ENGINEERED CHIMERIC FUSION PROTEIN COMPOSITIONS AND METHODS OF USE THEREOF

CROSS REFERNCE

[0001] This application claims the benefit of U.S. Provisional Application No. 62/841,190, filed on April 30, 2019, U.S. Provisional Application No. 62/841,183, filed on April 30, 2019, U.S. Non-Provisional Application No. 16/827,381, filed on March 23, 2020, and U.S. Non-Provisional Application No. 16/827,302, filed on March 23, 2020, each of which is incorporated herein by reference in its entirety.

SEQUENCE LISTING

[0001.1] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on May 8, 2020, is named 56371-701_601_SL.txt and is 56,537 bytes in size.

BACKGROUND

[0002] Cellular immunotherapy is a promising new technology for fighting difficult to treat diseases, such as cancer, and persistent infections and also certain diseases that are refractory to other forms of treatment. A major breakthrough has come across with the discovery of CAR-T cell and their potential use in immunotherapy. CAR-T cells are T lymphocytes expressing a chimeric antigen receptor which helps target the T cell to specific diseased cells such as cancer cells, and can induce cytotoxic responses intended to kill the target cancer cell or immunosuppression and/or tolerance depending on the intracellular domain employed and co-expressed immunosuppressive cytokines. However, several limitations along the way has slowed the progress on CAR-T cells and dampened its promise in clinical trials.

[0003] Understanding the limitations of CAR-T cells is the key to leveraging the technology and continue innovations towards better immunotherapy models. Specifically, in T cell malignancies, CAR-T cells appear to have faced a major problem. CAR-T cells and malignant T cells share surface antigen in most T cell lymphomas (TCL), therefore, CAR-T cells are subject to cytotoxicity in the same way as cancer cells. In some instances, the CAR-T products may be contaminated by malignant T cells. Additionally, T cell aplasia is a potential problem due to prolonged persistence of the CAR-T cells. Other limitations include the poor ability for CAR-T cells to penetrate into solid tumors and the potent tumor microenvironment which acts to downregulate their anti-tumor potential. CAR-T cell function is also negatively influenced by the immunosuppressive tumor microenvironment (TME) that leads to endogenous T cell inactivation and exhaustion.

[0004] Myeloid cells, including macrophages, are cells derived from the myeloid lineage and belong to the innate immune system. They are derived from bone marrow stem cells which egress into the blood and can migrate into tissues. Some of their main functions include phagocytosis, the activation of T cell responses, and clearance of cellular debris and extracellular matrices. They also play an

successfully transfect or transduce a myeloid cell, or otherwise induce a genetic modification in a myeloid cell, with the purpose of augmenting a functional aspect of a myeloid cell, additionally, without compromising the cell's differentiation capability, maturation potential, and/or its plasticity.

[0011a] In one aspect, provided herein is a pharmaceutical composition comprising: (A) a population of myeloid cells comprising CD14+ cells comprising a recombinant polynucleic acid encoding a chimeric fusion protein (CFP), wherein the CFP comprises : (i) an extracellular domain comprising an antigen binding domain that binds to a CD5 antigen, (ii) a transmembrane domain operatively linked to the extracellular domain; and (iii) an intracellular domain with at least one intracellular signaling domain; and (B) a pharmaceutically acceptable excipient.

[0011b] In one aspect, provided herein is a use of the pharmaceutical composition as described herein in the manufacture of a medicament for treating a cancer in a human subject in need thereof.

[0011c] In one aspect, provided herein is a method of making a population of cells comprising CD14+ cells comprising a recombinant polynucleic acid encoding a chimeric fusion protein (CFP), the method comprising: (a) selecting CD14+ cells from a biological sample from a human subject, thereby obtaining a population of CD14+ cells isolated from the biological sample from a human subject, and (b) contacting a recombinant polynucleic acid comprising a sequence encoding a CFP to the population of CD14+ cells isolated from the biological sample of (a), thereby introducing the recombinant polynucleic acid into the population of CD14+ cells isolated from the biological sample of (a) and obtaining the population of cells with CD14+ cells comprising a recombinant polynucleic acid encoding a CFP, wherein the CFP comprises (i) an extracellular domain, (ii) a transmembrane domain operatively linked to the extracellular domain; and (iii) an intracellular domain with at least one intracellular signaling domain; wherein the CFP is capable of binding CD5.

[0011d] In one aspect, provided herein is the pharmaceutical composition as described herein for use in therapy.

INCORPORATION BY REFERENCE

[0012] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings.

[0014] **FIG. 1A** depicts a diagram showing some of the potentially engineerable functions of myeloid cells.

[0015] **FIG. 1B** depicts a diagram, indicating the presence of various cell types in different types of cancer. Macrophages are the most abundant cells in the depicted cancer types.

[0016] **FIG. 2A** depicts a schematic showing an exemplary chimeric receptor fusion protein (CFP) containing an extracellular binding domain, a transmembrane domain, a first intracellular signaling domain and a second intracellular signaling domain. The signaling domains can be derived from other receptors and be designed to elicit any number of cell functions.

