

Background

[0004] Various strategies are available for stimulating or expanding cell populations *in vitro* or *ex vivo*, including for expanding antigen-specific T cells in vitro for use in adoptive cellular immunotherapy or cancer therapy. Improved strategies are needed for stimulating or expanding cell populations, including for research, diagnostic and therapeutic purposes. Provided are reagents, methods, and articles of manufacture and kits that meet such needs.

Summary

[0005] Provided herein is a method of expanding cells, including incubating an input composition containing cells expressing a recombinant antigen receptor containing an extracellular antigen-binding domain that specifically binds or recognizes an antigen with a plurality of particles, each of the plurality of particles containing a binding molecule that specifically binds to the antigen-binding domain, wherein binding of the binding molecule to the antigen-binding domain induces expansion of the cells containing the recombinant antigen receptor, thereby producing an output composition containing expanded cells. In some examples, the recombinant antigen receptor is a chimeric antigen receptor (CAR). In some examples, the antigen-binding domain contains an antibody or antigen-binding fragment thereof. In some cases, the antigen-binding fragment is or contains a single chain antibody fragment. In some examples, the antigen-binding fragment thereof contains antibody variable regions joined by a flexible linker. In some of any such examples, the antigen-binding fragment thereof is or contains an scFv.

[0006] In some of any such examples, the antigen is selected from among $\alpha v \beta 6$ integrin ($\alpha v \beta 6$ integrin), B cell maturation antigen (BCMA), B7-H3, B7-H6, carbonic anhydrase 9 (CA9, also known as CAIX or G250), a cancer-testis antigen, cancer/testis antigen 1B (CTAG, also known as NY-ESO-1 and LAGE-2), carcinoembryonic antigen (CEA), a cyclin, cyclin A2, C-C Motif Chemokine Ligand 1 (CCL-1), CD19, CD20, CD22, CD23, CD24, CD30, CD33, CD38, CD44, CD44v6, CD44v7/8, CD123, CD138, CD171, chondroitin sulfate proteoglycan 4 (CSPG4), epidermal growth factor protein (EGFR), type III epidermal growth factor receptor mutation (EGFR vIII), epithelial glycoprotein 2 (EPG-2), epithelial glycoprotein 40 (EPG-40), ephrinB2, ephrin receptor A2 (EPHa2), estrogen receptor, Fc receptor like 5 (FCRL5; also known as Fc receptor homolog 5 or FCRH5), fetal acetylcholine receptor (fetal AchR), a folate binding protein (FBP), folate receptor alpha, ganglioside GD2, O-acetylated GD2 (OGD2),

ganglioside GD3, glycoprotein 100 (gp100), G Protein Coupled Receptor 5D (GPRC5D), Her2/neu (receptor tyrosine kinase erb-B2), Her3 (erb-B3), Her4 (erb-B4), erbB dimers, Human high molecular weight-melanoma-associated antigen (HMW-MAA), hepatitis B surface antigen, Human leukocyte antigen A1 (HLA-A1), Human leukocyte antigen A2 (HLA-A2), IL-22 receptor alpha(IL-22Ra), IL-13 receptor alpha 2 (IL-13Ra2), kinase insert domain receptor (kdr), kappa light chain, L1 cell adhesion molecule (L1-CAM), CE7 epitope of L1-CAM, Leucine Rich Repeat Containing 8 Family Member A (LRRC8A), Lewis Y, Melanoma-associated antigen (MAGE)-A1, MAGE-A3, MAGE-A6, mesothelin, c-Met, murine cytomegalovirus (CMV), mucin 1 (MUC1), MUC16, natural killer group 2 member D (NKG2D) ligands, melan A (MART-1), neural cell adhesion molecule (NCAM), oncofetal antigen, Preferentially expressed antigen of melanoma (PRAME), progesterone receptor, a prostate specific antigen, prostate stem cell antigen (PSCA), prostate specific membrane antigen (PSMA), Receptor Tyrosine Kinase Like Orphan Receptor 1 (ROR1), survivin, Trophoblast glycoprotein (TPBG also known as 5T4), tumor-associated glycoprotein 72 (TAG72), vascular endothelial growth factor receptor (VEGFR), vascular endothelial growth factor receptor 2 (VEGFR2), Wilms Tumor 1 (WT-1), a pathogen-specific or pathogen-expressed antigen, or an antigen associated with a universal tag, and/or biotinylated molecules, and/or molecules expressed by HIV, HCV, HBV or other pathogens.

[0007] In some of any such examples, the antigen is selected from among ROR1, B cell maturation antigen (BCMA), carbonic anhydrase 9 (CAIX), Her2/neu (receptor tyrosine kinase erbB2), LI-CAM, CD19, CD20, CD22, mesothelin, CEA, and hepatitis B surface antigen, anti-folate receptor, CD23, CD24, CD30, CD33, CD38, CD44, EGFR, epithelial glycoprotein 2 (EPG-2), epithelial glycoprotein 40 (EPG-40), EPHA2, erb-B2, erb-B3, erb-B4, erbB dimers, EGFR vIII, folate binding protein (FBP), FCRL5, FCRH5, fetal acetylcholine receptor, GD2, GD3, HMW-MAA, IL-22R-alpha, IL-13R-alpha2, kinase insert domain receptor (kdr), kappa light chain, Lewis Y, L1-cell adhesion molecule, (L1-CAM), Melanoma-associated antigen (MAGE)-A1, MAGE-A3, MAGE-A6, Preferentially expressed antigen of melanoma (PRAME), survivin, TAG72, B7-H6, IL-13 receptor alpha 2 (IL-13Ra2), CA9, GD3, HMW-MAA, CD171, G250/CAIX, HLA-AI MAGE A1, HLA-A2 NY-ESO-1, PSCA, folate receptor-a, CD44v6, CD44v7/8, avb6 integrin, 8H9, NCAM, VEGF receptors, 5T4, Foetal AchR, NKG2D ligands, CD44v6, dual antigen, a cancer-testes antigen, mesothelin, murine CMV, mucin 1 (MUC1), MUC16, PSCA, NKG2D, NY-ESO-1, MART-1, gp100, oncofetal antigen, ROR1, TAG72,

VEGF-R2, carcinoembryonic antigen (CEA), prostate specific antigen, PSMA, Her2/neu, estrogen receptor, progesterone receptor, ephrinB2, CD123, c-Met, GD-2, O-acetylated GD2 (OGD2), CE7, Wilms Tumor 1 (WT-1), a cyclin, cyclin A2, CCL-1, CD138, a pathogen-specific antigen and an antigen associated with a universal tag.

[0008] In some of any such examples, the binding molecule does not bind or recognize a linker or spacer region of the recombinant antigen receptor, said linker or spacer region connecting the antigen-binding domain to the transmembrane domain of the antigen receptor. In some of any such examples, the binding molecule is an anti-idiotypic antibody or antigen-binding fragment thereof that specifically binds to the antigen-binding domain.

[0009] Provided herein is a method of expanding cells, including incubating an input composition containing cells expressing a chimeric antigen receptor (CAR) containing an antigen-binding domain that specifically binds or recognizes an antigen with a plurality of particles, each of the plurality of particles, e.g., beads, containing a binding molecule that is an anti-idiotypic antibody or antigen-binding fragment thereof that specifically binds to the antigen-binding domain, wherein binding of the anti-idiotypic antibody or antigen-binding fragment thereof to the antigen-binding domain induces expansion of the cells containing the chimeric antigen receptor, thereby producing an output composition containing expanded cells. In some of any such examples, the binding molecule contains a recombinant antigen or a portion thereof recognized by the antigen-binding domain.

[0010] Provided herein is a method of expanding cells, including incubating an input composition containing cells expressing a chimeric antigen receptor (CAR) containing an antigen-binding domain that specifically binds or recognizes an antigen with a plurality of particles, e.g., beads, each of the plurality of particles containing a binding molecule containing a recombinant antigen or a portion thereof recognized by the antigen-binding domain, wherein binding of the recombinant antigen or portion thereof to the antigen-binding domain induces expansion of the cells containing the chimeric antigen receptor, thereby producing an output composition containing expanded cells.

