid sequence of SEQ ID NO: 36. An exemplary CD40 transmembrane domain comprises or consists of an amino acid sequence of SEQ ID NO:37. An exemplary PD-1 transmembrane domain comprises or consists of an amino acid sequence of SEQ ID NO:38. An exemplary CD7 transmembrane domain comprises or consists of an amino acid sequence of SEQ ID NO: 39. An exemplary LIGHT transmembrane domain comprises or consists of an amino acid sequence of SEQ ID NO:40. An exemplary NKG2C transmembrane domain comprises or consists of an amino acid sequence of SEQ ID NO:41. An exemplary B7-H3 transmembrane domain comprises or consists of an amino acid sequence of SEQ ID NO: 42. In certain embodiments, the transmembrane domain comprises or consists of an amino acid sequence having at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% identity to any one of SEQ ID NOS: 28-42, and 59. In certain embodiments, the transmembrane domain comprises an amino acid sequence having at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acid modifications (e.g., deletion, additions, substitutions) to an amino acid sequence of any one of SEQ ID NOS: 28-42, and 59.

[0084] Transmembrane domains may derived from any mammalian species, including humans, primates, cows, horses, goats, sheep, dogs, cats, mice, rats, rabbits, guinea pigs, pigs, and transgenic species thereof.

[0085] In certain embodiments, a chimeric Tim4 receptor comprises polynucleotide sequences derived from any mammalian species, including humans, primates, cows, horses, goats, sheep, dogs, cats, mice, rats, rabbits, guinea pigs, pigs, transgenic species thereof, or any combination thereof. In certain embodiments, a chimeric Tim4 receptor is murine, chimeric, human, or humanized.

[0086] It is understood that direct fusion of one domain to another domain of a chimeric Tim4 receptor described herein does not preclude the presence of intervening junction amino acids. Junction amino acids may be natural or non-natural (e.g., resulting from the construct design of a chimeric protein). For example, junction amino acids may result from restriction enzyme sites used for joining one domain to another domain or cloning polynucleotides encoding chimeric Tim4 receptors into vectors.

Exemplary Chimeric Tim4 Receptors

[0087] The component parts of a chimeric Tim4 receptor as disclosed herein can be selected and arranged in various combinations to provide a desired specificity and effector phenotype to a host cell.

[0088] An exemplary chimeric Tim4 receptor of the present disclosure comprises an extracellular domain comprising a Tim4 binding domain; an intracellular signaling domain comprising a CD28 costimulatory signaling domain; and a CD28 transmembrane domain positioned between and

connecting the extracellular domain and the intracellular signaling domain. In certain embodiments, the chimeric Tim4 receptor comprises twht  tkan amino acid sequence of SEQ ID NO:43. In some embodiments, the chimeric Tim4 receptor comprises twht  tkan amino acid sequence of amino acids 23-347 of SEQ ID NO:43.

[0089] Another exemplary chimeric Tim4 receptor of the present disclosure comprises an extracellular domain comprising a Tim4 binding domain; an intracellular signaling domain comprising a CD28 costimulatory signaling domain; and a Tim4 transmembrane domain positioned between and connecting the extracellular domain and the intracellular signaling domain. In certain embodiments, the chimeric Tim4 receptor comprises twht  tkan amino acid sequence of SEQ ID NO:44. In some embodiments, the chimeric Tim4 receptor comprises twht  tkan amino acid sequence of amino acids 23-341 of SEQ ID NO:44.

[0090] Another exemplary chimeric Tim4 receptor of the present disclosure comprises an extracellular domain comprising a Tim4 binding domain; an intracellular signaling domain comprising a 4-1BB costimulatory signaling domain; and a Tim4 transmembrane domain positioned between and connecting the extracellular domain and the intracellular signaling domain.

[0091] Another exemplary chimeric Tim4 receptor of the present disclosure comprises an extracellular domain comprising a Tim4 binding domain; an intracellular signaling domain comprising a 4-1BB costimulatory signaling domain; and a 4-1BB transmembrane domain positioned between and connecting the extracellular domain and the intracellular signaling domain.

[0092] Another exemplary chimeric Tim4 receptor of the present disclosure comprises an extracellular domain comprising a Tim4 binding domain; an intracellular signaling domain comprising a CD28 costimulatory signaling domain and a CD3ζ ITAM-containing activating domain; and a Tim4 transmembrane domain positioned between and connecting the extracellular domain and the intracellular signaling domain.

[0093] Yet another exemplary chimeric Tim4 receptor of the present disclosure comprises an extracellular domain comprising a Tim4 binding domain; an intracellular signaling domain comprising a 4-1BB costimulatory signaling domain and a CD3ζ ITAM-containing activating domain; and a Tim4 transmembrane domain positioned between and connecting the extracellular domain and the intracellular signaling domain. In certain embodiments, the chimeric Tim4 receptor comprises twht  tkan amino acid sequence of SEQ ID NO:45. In some embodiments, the chimeric Tim4 receptor comprises twht  tkan amino acid sequence of amino acids 23-454 of SEQ ID NO:45.

Polynucleotides. Vectors, and Host Cells

[0094] In certain aspects, the present disclosure provides nucleic acid molecules that encode any one or more of the chimeric Tim4 receptors described herein. A nucleic acid may refer to a single- or double-stranded DNA, cDNA, or RNA, and may include a positive and a negative strand of the nucleic acid which complement one another, including antisense DNA, cDNA, and RNA. A nucleic acid may be naturally occurring or synthetic forms of DNA or RNA. The nucleic acid sequences encoding a desired chimeric Tim4 receptor can be obtained or produced using recombinant methods known in the art using standard techniques, such as by screening libraries from cells expressing the desired sequence or a portion thereof, by deriving the sequence from a vector known to include the same, or by isolating the sequence or a portion thereof directly from cells or tissues containing the same as described in, for example, Sambrook et al. (1989 and 2001 editions; *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, NY) and Ausubel et al. (Current Protocols in Molecular Biology, 2003). Alternatively, the sequence of interest can be produced synthetically, rather than being cloned.

[0095] Polynucleotides encoding the chimeric Tim4 receptor compositions provided herein may be derived from any animal, such as humans, primates, cows, horses, sheep, dogs, cats, mice, rats, rabbits, guinea pigs, pigs, or a combination thereof. In certain embodiments, a polynucleotide encoding the chimeric Tim4 receptor is from the same animal species as the host cell into which the

polynucleotide is inserted.
[0096] The polynucleotides encoding chimeric Tim4 receptors of the present disclosure may be operatively linked to expression control sequences. Expression control sequences may include appropriate transcription initiation, termination, promoter and enhancer sequences; efficient RNA processing signals such as splicing and polyadenylation signals; sequences that stabilize cytoplasmic mRNA; sequences that enhance translation efficiency (i.e., Kozak consensus sequences); sequences that enhance protein stability; and possibly sequences that enhance protein secretion.
[0097] In certain embodiments, a polynucleotide encoding a chimeric Tim4 receptor comprises a sequence encoding a signal peptide (also referred to as leader peptide or signal sequence) at the 5′-end for targeting of the precursor protein to the secretory pathway. The signal peptide is optionally cleaved from the N-terminus of the extracellular domain during cellular processing and localization of the chimeric Tim4 receptor to the host cell membrane. A polypeptide from which a signal peptide sequence has been cleaved or removed may also be called a mature polypeptide. Examples of signal peptides that may be used in the chimeric Tim4 receptors of the present disclosure include signal peptides derived from endogenous secreted proteins, including, e.g., GM-CSF (amino acid sequence of SEQ ID NO:46) or Tim4 (amino acid sequence of SEQ ID NO:47 or 61). In certain embodiments, a polynucleotide sequence encodes a mature chimeric Tim4 receptor polypeptide, or a polypeptide sequence comprises a mature chimeric Tim4 receptor polypeptide. It is understood by persons of skill in the art that for sequences disclosed herein that include a signal peptide sequence, the signal peptide sequence may be replaced with another signal peptide that is capable of trafficking the encoded protein to the extracellular membrane.
[0098] In certain embodiments, a chimeric Tim4 receptor encoding polynucleotide of the present disclosure is codon optimized for efficient expression in a target host cell comprising the polynucleotide (see, e.g, Scholten et al., *Clin. Immunol.* 119:135-145 (2006)). As used herein, a "codon optimized" polynucleotide comprises a heterologous polynucleotide having codons modified with silent mutations corresponding to the abundances of tRNA in a host cell of interest.
[0099] A single polynucleotide molecule may encode one, two, or more chimeric Tim4 receptors according to any of the embodiments disclosed herein. A polynucleotide encoding more than one gene may comprise a sequence (e.g., IRES, viral 2A peptide) disposed between each gene for multicistronic expression.
[0100] A polynucleotide encoding a desired chimeric Tim4 receptor can be inserted into an appropriate vector, e.g., a viral vector, non-viral plasmid vector, and non-viral vectors, such as lipid-based DNA vectors, modified mRNA (modRNA), self-amplifying mRNA, CELiD, and transposon-mediated gene transfer (PiggyBac, Sleeping Beauty), for introduction into a host cell of interest (e.g., an immune cell). Polynucleotides encoding a chimeric Tim4 receptor of the present disclosure can be cloned into any suitable vector, such as an expression vector, a replication vector, a probe generation vector, or a sequencing vector. In certain embodiments, a polynucleotide encoding the extracellular domain, a polynucleotide encoding the transmembrane domain, and a polynucleotide encoding the intracellular signaling domain are joined together into a single polynucleotide and then inserted into a vector. In other embodiments, a polynucleotide encoding the extracellular domain, a polynucleotide encoding the transmembrane domain, and a polynucleotide encoding the intracellular signaling domain may be inserted separately into a vector such that the expressed amino acid sequence produces a functional chimeric Tim4 receptor. A vector that encodes a chimeric Tim4 receptor is referred to herein as a "chimeric Tim4 receptor vector."
[0101] In certain embodiments, a vector comprises a polynucleotide encoding one chimeric Tim4 receptor. In certain embodiments, a vector comprises one polynucleotide encoding two or more chimeric Tim4 receptors. In certain embodiments, a single polynucleotide encoding two or more chimeric Tim4 receptors is cloned into a cloning site and expressed from a single promoter, with

each chimeric Tim4 receptor sequence separated from each other by an internal ribosomal entry site (IRES), furin cleavage site, or viral 2A peptide to allow for co-expression of multiple genes from a single open reading frame (e.g., a multicistronic vector). In certain embodiments, a viral 2A peptide is a porcine teschovirus-1 (P2A), Thosea asigna virus (T2A), equine rhinitis A virus (E2A), foot-and-mouth disease virus (F2A), or variant thereof. An exemplary T2A peptide comprises an amino acid sequence of SEQ ID NO: 48, 64, 65, or 66. An exemplary P2A peptide comprises an amino acid sequence of SEQ ID NO:49 or 67. An exemplary E2A peptide sequence comprises an amino acid sequence of SEQ ID NO:50. An exemplary F2A peptide sequence comprises an amino acid sequence of SEQ ID NO:51.

[0102] In certain embodiments, a vector comprises two or more polynucleotides, each polynucleotide encoding a chimeric Tim4 receptor. The two or more polynucleotides encoding chimeric Tim4 receptors may be cloned sequentially into a vector at different cloning sites, with each chimeric Tim4 receptor expressed under the regulation of different promoters. In certain embodiments, vectors that allow long-term integration of a transgene and propagation to daughter cells are utilized. Examples include viral vectors such as, adenovirus, adeno-associated virus, vaccinia virus, herpes viruses, cytomegalovirus, pox virus, or retroviral vectors, such as lentiviral vectors. Vectors derived from lentivirus can be used to achieve long-term gene transfer and have added advantages over vectors including the ability to transduce non-proliferating cells, such as hepatocytes, and low immunogenicity.