[0017] **FIG. 2B** depicts a schematic showing an exemplary CFP containing an extracellular binding domain, a transmembrane domain, and an intracellular signaling domain (left), and a CFP containing an extracellular binding domain, a transmembrane domain, a first intracellular signaling domain, a second intracellular signaling domain, a third intracellular signaling domain, and one or more additional intracellular signaling domains. The signaling domains can be derived from other receptors and be designed to elicit any number of cell functions.

[0018] **FIG. 2C** depicts a schematic showing an exemplary CFP dimer containing an anti-CD5 extracellular binding domain, a transmembrane domain, and an intracellular signaling domain containing an intracellular domain derived from FcRγ fused to a PI3K recruitment domain.

[0019] **FIG. 2D** depicts a schematic showing an exemplary CFP dimer containing an extracellular antigen binding domain, a transmembrane domain, and an intracellular signaling domain containing a phagocytosis domain a PI3K recruitment domain and a pro-inflammation domain.

some embodiments, the one or more growth factors may be a macrophage activating cytokine, such as IFNγ. In some embodiments the polymeric structures are capable of sustained release of the one or more growth factors in an in vivo environment, such as in a solid tumor.

[0197] In some embodiments, the recombinant nucleic acid comprises a sequence encoding a homeostatic regulator of inflammation. In some embodiments, the homeostatic regulator of inflammation is a sequence in an untranslated region (UTR) of an mRNA. In some embodiments, the sequence in the UTR is a sequence that binds to an RNA binding protein. In some embodiments, translation is inhibited or prevented upon binding of the RNA binding protein to the sequence in an untranslated region (UTR). In some embodiments, the sequence in the UTR comprises a consensus sequence of WWWU(AUUUA)UUUW (SEQ ID NO: 35), wherein W is A or U. In some embodiments, the recombinant nucleic acid is expressed on a bicistronic vector.

[0198] In some embodiments, the target cell is a mammalian cell. In some embodiments, the target cell is a human cell. In some embodiments, the target cell comprises a cell infected with a pathogen. In some embodiments, the target cell is a cancer cell. In some embodiments, the target cell is a cancer cell that is a lymphocyte. In some embodiments, the target cell is a cancer cell that is an ovarian cancer cell. In some embodiments, the target cell is a cancer cell that is a breast cell. In some embodiments, the target cell is a cancer cell that is a pancreatic cell. In some embodiments, the target cell is a cancer cell that is a glioblastoma cell.

[0199] In some embodiments, the recombinant nucleic acid is DNA. In some embodiments, the recombinant nucleic acid is RNA. In some embodiments, the recombinant nucleic acid is mRNA. In some embodiments, the recombinant nucleic acid is an unmodified mRNA. In some embodiments, the recombinant nucleic acid is a modified mRNA. In some embodiments, the recombinant nucleic acid is a circRNA. In some embodiments, the recombinant nucleic acid is a tRNA. In some embodiments, the recombinant nucleic acid is a microRNA.

[0200] Also provided herein is a vector comprising a recombinant nucleic acid sequence encoding a CFP described herein. In some embodiments, the vector is viral vector. In some embodiments, the viral vector is retroviral vector or a lentiviral vector. In some embodiments, the vector further comprises a promoter operably linked to at least one nucleic acid sequence encoding one or more polypeptides. In some embodiments, the vector is polycistronic. In some embodiments, each of the at least one nucleic acid sequence is operably linked to a separate promoter. In some embodiments, the vector further comprises one or more internal ribosome entry sites (IRESs). In some embodiments, the vector further comprises a 5'UTR and/or a 3'UTR flanking the at least one nucleic acid sequence encoding one or more polypeptides. In some embodiments, the vector further comprises one or more regulatory regions.

dSR-CI	Ac-LDL, G^+ and G^-bacteria, glucan, laminarin
CD-36	Thrombospondin, Collagen, AGE, Apoptotic cells, Ox-LDL, PfEMP protein on plasmodium infected RBC Diacylated lipids on bacteria
SR-BI	AGE-modified proteins, Apoptotic cells Ox-LDL
CLA-I/human SR-BI	Apoptotic cells,Ox-LDL, LPS, Hepatitis C virus E2 glycoprotein
gp-340	Surfactant protein-A, surfactant protein-D, G^+ and G^-bacteria Influenza A virus, gp-120

(ND, not defined)

[0284] In some embodiments, the recombinant nucleic acid encodes a chimeric antigenic receptor for phagocytosis (CAR-P). In some embodiments, the recombinant nucleic acid encodes a phagocytic receptor (PR) fusion protein.