[0011] In some of any such examples, the antigen is selected from among $\alpha\text{v}\beta\text{6}$ integrin ($\alpha\text{v}\beta\text{6}$ integrin), B cell maturation antigen (BCMA), B7-H3, B7-H6, carbonic anhydrase 9 (CA9, also known as CAIX or G250), a cancer-testis antigen, cancer/testis antigen 1B (CTAG, also known as NY-ESO-1 and LAGE-2), carcinoembryonic antigen (CEA), a cyclin, cyclin A2, C-C Motif Chemokine Ligand 1 (CCL-1), CD19, CD20, CD22, CD23, CD24, CD30, CD33, CD38,

CD44, CD44v6, CD44v7/8, CD123, CD133, CD138, CD171, chondroitin sulfate proteoglycan 4 (CSPG4), epidermal growth factor protein (EGFR), type III epidermal growth factor receptor mutation (EGFR vIII), epithelial glycoprotein 2 (EPG-2), epithelial glycoprotein 40 (EPG-40), ephrinB2, ephrin receptor A2 (EPHa2), estrogen receptor, Fc receptor like 5 (FCRL5; also known as Fc receptor homolog 5 or FCRH5), fetal acetylcholine receptor (fetal AchR), a folate binding protein (FBP), folate receptor alpha, ganglioside GD2, O-acetylated GD2 (OGD2), ganglioside GD3, glycoprotein 100 (gp100), glypican-3 (GPC3), G Protein Coupled Receptor 5D (GPRC5D), Her2/neu (receptor tyrosine kinase erb-B2), Her3 (erb-B3), Her4 (erb-B4), erbB dimers, Human high molecular weight-melanoma-associated antigen (HMW-MAA), hepatitis B surface antigen, Human leukocyte antigen A1 (HLA-A1), Human leukocyte antigen A2 (HLA-A2), IL-22 receptor alpha (IL-22R α), IL-13 receptor alpha 2 (IL-13R α 2), kinase insert domain receptor (kdr), kappa light chain, L1 cell adhesion molecule (L1-CAM), CE7 epitope of L1-CAM, Leucine Rich Repeat Containing 8 Family Member A (LRRC8A), Lewis Y, Melanoma-associated antigen (MAGE)-A1, MAGE-A3, MAGE-A6, MAGE-A10, mesothelin (MSLN), c-Met, murine cytomegalovirus (CMV), mucin 1 (MUC1), MUC16, natural killer group 2 member D (NKG2D) ligands, melan A (MART-1), neural cell adhesion molecule (NCAM), oncofetal antigen, Preferentially expressed antigen of melanoma (PRAME), progesterone receptor, a prostate specific antigen, prostate stem cell antigen (PSCA), prostate specific membrane antigen (PSMA), Receptor Tyrosine Kinase Like Orphan Receptor 1 (ROR1), survivin, Trophoblast glycoprotein (TPBG also known as 5T4), tumor-associated glycoprotein 72 (TAG72), Tyrosinase related protein 1 (TRP1, also known as TYRP1 or gp75), Tyrosinase related protein 2 (TRP2, also known as dopachrome tautomerase, dopachrome delta-isomerase or DCT), vascular endothelial growth factor receptor (VEGFR), vascular endothelial growth factor receptor 2 (VEGFR2), Wilms Tumor 1 (WT-1), a pathogen-specific or pathogen-expressed antigen, or an antigen associated with a universal tag, and/or biotinylated molecules, and/or molecules expressed by HIV, HCV, HBV or other pathogens.

[0012] In some examples, the recombinant antigen is selected from among ROR1, B cell maturation antigen (BCMA), carbonic anhydrase 9 (CAIX), Her2/neu (receptor tyrosine kinase erbB2), LI-CAM, CD19, CD20, CD22, mesothelin, CEA, and hepatitis B surface antigen, anti-folate receptor, CD23, CD24, CD30, CD33, CD38, CD44, EGFR, epithelial glycoprotein 2 (EPG-2), epithelial glycoprotein 40 (EPG-40), EPHA2, erb-B2, erb-B3, erb-B4, erbB dimers, EGFR vIII, folate binding protein (FBP), FCRL5, FCRH5, fetal acetylcholine receptor, GD2,

GD3, HMW-MAA, IL-22R-alpha, IL-13R-alpha2, kinase insert domain receptor (kdr), kappa light chain, Lewis Y, L1-cell adhesion molecule, (L1-CAM), Melanoma-associated antigen (MAGE)-A1, MAGE-A3, MAGE-A6, Preferentially expressed antigen of melanoma (PRAME), survivin, TAG72, B7-H6, IL-13 receptor alpha 2 (IL-13Ra2), CA9, GD3, HMW-MAA, CD171, G250/CAIX, HLA-AI MAGE A1, HLA-A2 NY-ESO-1, PSCA, folate receptor-a, CD44v6, CD44v7/8, avb6 integrin, 8H9, NCAM, VEGF receptors, 5T4, Foetal AchR, NKG2D ligands, CD44v6, dual antigen, a cancer-testes antigen, mesothelin, murine CMV, mucin 1 (MUC1), MUC16, PSCA, NKG2D, NY-ESO-1, MART-1, gp100, oncofetal antigen, ROR1, TAG72, VEGF-R2, carcinoembryonic antigen (CEA), prostate specific antigen, PSMA, Her2/neu, estrogen receptor, progesterone receptor, ephrinB2, CD123, c-Met, GD-2, O-acetylated GD2 (OGD2), CE7, Wilms Tumor 1 (WT-1), a cyclin, cyclin A2, CCL-1, CD138 and a pathogen-specific antigen or a portion of any of the foregoing recognized by the antigen-binding domain. In some cases, the recombinant antigen is BCMA, CD22 or ROR1, or is a portion thereof recognized by the antigen-binding domain.

[0013] In some of any such examples, the portion of the recombinant antigen recognized by the antigen-binding domain contains the extracellular domain or a portion of the extracellular domain of the antigen. In some of any such examples, the portion of the recombinant antigen recognized by the antigen-binding domain contains essentially of the extracellular domain or a portion of the extracellular domain of the antigen.

[0014] In some examples, provided herein is a method of expanding cells, comprising incubating an input composition, said input composition comprising cells expressing a chimeric antigen receptor comprising an extracellular antigen-binding domain that specifically binds or recognizes an antigen, with a plurality of particles that are or comprise beads having attached a binding molecule that specifically binds to or recognizes the antigen-binding domain, wherein (i) the plurality of particles are from a composition having a concentration of the binding molecule of between or between about 0.5 µg/mL and 500 µg/mL, inclusive, and, during the incubating, the ratio of total cells present in the input composition to the plurality of particles is from or from about 5:1 to 1:5, inclusive; and (ii) binding of the binding molecule to the antigen-binding domain induces expansion of the cells comprising the chimeric antigen receptor, thereby producing an output composition comprising expanded cells.

[0015] Also provided herein is a method of expanding cells, including incubating an input composition containing cells expressing a chimeric antigen receptor (CAR) containing an

antigen-binding domain that specifically binds or recognizes B cell maturation antigen (BCMA) with a plurality of particles, e.g., beads, each of the plurality of particles, e.g., beads, containing a binding molecule containing the extracellular domain of BCMA or a portion of the extracellular domain recognized by the antigen-binding domain, wherein binding of the extracellular domain of BCMA or portion thereof to the antigen-binding domain induces expansion of the cells containing the chimeric antigen receptor, thereby producing an output composition containing expanded cells. In some examples, the portion of BCMA consists essentially of the extracellular domain or a portion of the extracellular domain.

[0016] In some of any such examples, the binding molecule is a fusion polypeptide containing the recombinant antigen or the portion thereof linked to a moiety, optionally wherein the moiety facilitates attachment to the particle. In some cases, the moiety is linked to the C-terminus of the recombinant antigen. In some instances, the moiety is hydrophobic or is enriched in hydrophobic amino acids. In some examples, the moiety is or contains an Fc domain. In some examples, the Fc region is derived from human IgG. In some of any such examples, the antigen is CD19.

[0017] Also provided herein is a method of expanding cells, including incubating an input composition containing cells expressing a chimeric antigen receptor (CAR) containing an antigen-binding domain that specifically binds or recognizes CD19 with a plurality of particles, e.g., beads, each of the plurality of particles containing a binding molecule that is an anti-idiotypic antibody or antigen-binding fragment thereof that specifically binds to the antigen-binding domain, wherein binding of the anti-idiotypic antibody or antigen-binding fragment thereof to the antigen-binding domain induces expansion of the cells containing the chimeric antigen receptor, thereby producing an output composition containing expanded cells. In some of any such examples, the antigen-binding domain of the antigen receptor is or contains antibody SJ25C1 or an antigen-binding fragment thereof. In some of any such examples, the antigen-binding domain of the antigen receptor is or contains antibody FMC63 or an antigen-binding fragment thereof.