[0103] A vector that encodes a core virus is referred to herein as a “viral vector.” There are a large number of available viral vectors suitable for use with the compositions of the instant disclosure, including those identified for human gene therapy applications (see Pfeifer and Verme, *Ann. Rev. Genomics Hum. Genet.* 2:177, 2001). Suitable viral vectors include vectors based on RNA viruses, such as retrovirus-derived vectors, e.g., Maloney murine leukemia virus (MLV)-derived vectors, and include more complex retrovirus-derived vectors, e.g., lentivirus-derived vectors. HIV-1-derived vectors belong to this category. Other examples include lentivirus vectors derived from HIV-2, FIV, equine infectious anemia virus, SIV, and Maedi-Visna virus (ovine lentivirus). Methods of using retroviral and lentiviral viral vectors and packaging cells for transducing mammalian host cells with viral particles containing chimeric receptor transgenes are known in the art and have been previous described, for example, in U.S. Pat. No. 8,119,772; Walchli et al., *PLoS One* 6: 327930, 2011; Zhao et al., *J. Immunol.* 174: 4415, 2005; Engels et al., *Hum. Gene Ther.* 14: 1155, 2003; Frecha et al., *Mol. Ther.* 18: 1748, 2010; Verhoeyen et al., *Methods Mol. Biol.* 506: 97, 2009. Retroviral and lentiviral vector constructs and expression systems are also commercially available.

[0104] In certain embodiments, a viral vector is used to introduce a non-endogenous polynucleotide encoding a chimeric Tim4 receptor to a host cell. A viral vector may be a retroviral vector or a lentiviral vector. A viral vector may also include a nucleic acid sequence encoding a marker for transduction. Transduction markers for viral vectors are known in the art and include selection markers, which may confer drug resistance, or detectable markers, such as fluorescent markers or cell surface proteins that can be detected by methods such as flow cytometry. In particular embodiments, a viral vector further comprises a gene marker for transduction comprising a fluorescent protein (e.g., green, yellow), an extracellular domain of human CD2, or a truncated human EGFR (EGFRt or tEGFR; see Wang et al., *Blood* 118:1255, 2011). An exemplary tEGFR comprises an amino acid sequence of SEQ ID NO:52. When a viral vector genome comprises a plurality of genes to be expressed in a host cell as separate proteins from a single transcript, the viral vector may also comprise additional sequences between the two (or more) genes allowing for multicistronic expression. Examples of such sequences used in viral vectors include internal ribosome entry sites (IRES), furin cleavage sites, viral 2A peptides (e.g., T2A, P2A, E2A, F2A), or any combination thereof.

[0105] Other viral vectors also can be used for polynucleotide delivery including DNA viral

vectors, including, for example adenovirus-based vectors and adeno-associated virus (AAV)-based vectors; vectors derived from herpes simplex viruses (HSVs), including amplicon vectors, replication-defective HSV and attenuated HSV (Krisky et al., *Gene Ther.* 5:1517, 1998).
[0106] Other viral vectors recently developed for gene therapy uses can also be used with the compositions and methods of this disclosure. Such vectors include those derived from baculoviruses and α-viruses. (Jolly, D J. 1999. Emerging Viral Vectors. pp 209-40 in Friedmann T. ed. The Development of Human Gene Therapy. New York: Cold Spring Harbor Lab), or plasmid vectors (such as sleeping beauty or other transposon vectors).
[0107] In certain embodiments, a chimeric Tim4 receptor vector can be constructed to optimize spatial and temporal control. For example, a chimeric Tim4 receptor vector can include promoter elements to optimize spatial and temporal control. In some embodiments, a chimeric Tim4 receptor vector includes tissue specific promoters or enhancers that enable specific induction of a chimeric Tim4 receptor to an organ, a cell type (e.g., immune cell), or a pathologic microenvironment, such as a tumor or infected tissue. An "enhancer" is an additional promoter element that can function either cooperatively or independently to activate transcription. In certain embodiments, a chimeric Tim4 receptor vector includes a constitutive promoter. In certain embodiments, a chimeric Tim4 receptor vector includes an inducible promoter. In certain embodiments, a chimeric Tim4 receptor vector includes a tissue specific promoter.
[0108] In certain embodiments, a chimeric Tim4 receptor vector can include a gene encoding a homing receptor, such as CCR4 or CXCR4, to improve homing and antitumor activity in vivo.
[0109] Where temporal control is desired, a chimeric Tim4 receptor vector may include an element that allows for inducible depletion of transduced cells. For example, such a vector may include an inducible suicide gene. A suicide gene may be an apoptotic gene or a gene that confers sensitivity to an agent (e.g., a drug). Exemplary suicide genes include chemically inducible caspase 9 (iCASP9) (U.S. Patent Publication No. 2013/0071414), chemically inducible Fas, or Herpes simplex virus thymidine kinase (HSV-TK), which confers sensitivity to ganciclovir. In further embodiments, a chimeric Tim4 receptor vector can be designed to express a known cell surface antigen that, upon infusion of an associated antibody, enables depletion of transduced cells. Examples of cell surface antigens and their associated antibodies that may be used for depletion of transduced cells include CD20 and Rituximab, RQR8 (combined CD34 and CD20 epitopes, allowing CD34 selection and anti-CD20 deletion) and Rituximab, and EGFR and Cetuximab.
[0110] Inducible vector systems, such as the tetracycline (Tet)-On vector system which activates transgene expression with doxycycline (Heinz et al., Hum. Gene Ther. 2011, 22:166-76) may also be used for inducible chimeric Tim4 receptor expression. Inducible chimeric Tim4 receptor expression may be also accomplished via retention using a selective hook (RUSH) system based on streptavidin anchored to the membrane of the endoplasmic reticulum through a hook and a streptavidin binding protein introduced into the chimeric Tim4 receptor structure, where addition of biotin to the system leads to the release of the chimeric Tim4 receptor from the endoplasmic reticulum (Agaugue et al., 2015, Mol. Ther. 23 (Suppl. 1): S88).
[0111] In certain embodiments, a chimeric Tim4 receptor modified host cell may also be modified to co-express one or more small GTPases. Rho GTPases, a family of small (˜21 k Da) signaling G proteins and also a subfamily of the Ras superfamily, regulate actin cytoskeleton organization in various cell types and promote pseudopod extension and phagosome closure during phagocytosis (see, e.g., Castellano et al., 2000, J. Cell Sci. 113:2955-2961). Engulfment requires F-actin recruitment beneath tethered cells or particles, and F-actin rearrangement to allow membrane extension resulting in cell or particle internalization. RhoGTPases include RhoA, Rac1, Rac2, RhoG, and CDC42. Other small GTPases, such as Rap1, is involved in regulation of complement mediated phagocytosis. Co-expression of a small GTPase with the chimeric Tim4 receptor may promote target cell or particle internalization and/or phagosome formation by the host cell. In some embodiments, a recombinant nucleic acid molecule encoding a GTPase is encoded on a separate

vector than the chimeric Tim4 receptor-containing vector. In other embodiments, a recombinant nucleic acid molecule encoding a GTPase is encoded on the same vector as the chimeric Tim4 receptor. The GTPase and chimeric Tim4 receptor may be expressed under the regulation of different promoters on the same vector (e.g., at different multiple cloning sites). Alternatively, the chimeric Tim4 receptor and GTPase may be expressed under the regulation of one promoter in a multicistronic vector. The polynucleotide sequence encoding the chimeric Tim4 receptor and the polynucleotide sequence encoding the small GTPase(s) may be separated from each other by an IRES or viral 2A peptide in a multicistronic vector. Exemplary 2A peptides include T2A (SEQ ID NO:48), P2A (SEQ ID NO:49), E2A (SEQ ID NO:50), F2A (SEQ ID NO:51). Examples of GTPases that may be co-expressed with a chimeric Tim4 receptor include Rac1, Rac2, Rab5 (also referred to as Rab5a), Rab7, Rap1, RhoA, RhoG, CDC42, or any combination thereof. In specific embodiments, the GTPase comprises or is a sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to a Rac1 amino acid sequence of SEQ ID NO:53, a Rab5 amino acid sequence of SEQ ID NO:54, a Rab7 amino acid sequence of SEQ ID NO:55, a Rap1 amino acid sequence of SEQ ID NO:56, a RhoA amino acid sequence of SEQ ID NO:57, a CDC42 amino acid sequence of SEQ ID NO: 58, or any combination thereof.

[0112] In certain embodiments, a chimeric Tim4 receptor modified host cell may also be modified to co-express a chimeric antigen receptor (CAR). Chimeric antigen receptors are recombinant receptors generally composed of an scFv binding domain derived from an antibody, a transmembrane domain, and an intracellular signaling domain(s) usually derived from a TCR. In certain embodiments, a CAR is a first generation CAR, a second generation CAR, or a third generation CAR. A first generation CAR generally has an intracellular signaling domain comprising an intracellular signaling domain of CD3ζ or FcγRI or other ITAM-containing activating domain to provide a T cell activation signal. Second generation CARs further comprise a costimulatory signaling domain (e.g., a costimulatory signaling domain from an endogenous T cell costimulatory receptor, such as CD28, 4-1BB, or ICOS). Third generation CARs comprise an ITAM-containing activating domain, a first costimulatory signaling domain and a second costimulatory signaling domain.

[0113] In certain embodiments, a chimeric Tim4 receptor modified host cell may also be modified to co-express a recombinant TCR. In one embodiment, a recombinant TCR is an enhanced affinity TCR.

[0114] In certain embodiments, a chimeric Tim4 receptor modified host cell may also be modified to co-express a single chain TCR (scTCR) fusion protein. A scTCR fusion protein comprises a binding domain comprising a scTCR (a TCR Vα domain linked to a TCR VB domain), an optional extracellular spacer, a transmembrane domain, and an intracellular component comprising a single intracellular signaling domain providing an T cell activation signal (e.g., a CD3ζ ITAM-containing activating domain) and optionally a costimulatory signaling domain (see, Aggen et al., 2012, Gene Ther. 19:365-374; Stone et al., Cancer Immunol. Immunother. 2014, 63:1163-76).

[0115] In certain embodiments, a chimeric Tim4 receptor modified host cell may also be modified to co-express a T cell receptor-based chimeric antigen receptor (TCR-CAR). A TCR-CAR is a heterodimeric fusion protein generally comprising a soluble TCR (a polypeptide chain comprising a Vα domain and Ca domain and a polypeptide chain comprising a Vβ domain and a Cβ domain) wherein the VβCβ polypeptide chain is linked to a transmembrane domain and an intracellular signaling component (e.g., an ITAM-containing activating domain and optionally a costimulatory signaling domain) (see, e.g., Walseng et al., 2017 Scientific Reports 7: 10713).

[0116] In certain embodiments, a recombinant nucleic acid molecule encoding a cellular immunotherapy composition, e.g., CAR, TCR, scTCR fusion protein, or TCR-CAR, is encoded on a separate vector than the chimeric Tim4 receptor-containing vector within a host cell. In other embodiments, a recombinant nucleic acid molecule encoding a CAR, TCR, scTCR fusion protein,

or TCR-CAR is encoded on the same vector as the chimeric Tim4 receptor within a host cell. The CAR, TCR, scTCR fusion protein, or TCR-CAR, and the chimeric Tim4 receptor may be expressed under the regulation of different promoters on the same vector (e.g., at different multiple cloning sites). Alternatively, the chimeric Tim4 receptor and CAR, TCR, scTCR fusion protein, or TCR-CAR may be expressed under the regulation of one promoter in a multicistronic vector. The polynucleotide sequence encoding the chimeric Tim4 receptor and the polynucleotide sequence encoding the CAR, TCR, scTCR fusion protein, or TCR-CAR may be separated by an IRES or viral 2A peptide in a multicistronic vector.

[0117] In certain embodiments, a cell, such as an immune cell, obtained from a subject may be genetically modified into a non-natural or recombinant cell (e.g., a non-natural or recombinant immune cell) by introducing a polynucleotide that encodes a chimeric Tim4 receptor as described herein, whereby the cell expresses a cell surface localized chimeric Tim4 receptor. In certain embodiments, a host cell is an immune cell, such as a myeloid progenitor cell or a lymphoid progenitor cell. Exemplary immune cells that may be modified to comprise a polynucleotide encoding a chimeric Tim4 receptor or a vector comprising a polynucleotide encoding a chimeric Tim4 receptor include a T cell, a natural killer cell, a B cell, a lymphoid precursor cell, an antigen presenting cell, a dendritic cell, a Langerhans cell, a myeloid precursor cell, a mature myeloid cell, a monocyte, or a macrophage.