[0285] In some embodiments, the ICD of a CFP encoded by the recombinant nucleic acid comprises a domain from a protein selected from the group consisting of TNFR1, CD40, MDA5, lectin, dectin 1, mannose receptor (CD206), scavenger receptor A1 (SRA1), MARCO, CD36, CD163, MSR1, SCARA3, COLEC12, SCARA5, SCARB1, SCARB2, CD68, OLR1, SCARF1, SCARF2, CXCL16, STAB1, STAB2, SRCRB4D, SSC5D, CD205, CD207, CD209, RAGE, CD14, CD64, F4/80, CCR2, CX3CR1, CSF1R, Tie2, HuCRIg(L), and CD169 receptor.

[0286] In some embodiments, the ICD comprises the signaling domain derived from any one or more of: lectin, dectin 1, mannose receptor (CD206), scavenger receptor A1 (SRA1), MARCO (Macrophage Receptor with Collagenous Structure, aliases: SRA6, SCARA2), CD36 (Thrombospondin receptor, aliases: Scavenger Receptor class B, member 3), CD163 (Scavenger receptor, cysteine rich-type 1), MSR1, SCARA3, COLEC12 (aliases: Scavenger Receptor With C-Type Lectin, SCARA4, or Collectin 12), SCARA5, SCARB1, SCARB2, CD68 (SCARD, microsialin), OLR1 (Oxidized Low Density Lipoprotein Receptor 1, LOX1, or C-Type Lectin Domain Family 8 Member A), SCARF1, SCARF2, SRCRB4D, SSC5D, and CD169 (aliases, Sialoadhesin receptor, SIGLEC1).

[0287] In some embodiments, the recombinant nucleic acid encodes, for example, an intracellular domain of human MARCO. The PR subunit can compriss an intracellular domain having a 44 amino acid ICD of human MARCO having an amino acid sequence: MRNKKILKEDELLSETQQAAFHQIAMEPFEINVPKPKRRNGVNF (SEQ ID NO: 36). In some embodiments the PR

subunit comprises a variant which is at least 70%, 75%, 80%, 85%, 90% or 95% identical to the intracellular domain of MARCO. In some embodiments, the PR comprises a transmembrane region of human MARCO.

[0288] In some embodiments, the recombinant nucleic acid encodes an intracellular domain of human SRA1. The CFP comprises an intracellular domain having a 50 amino acid ICD of human SRA1 having an amino acid sequence: MEQWDHFHNQQEDTDSCSESVKFDARSMTA LLPPNPKNSPSLQEKLKSFK (SEQ ID NO: 37). In some embodiments the PR subunit comprises a variant which is at least 70%, 75%, 80%, 85%, 90% or 95% identical to the intracellular domain of human SRA1. The intracellular region of SRA has a phosphorylation site.

[0289] In some embodiments, the CFP comprises a transmembrane region of human SRA1.

[0290] In some embodiments, the recombinant nucleic acid comprises a sequence encoding an intracellular domain of CD36. In some embodiments, the recombinant nucleic acid comprises a sequence encoding TM domain of CD36. Naturally occurring full length CD36 has two TM domains and two short intracellular domains, and an extracellular domain of CD36 binds to oxidized LDL. Both of the intracellular domains contain pairs of cysteines that are fatty acid acylated. It lacks known signaling domains (e.g. kinase, phosphatase, g-protein binding, or scaffolding domains). N-terminal cytoplasmic domain is extremely short (5–7 amino acid residues) and is closely associated with the internal leaflet of the plasma membrane. The carboxy-terminal domain contains 13 amino acids, containing a CXCX5K motif homologous to a region in the intracellular domain of CD4 and CD8 that is known to interact with signaling molecules. The intracellular domain of CD36 is capable of assembling a signaling complex that activates lyn kinases, MAP kinases and Focal Adhesion Kinases (FAK), and inactivation of src homology 2-containing phosphotyrosine phosphatase (SHP-2). Members of the guanine nucleotide exchange factors (GEFs) have been identified as potential key signaling intermediates.

[0291] In some embodiments, the recombinant nucleic acid encodes for example, an intracellular domain of human SCARA3. The CFP may comprise an intracellular domain having a 56 amino acid ICD of human SCARA3 having an amino acid sequence: MKVRSAGGDGDALCVTEEDL AGDDEDMPTFPCTQKGRPGPRCSRCQKNLS LHTSVR (SEQ ID NO: 38). In some embodiments, the CFP comprises a variant which is at least 70%, 75%, 80%, 85%, 90% or 95% identical to an intracellular domain of human SCARA3. In some embodiments the CFP comprises a TM domain of SCARA3.

[0292] In some embodiments, the TM domain of a PR is about 20-30 amino acids long. In some embodiments, the TM domain comprises multiple transmembrane spans. In some embodiment, the TM domain comprises about 20-30, 30-40, 40-50, 50-60, 60-70, 70-80, 80-90, 90-100, 100-150, or

by a linker. In some embodiments, where there are more than one scFv at the extracellular antigen binding domain, the more than scFvs are linked with each other by linkers.

[0376] In some embodiments, linkers are short peptide sequences.