[0018] In some of any such examples, the antigen-binding fragment is or contains an scFv. In some of any such examples, the antigen or recombinant antigen is human. In some of any such examples, the anti-idiotypic antibody or antigen-binding fragment thereof contains at least a portion of an immunoglobulin constant region. In some examples, the at least a portion of an immunoglobulin constant region contains an Fc region or a portion of the Fc

containing the CH2 and CH3 domains. In some examples, the constant region or Fc region is derived from human IgG.

[0019] In some of any such examples, the anti-idiotypic antibody or antigen-binding fragment thereof is an intact antibody or full-length antibody. In some of any such examples, the binding molecule is covalently or non-covalently attached to the particles, e.g., beads. In some of any such examples, the binding molecule is attached to each of the plurality of particles, e.g., beads, at or near the C-terminal amino acid residue of the binding molecule and/or attachment of the binding molecule to each of the plurality of particles, e.g., beads, is carried out such that the region or epitope of the binding molecule recognized by the antigen-binding domain of the antigen receptor is oriented so that it is capable of being recognized by the antigen receptor.

[0020] In some of any such examples, the particles, e.g., beads, are synthetic particles, insoluble particles, solid particles or are non-cellular particles. In some of any such examples disclosed herein, the particles are beads. In some of any such examples, the plurality of particles contains beads. In some of any such examples, the particles are or comprise one or more polymers or oligomers and/or are polymeric and/or oligomeric. In some of any such examples, the plurality of particles contains a mean diameter of between or between about 1 μm and 10 μm or between or between about 2 μm and 5 μm . In some of any such examples, the plurality of particles, e.g., beads, includes a mean diameter of about 2.8 μm . In some of any such examples, the plurality of particles, e.g., beads, includes a mean diameter of about 4.5 μm . In some of any such examples, the plurality of particles, e.g., beads, includes a mean density of between about 0.5 g/cm^3 and 5.0 g/cm^3 or between or between about 1 g/cm^3 and about 2 g/cm^3 . In some of any such examples, the plurality of particles, e.g., beads, includes a mean density of about 1.3 g/cm^3 . In some of any such examples, the plurality of particles, e.g., beads, includes a mean density of about 1.5 g/cm^3 .

[0021] In some of any such examples, the plurality of particles, e.g., beads, is monodisperse. In some of any such examples, the binding molecule is covalently attached to the particles. In some of any such examples, the particle contains a surface exposed functional group for attachment of the binding molecule and/or wherein the binding molecule is covalently attached to the particle via a surface exposed functional group.

[0022] In some of any such examples, the surface exposed functional group is an amino group, a carboxyl group, a thiol group, an aldehyde group, a chloromethyl group, an epoxy

group, a hydroxyl group, a tosyl group or a hydrazine group. In some examples, the surface exposed functional group is a tosyl group.

[0023] In some of any such examples, the plurality of particles, e.g., beads, are biocompatible or non-toxic to cells. In some of any such examples, the plurality of particles, e.g., beads, contains particles including glass, silica, polyesters of hydroxy carboxylic acids, polyanhydrides of dicarboxylic acids, copolymers of hydroxy carboxylic acids, copolymers dicarboxylic acids, or metal. In some of any such examples, the particles, e.g., beads, contain a surface including a polymer, a polysaccharide, a silica, a fatty acid, a carbon or a combination thereof. In some examples, the polymer is polyethylene glycol, poly(lactic-co-glycolic acid), polyglutaraldehyde, polyurethane, polystyrene, and polyvinyl alcohol or combinations thereof. In some of any such examples, the plurality of particles contain particles including a hydrophobic surface. In some of any such examples, the plurality of particles, e.g., beads, contain particles including a polystyrene surface.

[0024] In some of any such examples, the plurality of particles, e.g., beads, contains particles that are magnetic and/or include a magnetic core, a paramagnetic core or a superparamagnetic core.

[0025] In some of any such examples, the particles are from a composition having a concentration of the binding molecule is between or between about 0.5 $\mu\text{g/mL}$ and 500 $\mu\text{g/mL}$, 1 $\mu\text{g/mL}$ and 200 $\mu\text{g/mL}$ or 5 $\mu\text{g/mL}$ and 100 $\mu\text{g/mL}$, inclusive. In some of any such examples, the particles are from a composition having a concentration of the binding molecule is at least or at least about 1 $\mu\text{g/mL}$, 5 $\mu\text{g/mL}$, 10 $\mu\text{g/mL}$, 25 $\mu\text{g/mL}$, 50 $\mu\text{g/mL}$, 100 $\mu\text{g/mL}$ or 200 $\mu\text{g/mL}$. In some of any such examples, each of the plurality of particles, e.g., beads, contains at least or about at least 10 copies, 10^2 copies, 10^3 copies, 10^4 copies, 10^5 copies or 10^6 copies of the binding molecule.

[0026] In some of any such examples, at least a portion of the incubation is performed in the presence of an agent that specifically binds to an additional molecule on the cell to provide an accessory signal and/or to block an inhibitory signal. In some of any such examples, the agent is provided together with the particles, e.g., beads, optionally the agent is contained by each of the plurality of particles or a subset thereof. In some of any such examples, the agent is provided separately from the plurality of particles, e.g., beads.

[0027] In some of any such examples, the particles, e.g., beads, further include an agent that specifically binds to an additional molecule on the cell to provide an accessory signal and/or

to block an inhibitory signal. In some of any such examples, the agent is a ligand or is an antibody or antigen-binding fragment thereof. In some of any such examples, the molecule is a costimulatory molecule or is an activating co-receptor. In some of any such examples, the costimulatory molecule or activating co-receptor is OX-40, ICOS, DAP10, CD28 or 4-1BB. In some examples, the molecule is a ligand of an activating receptor or co-receptor, such as OX-40L, ICOSL, B7-1, B7-2 or 4-1BBL. In some of any such examples, the molecule is an inhibitory receptor. In some examples, the inhibitory receptor is CTLA-4, PD-1, LAG-3, Tim-3, BTLA or TIGIT. In some of any such examples, the agent is covalently attached to the particles, e.g., beads. In some examples, the molecule is a ligand of an inhibitory receptor, such as is PD-L1, PD-L2, CD155, CD112 or LIGHT.

[0028] In some of any such examples, the ratio, optionally molar or weight ratio, of the binding molecule and the agent contained by the particles, e.g., beads, is or is about 1:1. In some of any such examples, the ratio of total cells present in the input composition to particles, e.g., beads, is from or from about 5:1 to 1:5, 3:1 to 1:3 or 2:1 to 1:2. In some of any such examples, the ratio of total cells present in the input composition to particles, e.g., beads, is from or from about 1:0.1 to 1:5. In some of any such examples, the ratio of total cells present in the input composition to particles, e.g., beads, is or is about 1:1.

[0029] In some of any such examples, the incubation is carried out for at least or greater than or greater than about 2 hours, 4 hours, 8 hours, 12 hours, 24 hours, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, or 14 days. In some of any such examples, the incubation is carried out at a temperature between or between about 30 °C and 39 °C, inclusive. In some of any such examples, the incubation is carried out at a temperature of $37^{\circ} \pm 2.0^{\circ} \text{C}$.

[0030] In some of any such examples, the cells include immune cells or induced pluripotent stem cells (iPSC). In some of any such examples, the immune cell is a T cell or an NK cell. In some of any such examples, the cells contain CD4+ and/or CD8+ T cells. In some of any such examples, the ratio of the CD4+ cells to the CD8+ cells is or is about 1:1, 1:2, 2:1, 1:3 or 3:1. In some of any such examples, the cells are primary cells obtained from a subject, optionally a human subject. In some of any such examples, the cells are human.

[0031] In some of any such examples, the input composition is produced by a method including contacting a composition of cells with a nucleic acid molecule encoding the

recombinant antigen receptor under conditions to introduce the nucleic acid molecule into one or more cells in the composition.

[0032] Also provided herein is a method of genetically engineering a cell, including contacting a composition of cells with a nucleic acid molecule encoding a recombinant antigen receptor under conditions to introduce the nucleic acid molecule into one or more cells in the composition, thereby producing an input composition; and incubating cells of the input composition according to the methods described herein. In some examples, at least a portion of the contacting and incubating are carried out simultaneously.