[0118] In certain embodiments, a B cell is genetically modified to express one or more chimeric Tim4 receptors. B cells possess certain properties that may be advantageous as host cells, including: trafficking to sites of inflammation, capable of internalizing and presenting antigen, capable of costimulating T cells, highly proliferative, and self-renewing (persist for life). In certain embodiments, a chimeric Tim4 receptor modified B cell is capable of digesting an engulfed target cell or engulfed target particle into smaller peptides and presenting them to T cells via an MHC molecule. Antigen presentation by a chimeric Tim4 receptor modified B cell may contribute to antigen spreading of the immune response to non-targeted antigens. B cells include progenitor or precursor cells committed to the B cell lineage (e.g., pre-pro-B cells, pro-B cells, and pre-B cells); immature and inactivated B cells; or mature and functional or activated B cells. In certain embodiments, B cells may be naïve B cells, plasma cells, regulatory B cells, marginal zone B cells, follicular B cells, lymphoplasmacytoid cell, plasmablast cell, memory B cells, or any combination thereof. Memory B cells may be distinguished from naïve B cells by expression of CD27, which is absent on naïve B cells. In certain embodiments, the B cells can be primary cells or cell lines derived from human, mouse, rat, or other mammals. B cell lines are well known in the art. If obtained from a mammal, a B cell can be obtained from numerous sources, including blood, bone marrow, spleen, lymph node, or other tissues or fluids. A B cell composition may be enriched or purified.

[0119] In certain embodiments, a T cell is genetically modified to express one or more chimeric Tim4 receptors. Exemplary T cells include CD4.sup.+ helper, CD8.sup.+ effector (cytotoxic), naïve (CD45 RA+, CCR7+, CD62L+, CD27+, CD45RO−), central memory (CD45RO.sup.+, CD62L.sup.+, CD8.sup.+), effector memory (CD45RA+, CD45RO−, CCR7−, CD62L−, CD27−), T memory stem, regulatory, mucosal-associated invariant (MAIT), γδ (gd), tissue resident T cells, natural killer T cells, or any combination thereof. In certain embodiments, the T cells can be primary cells or cell lines derived from human, mouse, rat, or other mammals. If obtained from a mammal, a T cell can be obtained from numerous sources, including blood, bone marrow, lymph node, thymus, or other tissues or fluids. A T cell composition may be enriched or purified. T cell lines are well known in the art, some of which are described in Sandberg et al., *Leukemia* 21:230, 2000. In certain embodiments, the T cells lack endogenous expression of a TCRα gene, TCRβ gene, or both. Such T cells may naturally lack endogenous expression of TCRα and β chains, or may have been modified to block expression (e.g., T cells from a transgenic mouse that does not express TCR α and β chains or cells that have been manipulated to inhibit expression of TCR α and

β chains) or to knockout a TCRα chain, a TCRβ chain, or both genes.
[0120] In certain embodiments, host cells expressing a chimeric Tim4 protein of this disclosure on the cell surface are not T cells or cells of a T cell lineage, but cells that are progenitor cells, stem cells or cells that have been modified to express cell surface anti-CD3.
[0121] In certain embodiments, gene editing methods are used to modify the host cell genome to comprise a polynucleotide encoding a chimeric Tim4 receptor of the present disclosure. Gene editing, or genome editing, is a method of genetic engineering wherein DNA is inserted, replaced, or removed from a host cell's genome using genetically engineered endonucleases. The nucleases create specific double-stranded breaks at targeted loci in the genome. The host cell's endogenous DNA repair pathways then repair the induced break(s), e.g., by non-homologous ending joining (NHEJ) and homologous recombination. Exemplary endonucleases useful for gene editing include a zinc finger nuclease (ZFN), a transcription activator-like effector (TALE) nuclease, a clustered regularly interspaced short palindromic repeats (CRISPR)/Cas nuclease system (e.g., CRISPR-Cas9), a meganuclease, or combinations thereof. Methods of disrupting or knocking out genes or gene expression in immune cells including B cells and T cells, using gene editing endonucleases are known in the art and described, for example, in PCT Publication Nos. WO 2015/066262; WO 2013/074916; WO 2014/059173; Cheong et al., Nat. Comm. 2016 7: 10934; Chu et al., Proc. Natl. Acad. Sci. USA 2016 113: 12514-12519; methods from each of which are incorporated herein by reference in their entirety.
[0122] In certain embodiments, expression of an endogenous gene of the host cell is inhibited, knocked down, or knocked out. Examples of endogenous genes that may be inhibited, knocked down, or knocked out in a B cell include IGH, IGκ, IGλ, or any combination thereof. Examples of endogenous genes that may be inhibited, knocked down, or knocked out in a T cell include a TCR gene (TRA or TRB), an HLA gene (HLA class I gene or HLA class II gene), an immune checkpoint molecule (PD-L1, PD-L2, CD80, CD86, B7-H3, B7-H4, HVEM, adenosine, GAL9, VISTA, CEACAM-1, CEACAM-3, CEACAM-5, PVRL2, PD-1, CTLA-4, BTLA, KIR, LAG3, TIM3, A2aR, CD244/2B4, CD160, TIGIT, LAIR-1, or PVRIG/CD112R), or any combination thereof. Expression of an endogenous gene may be inhibited, knocked down, or knocked out at the gene level, transcriptional level, translational level, or a combination thereof. Methods of inhibiting, knocking down, or knocking out an endogenous gene may be accomplished, for example, by an RNA interference agent (e.g., siRNA, shRNA, miRNA, etc.) or an engineered endonuclease (e.g., CRISPR/Cas nuclease system, a zinc finger nuclease (ZFN), a Transcription Activator Like Effector nuclease (TALEN), a meganuclease), or any combination thereof. In certain embodiments, an endogenous B cell gene (e.g., IGH, IGK, or IGA) is knocked out by insertion of a polynucleotide encoding a chimeric Tim4 receptor of the present disclosure into the locus of the endogenous B cell gene, such as via an engineered endonuclease. In certain embodiments, an endogenous T cell gene (e.g., a TCR gene, an HLA gene, or an immune checkpoint molecule gene) is knocked out by insertion of a polynucleotide encoding a chimeric Tim4 receptor of the present disclosure into the locus of the endogenous T cell gene, such as via an engineered endonuclease.
[0123] In certain embodiments, a host cell may be genetically modified to express one type of chimeric Tim4 receptor. In other embodiments, a host cell may express at least two or more different chimeric Tim4 receptors.
[0124] The present disclosure also provides a composition comprising a population of chimeric Tim4 receptor modified host cells. In certain embodiments, the population of chimeric Tim4 receptor modified host cells may be a population of B cells, a population of T cells, a population of natural killer cells, a population of lymphoid precursor cells, a population of antigen presenting cells, a population of dendritic cells, a population of Langerhans cells, a population of myeloid precursor cells, a population of mature myeloid cells, or any combination thereof. Furthermore, a population of chimeric Tim4 receptor modified host cells of a particular cell type may be composed of one or more subtypes. For example, a population of B cells may be composed of chimeric Tim4

receptor modified naïve B cells, plasma cells, regulatory B cells, marginal zone B cells, follicular B cells, lymphoplasmacytoid cells, plasmablast cells, memory B cells, or any combination thereof. In another example, a population of T cells may be composed of chimeric Tim4 receptor modified CD4.sup.+ helper T cells, CD8.sup.+ effector (cytotoxic) T cells, naïve (CD45 RA+, CCR7+, CD62L+, CD27+, CD45RO−) T cells, central memory (CD45RO.sup.+, CD62L.sup.+, CD8.sup.+) T cells, effector memory (CD45RA+, CD45RO−, CCR7−, CD62L−, CD27−) T cells, T memory stem cells, regulatory T cells, mucosal-associated invariant T cells (MAIT), γδ (gd) cells, tissue resident T cells, natural killer T cells, or any combination thereof.

[0125] In certain embodiments, a population of host cells is composed of cells that each expresses the same chimeric Tim4 receptor(s). In other embodiments, a population of host cells is composed of a mixture of two or more subpopulation of host cells, wherein each subpopulation expresses a different chimeric Tim4 receptor or set of chimeric Tim4 receptors.

[0126] In certain embodiments, when preparing chimeric Tim4 receptor modified host cells, e.g., B cells or T cells, one or more growth factor cytokines that promotes proliferation of the host cells, e.g., B cells or T cells, may be added to the cell culture. The cytokines may be human or non-human. Exemplary growth factor cytokines that may be used to promote T cell proliferation include IL-2, IL-15, or the like. Exemplary growth factor cytokines that may be used to promote B cell proliferation include CD40L, IL-2, IL-4, IL-15, IL-21, BAFF, or the like.

[0127] Prior to genetic modification of the host cells with a chimeric Tim4 receptor vector, a source of host cells (e.g., T cells, B cells, natural killer cells, etc.) is obtained from a subject (e.g., whole blood, peripheral blood mononuclear cells, bone marrow, lymph node tissue, cord blood, thymus tissue, tissue from a site of infection, ascites, pleural effusion, spleen tissue), from which host cells are isolated using methods known in the art. Specific host cell subsets can be collected in accordance with known techniques and enriched or depleted by known techniques, such as affinity binding to antibodies, flow cytometry and/or immunomagnetic selection. After enrichment and/or depletion steps and introduction of a chimeric Tim4 receptor, in vitro expansion of the desired modified host cells can be carried out in accordance with known techniques, or variations thereof that will be apparent those skilled in the art.

[0128] Chimeric Tim4 receptors of the present disclosure confer cytotoxic activity to host cells expressing the chimeric Tim4 receptors that is specific for phosphatidylserine. Thus, upon binding phosphatidylserine exposed on the surface of a target cell, a host cell expressing a chimeric Tim4 receptor is capable of inducing apoptosis of the target cell. In certain embodiments, the host cell expressing the chimeric Tim4 receptor induces apoptosis of the target cell via: release of granzymes, perforins, granulysin, or any combination thereof; Fas ligand-Fas interaction; or both. In further embodiments, the chimeric Tim4 receptor further confers phosphatidylserine specific engulfment activity to host cells expressing the chimeric Tim4 receptor. In yet further embodiments, the host cell does not naturally exhibit an engulfment phenotype prior to modification with the chimeric Tim4 receptor.

[0129] Chimeric Tim4 receptors of the present disclosure may also be capable of costimulating T cells via at least one signaling pathway. In certain embodiments, chimeric Tim4 receptors provide costimulatory signals to T cells via at least two distinct signaling pathways (e.g., via the selected costimulatory signaling domain(s) in the chimeric Tim4 receptor and Tim1). For example, a chimeric Tim4 receptor comprising a CD28 costimulatory signaling domain may be capable of providing a costimulatory signal via CD28 and Tim1. In another example, a chimeric Tim4 receptor comprising a 4-1BB costimulatory signaling domain may be capable of providing a costimulatory signal via 4-1BB and Tim1. In yet another example, a chimeric Tim4 receptor comprising a 4-1BB costimulatory signaling domain and CD28 costimulatory signaling domain may be capable of providing a costimulatory signal via 4-1BB, CD28, and Tim1.

[0130] In certain embodiments, host immune cells expressing the chimeric Tim4 receptors exhibit reduction or inhibition of immune cell exhaustion. In certain embodiments, the host immune cell is

a T cell or NK cells. In certain embodiments, exhausted T cells exhibit; (a) increased expression of PD-1, TIGIT, LAG3, TIM3, or any combination thereof; (b) decreased production of IFN-γ, IL-2, TNF-α, or any combination thereof; or both (a) and (b). In certain embodiments, exhausted NK cells exhibit; (a) increased expression of PD-1, NKG2A, TIM3, or any combination thereof; (b) decreased production of IFN-γ, TNF-α, or both; or both (a) and (b).

[0131] In certain embodiments, host cells expressing the chimeric Tim4 receptors exhibit an enhanced effector response (e.g., tumor specific). In certain embodiments, the effector response is enhanced T cell proliferation, cytokine production (e.g., IFN-γ, IL-2, TNF-α), cytotoxic activity, persistence, or any combination thereof. Host cells expressing chimeric Tim4 receptors may be administered to a subject alone, or in combination with other therapeutic agents, including for example CAR-T cells, TCRs, antibodies, radiation therapy, chemotherapies, small molecules, oncolytic viruses, electropulse therapy, etc.