[0377] The linker may be as simple as a covalent bond, or it may be a polymeric linker many atoms in length. In certain embodiments, the linker is a polypeptide or based on amino acids. In other embodiments, the linker is not peptide-like. In certain embodiments, the linker is a covalent bond (e.g., a carbon-carbon bond, disulfide bond, carbon-heteroatom bond, etc.).

[0378] In some embodiments, the linker is an amino acid or a plurality of amino acids (e.g., a peptide or protein). In some embodiments, the linker is a bond (e.g., a covalent bond), an organic molecule, group, polymer, or chemical moiety. In some embodiments, the linker is about 3 to about 104 (e.g., 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, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100) amino acids in length. In some embodiments the linkers are stretches of Glycine and one or more Serine residues. Other amino acids preferred for short peptide linkers include but are not limited to threonine (Thr), serine (Ser), proline (Pro), glycine (Gly), aspartic acid (Asp), lysine (Lys), glutamine (Gln), asparagine (Asn), and alanine (Ala) arginine (Arg), phenylalanine (Phe), glutamic acid (Glu). Of these Pro, Thr, and Gln are frequently used amino acids for natural linkers. Pro is a unique amino acid with a cyclic side chain which causes a very restricted conformation. Pro-rich sequences are used as interdomain linkers, including the linker between the lipoyl and E3 binding domain in pyruvate dehydrogenase ($GA_2PA_3PAKQEA_3PAPA_2KAEAPA_3PA_2KA$ (SEQ ID NO: 39)). For the purpose of the disclosure, the empirical linkers may be flexible linkers, rigid linkers, and cleavable linkers. Sequences such as (G4S)x (where x is multiple copies of the moiety, designated as 1, 2, 3, 4, and so on) (SEQ ID NO: 40) comprise a flexible linker sequence. Other flexible sequences used herein include several repeats of glycine, e.g., (Gly)6 (SEQ ID NO: 41) or (Gly)8 (SEQ ID NO: 42). On the other hand, a rigid linker may be used, for example, a linker (EAAAK)x, where x is an integer, 1, 2, 3, 4 etc. (SEQ ID NO: 43) gives rise to a rigid linker. Various linker lengths and flexibilities between domains or subunits of the fusion proteins provided herein can be employed, e.g., ranging from very flexible linkers of the form (GGGS)n (SEQ ID NO: 44), (GGGGS)n (SEQ ID NO: 45), and (G)n to more rigid linkers of the form (EAAAK)n (SEQ ID NO: 46), (SGGS)n (SEQ ID NO: 47), SGSETPGTSESATPES (SEQ ID NO: 48) (see, e.g., Guilinger JP, Thompson DB, Liu DR. Fusion of catalytically inactive Cas9 to FokI nuclease improves the specificity of genome modification. Nat. Biotechnol. 2014; 32(6): 577-82; the entire contents are incorporated herein by reference) and (XP)n) in order to achieve the optimal length. In some embodiments, n is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15. In some embodiments, the linker comprises a (GGS)n motif, wherein

n is 1, 3, or 7 (SEQ ID NO: 49). In some embodiments, the linker comprises amino acid sequence SGGGGSG (SEQ ID NO: 11). In some embodiments, the linker comprises amino acid sequence GSGS (SEQ ID NO: 13).

[0379] In some embodiments the linkers are flexible. In some embodiments the linkers comprise a hinge region. 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. 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. In certain embodiments, an extracellular spacer domain is an immunoglobulin hinge region (e.g., IgGl, 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. In some embodiments, a linker or a spacer used herein comprises an IgG4 hinge region, having a sequence: ESKYGPPCPPCP (SEQ ID NO: 50). In some embodiments, the hinge region comprises a hinge or a spacer comprising a sequence present in the extracellular regions of type 1 membrane proteins, such as CD8a, CD4, CD28 and CD7, which may be wild-type or variants thereof. In some 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. In some embodiments the spacer or the linker may be further modified by post-translation modifications, such as glycosylation.

[0380] In some 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. In yet further embodiments, an extracellular spacer domain may be derived from scavenger receptor MERTK.

[0381] In some embodiments, the linker comprises at least 2, or at least 3 amino acids. In some embodiments, the linker comprises 4 amino acids. In some embodiments, the linker comprises 5 amino acids. In some embodiments, the linker comprises 6 amino acids. In some embodiments, the linker comprises 7 amino acids. In some embodiments, the linker comprises 8 amino acids. In some embodiments, the linker comprises 9 amino acids. In some embodiments, the linker comprises 8 amino acids. In some embodiments, the linker comprises 10 amino acids. In some embodiments the linker comprises greater than 10 amino acids. In some embodiments, the linker comprises 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acids. In some embodiments there are 12 or more amino acids in the linker. In some embodiments, there are 14 or more amino acids in the linker. In some embodiments, there are 15 or more amino acids in the linker.