[0032a] In one aspect, the present disclosure refers to a method of genetically engineering a cell, comprising: (a) contacting a composition of cells with a nucleic acid molecule encoding a chimeric antigen receptor under conditions to introduce the nucleic acid molecule into one or more cells in the composition, thereby producing an input composition; and (b) incubating cells of the input composition according to the method as disclosed herein.

[0033] In some of any such examples, the nucleic acid molecule is included in a viral vector, an episomal vector or a transposon. In some of any such examples, the contacting is carried out by transposon/transposase gene transfer. In some of any such examples, the contacting is carried out by transduction with a viral vector. In some examples, the viral vector is a retrovirus, which optionally is a gamma-retroviral vector or a lentiviral vector.

[0034] In some instances, the contacting includes a step of spinoculating the viral vector with the composition of cells. In some cases, spinoculating includes rotating, in an internal cavity of a centrifugal chamber, the viral vector particles and composition of cells, wherein the rotation is at a relative centrifugal force at an internal surface of the side wall of the cavity that is between or between about 500 g and 2500 g, 500 g and 2000 g, 500 g and 1600 g, 500 g and 1000 g, 600 g and 1600 g, 600 g and 1000 g, 1000 g and 2000 g or 1000 g and 1600 g, each inclusive; or at least or at least about 600 g, 800 g, 1000 g, 1200 g, 1600 g, or 2000 g. In some examples, spinoculating is for a time that is greater than or about 5 minutes, greater than or about 10 minutes, greater than or about 15 minutes, greater than or about 20 minutes, greater than or about 30 minutes, greater than or about 45 minutes, greater than or about 60 minutes, greater than or about 90 minutes or greater than or about 120 minutes; or between or between about 5 minutes and 60 minutes, 10 minutes and 60 minutes, 15 minutes and 60 minutes, 15 minutes and 45 minutes, 30 minutes and 60 minutes or 45 minutes and 60 minutes, each inclusive.

[0035] In some of any such examples, the contacting is carried out in the presence of a transduction adjuvant. In some of any such examples, the composition of cells contains a plurality of T cells and, prior to the contacting, the method does not include stimulating or activating the T cells.

[0036] In some of any such examples, the composition of cells contains a plurality of T cells and, prior to the contacting, the method does not include incubating the composition in the presence of an agent or agents capable of inducing a signal through a TCR complex and/or incubation in the presence of an agent or agents capable of inducing proliferation of T cells, CD4+ T cells, and/or CD8+ T cells; and/or CD3-binding molecules, CD28-binding molecules, recombinant IL-2, recombinant IL-15, and recombinant IL-7 or a combination thereof. In some examples, prior to the contacting, the method does not include stimulating the T cells in the presence of an anti-CD3 antibody and/or an anti-CD28 antibody.

[0037] In some of any such examples, the composition of cells contains a plurality of T cells, said plurality of cells having been obtained from a sample from a subject, wherein the contacting is initiated no more than 24 hours after obtaining the sample from the subject; and/or prior to the contacting, the T cells have not been subjected to a temperature greater than or greater than about 15° C, about 18 ° C, about 22 ° C or about 25 ° C for a duration of more than 1 hour, 2 hours, 4 hours, 6 hours, 8 hours, 12 hours, or 24 hours after obtaining the sample from the subject; and/or prior to the contacting, the T cells have not been subjected to a temperature of, of about, greater than, or greater than about 37 ° ± 2.0 ° C for a duration of more than 15 minutes, 30 minutes, 1 hour or 2 hours after obtaining the sample from the subject. In some of any such examples, prior to said contacting, no more than 5 %, 10 %, 20 %, 30 %, or 40 % of the T cells are activated cells, express a surface marker selected from the group consisting of HLA-DR, CD25, CD69, CD71, CD40L and 4-1BB; include intracellular expression of a cytokine selected from the group consisting of IL-2, IFN-gamma, TNF-alpha, are in the G1 or later phase of the cell cycle and/or are capable of proliferating.

[0038] In some of any such examples, the method further includes, prior to the incubation or the contacting, obtaining a biological sample from the subject containing the cells and, optionally, selecting or enriching the cells, optionally T cells, from the sample. In some of any such examples, the percent of cells expressing the recombinant antigen receptor in the input composition is less than or less than about 75%, 70%, 60%, 50%, 40%, 30%, 20%, 15%, 10% or less. In some of any such examples, the composition of cells or the input composition contains at least or at least about 1×10^2 cells, 1×10^3 cells, 1×10^4 cells, 1×10^5 cells, 1×10^6 cells or 1×10^7 cells.

[0039] In some of any such examples, the surface expression of an activation marker or exhaustion marker of cells present in the output composition is less than surface expression of

the marker in a composition of cells produced after a similar incubation but in the presence of polyclonal stimulatory molecule capable of activating one or more intracellular signaling domains of one or more components of a TCR complex. In some cases, the exhaustion marker is an inhibitory receptor. In some instances, the exhaustion marker is PD-1, CTLA-4, TIM-3, LAG-3, BTLA or TIGIT. In some examples, the activation marker is HLA-DR, CD25, CD69, CD71, CD40L or 4-1BB. In some of any such examples, the surface expression is at least or at least about 1.2-fold, 1.5-fold, 2.0-fold, 3.0-fold, 4.0-fold, 5.0-fold, 10.0-fold or more.

[0040] In some of any such examples, the number of cells in the output composition is substantially the same or is greater than the number of cells in a composition of cells produced by a similar incubation but in the presence of a polyclonal stimulatory molecule capable of activating one or more intracellular signaling domains of one or more components of a TCR complex. In some of any such examples, the polyclonal stimulatory molecule contains an anti-CD3 antibody or fragment and/or an anti-CD28 antibody or fragment.

[0041] In some of any such examples, the number of the cells in the output composition is greater than the number of the cells in the input composition by greater than or greater than about 1.2-fold, 1.5-fold, 2.0-fold, 3.0-fold, 4.0-fold, 5.0-fold, 10.0-fold, 25-fold, 50-fold, 100-fold or more. In some of any such examples, the percent of cells containing the recombinant antigen receptor in the output composition is greater than or greater than about 50%, 60%, 70%, 80%, 90%, 95% or more. In some of any such examples, the number of cells in the output composition containing the recombinant antigen receptor is increased or enriched by 1.2-fold, 2.0-fold, 3.0-fold, 4.0-fold, 5.0-fold, 6.0-fold, 7.0-fold, 8.0-fold, 9.0-fold, 10-fold or more compared to the number of the cells containing the antigen receptor in the input composition.

[0042] In some of any such examples, at least a portion of the incubation is carried out in the presence of one or more additional agents that modulate cell expansion or activity. In some cases, the one or more additional agent is lenalidomide.

[0043] In some of any such examples, the method is performed in vitro or ex vivo. In some of any such examples, the antigen receptor is a CAR and the CAR further an intracellular signaling domain containing an ITAM. In some cases, the intracellular signaling domain contains an intracellular domain of a CD3-zeta (CD3 ζ) chain. In some examples, the CAR further contains a costimulatory signaling region. In some examples, the costimulatory signaling region contains a signaling domain of CD28 or 4-1BB. In some examples, the

costimulatory domain is CD28. In some of any such examples, the method further includes removing the plurality of particles, e.g., beads, from the output composition.

[0044] Provided herein is a composition of cells produced by any of the methods provided herein. Also provided herein is a surface modified particle containing a particle and a binding molecule bound to the surface of the particle, wherein the binding molecule specifically binds to an extracellular antigen-binding domain of an antigen receptor. In some examples, the antigen receptor is a chimeric antigen receptor (CAR). In some examples, the binding molecule does not bind or recognize a linker or spacer region of the recombinant antigen receptor, said linker or spacer region connecting the antigen-binding domain to the transmembrane domain of the antigen receptor.

[0044a] In another aspect, the present disclosure refers to a surface modified particle, comprising (i) a particle that is a bead comprising a diameter of between or between about 2 μm and 5 μm and (ii) and a binding molecule attached to the surface of the bead, wherein the binding molecule specifically binds to an extracellular antigen-binding domain of a chimeric antigen receptor.