[0132] In certain embodiments host cells expressing the chimeric Tim4 receptors exhibit a reduced immunosuppressive response to phosphatidylserine. Phosphatidylserine is one of the primary apoptotic cell ligands that signal “eat me” to phagocytes. The removal of apoptotic cells by phagocytes generally reduces or prevents an inflammatory response via secretion of anti-inflammatory cytokines IL-10 and TGF-β and the decrease of secretion of inflammatory cytokines TNF-α, IL-1β, and IL-12. Thus, phosphatidylserine may act as an immunosuppressive signal during the clearance of apoptotic cells. In certain embodiments, upon binding phosphatidylserine, a chimeric Tim4 receptor modified host cell exhibits increased antigen-specific cytokine production (e.g., IFN-γ, IL-2, TNF-α), thereby reducing the immunosuppressive response to phosphatidylserine.

[0133] The expression of chimeric Tim4 receptors on host cells may be functionally characterized according to any of a large number of art-accepted methodologies for assaying host cell (e.g., T cell) activity, including determination of T cell binding, activation or induction and also including determination of T cell responses that are antigen-specific. Examples include determination of T cell proliferation, T cell cytokine release, antigen-specific T cell stimulation, CTL activity (e.g., by detecting 51Cr or Europium release from pre-loaded target cells), changes in T cell phenotypic marker expression, and other measures of T cell functions. Procedures for performing these and similar assays are may be found, for example, in Lefkovits (*Immunology Methods Manual: The Comprehensive Sourcebook of Techniques*, 1998). See, also, *Current Protocols in Immunology*; Weir, *Handbook of Experimental Immunology*, Blackwell Scientific, Boston, MA (1986); Mishell and Shigii (eds.) *Selected Methods in Cellular Immunology*, Freeman Publishing, San Francisco, CA (1979); Green and Reed, *Science* 281:1309 (1998) and references cited therein. Cytokine levels may be determined according to methods known in the art, including for example, ELISA, ELISPOT, intracellular cytokine staining, flow cytometry, and any combination thereof (e.g., intracellular cytokine staining and flow cytometry). Immune cell proliferation and clonal expansion resulting from an antigen-specific elicitation or stimulation of an immune response may be determined by isolating lymphocytes, such as circulating lymphocytes in samples of peripheral blood cells or cells from lymph nodes, stimulating the cells with antigen, and measuring cytokine production, cell proliferation and/or cell viability, such as by incorporation of tritiated thymidine or non-radioactive assays, such as MTT assays and the like.

[0134] In certain embodiments, a chimeric Tim4 receptor modified host cell has a phagocytic index of about 20 to about 1,500 for a target cell. A “phagocytic index” is a measure of phagocytic activity of the transduced host cell as determined by counting the number of target cells or particles ingested per chimeric Tim4 receptor modified host cell during a set period of incubation of a suspension of target cells or particles and chimeric Tim4 receptor modified host cells in media. Phagocytic index may be calculated by multiplying [total number of engulfed target cells/total number of counted chimeric Tim4 receptor modified cells (e.g., phagocytic frequency)]×[average area of target cell or particle staining per chimeric Tim4 receptor.sup.+ host cell×100 (e.g., hybrid

capture)] or [total number of engulfed particles/total number of counted chimeric Tim4 receptor modified host cells]×[number of chimeric Tim4 receptor modified host cells containing engulfed particles/total number of counted chimeric Tim4 receptor cells]×100. In certain embodiments, a chimeric Tim4 receptor modified cell has a phagocytic index of about 30 to about 1,500; about 40 to about 1,500; about 50 to about 1,500; about 75 to about 1,500; about 100 to about 1,500; about 200 to about 1,500; about 300 to about 1,500; about 400 to about 1,500; about 500 to about 1,500; about 20 to about 1,400; about 30 to about 1,400; about 40 to about 1,400; about 50 to about 1,400; about 100 to about 1,400; about 200 to about 1,400; about 300 to about 1,400; about 400 to about 1,400; about 500 to about 1,400; about 20 to about 1,300; about 30 to about 1,300; about 40 to about 1,300; about 50 to about 1,300; about 100 to about 1,300; about 200 to about 1,300; about 300 to about 1,300; about 400 to about 1,300; about 500 to about 1,300; about 20 to about 1,200; about 30 to about 1,200; about 40 to about 1,200; about 50 to about 1,200; about 100 to about 1,200; about 200 to about 1,200; about 300 to about 1,200; about 400 to about 1,200; about 500 to about 1,200; about 20 to about 1,100; about 30 to about 1,100; about 40 to about 1,100; about 50 to about 1,100; about 100 to about 1,100; about 200 to about 1,100; about 300 to about 1,100; about 400 to about 1,100; or about 500 to about 1,100; about 20 to about 1,000; about 30 to about 1,000; about 40 to about 1,000; about 50 to about 1,000; about 100 to about 1,000; about 200 to about 1,000; about 300 to about 1,000; about 400 to about 1,000; or about 500 to about 1,000; about 20 to about 750; about 30 to about 750; about 40 to about 750; about 50 to about 750; about 100 to about 750; about 200 to about 750; about 300 to about 750; about 400 to about 750; or about 500 to about 750; about 20 to about 500; about 30 to about 500; about 40 to about 500; about 50 to about 500; about 100 to about 500; about 200 to about 500; or about 300 to about 500. In further embodiments, the incubation time is from about 2 hours to about 4 hours, about 2 hours, about 3 hours, or about 4 hours. In yet further embodiments, a chimeric Tim4 receptor modified cell exhibits phagocytic index that is statistically significantly higher than a cell transduced with truncated EGFR control. Phagocytic index may be calculated using methods known in the art and as further described in the Examples and PCT Application No. PCT/US2017/053553 (incorporated herein by reference in its entirety), including quantification by flow cytometry or fluorescence microscopy.

[0135] Host cells may be from an animal, such as a human, primate, cow, horse, sheep, dog, cat, mouse, rat, rabbit, guinea pig, pig, or a combination thereof. In a preferred embodiment, the animal is a human. Host cells may be obtained from a healthy subject or a subject having a disease associated with expression or overexpression of an antigen.

Methods of Use

[0136] In one aspect, the present disclosure provides methods for conferring or enhancing phosphatidylserine-specific cytotoxic activity of a cell comprising introducing into a host cell a nucleic acid molecule encoding at least one chimeric Tim4 receptor or a chimeric Tim4 receptor vector according to any of the embodiments described herein; and expressing the at least one chimeric Tim4 receptor in the host cell, wherein the at least one chimeric Tim4 receptor enhances the phosphatidylserine-specific cytotoxic activity of the host cell as compared to a the host cell prior to modification to express a chimeric Tim4 receptor. In certain embodiments, the cytotoxic activity of the host cell is increased at least about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 190%, 200% or more as compared to the host cell prior to modification with a nucleic acid molecule encoding a chimeric Tim4 receptor or a chimeric Tim4 receptor vector. In some embodiments, the host cell is a T cell or an NK cell. Methods of measuring cytotoxic activity of host cells, particularly immune cells such as T cells and NK cells, include a chromium (.sup.51Cr)-release assay, a β-gal or firefly luciferase release assay, flow cytometric methods of mediating target cell death and effector cell activity (see, e.g., Expert Rev. Vaccines, 2010, 9:601-616).

[0137] In certain embodiments, methods for conferring or enhancing phosphatidylserine-specific cytotoxic activity of a cell further comprise conferring or enhancing phosphatidylserine-specific engulfment activity of the host cell expressing the at least one chimeric Tim4 receptor. In certain such embodiments, the host cell does not naturally exhibit an engulfment phenotype prior to modification with the chimeric Tim4 receptor. For example in certain such embodiments, the engulfment activity of the host cell is increased at least about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 190%, 200% or more as compared to the host cell prior to modification to express the chimeric Tim4 receptor vector. In certain embodiments, the host cell does not naturally possess engulfment activity. In some embodiments, the host cell is a T cell or an NK cell. Methods of measuring engulfment activity of host cells include methods as described in PCT/US2017/053553 (incorporated herein by reference in its entirety).

[0138] In another aspect, a chimeric Tim4 receptor, a polynucleotide encoding a chimeric Tim4 receptor, a chimeric Tim4 receptor vector, or a host cell that expresses a chimeric Tim4 receptor according to any of the embodiments provided herein may be used in a method of enhancing effector function of the host cell. In certain embodiments, enhanced effector function comprises increased cytotoxic activity, increased antigen specific cytokine production (e.g., IFN-γ, IL-2, TNF-α, or any combination thereof), increased anti-apoptotic signaling, increased persistence, increased expansion, increased proliferation, or any combination thereof. In certain embodiments, the effector function of the host cell is enhanced at least about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 190%, 200% or more as compared to a host cell that is not modified with a nucleic acid molecule encoding a chimeric Tim4 receptor or a chimeric Tim4 receptor vector. In certain embodiments, the host cell is a T cell or an NK cell.

[0139] In another aspect, host cells modified with chimeric Tim4 receptors of the present disclosure can be used in methods for inhibiting or reducing immune cell exhaustion. In certain embodiments, reduced exhaustion in T cells comprises; (a) decreased expression of PD-1, TIGIT, LAG3, TIM3, or any combination thereof in T cells; (b) increased production of IFN-γ, IL-2, TNF-α, or any combination thereof in T cells; or both (a) and (b). In certain embodiments, reduced exhaustion in NK cells comprises; (a) decreased expression of PD-1, NKG2A, TIM3, or any combination thereof in NK cells; (b) increased production of IFN-γ, TNF-α, or both in NK cells; or both (a) and (b). In certain embodiments, the expression of an immune checkpoint molecule is decreased at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% in a host immune cell expressing the chimeric Tim4 receptor as compared to a host immune cell that is not modified with a nucleic acid molecule encoding a chimeric Tim4 receptor or a chimeric Tim4 receptor vector. In certain embodiments, the expression of the cytokine is increased at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 190%, 200% or more in a host immune cell expressing the chimeric Tim4 receptor as compared to a host immune cell that is not modified with a nucleic acid molecule encoding a chimeric Tim4 receptor or a chimeric Tim4 receptor vector.

[0140] In another aspect, a chimeric Tim4 receptor, a polynucleotide encoding a chimeric Tim4 receptor, a chimeric Tim4 receptor vector, or a host cell that expresses a chimeric Tim4 receptor according to any of the embodiments provided herein may be used in a method of reducing an immunosuppressive response to phosphatidylserine in a host cell. In certain embodiments, the immunosuppressive response comprises secretion of anti-inflammatory cytokines (e.g., IL-10, TGF-β, or both), the decrease in secretion of inflammatory cytokines (e.g., TNF-α, IL-1β, and IL-12), or both. In certain embodiments, the immunosuppressive response of the host cell to phosphatidylserine is decreased at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100% as compared to a host cell that is not

modified with a nucleic acid molecule encoding a chimeric Tim4 receptor or a chimeric Tim4 receptor vector. In certain embodiments, the host cell is a T cell or an NK cell.

[0141] In yet another aspect, a chimeric Tim4 receptor, a polynucleotide encoding a chimeric Tim4 receptor, a chimeric Tim4 receptor vector, or a host cell that expresses a chimeric Tim4 receptor according to any of the embodiments provided herein may be used in methods for eliminating target cells bearing surface exposed phosphatidylserine, e.g., for the elimination of cancer cells bearing surface presented phosphatidylserine. In certain embodiments, the target cells are damaged, stressed, apoptotic, necrotic cells (e.g., tumor cells) bearing surface exposed phosphatidylserine. In certain embodiments, host cells expressing chimeric Tim4 receptors clear damaged, stressed, apoptotic, or necrotic target cells bearing surface exposed phosphatidylserine via inducing apoptosis, or both inducing apoptosis and engulfment. Host cells expressing chimeric Tim4 receptors may be administered to a subject alone, or in combination with other therapeutic agents, including for example CAR-T cells, TCRs, antibodies, radiation therapy, chemotherapy, small molecules, oncolytic viruses, electropulse therapy, etc.