Other Fusion proteins for Enhancement of Phagocytosis

[00616] In some embodiments, the cytokine is selected from the group consisting of IL-1, IL3, IL5, IL-6, IL-12, IL-13, IL-23, TNF, IL-18, IL-23, IL-27, CSF, MCSF, GMCSF, IL17, IP-10, RANTES, an interferon.

[00617] In some embodiments, the recombinant nucleic acid comprises a homeostatic regulator of inflammation.

[00618] In some embodiments, the homeostatic regulator of inflammation is a sequence in an untranslated region (UTR) of an mRNA. In some embodiments, the sequence in the UTR is a sequence that binds to an RNA binding protein. In some embodiments, translation is inhibited or prevented upon binding of the RNA binding protein to the sequence in an untranslated region (UTR). In some embodiments, the sequence in the UTR comprises a consensus sequence of WWWU(AUUUA)UUUW (SEQ ID NO: 35), wherein W is A or U.

[00619] In some embodiments, the recombinant nucleic acid is expressed on a bicistronic vector.

[00620] In some embodiments, the target cell is a mammalian cell. In some embodiments, the target cell is a human cell. In some embodiments, the target cell comprises a cell infected with a pathogen. In some embodiments, the target cell is a cancer cell. In some embodiments, the target cell is a cancer cell that is a lymphocyte. In some embodiments, the target cell is a cancer cell that is an ovarian cancer cell. In some embodiments, the target cell is a cancer cell that is an ovarian pancreatic cell. In some embodiments, the target cell is a cancer cell that is a glioblastoma cell.

[00621] In some embodiments, the recombinant nucleic acid is DNA. In some embodiments, the recombinant nucleic acid is RNA. In some embodiments, the recombinant nucleic acid is mRNA. In some embodiments, the recombinant nucleic acid is a circRNA. In some embodiments, the recombinant nucleic acid is a tRNA. In some embodiments, the recombinant nucleic acid is a microRNA.

[00622] Provided herein is a vector comprising the composition described above. In some embodiments, vector is viral vector. In some embodiments, the viral vector is retroviral vector or a lentiviral vector. In some embodiments, the vector further comprises a promoter operably linked to at least one nucleic acid sequence encoding one or more polypeptides. In some embodiments, the vector is polycistronic. In some embodiments, each of the at least one nucleic acid sequence is operably linked to a separate promoter. In some embodiments, the vector further comprises one or more internal ribosome entry sites (IRESs). In some embodiments, the vector further comprises a 5'UTR and/or a 3'UTR flanking the at least one nucleic acid sequence encoding one or more polypeptides. In some embodiments, the vector further comprises one or more regulatory regions.

171. The composition of embodiment 169 or 170, wherein the sequence in the UTR comprises a consensus sequence of WWWU(AUUUA)UUUW (SEQ ID NO: 35), wherein W is A or U.

172. The composition of any one of the preceding embodiments, wherein the recombinant nucleic acid is expressed on a bicistronic vector.

173. The composition of any one of the preceding embodiments, wherein the target cell is a mammalian cell.

174. The composition of any one of the preceding embodiments, wherein the target cell is a human cell.

175. The composition of any one of the preceding embodiments, wherein the target cell comprises a cell infected with a pathogen.

176. The composition of any one of the preceding embodiments, wherein the target cell is a cancer cell.

177. The composition of any one of the preceding embodiments, wherein the target cell is a cancer cell that is a lymphocyte.

178. The composition of any one of the preceding embodiments, wherein the target cell is a cancer cell that is an ovarian cancer cell.

179. The composition of any one of the preceding embodiments, wherein the target cell is a cancer cell that is an ovarian pancreatic cell.

180. The composition of any one of the preceding embodiments, wherein the target cell is a cancer cell that is an glioblastoma cell.

181. The composition of any one of the preceding embodiments, wherein the recombinant nucleic acid is DNA.

182. The composition of any one of the preceding embodiments, wherein the recombinant nucleic acid is RNA.

183. The composition of any one of the preceding embodiments, wherein the recombinant nucleic acid is mRNA.

184. The composition of any one of the preceding embodiments, wherein the recombinant nucleic acid is a circRNA.

185. The composition of any one of the preceding embodiments, wherein the recombinant nucleic acid is a tRNA.

186. The composition of any one of the preceding embodiments, wherein the recombinant nucleic acid is a microRNA.

187. A vector comprising the composition of any one of embodiments 1-186.

and/or tumor cell killing in vitro and/or in vivo. A microparticle based phagocytosis assay was used to examine changes in phagocytosis. Briefly, streptavidin coupled fluorescent polystyrene microparticles (6 µm diameter) are conjugated with biotinylated recombinantly expressed and purified cancer ligand. Myeloid cells expressing the novel CFP were incubated with the ligand coated microparticles for 1-4 h and the amount of phagocytosis was analyzed and quantified using flow cytometry. Plasmid or lentiviral constructions of the designer CFPs are then prepared and tested in macrophage cells for cancer cell lysis.

[0707] Exemplary functional domain containing CFPs are described in the following sections.