[0045] In some of any such examples, the recombinant antigen is selected from among $\alpha\text{v}\beta 6$ integrin ($\alpha\text{v}\beta 6$ integrin), B cell maturation antigen (BCMA), B7-H3, B7-H6, carbonic anhydrase 9 (CA9, also known as CAIX or G250), a cancer-testis antigen, cancer/testis antigen 1B (CTAG, also known as NY-ESO-1 and LAGE-2), carcinoembryonic antigen (CEA), a cyclin, cyclin A2, C-C Motif Chemokine Ligand 1 (CCL-1), CD19, CD20, CD22, CD23, CD24, CD30, CD33, CD38, CD44, CD44v6, CD44v7/8, CD123, CD133, CD138, CD171, chondroitin sulfate proteoglycan 4 (CSPG4), epidermal growth factor protein (EGFR), type III epidermal growth factor receptor mutation (EGFR vIII), epithelial glycoprotein 2 (EPG-2), epithelial glycoprotein 40 (EPG-40), ephrinB2, ephrin receptor A2 (EPHa2), estrogen receptor, Fc receptor like 5 (FCRL5; also known as Fc receptor homolog 5 or FCRH5), fetal acetylcholine receptor (fetal AchR), a folate binding protein (FBP), folate receptor alpha, ganglioside GD2, O-acetylated GD2 (OGD2), ganglioside GD3, glycoprotein 100 (gp100), glypican-3 (GPC3), G Protein Coupled Receptor 5D (GPRC5D), Her2/neu (receptor tyrosine kinase erb-B2), Her3 (erb-B3), Her4 (erb-B4), erbB dimers, Human high molecular weight-melanoma-associated antigen (HMW-MAA), hepatitis B surface antigen, Human leukocyte antigen A1 (HLA-A1), Human leukocyte antigen A2 (HLA-A2), IL-22 receptor alpha (IL-22R α), IL-13 receptor alpha 2 (IL-13R α 2), kinase insert domain receptor (kdr), kappa light chain, L1 cell adhesion molecule (L1-CAM), CE7 epitope of L1-CAM, Leucine Rich Repeat Containing 8 Family Member A (LRRC8A), Lewis Y, Melanoma-associated antigen (MAGE)-A1, MAGE-A3, MAGE-A6, MAGE-A10, mesothelin (MSLN), c-Met, murine cytomegalovirus (CMV), mucin 1 (MUC1), MUC16, natural killer group 2 member D (NKG2D) ligands, melan A (MART-1), neural cell adhesion molecule (NCAM), oncofetal antigen, Preferentially expressed antigen of melanoma

(PRAME), progesterone receptor, a prostate specific antigen, prostate stem cell antigen (PSCA), prostate specific membrane antigen (PSMA), Receptor Tyrosine Kinase Like Orphan Receptor 1 (ROR1), survivin, Trophoblast glycoprotein (TPBG also known as 5T4), tumor-associated glycoprotein 72 (TAG72), Tyrosinase related protein 1 (TRP1, also known as TYRP1 or gp75), Tyrosinase related protein 2 (TRP2, also known as dopachrome tautomerase, dopachrome delta-isomerase or DCT), vascular endothelial growth factor receptor (VEGFR), vascular endothelial growth factor receptor 2 (VEGFR2), Wilms Tumor 1 (WT-1), a pathogen-specific or pathogen-expressed antigen, or an antigen associated with a universal tag, and/or biotinylated molecules, and/or molecules expressed by HIV, HCV, HBV or other pathogens.

[0046] In some of any such examples, the binding molecule contains a recombinant antigen or a portion thereof recognized by the antigen-binding domain. In some examples, the recombinant antigen is selected from among ROR1, B cell maturation antigen (BCMA), carbonic anhydrase 9 (CAIX), Her2/neu (receptor tyrosine kinase erbB2), L1-CAM, CD19, CD20, CD22, mesothelin, CEA, and hepatitis B surface antigen, anti-folate receptor, CD23, CD24, CD30, CD33, CD38, CD44, EGFR, epithelial glycoprotein 2 (EPG-2), epithelial glycoprotein 40 (EPG-40), EPHA2, erb-B2, erb-B3, erb-B4, erbB dimers, EGFR vIII, folate binding protein (FBP), FCRL5, FCRH5, fetal acetylcholine receptor, GD2, GD3, HMW-MAA, IL-22R-alpha, IL-13R-alpha2, kinase insert domain receptor (kdr), kappa light chain, Lewis Y, L1-cell adhesion molecule, (L1-CAM), Melanoma-associated antigen (MAGE)-A1, MAGE-A3, MAGE-A6, Preferentially expressed antigen of melanoma (PRAME), survivin, TAG72, B7-H6, IL-13 receptor alpha 2 (IL-13Ra2), CA9, GD3, HMW-MAA, CD171, G250/CAIX, HLA-AI MAGE A1, HLA-A2 NY-ESO-1, PSCA, folate receptor-a, CD44v6, CD44v7/8, avb6 integrin, 8H9, NCAM, VEGF receptors, 5T4, Foetal AchR, NKG2D ligands, CD44v6, dual antigen, a cancer-testes antigen, mesothelin, murine CMV, mucin 1 (MUC1), MUC16, PSCA, NKG2D, NY-ESO-1, MART-1, gp100, oncofetal antigen, ROR1, TAG72, VEGF-R2, carcinoembryonic antigen (CEA), prostate specific antigen, PSMA, Her2/neu, estrogen receptor, progesterone receptor, ephrinB2, CD123, c-Met, GD-2, O-acetylated GD2 (OGD2), CE7, Wilms Tumor 1 (WT-1), a cyclin, cyclin A2, CCL-1, CD138 and a pathogen-specific antigen or a portion of any of the foregoing recognized by the antigen-binding domain.

[0047] In some examples, the recombinant antigen is CD19, BCMA, CD22 or ROR1, or is a portion thereof recognized by the antigen-binding domain. In some of any such examples, the portion of the recombinant antigen recognized by the antigen-binding domain

contains the extracellular domain or a portion of the extracellular domain of the antigen. In some of any such examples, the portion of the recombinant antigen recognized by the antigen-binding domain consists essentially of the extracellular domain or a portion of the extracellular domain of the antigen.

[0048] Provided herein is a surface modified particle, containing a particle and a binding molecule bound to the surface of the particle, wherein the binding molecule contains an extracellular domain or a portion thereof of B cell maturation antigen (BCMA). In some of any such examples, the binding molecule is a fusion polypeptide containing the recombinant antigen or the portion thereof linked to a moiety, optionally wherein the moiety facilitates attachment to the particle. In some cases, the moiety is linked to the C-terminus of the recombinant antigen. In some examples, the moiety is hydrophobic or is enriched in hydrophobic amino acids.

[0049] In some of any such examples, the moiety is or contains an Fc domain. In some instances, the Fc region is derived from human IgG. In some of any such examples, the recombinant antigen is human.

[0050] In some of any such examples, the binding molecule contains an anti-idiotypic antibody or antigen-binding fragment thereof. In some of any such examples, the antigen recognized by the antigen-binding domain is CD19. In some of any such examples, the antigen-binding domain of the antigen receptor is or contains antibody SJ25C1 or an antigen-binding fragment thereof. In some examples, the antigen-binding domain of the antigen receptor is or contains antibody FMC63 or an antigen-binding fragment thereof. In some examples, the antigen-binding fragment is or contains an scFv.

[0051] In some of any such examples, the anti-idiotypic antibody or antigen-binding fragment thereof contains at least a portion of an immunoglobulin constant region. In some instances, the at least a portion of an immunoglobulin constant region contains an Fc region or a portion of the Fc containing the CH2 and CH3 domains. In some examples, the constant region or Fc region is derived from human IgG. In some of any such examples, the anti-idiotypic antibody or antigen-binding fragment thereof is an intact antibody or full-length antibody.

[0052] In some of any such examples, the binding molecule is covalently or non-covalently attached to the particles, e.g., beads. In some of any such examples, the binding molecule is attached to each of the plurality of particles, e.g., beads, at or near the C-terminal

amino acid residue of the binding molecule and/or attachment of the binding molecule to each of the plurality of particles, e.g., beads, is carried out such that the region or epitope of the binding molecule recognized by the antigen-binding domain of the antigen receptor is oriented so that it is capable of being recognized by the antigen receptor. In some of any such examples, the particles, e.g., beads, are synthetic particles, insoluble particles, solid particles or are non-cellular particles.