[0142] In another aspect, a chimeric Tim4 receptor, a polynucleotides encoding a chimeric Tim4 receptor, a chimeric Tim4 receptor vector, or a host cell that expresses a chimeric Tim4 receptor according to any of the embodiments provided herein may be used in methods to enhance the effect of a therapeutic agent that induces cellular stress, damage, necrosis, or apoptosis. Certain therapies, such as chemotherapy, radiation therapy, UV light therapy, electropulse therapy, adoptive cellular immunotherapy (e.g., CAR-T cells, TCRs) and oncolytic viral therapy, can induce cell damage or death to tumor cells, diseased cells, and cells in their surrounding environment. Cells expressing chimeric Tim4 receptors can be administered in combination with the cell damaging/cytotoxic therapy to bind to the phosphatidylserine moieties exposed on the outer leaflet of targeted cells and clear stressed, damaged, diseased, apoptotic, necrotic cells.

[0143] In another aspect, a chimeric Tim4 receptor, a polynucleotides encoding a chimeric Tim4 receptor, a chimeric Tim4 receptor vector, or a host cell that expresses a chimeric Tim4 receptor according to any of the embodiments provided herein may be used in a method of treating a subject suffering from a disease, disorder or undesired condition. Embodiments of these methods include administering to a subject a therapeutically effective amount of a pharmaceutical composition including one or more chimeric Tim4 receptors, polynucleotides encoding one or more chimeric Tim4 receptors, vectors comprising polynucleotides encoding one or more chimeric Tim4 receptors, or a population of host cells genetically modified to express one or more chimeric Tim4 receptors according to the present description.

[0144] Diseases that may be treated with cells expressing a chimeric Tim4 receptor as described in the present disclosure include cancer and infectious diseases (viral, bacterial, fungal, protozoan infections). Adoptive immune and gene therapies are promising treatments for various types of cancer (Morgan et al., *Science* 314:126, 2006; Schmitt et al., *Hum. Gene Ther.* 20:1240, 2009; June, *J. Clin. Invest.* 117:1466, 2007) and infectious disease (Kitchen et al., *PLoS One* 4: 38208, 2009; Rossi et al., *Nat. Biotechnol.* 25:1444, 2007; Zhang et al., *PLoS Pathog.* 6: e1001018, 2010; Luo et al., *J. Mol. Med.* 89:903, 2011).

[0145] A wide variety of cancers, including solid tumors and leukemias are amenable to the compositions and methods disclosed herein. Exemplary cancers that may be treated using the receptors, modified host cells, and composition described herein include adenocarcinoma of the breast, prostate, and colon; all forms of bronchogenic carcinoma of the lung; myeloid leukemia; melanoma; hepatoma; neuroblastoma; papilloma; apudoma; choristoma; branchioma; malignant carcinoid syndrome; carcinoid heart disease; and carcinoma (e.g., Walker, basal cell, basosquamous, Brown-Pearce, ductal, Ehrlich tumor, Krebs 2, Merkel cell, mucinous, non-small cell lung, oat cell, papillary, scirrhous, bronchiolar, bronchogenic, squamous cell, and transitional cell). Additional types of cancers that may be treated using the receptors, modified host cells, and composition described herein include histiocytic disorders; malignant histiocytosis; leukemia;

Hodgkin's disease; immunoproliferative small; non-Hodgkin's lymphoma; plasmacytoma; multiple myeloma; plasmacytoma; reticuloendotheliosis; melanoma; chondroblastoma; chondroma; chondrosarcoma; fibroma; fibrosarcoma; giant cell tumors; histiocytoma; lipoma; liposarcoma; mesothelioma; myxoma; myxosarcoma; osteoma; osteosarcoma; chordoma; craniopharyngioma; dysgerminoma; hamartoma; mesenchymoma; mesonephroma; myosarcoma; ameloblastoma; cementoma; odontoma; teratoma; thymoma; trophoblastic tumor. Further, the following types of cancers are also contemplated as amenable to treatment using the receptors, modified host cells, and composition described herein: adenoma; cholangioma; cholesteatoma; cyclindroma; cystadenocarcinoma; cystadenoma; granulosa cell tumor; gynandroblastoma; hepatoma; hidradenoma; islet cell tumor; Leydig cell tumor; papilloma; sertoli cell tumor; theca cell tumor; leimyoma; leiomyosarcoma; myoblastoma; myomma; myosarcoma; rhabdomyoma; rhabdomyosarcoma; ependymoma; ganglioneuroma; glioma; medulloblastoma; meningioma; neurilemmoma; neuroblastoma; neuroepithelioma; neurofibroma; neuroma; paraganglioma; paraganglioma nonchromaffin. The types of cancers that may be treated also include angiokeratoma; angiolymphoid hyperplasia with eosinophilia; angioma sclerosing; angiomatosis; glomangioma; hemangioendothelioma; hemangioma; hemangiopericytoma; hemangiosarcoma; lymphangioma; lymphangiomyoma; lymphangiosarcoma; pinealoma; carcinosarcoma; chondrosarcoma; cystosarcoma phyllodes; fibrosarcoma; hemangiosarcoma; leiomyosarcoma; leukosarcoma; liposarcoma; lymphangiosarcoma; myosarcoma; myxosarcoma; ovarian carcinoma; rhabdomyosarcoma; sarcoma; neoplasms; nerofibromatosis; and cervical dysplasia.

[0146] Examples of hyperproliferative disorders amenable to therapy using the receptors, modified host cells, and composition described herein include B-cell cancers, including B-cell lymphomas (such as various forms of Hodgkin's disease, non-Hodgkin's lymphoma (NHL) or central nervous system lymphomas), leukemias (such as acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), Hairy cell leukemia, B cell blast transformation of chronic myeloid leukemia) and myelomas (such as multiple myeloma). Additional B cell cancers that may be treated using the receptors, modified host cells, and composition described herein include small lymphocytic lymphoma, B-cell prolymphocytic leukemia, lymphoplasmacytic lymphoma, splenic marginal zone lymphoma, plasma cell myeloma, solitary plasmacytoma of bone, extraosseous plasmacytoma, extra-nodal marginal zone B-cell lymphoma of mucosa-associated (MALT) lymphoid tissue, nodal marginal zone B-cell lymphoma, follicular lymphoma, mantle cell lymphoma, diffuse large B-cell lymphoma, mediastinal (thymic) large B-cell lymphoma, intravascular large B-cell lymphoma, primary effusion lymphoma, Burkitt's lymphoma/leukemia, B-cell proliferations of uncertain malignant potential, lymphomatoid granulomatosis, and post-transplant lymphoproliferative disorder.

[0147] Infectious diseases include those associated with infectious agents and include any of a variety of bacteria (e.g., pathogenic *E. coli, S. typhimurium, P. aeruginosa, B. anthracis, C. botulinum, C. difficile, C. perfringens, H. pylori, V. cholerae, Listeria* spp., *Rickettsia* spp., *Chlamydia* spp., and the like), mycobacteria, and parasites (including any known parasitic member of the Protozoa). Infectious viruses include eukaryotic viruses, such as adenovirus, bunyavirus, herpesvirus, papovavirus, papillomavirus (e.g., HPV), paramyxovirus, picornavirus, rhabdovirus (e.g., Rabies), orthomyxovirus (e.g., influenza), poxvirus (e.g., Vaccinia), reovirus, retrovirus, lentivirus (e.g., HIV), flavivirus (e.g., HCV, HBV) or the like. In certain embodiments, a composition comprising a chimeric Tim4 receptor according to the present disclosure is used for treating infection with a microbe capable of establishing a persistent infection in a subject.

[0148] A chimeric Tim4 receptor of the present disclosure may be administered to a subject in cell-bound form (e.g., gene therapy of target cell population). Thus, for example, a chimeric Tim4 receptor of the present disclosure may be administered to a subject expressed on the surface of T cells, Natural Killer Cells, Natural Killer T cells, B cells, lymphoid precursor cells, antigen presenting cells, dendritic cells, Langerhans cells, myeloid precursor cells, mature myeloid cells,

including subsets thereof, or any combination thereof. In certain embodiments, methods of treating a subject comprise administering an effective amount of chimeric Tim4 receptor modified cells (i.e., recombinant cells that express one or more chimeric Tim4 receptors). The chimeric Tim4 receptor modified cells may be xenogeneic, syngeneic, allogeneic, or autologous to the subject.
[0149] Pharmaceutical compositions including chimeric Tim4 receptor modified cells may be administered in a manner appropriate to the disease or condition to be treated (or prevented) as determined by persons skilled in the medical art. An appropriate dose, suitable duration, and frequency of administration of the compositions will be determined by such factors as the condition of the patient, size, weight, body surface area, age, sex, type and severity of the disease, particular therapy to be administered, particular form of the active ingredient, time and the method of administration, and other drugs being administered concurrently. The present disclosure provides pharmaceutical compositions comprising chimeric Tim4 receptor modified cells and a pharmaceutically acceptable carrier, diluent, or excipient. Suitable excipients include water, saline, dextrose, glycerol, or the like and combinations thereof. Other suitable infusion medium can be any isotonic medium formulation, including saline, Normosol R (Abbott), Plasma-Lyte A (Baxter), 5% dextrose in water, or Ringer's lactate.
[0150] A treatment effective amount of cells in a pharmaceutical composition is at least one cell (for example, one chimeric Tim4 receptor modified T cell) or is more typically greater than 10.sup.2 cells, for example, up to 10.sup.6, up to 10.sup.7, up to 10.sup.8 cells, up to 10.sup.9 cells, up to 10.sup.10 cells, or up to 10.sup.11 cells or more. In certain embodiments, the cells are administered in a range from about 10.sup.6 to about 10.sup.10 cells/m.sup.2, preferably in a range of about 10.sup.7 to about 10.sup.9 cells/m.sup.2. The number of cells will depend upon the ultimate use for which the composition is intended as well the type of cells included therein. For example, a composition comprising cells modified to contain a chimeric Tim4 receptor will comprise a cell population containing from about 5% to about 95% or more of such cells. In certain embodiments, a composition comprising chimeric Tim4 receptor modified cells comprises a cell population comprising at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more of such cells. For uses provided herein, the cells are generally in a volume of a liter or less, 500 mls or less, 250 mls or less, or 100 mls or less. Hence the density of the desired cells is typically greater than 10.sup.4 cells/ml and generally is greater than 10.sup.7 cells/ml, generally 10.sup.8 cells/ml or greater. The cells may be administered as a single infusion or in multiple infusions over a range of time. Repeated infusions of chimeric Tim4 receptor modified cells may be separated by days, weeks, months, or even years if relapses of disease or disease activity are present. A clinically relevant number of immune cells can be apportioned into multiple infusions that cumulatively equal or exceed 10.sup.6, 10.sup.7, 10.sup.8, 10.sup.9, 10.sup.10, or 10.sup.11 cells. A preferred dose for administration of a host cell comprising a recombinant expression vector as described herein is about 10.sup.7 cells/m.sup.2, about 5×10.sup.7 cells/m.sup.2, about 10.sup.8 cells/m.sup.2, about 5×10.sup.8 cells/m.sup.2, about 10.sup.9 cells/m.sup.2, about 5×10.sup.9 cells/m.sup.2, about 10.sup.10 cells/m.sup.2, about 5×10.sup.10 cells/m.sup.2, or about 10.sup.11 cells/m.sup.2.
[0151] Chimeric Tim4 receptor compositions as described herein may be administered intravenously, intraperitoneally, intranasally, intratumorly, into the bone marrow, into the lymph node, and/or into cerebrospinal fluid.
[0152] Chimeric Tim4 receptor compositions may be administered to a subject in combination with one or more additional therapeutic agents. Examples of therapeutic agents that may be administered in combination with a chimeric Tim4 compositions according to the present description include radiation therapy, adoptive cellular immunotherapy agent (e.g., recombinant TCR, enhanced affinity TCR, CAR, TCR-CAR, scTCR fusion protein, dendritic cell vaccine), antibody therapy, immune checkpoint molecule inhibitor therapy, UV light therapy, electric pulse therapy, high intensity focused ultrasound therapy, oncolytic virus therapy, or a pharmaceutical therapy, such as a

chemotherapeutic agent, a therapeutic peptide, a hormone, an aptamer, antibiotic, anti-viral agent, anti-fungal agent, anti-inflammatory agent, a small molecule therapy, or any combination thereof. In certain embodiments, the chimeric Tim4 receptor modified host cells may clear stressed, damaged, apoptotic, necrotic, infected, dead cells displaying surface phosphatidylserine induced by the one or more additional therapeutic agents.