Example 2. Generation of recombinant CFP having scavenger receptor ECD, TM and ICD (SR-CAR)

[0708] A CFP designed for the purpose of the present application is modular, having an extracellular target binding domain primarily comprising of an scFv, or an Fab region or V_{HH} domain, that can bind to a target, e.g. CD5, a short hinge, a transmembrane domain, and an intracellular domain comprising one or two or more signaling domains (**FIGs. 2A-2C**). Additionally, the extracellular domain can be designed to bind to a single or a multiple target (**FIG. 3**). An exemplary design of a phagocytic scavenger receptor is illustrated in **FIGs 4A** and **4B**. The recombinant nucleic acid encoding the CFP is constructed as follows: a signal peptide sequence which encodes for the membrane localization signal for the recombinant protein is placed upstream of the coding sequence of the extracellular antigen binding domain. Then the nucleic acid sequence encoding extracellular antigen binding scFv domain is synthesized and cloned into an expression vector, downstream of the signal peptide sequence. The CFP is made up of the sequence encoding the extracellular domain, the TM domain and the intracellular domain of the scavenger receptor of choice is ligated at the 3'end of the scFv, and preferably with a linker peptide sequence in between the 3'end of the scFv and the 5'end of the scavenger receptor ECD. An exemplary linker peptide is GGGS (SEQ ID NO: 44), and optionally the linker is a sequence that has two or more repeats of the tetramer. Once expressed, the scavenger receptor TM domain is incorporated in the cell membrane.

[0709] Lentiviral constructs of SR-CAR are prepared and purified for use in transduction studies.

Example 3. Expression and functional analysis of the recombinant CFG (SR-CAR)

[0710] To test the function of the CFP, human macrophages are transduced with pCMV-SRCAR using lipofectamine. In parallel, control cells are transfected with an empty vector. After stabilization of the cells for 48 hours, the cells are subjected to phagocytosis assay. **FIG. 4C** shows the expected result in an in vitro phagocytosis assay. Human primary macrophage transduced with control empty

in glycosylation state of GAPDH. Exemplary GAPDH ARE binding sequence is: WWWU(AUUUA)UUUW (SEQ ID NO: 35) (where W is A or U). GAPDH is an mRNA binding protein. When GAPDH remains bound to the mRNA, the mRNA is prevented from transcription and is therefore silent. Change of glyocylation activates the GAPDH and dissociates it from the mRNA. This triggers transcription of the mRNA. Hypoxic conditions can trigger the change in glycosylation state. Therefore this construct can be activated an expressed in hypoxic conditions, such as in tumor microenvironment.

Example 25. Design and functional analysis of recombinant chimeric protein expressing an FcRalpha receptor.

[00775] In this example, an exemplary design of a vector expressing the cancer antigen targeted receptor with a macrophage specific expression is described. In this exemplary construct the extracellular antigen binding domain is a scFv that can specifically bind to a cancer antigen. The extracellular transmembrane and intracellular domains of an FcRα chain is fused with the scFv through a linker (CAR-FcRγ. The FcRγ chain heterodimerizes with endogenous transmembrane domain of FcRγ which are expressed specifically in macrophages. **FIG. 31A** shows illustrative schematics of the cancer targeting FcRγ receptor.

[00776] **FIG. 31B** shows the expected result of relative phagocytoses of the dye loaded target tumor cells, quantified by flow cytometry. Cells expressing the CAR-FcRγ exhibit enhanced phagocytosis over cells expressing the control vector. **FIG. 31C** shows the expected result of relative lysis of the dye loaded target tumor cells. Cells expressing FcRγ exhibit higher target cell lysis over cells expressing the control vector. When NSG mice are injected with tumor cells expressing luciferase on day 0. Mice are either untreated or injected with human primary macrophage co-expressing CAR-FcRγ and a survival curve is generated (**Fig. 31D**).

Example 26. Design and functional analysis of recombinant CFP (CAR-TREM)

[00777] In this example, an exemplary design of a vector expressing the cancer antigen targeted receptor with a myeloid cell specific expression is described. In this exemplary construct the extracellular antigen binding domain is a scFv, which can specifically bind to a cancer antigen. The extracellular transmembrane and intracellular domains of a TREM chain are fused with the scFv through a linker (CAR-TREM□. The TREM chain heterodimerizes with endogenous DAP12 transmembrane domain of DAP12 and generates pro-inflammatory signal that promote phagocytosis. **FIG. 32A** shows illustrative schematics of the cancer targeting CAR-TREM.

[00778] **FIG. 32B** shows the expected result of relative phagocytoses of the dye loaded target tumor cells, quantified by flow cytometry. Cells expressing the CAR-TREM exhibit enhanced phagocytosis over cells expressing the control vector. **FIG. 32C** shows the expected result of relative lysis of the

[00780] In this example, specific enhancements of the constructs are described. The designs described in this section can be adapted to any of the constructs described in the disclosure, as is understood by one of skill in the art. As shown in **FIG. 34A** (upper panel), the design exemplifies a generic construct of a CAR, with insertion of an AU rich element (ARE) sequence in the 3' UTR that result in RNA binding proteins (eg GAPDH) binding to mature mRNA strand preventing translation. The GAPDH binding sequence can be designated as WWWU(AUUUA)UUUW (SEQ ID NO: 35), where W is A or U. Glycolysis results in the uncoupling of the RNA binding proteins (eg GAPDH) allowing for mRNA strand translation.