[0053] In some of any such examples, the plurality of particles, e.g., beads, contains beads. In some of any such examples, the particle has a diameter of between or between about 1 μm and 10 μm or between or between about 2 μm and 5 μm , each inclusive. In some of any such examples, the particle has a diameter of about 2.8 μm . In some of any such examples, the particle has a diameter of about 4.5 μm . In some of any such examples, the binding molecule is covalently attached to the particles, e.g., beads.

[0054] In some of any such examples, the particle contains a surface exposed functional group for attachment of the binding molecule and/or wherein the binding molecule is covalently attached to the particle via a surface exposed functional group. In some examples, the surface exposed functional group is an amino group, a carboxyl group, a thiol group, an aldehyde group, a chloromethyl group, an epoxy group, a hydroxyl group, a tosyl group or a hydrazine group. In some cases, the surface exposed functional group is a tosyl group.

[0055] In some of any such examples, the particle is biocompatible or non-toxic to cells. In some of any such examples, the plurality of particles, e.g., beads, include particles, e.g., beads, containing glass, silica, polyesters of hydroxy carboxylic acids, polyanhydrides of dicarboxylic acids, copolymers of hydroxy carboxylic acids, copolymers dicarboxylic acids, or metal. In some of any such examples, the particles, e.g., beads, contain a surface including a polymer, a polysaccharide, a silica, a fatty acid, a carbon or a combination thereof. In some instances, the polymer is polyethylene glycol, poly(lactic-co-glycolic acid), polyglutaraldehyde, polyurethane, polystyrene, and polyvinyl alcohol or combinations thereof.

[0056] In some of any such examples, the particle contains a hydrophobic surface. In some of any such examples, the particles, e.g., beads, include a polystyrene surface. In some of any such examples, the particle is magnetic and/or contains a magnetic core, a paramagnetic core or a superparamagnetic core. In some of any such examples, the particle contains at least or about at least 10 copies, 10^2 copies, 10^3 copies, 10^4 copies, 10^5 copies or 10^6 copies of the binding molecule.

[0057] In some of any such examples, the particle further contains an agent that specifically binds to an additional molecule on the cell to provide an accessory signal and/or to block an inhibitory signal, thereby modulating expansion of the cells. In some of any such examples, the agent is a ligand or is an antibody or antigen-binding fragment thereof. In some examples, the molecule is a costimulatory molecule or is an activating co-receptor. In some examples, the costimulatory molecule or activating co-receptor is OX-40, ICOS, DAP10, CD28 or 4-1BB. In some examples, the molecule is a ligand of an activating receptor or co-receptor, such as OX-40L, ICOSL, B7-1, B7-2 or 4-1BBL. In some cases, the molecule is an inhibitory receptor. In some examples, the inhibitory receptor is CTLA-4, PD-1, LAG-3, Tim-3, BTLA or TIGIT. In some examples, the molecule is a ligand of an inhibitory receptor, such as is PD-L1, PD-L2, CD155, CD112 or LIGHT.

[0058] In some of any such examples, the agent is covalently attached to the particles, e.g., beads. In some of any such examples, the ratio, optionally molar or weight ratio, of the binding molecule and the agent contained by the particle is or is about 1:1.

[0059] Provided herein is a composition containing a plurality of the surface modified particles, e.g., beads, described herein. In some examples, the particles are from a composition having a concentration of the binding molecule is between or between about 0.5 $\mu\text{g/mL}$ and 500 $\mu\text{g/mL}$, 1 $\mu\text{g/mL}$ and 200 $\mu\text{g/mL}$ or 5 $\mu\text{g/mL}$ and 100 $\mu\text{g/mL}$, each inclusive. In some examples, the particles are from a composition having a concentration of the binding molecule that is at least or at least about 1 $\mu\text{g/mL}$, 5 $\mu\text{g/mL}$, 10 $\mu\text{g/mL}$, 25 $\mu\text{g/mL}$, 50 $\mu\text{g/mL}$, 100 $\mu\text{g/mL}$ or 200 $\mu\text{g/mL}$. In some of any such examples, the composition is monodisperse.

[0059a] In another aspect, the present disclosure refers to a composition, comprising a plurality of the surface modified particles as disclosed herein.

[0060] Also provided herein is a kit containing any of the particles, e.g., beads, described herein or the any of the composition described herein and instructions for use. In some cases, instructions are for selecting or enriching, from a population of cells, cells expressing an antigen receptor containing an antigen-binding domain specifically recognized by the binding molecule. In some instances, instructions are for expanding, from a population of cells, cells expressing an antigen receptor containing an antigen-binding domain specifically recognized by the binding molecule. In some examples, the percent of cells expressing a recombinant antigen receptor in the population of cells is less than or less than about 75%, 70%, 60%, 50%, 40%, 30%, 20%, 15%, 10% or less.

[0060a] In another aspect, the present disclosure refers to a kit, comprising the surface modified particle as disclosed herein or the composition as disclosed herein and instructions for use, optionally wherein: instructions are for selecting or enriching, from a population of cells, cells expressing a chimeric antigen receptor comprising an antigen-binding domain specifically recognized by the binding molecule; or instructions are for stimulating or expanding, from a population of cells, cells expressing a chimeric antigen receptor comprising an antigen-binding domain specifically recognized by the binding molecule.

[0061] Provided here is a method for expanding cells including incubating a population of cells with any of the particles, e.g., beads, described herein or any of the compositions described

herein. Also provided is a method of selecting or enriching cells including contacting a population of cells with any of the particles, e.g., beads, described herein or any of the compositions described herein.

[0061a] In another aspect, the present disclosure refers to a method for expanding cells, comprising incubating a population of cells with the surface modified particle as disclosed herein or the composition as disclosed herein.

[0061b] In another aspect, the present disclosure refers to a method of selecting or enriching cells, comprising contacting a population of cells with the surface modified particle as disclosed herein or the composition as disclosed herein.

[0062] Provided herein is a long-term stimulation method for assessing a cell composition including incubating, for a period of time of at least 10 days, an input composition under conditions to stimulate a CAR-dependent activity in cells in the input composition, said input composition containing T cells expressing a chimeric antigen receptor (CAR) containing an extracellular antigen-binding domain that specifically binds or recognizes an antigen, thereby producing an output composition; and assessing one or more phenotype or activity of one or more cells of the output composition.

[0062a] In another aspect, the present disclosure refers to a long-term stimulation method for assessing a cell composition, the method comprising:

incubating, for a period of time of at least 10 days, an input composition under conditions to stimulate a CAR-dependent activity in cells in the input composition, said input composition comprising T cells expressing a chimeric antigen receptor (CAR) comprising an extracellular antigen-binding domain that specifically binds or recognizes an antigen, thereby producing an output composition; and

assessing one or more phenotype or activity of one or more cells of the output composition; wherein:

the conditions to stimulate a CAR-dependent activity comprises the presence of a binding molecule that specifically binds to the antigen-binding domain of the CAR; and
the binding molecule is attached to a solid support.

[0063] In some of any of the examples of a long-term stimulation method, the conditions to stimulate a CAR-dependent activity includes the presence of a binding molecule that specifically binds to the antigen-binding domain of the CAR. In some examples, the binding molecule is attached to a support. In some

examples, the support is a solid support. In some examples, the solid support is the surface of a well of a microplate or a bead. In some examples, the solid support is a microplate having the binding molecule attached to the microplate, and the incubation is carried out in the microplate. In some examples, the solid support is a bead having attached the binding molecule, and the incubation is carried out in the presence of a plurality of the beads.

[0064] In some of any of the examples of a long-term stimulation method, the binding molecule is or comprises a recombinant antigen or a portion thereof recognized by the antigen-binding domain. In some examples, the recombinant antigen or portion thereof is BCMA, or is a portion thereof recognized by the antigen-binding domain. In some examples, the binding molecule is or includes an anti-idiotypic antibody or antigen-binding fragment thereof that specifically binds to the antigen-binding domain. In some examples, the antigen-binding domain of the antigen receptor is or comprises antibody SJ25C1 or an antigen-binding fragment thereof. In some examples, the antigen-binding domain of the antigen receptor is or includes antibody FMC63 or an antigen-binding fragment thereof.

[0065] In some of any of the examples of a long-term stimulation method, the method is carried out *in vitro* or *ex vivo*.