[0153] In certain embodiments, the chimeric Tim4 receptor and adoptive cellular immunotherapy agent are administered to the subject in the same host cell or different host cells. In certain embodiments, the chimeric Tim4 receptor and adoptive cellular immunotherapy agent are expressed in the same host cell from the same vector or from separate vectors. In certain embodiments, the chimeric Tim4 receptor and adoptive cellular immunotherapy agent are expressed in the same host cell from a multicistronic vector. In certain embodiments, the chimeric Tim4 receptor is expressed in the same host cell type as the adoptive cellular immunotherapy agent (e.g., the chimeric Tim4 receptor is expressed CD4 T cells and the CAR/or TCR is expressed in CD4 T cells). In other embodiments, the chimeric Tim4 receptor is expressed in a different host cell type as the adoptive immunotherapy agent (e.g., the chimeric Tim4 receptor is expressed CD4 T cells and the CAR/or TCR is expressed in CD8 T cells).

[0154] Exemplary antigens that a recombinant TCR, enhanced affinity TCR, CAR, TCR-CAR, or scTCR fusion protein may target include WT-1, mesothelin, MART-1, NY-ESO-1, MAGE-A3, HPV E7, survivin, a Fetoprotein, and a tumor-specific neoantigen.

[0155] Exemplary antigens that a CAR may target include CD138, CD38, CD33, CD123, CD72, CD79a, CD79b, mesothelin, PSMA, BCMA, ROR1, MUC-16, L1CAM, CD22, CD19, CD20, CD23, CD24, CD37, CD30, CA125, CD56, c-Met, EGFR, GD-3, HPV E6, HPV E7, MUC-1, HER2, folate receptor α, CD97, CD171, CD179a, CD44v6, WT1, VEGF-α, VEGFR1, IL-13Rα1, IL-13Rα2, IL-11Rα, PSA, FcRH5, NKG2D ligand, NY-ESO-1, TAG-72, CEA, ephrin A2, ephrin B2, Lewis A antigen, Lewis Y antigen, MAGE, MAGE-A1, RAGE-1, folate receptor β, EGFRviii, VEGFR-2, LGR5, SSX2, AKAP-4, FLT3, fucosyl GM1, GM3, o-acetyl-GD2, and GD2.

[0156] Radiation therapy includes external beam radiation therapy (e.g., conventional external beam radiation therapy, stereotactic radiation, 3-dimensional conformal radiation therapy, intensity-modulated radiation therapy, volumetric modulated arc therapy, particle therapy, proton therapy, and auger therapy), brachytherapy, systemic radioisotope therapy, intraoperative radiotherapy, or any combination thereof.

[0157] Exemplary antibodies for use in conjunction with the chimeric Tim4 compositions described herein include rituxmab, pertuzumab, trastuzumab, alemtuzumab, Ibritumomab tiuxetan, Brentuximab vedotin, cetuximab, bevacizumab, abciximab, adalimumab, alefacept, basilizimab, belimumab, bezlotoxumab, canakinumab, certolizumab pegol, daclizumab, denosumab, efalizumab, golimumab, olaratumab, palivizumab, panitumumab, and tocilizumab.

[0158] Exemplary inhibitors of immune checkpoint molecules that may be for use in conjunction with the chimeric Tim4 compositions described herein include checkpoint inhibitors targeting PD-L1, PD-L2, CD80, CD86, B7-H3, B7-H4, HVEM, adenosine, GAL9, VISTA, CEACAM-1, CEACAM-3, CEACAM-5, PVRL2, PD-1, CTLA-4, BTLA, KIR, LAG3, TIM3, A2aR, CD244/2B4, CD160, TIGIT, LAIR-1, PVRIG/CD112R, or any combination thereof. In certain embodiments, an immune checkpoint inhibitor may be an antibody, a peptide, an RNAi agent, or a small molecule. An antibody specific for CTLA-4 may be ipilimumab or tremelimumab. An antibody specific for PD-1 may be pidilizumab, nivolumab, or pembrolizumab. An antibody specific for PD-L1 may be durvalumab, atezolizumab, or avelumab.

[0159] Exemplary chemotherapeutics for use in conjunction with the chimeric Tim4 receptor compositions described herein may include an alkylating agent, a platinum based agent, a cytotoxic agent, an inhibitor of chromatin function, a topoisomerase inhibitor, a microtubule inhibiting drug, a DNA damaging agent, an antimetabolite (such as folate antagonists, pyrimidine analogs, purine analogs, and sugar-modified analogs), a DNA synthesis inhibitor, a DNA interactive agent (such as

an intercalating agent), and a DNA repair inhibitor.
[0160] A chemotherapeutic includes non-specific cytotoxic agents that inhibit mitosis or cell division, as well as molecularly targeted therapy that blocks the growth and spread of cancer cells by targeting specific molecules that are involved in tumor growth, progression, and metastasis (e.g., oncogenes). Exemplary non-specific chemotherapeutics for use in conjunction with the tandem expression cassette compositions described herein may include an alkylating agent, a platinum based agent, a cytotoxic agent, an inhibitor of chromatin function, a topoisomerase inhibitor, a microtubule inhibiting drug, a DNA damaging agent, an antimetabolite (such as folate antagonists, pyrimidine analogs, purine analogs, and sugar-modified analogs), a DNA synthesis inhibitor, a DNA interactive agent (such as an intercalating agent), and a DNA repair inhibitor.
[0161] Examples of chemotherapeutic agents considered for use in combination therapies contemplated herein include vemurafenib, dabrafenib, trametinib, cobimetinib, anastrozole (Arimidex®), bicalutamide (Casodex®), bleomycin sulfate (Blenoxane®), busulfan (Myleran®), busulfan injection (Busulfex®), capecitabine (Xeloda®), N4-pentoxycarbonyl-5-deoxy-5-fluorocytidine, carboplatin (Paraplatin®), carmustine (BiCNU®), chlorambucil (Leukeran®), cisplatin (Platinol®), cladribine (Leustatin®), cyclophosphamide (Cytoxan® or Neosar®), cytarabine, cytosine arabinoside (Cytosar-U®), cytarabine liposome injection (DepoCyt®), dacarbazine (DTIC-Dome®), dactinomycin (Actinomycin D, Cosmegan), daunorubicin hydrochloride (Cerubidine®), daunorubicin citrate liposome injection (DaunoXome®), dexamethasone, docetaxel (Taxotere®), doxorubicin hydrochloride (Adriamycin®, Rubex®), etoposide (Vepesid®), fludarabine phosphate (Fludara®), 5-fluorouracil (Adrucil®, Efudex®), flutamide (Eulexin®), tezacitibine, Gemcitabine (difluorodeoxycitidine), hydroxyurea (Hydrea®), Idarubicin (Idamycin®), ifosfamide (IFEX®), irinotecan (Camptosar®), L-asparaginase (ELSPAR®), leucovorin calcium, melphalan (Alkeran®), 6-mercaptopurine (Purinethol®), methotrexate (Folex®), mitoxantrone (Novantrone®), mylotarg, paclitaxel (Taxol®), phoenix (Yttrium90/MX-DTPA), pentostatin, polifeprosan 20 with carmustine implant (Gliadel®), fdabra tamoxifen citrate (Nolvadex®), teniposide (Vumon®), 6-thioguanine, thiotepa, tirapazamine (Tirazone®), topotecan hydrochloride for injection (Hycamptin®), vinblastine (Velban®), vincristine (Oncovin®), ibrutinib, venetoclax, crizotinib, alectinib, brigatinib, ceritinib, and vinorelbine (Navelbine®).
[0162] Exemplary alkylating agents for use in combination therapies contemplated herein include nitrogen mustards, ethylenimine derivatives, alkyl sulfonates, nitrosoureas and triazenes): uracil mustard (Aminouracil Mustard®, Chlorethaminacil®, Demethyldopan®, Desmethyldopan®, Haemanthamine®, Nordopan®, Uracil nitrogen Mustard®, Uracillost®, Uracilmostaza®, Uramustin®, Uramustine®), chlormethine (Mustargen®), cyclophosphamide (Cytoxan®, Neosar®, Clafen®, Endoxan®, Procytox®, Revimmune™), ifosfamide (Mitoxana®), melphalan (Alkeran®), Chlorambucil (Leukeran®), pipobroman (Amedel®, Vercyte®), triethylenemelamine (Hemel®, Hexalen®, Hexastat®), triethylenethiophosphoramine, Temozolomide (Temodar®), thiotepa (Thioplex®), busulfan (Busilvex®, Myleran®), carmustine (BiCNU®), lomustine (CeeNUR), streptozocin (Zanosar®), and Dacarbazine (DTIC-Dome®). Additional exemplary alkylating agents for use in combination therapies contemplated herein include, without limitation, Oxaliplatin (Eloxatin®); Temozolomide (Temodar® and Temodal®); Dactinomycin (also known as actinomycin-D, Cosmegen®); Melphalan (also known as L-PAM, L-sarcolysin, and phenylalanine mustard, Alkeran®); Altretamine (also known as hexamethylmelamine (HMM), Hexalen®); Carmustine (BiCNUR); Bendamustine (Treanda®); Busulfan (Busulfex® and Myleran®); Carboplatin (Paraplatin®); Lomustine (also known as CCNU, CeeNUR); Cisplatin (also known as CDDP, Platinol® and Platinol®-AQ); Chlorambucil (Leukeran®); Cyclophosphamide (Cytoxan® and Neosar®); Dacarbazine (also known as DTIC, DIC and imidazole carboxamide, DTIC-Dome®); Altretamine (also known as hexamethylmelamine (HMM), Hexalen®); Ifosfamide (Ifex®); Prednumustine; Procarbazine (Matulane®); Mechlorethamine (also known as nitrogen