[00781] Other exemplary ARE sequences can be used to replace the GAPDH binding sequence. Such sequences may be the ARE sequence that bind to ADK, ALDH18A1, ALDH6A1, ALDOA, ASS1, CCBL2, CS, DUT, ENO1,FASN, FDPS, GOT2,HADHB, HK2, HSD17B10, MDH2, NME1, NQ01, PKM2, PPP1CC, SUCLG1, TP11, GAPDH, LDH. The modified ARE is used at the 3'end of the expression constructs for a CARP, as shown in **FIG. 10A** (lower panel).

[00782] **FIG 34B** exemplifies a generic construct that is expresses a proinflammatory protein, either as part of a monocistronic construct or a polysictronic construct for expression a CARP along with the expression of the pro-inflammatory protein described herein; or the construct described herein can be used for co-expression with any other chimeric antigenic construct. Not shown in FIG. 34B, are the remaining parts of the construct of the section shown is used as part of the construct encoding the CAR proteins. Any protein can be expressed as "protein of interest," which includes but is not limited to Interleukin 12, Type 2 interferon, Type 1 interferon, proinflammatory mediators, soluble factors, granules, lytic proteins, Nitric oxide, etc etc – anything that triggers anti-tumor activity (anti-PD1 antibody, etc), FMLP ligand to attract neutrophils. The 3'end of the coding sequences contain one or more ARE sequences that can bind to any one of ADK, ALDH18A1, ALDH6A1, ALDOA, ASS1, CCBL2, CS, DUT, ENO1,FASN, FDPS, GOT2,HADHB, HK2, HSD17B10, MDH2, NME1, NQ01, PKM2, PPP1CC, SUCLG1, TP11, GAPDH, LDH etc

[00783] **FIG. 34C** shows several exemplary modular constructs applicable to the designs disclosed herein. **FIG. 34C** depicts an exemplary construct where two or more coding sequences are separated by a T2A or a P2A cleavage site encoded by the construct ((i)). Endogenous proteins cleave the newly translated proteins at the site, releasing two independent proteins, generated from a single construct. **FIG. 34C** also depicts an exemplary bicistronic construct comprising two coding sequences, one for the fused protein comprising the antigen specific binder and an intracellular domain (ICD), under the influence of promoter P1; and the other coding the inflammatory gene under the influence of the second promoter P2, and the 3'regulatory element. The two coding sequences are designed to be in opposite

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:

(A) a population of myeloid cells comprising CD14+ cells comprising a recombinant polynucleic acid encoding a chimeric fusion protein (CFP), wherein the CFP comprises :

(i) an extracellular domain comprising an antigen binding domain that binds to a CD5 antigen, (ii) a transmembrane domain operatively linked to the extracellular domain; and

(iii) an intracellular domain with at least one intracellular signaling domain; and

(B) a pharmaceutically acceptable excipient.

2. The pharmaceutical composition of claim 1, wherein the CD5 antigen binding domain is an antibody domain or a fragment thereof, a single chain variable fragment (ScFv), an Fab, a single domain antibody, a nanobody, a V_H domain, a V_L domain, a V_{HH} domain, a VNAR domain, a bispecific antibody, a diabody or a combination thereof, wherein the antigen binding domain of the CFP binds to CD5 antigen with an affinity of 250 nM or less.

3. The pharmaceutical composition of claim 1, wherein the intracellular domain further comprises one or more additional intracellular signaling domains.

4. The pharmaceutical composition of claim 3, wherein the one or more additional intracellular signaling domains comprises an intracellular signaling domain from a receptor other than Megf10, MerTk, Fc α R, and Bai1.

5. The pharmaceutical composition of claim 1, wherein the intracellular domain of the CFP comprises an intracellular signaling domain of an FcR gamma or a fragment thereof.

6. The pharmaceutical composition of claim 5, wherein the intracellular domain comprises an intracellular signaling domain with at least 90% sequence identity to SEQ ID NO: 3.

7. The pharmaceutical composition of claim 3, wherein the one or more additional intracellular signaling domains comprises a proinflammatory signaling domain, wherein the proinflammatory signaling domain is a PI3K recruitment domain.

8. The pharmaceutical composition of claim 7, wherein the PI3K recruitment domain comprises a sequence with at least 90% sequence identity to SEQ ID NO: 4.

9. The pharmaceutical composition of claim 3, wherein the one or more additional intracellular signaling domains comprise an intracellular signaling domain from an intracellular signaling domain of CD40.