[0066] In some of any of the examples of a long-term stimulation method, the input composition is incubated in the presence of a media that does not comprise recombinant

cytokines. In some examples, the incubation is carried out continuously or is not interrupted for the period of time. In some examples, during the incubation, cells are not replated, media is not changed and binding molecule is not added.

[0067] In some of any of the examples of a long-term stimulation method, the method includes assessing one or more phenotypes of activation, exhaustion or differentiation state of the one or more cells of the output composition. In some examples, the phenotype is exhaustion and the assessing includes measuring the expression, optionally surface expression, of one or more markers selected from CTLA-4, FOXP3, PD-1, TIGIT, LAB-3, 2B4, BTLA, TIM3, VISTA, or CD96. In some examples, the phenotype is activation and the assessing includes measuring the expression, optionally surface expression, of one or more markers selected from CD25, CD26, CD27, CD28, CD30, CD71, CD154, CD40L, CD127, LAG3, or Ki67. In some examples, the phenotype is differentiation state and the assessing includes measuring one or more markers selected from (i) one or more of CD25, CD45RO, CD56, KLRG1, CD95 and/or (ii) one or of CD45RA, CD27, CD28, CD62L, and CCR7, optionally wherein the one or more markers are markers are positively or inversely associated with naïve-like T cells.

[0068] In some of any of the examples of a long-term stimulation method, the method includes assessing one or more activities of the one or more cells of the output composition. In some examples, the one or more activities comprises a CAR-dependent activity, optionally an antigen-stimulated activity. In some of any of the examples of a long-term stimulation method, the one or more activities comprises cytolytic activity or cytokine production.

[0069] In some examples of any of the long-term stimulation method, the period of time is at least or at least about 11 days, 12 days, 13 days, 14 days, or 15 days. In some examples, the period of time is or is about 11 days, 12 days, 13 days, 14 days or 15 days.

[0070] In some of any of the examples of a long-term stimulation method, the input composition contains cells that have been exposed or contacted with a test agent or compound prior to the incubation, optionally wherein the exposing or contacting is carried out during one or more steps of a process for producing the input composition comprising the T cells expressing the CAR. In some examples, the method is carried out on a plurality of input compositions, each of said input compositions of the plurality being produced by a different process.

[0071] In some of any of the examples of a long-term stimulation method, the method further includes comparing the phenotype or activity of the output composition to the phenotype

or activity of a control composition, optionally wherein the control composition is a composition of T cells that have been incubated for the at least 10 days under the same conditions to stimulate the CAR-dependent activity, said composition of T cells having not been produced in the presence of the test agent or compound or having been produced by an alternative process compared to the input composition. In some examples, the method further includes identifying an output composition that exhibits reduced exhaustion, reduced activation or decreased differentiation, such as compared to the control composition. In some examples, the decreased differentiation comprises increased expression of one more naïve-like T cell markers.

Brief Description of the Drawings

[0072] **FIG. 1** shows a histogram of Cell Trace Violet (CTV) staining in CD3⁺ T cells expressing anti-BCMA CAR (CAR T cells) and CD3⁺ cells that do not express a CAR (Mock) following incubation with BCMA conjugated-beads.

[0073] **FIG. 2A** shows dot plots for CD25 surface expression (y-axis) and CTV staining intensity (x-axis) in cells following incubation with anti-CD3/ anti-CD28 antibody-conjugated beads (left) or BCMA-conjugated beads (right).

[0074] **FIG. 2B** shows a histogram of CTV staining of cells following treatment with anti-CD3/ anti-CD28 antibody-conjugated beads or BCMA-conjugated beads.

[0075] **FIG. 2C** shows histograms of PD-1 expression in CD4⁺ cells (left) or CD8⁺ cells (right) following incubation cells with anti-CD3/ anti-CD28 antibody-conjugated beads or BCMA-conjugated beads.

[0076] **FIG. 3A** shows the percentage of anti-BCMA CAR⁺ T cells following incubation of each of three different T cell compositions containing the CAR-expressing T cells (each generated from a different donor) with BCMA-conjugated beads for up to 14 days. Results from each cell composition are separately plotted with triangles, squares, and circles.

[0077] **FIG. 3B** shows the surface expression as determined by mean fluorescence intensity (MFI) for detection of CD25 (left), CCR7 (center), and CD27 (right) in CD8⁺ cells following four, seven, or fourteen days of incubation with BCMA conjugated beads for up to 14 days. Results are shown for each of the three different cell compositions containing anti-BCMA CAR expressing cells described in **FIG. 3A** and are separately plotted with triangles, squares, and circles.

CLAIMS

1. A surface modified particle, comprising (i) a particle that is a bead comprising a diameter of between or between about 2 μm and 5 μm and (ii) and a binding molecule attached to the surface of the bead, wherein the binding molecule specifically binds to an extracellular antigen-binding domain of a chimeric antigen receptor.
2. The surface modified particle of claim 1, wherein the binding molecule does not bind or recognize a linker or spacer region of the recombinant antigen receptor, said linker or spacer region connecting the antigen-binding domain to the transmembrane domain of the antigen receptor.
3. The surface modified particle of claim 1 or claim 2, wherein the binding molecule comprises a recombinant antigen or a portion thereof recognized by the antigen-binding domain.
4. The surface modified particle of claim 3, wherein the recombinant antigen is BCMA, or is a portion thereof recognized by the antigen-binding domain.
5. The surface modified particle of claim 3 or claim 4, wherein the binding molecule is a fusion polypeptide comprising the recombinant antigen or the portion thereof linked to a moiety, wherein the moiety facilitates attachment to the particle.
6. The surface modified particle of claim 5, wherein:
the moiety is linked to the C-terminus of the recombinant antigen; and/or
the moiety is or comprises an Fc domain.
7. The surface modified particle of claim 1 or claim 2, wherein the binding molecule comprises an anti-idiotypic antibody or antigen-binding fragment thereof.

8. The surface modified particle of any of claims 1, 2, and 7, wherein the antigen recognized by the antigen-binding domain is CD19.

9. The surface modified particle of claim 7 or claim 8, wherein the antigen-binding domain of the chimeric antigen receptor is or comprises antibody SJ25C1 or an antigen-binding fragment thereof.

10. The surface modified particle of claim 7 or claim 8, wherein the antigen-binding domain of the chimeric antigen receptor is or comprises antibody FMC63 or an antigen-binding fragment thereof.

11. The surface modified particle of claim 9, wherein the antigen-binding fragment is or comprises an scFv.

12. The surface modified particle of claim 10, wherein the antigen-binding fragment is or comprises an scFv.

13. The surface modified particle of any of claims 7-12, wherein:
the anti-idiotypic antibody or antigen-binding fragment thereof comprises at least a portion of an immunoglobulin constant region; and/or
the anti-idiotypic antibody or antigen-binding fragment thereof comprises at least a portion of an Fc region or a portion of the Fc comprising the CH2 and CH3 domains; and/or
the anti-idiotypic antibody or antigen-binding fragment thereof is an intact antibody or full-length antibody.

14. The surface modified particle of any of claims 1-13, wherein:
the binding molecule is attached to the particle at or near the C-terminal amino acid residue of the binding molecule; and/or
the binding molecule is covalently attached to the particles; and/or
the binding molecule is covalently attached to the particles via a surface exposed functional group selected from an amino group, a carboxyl group, a thiol group, an aldehyde group, a

chloromethyl group, an epoxy group, a hydroxyl group, a tosyl group or a hydrazine group;
and/or

the binding molecule is covalently attached to the particles via a surface exposed functional group that is a tosyl group.

15. The surface modified particle of any of claims 1-14, wherein the particle has a diameter of or of about 2.8 μm or 4.5 μm .

16. The surface modified particle of any of claims 1-15, wherein:
the particle is or comprises glass, silica, polyesters of hydroxy carboxylic acids, polyanhydrides of dicarboxylic acids, copolymers of hydroxy carboxylic acids, copolymers dicarboxylic acids, or a metal; and/or
the particle comprises a surface comprising a polymer, a polysaccharide, a silica, a fatty acid, a carbon, or a combination thereof; and/or
the particle comprises a surface comprising a polymer that is polyethylene glycol, poly(lactic-co-glycolic acid), polyglutaraldehyde, polyurethane, polystyrene, polyvinyl alcohol, or combinations thereof; and/or
the particle comprises a hydrophobic surface; and/or
the particle comprises a polystyrene surface; and/or
the particle is magnetic and/or comprises a magnetic core, a paramagnetic core or a superparamagnetic core.