mustard, mustine and mechloroethamine hydrochloride, Mustargen®); Streptozocin (Zanosar®); Thiotepa (also known as thiophosphoamide, TESPA and TSPA, Thioplex®); Cyclophosphamide (Endoxan®, Cytoxan®, Neosar®, Procytox®, Revimmune®); and Bendamustine HCl (Treanda®).
[0163] Exemplary platinum based agents for use in combination therapies contemplated herein include carboplatin, cisplatin, oxaliplatin, nedaplatin, picoplatin, satraplatin, phenanthriplatin, and triplatin tetranitrate.
[0164] Exemplary molecularly targeted inhibitors for use in conjunction with the chimeric Tim4 receptor compositions described herein include small molecules that target molecules involved in cancer cell growth and survival, including for example, hormone antagonists, signal transduction inhibitors, gene expression inhibitors (e.g., translation inhibitors), apoptosis inducers, angiogenesis inhibitors (e.g., a VEGF pathway inhibitor), tyrosine kinase inhibitors (e.g., an EGF/EGFR pathway inhibitor), growth factor inhibitors, GTPase inhibitors, serine/threonine kinase inhibitors, transcription factor inhibitors, inhibitors of driver mutations associated with cancer, B-Raf inhibitors, a MEK inhibitors, mTOR inhibitors, adenosine pathway inhibitors, EGFR inhibitors, PI3K inhibitors, BCL2 inhibitors, VEGFR inhibitors, MET inhibitors, MYC inhibitors, BCR-ABL inhibitors, HER2 inhibitors, H-RAS inhibitors, K-RAS inhibitors, PDGFR inhibitors, ALK inhibitors, ROS1 inhibitors, and BTK inhibitors. In certain embodiments, use of molecularly targeted therapy comprises administering a molecularly targeted therapy specific for the molecular target to a subject identified as having a tumor that possesses the molecular target (e.g., driver oncogene). In certain embodiments, the molecular target has an activating mutation. In certain embodiments, use of chimeric Tim4 receptor modified cells in combination with a molecularly targeted inhibitor increases the magnitude of anti-tumor response, the durability of anti-tumor response, or both. In certain embodiments, a lower than typical dose of molecularly targeted therapy is used in combination with chimeric Tim4 receptor modified cells.
[0165] Exemplary angiogenesis inhibitors for use in conjunction with the chimeric Tim4 receptor compositions described herein may include, without limitation A6 (Angstrom Pharmaceuticals), ABT-510 (Abbott Laboratories), ABT-627 (Atrasentan) (Abbott Laboratories/Xinlay), ABT-869 (Abbott Laboratories), Actimid (CC4047, Pomalidomide) (Celgene Corporation), AdGVPEDF.11D (GenVec), ADH-1 (Exherin) (Adherex Technologies), AEE788 (Novartis), AG-013736 (Axitinib) (Pfizer), AG3340 (Prinomastat) (Agouron Pharmaceuticals), AGX1053 (AngioGenex), AGX51 (AngioGenex), ALN-VSP (ALN-VSP 02) (Alnylam Pharmaceuticals), AMG 386 (Amgen), AMG706 (Amgen), Apatinib (YN968D1) (Jiangsu Hengrui Medicine), AP23573 (Ridaforolimus/MK8669) (Ariad Pharmaceuticals), AQ4N (Novavea), ARQ 197 (ArQule), ASA404 (Novartis/Antisoma), Atiprimod (Callisto Pharmaceuticals), ATN-161 (Attenuon), AV-412 (Aveo Pharmaceuticals), AV-951 (Aveo Pharmaceuticals), Avastin (Bevacizumab) (Genentech), AZD2171 (Cediranib/Recentin) (AstraZeneca), BAY 57-9352 (Telatinib) (Bayer), BEZ235 (Novartis), BIBF1120 (Boehringer Ingelheim Pharmaceuticals), BIBW 2992 (Boehringer Ingelheim Pharmaceuticals), BMS-275291 (Bristol-Myers Squibb), BMS-582664 (Brivanib) (Bristol-Myers Squibb), BMS-690514 (Bristol-Myers Squibb), Calcitriol, CCI-779 (Torisel) (Wyeth), CDP-791 (ImClone Systems), Ceflatonin (Homoharringtonine/HHT) (ChemGenex Therapeutics), Celebrex (Celecoxib) (Pfizer), CEP-7055 (Cephalon/Sanofi), CHIR-265 (Chiron Corporation), NGR-TNF, COL-3 (Metastat) (Collagenex Pharaceuticals), Combretastatin (Oxigene), CP-751,871 (Figitumumab) (Pfizer), CP-547,632 (Pfizer), CS-7017 (Daiichi Sankyo Pharma), CT-322 (Angiocept) (Adnexus), Curcumin, Dalteparin (Fragmin) (Pfizer), Disulfiram (Antabuse), E7820 (Eisai Limited), E7080 (Eisai Limited), EMD 121974 (Cilengitide) (EMD Pharmaceuticals), ENMD-1198 (EntreMed), ENMD-2076 (EntreMed), Endostar (Simcere), Erbitux (ImClone/Bristol-Myers Squibb), EZN-2208 (Enzon Pharmaceuticals), EZN-2968 (Enzon Pharmaceuticals), GC1008 (Genzyme), Genistein, GSK1363089 (Foretinib) (GlaxoSmithKline), GW786034 (Pazopanib) (GlaxoSmithKline), GT-111 (Vascular Biogenics Ltd.), IMC-1121B (Ramucirumab) (ImClone Systems), IMC-18F1 (ImClone Systems), IMC-3G3 (ImClone LLC),

INCB007839 (Incyte Corporation), INGN 241 (Introgen Therapeutics), Iressa (ZD1839/Gefitinib), LBH589 (Faridak/Panobinostst) (Novartis), Lucentis (Ranibizumab) (Genentech/Novartis), LY317615 (Enzastaurin) (Eli Lilly and Company), Macugen (Pegaptanib) (Pfizer), MEDI522 (Abegrin) (MedImmune), MLN518 (Tandutinib) (Millennium), Neovastat (AE941/Benefin) (Aeterna Zentaris), Nexavar (Bayer/Onyx), NM-3 (Genzyme Corporation), Noscapine (Cougar Biotechnology), NPI-2358 (Nereus Pharmaceuticals), OSI-930 (OSI), Palomid 529 (Paloma Pharmaceuticals, Inc.), Panzem Capsules (2ME2) (EntreMed), Panzem NCD (2ME2) (EntreMed), PF-02341066 (Pfizer), PF-04554878 (Pfizer), PI-88 (Progen Industries/Medigen Biotechnology), PKC412 (Novartis), Polyphenon E (Green Tea Extract) (Polypheno E International, Inc), PPI-2458 (Praecis Pharmaceuticals), PTC299 (PTC Therapeutics), PTK787 (Vatalanib) (Novartis), PXD101 (Belinostat) (CuraGen Corporation), RAD001 (Everolimus) (Novartis), RAF265 (Novartis), Regorafenib (BAY73-4506) (Bayer), Revlimid (Celgene), Retaane (Alcon Research), SN38 (Liposomal) (Neopharm), SNS-032 (BMS-387032) (Sunesis), SOM230 (Pasireotide) (Novartis), Squalamine (Genaera), Suramin, Sutent (Pfizer), Tarceva (Genentech), TB-403 (Thrombogenics), Tempostatin (Collard Biopharmaceuticals), Tetrathiomolybdate (Sigma-Aldrich), TG100801 (TargeGen), Thalidomide (Celgene Corporation), Tinzaparin Sodium, TKI258 (Novartis), TRC093 (Tracon Pharmaceuticals Inc.), VEGF Trap (Aflibercept) (Regeneron Pharmaceuticals), VEGF Trap-Eye (Regeneron Pharmaceuticals), Veglin (VasGene Therapeutics), Bortezomib (Millennium), XL184 (Exelixis), XL647 (Exelixis), XL784 (Exelixis), XL820 (Exelixis), XL999 (Exelixis), ZD6474 (AstraZeneca), Vorinostat (Merck), and ZSTK474.

[0166] Exemplary Vascular Endothelial Growth Factor (VEGF) receptor inhibitors for use in conjunction with the chimeric Tim4 receptor compositions described herein may include, but are not limited to, Bevacizumab (Avastin®), axitinib (Inlyta®); Brivanib alaninate (BMS-582664, (S)-((R)-1-(4-(4-Fluoro-2-methyl-1H-indol-5-yloxy)-5-methylpyrrolo[2,1-f][1,2,4]triazin-6-yloxy) propan-2-yl) 2-aminopropanoate); Sorafenib (Nexavar®); Pazopanib (Votrient®); Sunitinib malate (Sutent®); Cediranib (AZD2171, CAS 288383-20-1); Vargatef (BIBF1120, CAS 928326-83-4); Foretinib (GSK1363089); Telatinib (BAY57-9352, CAS 332012-40-5); Apatinib (YN968D1, CAS 811803 May 1); Imatinib (Gleevec®); Ponatinib (AP24534, CAS 943319-70-8); Tivozanib (AV951, CAS 475108-18-0); Regorafenib (BAY73-4506, CAS 755037-03-7); Vatalanib dihydrochloride (PTK787, CAS 212141-51-0); Brivanib (BMS-540215, CAS 649735-46-6); Vandetanib (Caprelsa® or AZD6474); Motesanib diphosphate (AMG706, CAS 857876-30-3, N-(2,3-dihydro-3,3-dimethyl-1H-indol-6-yl)-2-[(4-pyridinylmethyl)amino]-3-pyridinecarboxamide, described in PCT Publication No. WO 02/066470); Dovitinib dilactic acid (TKI258, CAS 852433-84-2); Linfanib (ABT869, CAS 796967-16-3); Cabozantinib (XL184, CAS 849217-68-1); Lestaurtinib (CAS 111358-88-4); N-[5-[[[5-(1,1-Dimethylethyl)-2-oxazolyl]methyl]thio]-2-thiazolyl]-4-piperidinecarboxamide (BMS38703, CAS 345627-80-7); (3R,4R)-4-Amino-1-((4-((3-methoxyphenyl)amino) pyrrolo[2,1-f][1,2,4]triazin-5-yl)methyl) piperidin-3-ol (BMS690514); N-(3,4-Dichloro-2-fluorophenyl)-6-methoxy-7-[[(3aα,5β,6aα)-octahydro-2-methylcyclopenta[c]pyrrol-5-yl]methoxy]-4-quinazolinamine (XL647, CAS 781613-23-8); 4-Methyl-3-[[1-methyl-6-(3-pyridinyl)-1H-pyrazolo[3,4-d]pyrimidin-4-yl]amino]-N-[3-(trifluoromethyl)phenyl]-benzamide (BHG712, CAS 940310-85-0); and Aflibercept (Eylea®).

[0167] Exemplary EGF pathway inhibitors for use in conjunction with the chimeric Tim4 receptor compositions described herein may include, without limitation tyrphostin 46, EKB-569, erlotinib (Tarceva®), gefitinib (Iressa®), erbitux, nimotuzumab, lapatinib (Tykerb®), cetuximab (anti-EGFR mAb), .sup.188Re-labeled nimotuzumab (anti-EGFR mAb), and those compounds that are generically and specifically disclosed in WO 97/02266, EP 0 564 409, WO 99/03854, EP 0 520 722, EP 0 566 226, EP 0 787 722, EP 0 837 063, U.S. Pat. No. 5,747,498, WO 98/10767, WO 97/30034, WO 97/49688, WO 97/38983 and WO 96/33980. Exemplary EGFR antibodies include, but are not limited to, Cetuximab (Erbitux®); Panitumumab (Vectibix®); Matuzumab (EMD-72000); Trastuzumab (Herceptin®); Nimotuzumab (hR3); Zalutumumab; TheraCIM h-R3;

MDX0447 (CAS 339151-96-1); and ch806 (mAb-806, CAS 946414-09-1). Exemplary Epidermal growth factor receptor (EGFR) inhibitors include, but not limited to, Osimertinib (Tagrisso®), Erlotinib hydrochloride (Tarceva®), Gefitnib (Iressa®); N-[4-[(3-Chloro-4-fluorophenyl)amino]-7-[[(3″S″)-tetrahydro-3-furanyl]oxy]-6-quinazolinyl]-4 (dimethylamino)-2-butenamide, Tovok®); Vandetanib (Caprelsa®); Lapatinib (Tykerb®); (3R,4R)-4-Amino-1-((4-((3-methoxyphenyl)amino) pyrrolo[2,1-f][1,2,4]triazin-5-yl)methyl) piperidin-3-ol (BMS690514); Canertinib dihydrochloride (CI-1033); 6-[4-[(4-Ethyl-1-piperazinyl)methyl]phenyl]-N-[(1R)-1-phenylethyl]-7H-Pyrrolo[2,3-d]pyrimidin-4-amine (AEE788, CAS 497839-62-0); Mubritinib (TAK165); Pelitinib (EKB569); Afatinib (BIBW2992); Neratinib (HKI-272); N-[4-[[1-[(3-Fluorophenyl)methyl]-1H-indazol-5-yl]amino]-5-methylpyrrolo[2,1-f][1,2,4]triazin-6-yl]-carbamic acid, (3S)-3-morpholinylmethyl ester (BMS599626); N-(3,4-Dichloro-2-fluorophenyl)-6-methoxy-7-[[(3aα,5β,6aα)-octahydro-2-methylcyclopenta[c]pyrrol-5-yl]methoxy]-4-quinazolinamine (XL647, CAS 781613-23-8); and 4-[4-[[(1R)-1-Phenylethyl]amino]-7H-pyrrolo[2,3-d]pyrimidin-6-yl]-phenol (PKI166, CAS 187724-61-4).

[0168] Exemplary mTOR inhibitors for use in conjunction with the chimeric Tim4 receptor compositions described herein may include, without limitation, rapamycin (Rapamune®), and analogs and derivatives thereof; SDZ-RAD; Temsirolimus (Torisel®; also known as CCI-779); Ridaforolimus (formally known as deferolimus, (1R,2R,4S)-4-[(2R)-2 [(1R,9S,12S,15R,16E,18R,19R,21R,23S,24E,26E,28Z,30S,32S,35R)-1,18-dihydroxy-19,30-dimethoxy-15,17,21,23,29,35-hexamethyl-2,3,10,14,20-pentaoxo-11,36-dioxa-4-azatricyclo[30.3.1.0.sup.4,9]hexatriaconta-16,24,26,28-tetraen-12-yl]propyl]-2-methoxycyclohexyl dimethylphosphinate, also known as AP23573 and MK8669, and described in PCT Publication No. WO 03/064383); Everolimus (Afinitor® or RAD001); Rapamycin (AY22989, Sirolimus®); Simapimod (CAS 164301-51-3); (5-{2,4-Bis[(3S)-3-methylmorpholin-4-yl]pyrido[2,3-d]pyrimidin-7-yl}-2-methoxyphenyl) methanol (AZD8055); 2-Amino-8-[trans-4-(2-hydroxyethoxy)cyclohexyl]-6-(6-methoxy-3-pyridinyl)-4-methyl-pyrido[2,3-d]pyrimidin-7 (8H)-one (PF04691502, CAS 1013101-36-4); and N.sup.2-[1,4-dioxo-[[4-(4-oxo-8-phenyl-4H-1-benzopyran-2-yl) morpholinium-4-yl]methoxy]butyl]-L-arginylglycyl-L-α-aspartylL-serine-, inner salt (SF1126, CAS 936487-67-1).