10. The pharmaceutical composition of claim 9, wherein the intracellular signaling domain from an intracellular signaling domain of CD40 comprises a sequence with at least 90% sequence identity to SEQ ID NO: 5

11. The pharmaceutical composition of claim 1, wherein the transmembrane domain comprises a CD8 transmembrane domain, comprising an amino acid sequence with at least 90% sequence identity to SEQ ID NO: 6 or SEQ ID NO: 7 or SEQ ID NO: 29; or

wherein the transmembrane domain comprises a transmembrane domain from CD2, CD28 or CD68, comprising an amino acid sequence with at least 90% sequence identity to SEQ ID NO: 34, SEQ ID NO: 18 or SEQ ID NO: 19.

12. The pharmaceutical composition of claim 1 or 11, wherein the extracellular domain further comprises a hinge domain from CD8, wherein the hinge domain is operatively linked to the transmembrane domain and the CD5 binding domain.

13. The pharmaceutical composition of claim 1, wherein the recombinant polynucleic acid is an mRNA.

14. The pharmaceutical composition of claim 1, wherein the CFP comprises a sequence having at least 90% sequence identity to SEQ ID NO: 14.

15. The pharmaceutical composition of claim 1, wherein the pharmaceutical composition comprises at least 6x10^6 myeloid cells.

16. Use of the pharmaceutical composition of any one of claims 1-15 in the manufacture of a medicament for treating a cancer in a human subject in need thereof.

17. The use of claim 21, wherein the cancer is T cell lymphoma.

18. A method of making a population of cells comprising CD14+ cells comprising a recombinant polynucleic acid encoding a chimeric fusion protein (CFP), the method comprising:

(a) selecting CD14+ cells from a biological sample from a human subject, thereby obtaining a population of CD14+ cells isolated from the biological sample from a human subject, and

(b) contacting a recombinant polynucleic acid comprising a sequence encoding a CFP to the population of CD14+ cells isolated from the biological sample of (a), thereby introducing the recombinant polynucleic acid into the population of CD14+ cells isolated from the biological sample of (a) and obtaining the population of cells with CD14+ cells comprising a recombinant polynucleic acid encoding a CFP, wherein the CFP comprises (i) an extracellular domain, (ii) a transmembrane domain operatively linked to the extracellular

domain; and (iii) an intracellular domain with at least one intracellular signaling domain; wherein the CFP is capable of binding CD5.

19. The method of claim 18, wherein selecting CD14+ cells comprises negative selection.

20. The method of claim 18, wherein negative selection comprises contacting the biological sample with an antibody targeting a cell surface molecule selected from the group consisting of CD16, CD3, CD8, CD11c, CD40, CD206 and any combination thereof; and

wherein selecting CD14+ cells comprises selecting CD14+ cells without contacting the biological sample with an antibody targeting CD14.

21. The method of claim 18, wherein selecting CD14+ cells comprises selecting CD14+ cells by density centrifugation.

22. The method of claim 18, wherein selecting CD14+ cells comprises selecting CD14+ cells by elutriation.

23. The method of claim 18, wherein the population of cells with CD14+ cells comprising a recombinant polynucleic acid encoding a CFP is a population of cells with at least $1x10^6$ CD14+/CD16- cells containing a recombinant polynucleic acid encoding a CFP.

24. The method of claim 18, wherein selecting CD14+ cells comprises fluorescence activated cell sorting (FACS) or magnetic activated cell sorting (MACS).

25. The method of claim 18, wherein the recombinant polynucleic acid is RNA.

26. The method of claim 25, wherein the RNA is mRNA.

27. The method of claim 18, wherein contacting a recombinant polynucleic acid comprising a sequence encoding a CFP into the population of CD14+ cells isolated from the biological sample of (a) comprises electroporating the population of CD14+ cells isolated from the biological sample of (a) in the presence of the recombinant polynucleic acid.

28. The method of claim 18, further comprising expressing the CFP in the CD14+ cells of (b) containing a recombinant polynucleic acid encoding a CFP.

29. The method of claim 18, wherein the population of cells with CD14+ cells containing a recombinant polynucleic acid encoding a CFP comprises at least $1x10^6$ cells.

30. The method of claim 18, wherein the biological sample from a human subject is a peripheral blood sample or an apheresis sample.

31. The method of claim 18, wherein the intracellular domain comprises at least two intracellular signaling domains.

32. The method of claim 18, wherein the intracellular signaling domain comprises an intracellular signaling domain derived from FcγR or FcεR.
33. The method of claim 32, wherein the intracellular domain comprises a PI3K recruitment domain.
34. The method of claim 18, wherein the transmembrane domain comprise a CD8 transmembrane domain, a CD28 transmembrane domain, a CD2 transmembrane domain, or a CD68 transmembrane domain.
35. The method of claim 18, wherein at least 50% of the population of CD14+ cells isolated from the biological sample of (a) express the CFP 24 hours after (b).
36. The pharmaceutical composition of any one of claims 1-15 for use in therapy.