17. The surface modified particle of any of claims 1-16, wherein the particle comprises at least or about at least 10 copies, 10^2 copies, 10^3 copies, 10^4 copies, 10^5 copies or 10^6 copies of the binding molecule.

18. The surface modified particle of any of claims 1-17, wherein the particle further comprises at least one agent attached to the surface of the particle, wherein the at least one agent specifically binds to an additional molecule on a cell to provide an accessory signal and/or to block an inhibitory signal.

19. The surface modified particle of claim 18, wherein:

the at least one agent is a ligand or is an antibody or antigen-binding fragment thereof; and/or
the at least one agent is covalently attached to the particle; and/or
the ratio of the binding molecule and the at least one agent attached to the particle is or is about 1:1.

20. The surface modified particle of claim 18 or claim 19, wherein:
the additional molecule is a costimulatory molecule or an activating co-receptor; the additional molecule is OX-40, ICOS, DAP10, B7-1, B7-2, CD28 or 4-1BB; or
the additional molecule is an inhibitory receptor; or
the additional molecule is CTLA-4, PD-1, LAG-3, Tim-3, BTLA or TIGIT; or
the additional molecule is a T cell surface protein; or
the additional molecule is CD2 or CD3; or
the additional molecule is CD2 or CD28.

21. The surface modified particle of claim 20, wherein the particle comprises one or both of an anti-CD2 antibody and an anti-CD28 antibody.

22. A composition, comprising a plurality of the surface modified particles of any of claims 1-21.

23. The composition of claim 22, wherein:
the concentration of the binding molecule in the composition is between or between about 0.5 $\mu\text{g/mL}$ and 500 $\mu\text{g/mL}$, 1 $\mu\text{g/mL}$ and 200 $\mu\text{g/mL}$ or 5 $\mu\text{g/mL}$ and 100 $\mu\text{g/mL}$ L; or
the concentration of the binding molecule in the composition is at least or at least about 1 $\mu\text{g/mL}$, 5 $\mu\text{g/mL}$, 10 $\mu\text{g/mL}$, 25 $\mu\text{g/mL}$, 50 $\mu\text{g/mL}$, 100 $\mu\text{g/mL}$ or 200 $\mu\text{g/mL}$.

24. The composition of claim 22 or claim 23, wherein:
the plurality of surface modified particles comprises a mean diameter of between or between about 2 μm and 5 μm ; or
the plurality of surface modified particles comprises a mean density of between or between about 1 g/cm^3 and about 2 g/cm^3 .

25. A kit, comprising the surface modified particle of any of claims 1-21 or the composition of any of claims 22-24 and instructions for use, optionally wherein:

instructions are for selecting or enriching, from a population of cells, cells expressing a chimeric antigen receptor comprising an antigen-binding domain specifically recognized by the binding molecule; or

instructions are for stimulating or expanding, from a population of cells, cells expressing a chimeric antigen receptor comprising an antigen-binding domain specifically recognized by the binding molecule.

26. A method for expanding cells, comprising incubating a population of cells with the surface modified particle of any of claims 1-21 or the composition of any of claims 22-24.

27. The method of claim 26, wherein:

the population comprises cells expressing a chimeric antigen receptor comprising an extracellular antigen-binding domain that specifically binds or recognizes an antigen; and

the binding molecule of the surface modified particle or each of the surface modified particles of the composition specifically binds to or recognizes the extracellular antigen-binding domain of the chimeric antigen receptor; and

binding of the binding molecule to the extracellular antigen-binding domain induces expansion of the cells comprising the chimeric antigen receptor.

28. The method of claim 27, wherein:

the extracellular antigen-binding domain specifically binds or recognizes B cell maturation antigen (BCMA); and

the binding molecule comprises the extracellular domain of BCMA or a portion of the extracellular domain recognized by the extracellular antigen-binding domain.

29. The method of claim 27, wherein:

the extracellular antigen-binding domain specifically binds or recognizes CD19; and

the binding molecule is an anti-idiotypic antibody or antigen-binding fragment thereof that specifically binds to the antigen-binding domain.

30. The method of any of claims 26-29, wherein during the incubating, the ratio of total cells in the population to the plurality of surface modified particles of the composition is from or from about 5:1 to 1:5, 3:1 to 1:3, or 2:1 to 1:2, each inclusive.

31. The method of claim 30, wherein the ratio is or is about 1:1.

32. The method of any of claims 26-31, wherein the incubation is carried out for greater than or greater than about 2 hours, 4 hours, 8 hours, 12 hours, 24 hours, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, or 16 days.

33. The method of any of claims 26-32, wherein:
the population comprises immune cells; and/or
the population comprises T cells or NK cells; and/or
the population comprises induced pluripotent stem cells (iPSC); and/or
the population comprises one or both of CD4+ T cells and CD8+ T cells, and/or
the population comprises primary cells obtained from a subject, optionally a human subject.

34. The method of claim 33, wherein the ratio of the CD4+ cells to the CD8+ cells is between or between about 1:2 and 2:1, or 1:3 and 3:1, or is or is about 1:1.

35. A method of genetically engineering a cell, comprising:
(a) contacting a composition of cells with a nucleic acid molecule encoding a chimeric antigen receptor under conditions to introduce the nucleic acid molecule into one or more cells in the composition, thereby producing an input composition; and
(b) incubating cells of the input composition according to the method of any of claims 26-34.

36. The method of claim 35, wherein at least a portion of the contacting and incubating are carried out simultaneously.

37. The method of claim 35 or claim 36, wherein the contacting is carried out by transduction with a viral vector.

38. The method of claim 37, wherein the viral vector is a retroviral vector, a gamma-retroviral vector, or a lentiviral vector.

39. The method of any of claims 35-38, wherein the number of cells in the output composition comprising the chimeric antigen receptor is increased or enriched by 1.2-fold, 2.0-fold, 3.0-fold, 4.0-fold, 5.0-fold, 6.0-fold, 7.0-fold, 8.0-fold, 9.0-fold, 10-fold or more compared to the number of the cells comprising the antigen receptor in the input composition.

40. A method of selecting or enriching cells, comprising contacting a population of cells with the surface modified particle of any of claims 1-21 or the composition of any of claims 22-24.

41. A long-term stimulation method for assessing a cell composition, the method comprising:

incubating, for a period of time of at least 10 days, an input composition under conditions to stimulate a CAR-dependent activity in cells in the input composition, said input composition comprising T cells expressing a chimeric antigen receptor (CAR) comprising an extracellular antigen-binding domain that specifically binds or recognizes an antigen, thereby producing an output composition; and

assessing one or more phenotype or activity of one or more cells of the output composition; wherein:

the conditions to stimulate a CAR-dependent activity comprises the presence of a binding molecule that specifically binds to the antigen-binding domain of the CAR; and the binding molecule is attached to a solid support.

42. The method of claim 41, wherein:

the solid support is the surface of a well of a microplate or a bead; or

the solid support is a microplate having the binding molecule attached to the microplate, and the incubation is carried out in the microplate; or

the solid support is a bead having attached the binding molecule, and the incubation is carried out in the presence of a plurality of the beads.

43. The method of claim 41 or claim 42, wherein the binding molecule is or comprises a recombinant antigen or a portion thereof recognized by the antigen-binding domain.

44. The method of claim 43, wherein the recombinant antigen or portion thereof is BCMA, or is a portion thereof recognized by the antigen-binding domain.

45. The method of claim 41 or claim 42, wherein the binding molecule is or comprises an anti-idiotypic antibody or antigen-binding fragment thereof that specifically binds to the antigen-binding domain.

46. The method of claim 45, wherein:
the antigen-binding domain of the antigen receptor is or comprises antibody SJ25C1 or an antigen-binding fragment thereof; or
the antigen-binding domain of the antigen receptor is or comprises antibody FMC63 or an antigen-binding fragment thereof.

47. The method of any of claims 41-46, comprising:
assessing one or more phenotypes of activation, exhaustion or differentiation state of the one or more cells of the output composition; and/or
assessing one or more activities of the one or more cells of the output composition; and/or
assessing CAR-dependent activity; and/or
assessing cytolytic activity or cytokine production.

48. The method of any of claims 41-47, wherein:
the period of time is at least or at least about 11 days, 12 days, 13 days, 14 days, or 15 days; or
the period of time is or is about 11 days, 12 days, 13 days, 14 days or 15 days.