[0169] Exemplary Phosphoinositide 3-kinase (PI3K) inhibitors for use in conjunction with the chimeric Tim4 receptor compositions described herein may include, but are not limited to, 4-[2-(1H-Indazol-4-yl)-6-[[4-(methylsulfonyl) piperazin-1-yl]methyl]thieno[3,2-d]pyrimidin-4-yl]morpholine (also known as GDC 0941 and described in PCT Publication Nos. WO 09/036082 and WO 09/055730); 2-Methyl-2-[4-[3-methyl-2-oxo-8-(quinolin-3-yl)-2,3-dihydroimidazo[4,5-c]quinolin-1-yl]phenyl]propionitrile (also known as BEZ 235 or NVP-BEZ 235, and described in PCT Publication No. WO 06/122806); 4-(trifluoromethyl)-5-(2,6-dimorpholinopyrimidin-4-yl) pyridin-2-amine (also known as BKM120 or NVP-BKM120, and described in PCT Publication No. WO2007/084786); Tozasertib (VX680 or MK-0457, CAS 639089-54-6); (5Z)-5-[[4-(4-Pyridinyl)-6-quinolinyl]methylene]-2,4-thiazolidinedione (GSK1059615, CAS 958852-01-2); (1E,4S,4aR,5R,6aS,9aR)-5-(Acetyloxy)-1-[(di-2-propenylamino)methylene]-4,4a,5,6,6a,8,9,9a-octahydro-11-hydroxy-4-(methoxymethyl)-4a,6a-dimethyl-cyclopenta[5,6]naphtho[1,2-c]pyran-2,7,10 (1H)-trione (PX866, CAS 502632-66-8); and 8-Phenyl-2-(morpholin-4-yl)-chromen-4-one (LY294002, CAS 154447-36-6). Exemplary Protein Kinase B (PKB) or AKT inhibitors include, but are not limited to. 8-[4-(1-Aminocyclobutyl)phenyl]-9-phenyl-1,2,4-triazolo[3,4-f] [1,6]naphthyridin-3 (2H)-one (MK-2206, CAS 1032349-93-1); Perifosine (KRX0401); 4-Dodecyl-N-1,3,4-thiadiazol-2-yl-benzenesulfonamide (PHT-427, CAS 1191951-57-1); 4-[2-(4-Amino-1,2,5-oxadiazol-3-yl)-1-ethyl-7-[(3S)-3-piperidinylmethoxy]-1H-imidazo[4,5-c]pyridin-4-yl]-2-methyl-3-butyn-2-ol (GSK690693, CAS 937174-76-0); 8-(1-Hydroxyethyl)-2-methoxy-3-[(4-methoxyphenyl) methoxy]-6H-dibenzo[b,d]pyran-6-one (palomid 529, P529, or SG-00529); Tricirbine (6-Amino-4-methyl-8-(β-D-ribofuranosyl)-4H,8H-pyrrolo[4,3,2-de]pyrimido[4,5-

c]pyridazine); (αS)-α-[[[5-(3-Methyl-1H-indazol-5-yl)-3-pyridinyl]oxy]methyl]-benzeneethanamine (A674563, CAS 552325-73-2); 4-[(4-Chlorophenyl)methyl]-1-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-4-piperidinamine (CCT128930, CAS 885499-61-6); 4-(4-Chlorophenyl)-4-[4-(1H pyrazol-4-yl)phenyl]-piperidine (AT7867, CAS 857531-00-1); and Archexin (RX-0201, CAS 663232-27-7).

[0170] In certain embodiments, a tyrosine kinase inhibitor used in combination with chimeric Tim4 receptor modified cells is an anaplastic lymphoma kinase (ALK) inhibitor. Exemplary ALK inhibitors include crizotinib, ceritinib, alectinib, brigatinib, dalantercept, entrectinib, and lorlatinib.

[0171] In certain embodiments where chimeric Tim4 receptor modified cells are administered in combination with one or more additional therapies, the one or more additional therapies may be administered at a dose that might otherwise be considered subtherapeutic if administered as a monotherapy. In such embodiments, the chimeric Tim4 receptor composition may provide an additive or synergistic effect such that the one or more additional therapies can be administered at a lower dose. Combination therapy includes administration of a chimeric Tim4 receptor compositions as described herein before an additional therapy (e.g., 1 day to 30 days or more before the additional therapy), concurrently with an additional therapy (on the same day), or after an additional therapy (e.g., 1 day-30 days or more after the additional therapy). In certain embodiments, the chimeric Tim4 receptor modified cells are administered after administration of the one or more additional therapies. In further embodiments, the chimeric Tim4 receptor modified cells are administered 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 days after administration of the one or more additional therapies. In still further embodiments, the chimeric Tim4 receptor modified cells are administered within 4 weeks, within 3 weeks, within 2 weeks, or within 1 week after administration of the one or more additional therapies. Where the one or more additional therapies involves multiple doses, the chimeric Tim4 receptor modified cells may be administered after the initial dose of the one or more additional therapies, after the final dose of the one or more additional therapies, or in between multiple doses of the one or more additional therapies.

[0172] In certain embodiments, methods of the present disclosure include a depletion step. A depletion step to remove chimeric Tim4 receptors from the subject may occur after a sufficient amount of time for therapeutic benefit in order to mitigate toxicity to a subject. In such embodiments, the chimeric Tim4 receptor vector may include an inducible suicide gene, such as iCASP9, inducible Fas, or HSV-TK. Similarly, a chimeric Tim4 receptor vector may be designed for expression of a known cell surface antigen such as CD20 or truncated EGFR (SEQ ID NO:52) that facilitates depletion of transduced cells through infusion of an associated monoclonal antibody (mAb), for example, Rituximab for CD20 or Cetuximab for EGFR. Alemtuzumab, which targets CD52 present on the surface of mature lymphocytes, may also be used to deplete transduced B cells, T cells, or natural killer cells.

[0173] Subjects that can be treated by the compositions and methods of the present disclosure include animals, such as humans, primates, cows, horses, sheep, dogs, cats, mice, rats, rabbits, guinea pigs, or pigs. The subject may be male or female, and can be any suitable age, including infant, juvenile, adolescent, adult, and geriatric subjects.

[0174] The various embodiments described above can be combined to provide further embodiments. All of the U.S. patents, U.S. patent application publications, U.S. patent applications, foreign patents, foreign patent applications and non-patent publications referred to in this specification and/or listed in the Application Data Sheet, including U.S. Provisional Patent Application No. 62/649,491, filed Mar. 28, 2018, are incorporated herein by reference, in their entirety. Aspects of the embodiments can be modified, if necessary to employ concepts of the various patents, applications and publications to provide yet further embodiments.

[0175] These and other changes can be made to the embodiments in light of the above-detailed description. In general, in the following claims, the terms used should not be construed to limit the

claims to the specific embodiments disclosed in the specification and the claims, but should be construed to include all possible embodiments along with the full scope of equivalents to which such claims are entitled. Accordingly, the claims are not limited by the disclosure.

Claims

1. A polynucleotide encoding a chimeric Tim4 receptor comprising a single chain chimeric protein, the single chain chimeric protein comprising: a receptor binding domain comprising a Tim4 binding domain; an intracellular signaling domain comprising a CD28 costimulatory signaling domain and a CD3ζ signaling domain; and a transmembrane domain positioned between and connecting the receptor binding domain and the intracellular signaling domain.
2. The polynucleotide of claim 1, wherein the Tim4 binding domain comprises the amino acid sequence of SEQ ID NO:2 or amino acids 25-314 of SEQ ID NO:2.
3. The polynucleotide of claim 1, wherein the receptor binding domain further comprises an extracellular spacer domain positioned between the Tim4 binding domain and the transmembrane domain.
4. The polynucleotide of claim 3, wherein the extracellular spacer domain comprises an immunoglobulin hinge region, a hinge region of a type 1 membrane protein, a stalk region of a type II C-lectin, an immunoglobulin constant domain, or a fragment thereof.
5. The polynucleotide of claim 4, wherein the extracellular spacer domain comprises: (a) an IgG1, IgG2, IgG3, IgG4, IgA, or IgD hinge region; (b) a modified IgG4 hinge region comprising the amino acid sequence of SEQ ID NO: 3; (c) a stalk region of a type II C-lectin selected from CD23, CD69, CD72, CD94, NKG2A, and NKG2D; (d) a hinge region of a type 1 membrane protein selected from CD8a, CD4, CD28 and CD7; or (e) an immunoglobulin constant region domain selected from a CH1 domain, a CH2 domain, a CH3 domain, or any combination thereof.
6. The polynucleotide of claim 1, wherein the transmembrane domain comprises a Tim4, CD27, CD28, CD8, 4-1BB, OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, or B7-H3 transmembrane domain.
7. The polynucleotide of claim 6, wherein the transmembrane domain comprises a Tim4 transmembrane domain comprising the amino acid sequence of SEQ ID NO:28, a CD27 transmembrane domain comprising the amino acid sequence of SEQ ID NO:32, a CD28 transmembrane domain comprising the amino acid sequence of SEQ ID NO:29, a 4-1BB transmembrane domain comprising the amino acid sequence of SEQ ID NO:30, an OX40 transmembrane domain comprising the amino acid sequence of SEQ ID NO: 31, a CD30 transmembrane domain comprising the amino acid sequence of SEQ ID NO: 36, a CD40 transmembrane domain comprising the amino acid sequence of SEQ ID NO: 37, a PD-1 transmembrane domain comprising the amino acid sequence of SEQ ID NO: 38, an ICOS transmembrane domain comprising the amino acid sequence of SEQ ID NO: 33, a LFA-1 transmembrane domain comprising the amino acid sequence of SEQ ID NO:35, a CD2 transmembrane domain comprising the amino acid sequence of SEQ ID NO:34, or a CD7 transmembrane domain comprising the amino acid sequence of SEQ ID NO:39, a LIGHT transmembrane domain comprising the amino acid sequence of SEQ ID NO:40, a NKG2C transmembrane domain comprising the amino acid sequence of SEQ ID NO:41, or a B7-H3 transmembrane domain comprising the amino acid sequence of SEQ ID NO:42.
8. The polynucleotide of claim 1, wherein the CD28 costimulatory signaling domain comprises the amino acid sequence of SEQ ID NO:4 or 62.
9. The polynucleotide of claim 1, wherein the transmembrane domain is a CD28 transmembrane domain.
10. The polynucleotide of claim 1, wherein the CD3ζ signaling domain comprises the amino acid sequence of SEQ ID NO:63 or 19.

11. The polynucleotide of claim 1, wherein the chimeric Tim4 receptor comprises the amino acid sequence of SEQ ID NO:69 or amino acids 25-495 of SEQ ID NO:69.
12. The polynucleotide of claim 1, wherein the polynucleotide is codon optimized for expression in a host cell.
13. An expression vector comprising a polynucleotide of claim 1.
14. The expression vector of claim 13, wherein the expression vector is a viral vector.
15. The expression vector of claim 13, wherein the expression vector is a lentiviral vector.
16. A modified T cell comprising an expression vector according to claim 13.
17. The modified T cell of claim 16, wherein the T cell is a CD4+ T cell.
18. The modified T cell of claim 16, wherein the T cell is a CD8+ T cell.
19. A pharmaceutical composition comprising a modified T cell according to claim 16, and a pharmaceutically acceptable carrier, diluent, or excipient.