

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 an immune 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.

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 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; the binding molecule is a recombinant antigen BCMA, or is a portion thereof recognized by the antigen-binding domain.

[0044b] 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 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; the binding molecule comprises an anti-idiotypic antibody or antigen-binding fragment thereof; and 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.

[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

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 immune 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 immune 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 example, 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

CLAIMS

1. A surface modified particle, comprising (i) a particle that is a bead comprising a diameter of between 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;

the binding molecule is a recombinant antigen BCMA, or is a portion thereof recognized by the antigen-binding domain.

2. A surface modified particle, comprising (i) a particle that is a bead comprising a diameter of between 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;

the binding molecule comprises an anti-idiotypic antibody or antigen-binding fragment thereof; and

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.

3. The surface modified particle of claim 1 or 2, 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.

4. The surface modified particle of claim 1, 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.

5. The surface modified particle of claim 4, wherein:
the moiety is linked to the C-terminus of the recombinant antigen; and/or

the moiety is or comprises an Fc domain.

6. The surface modified particle of claim 2 or claim 3, wherein the antigen recognized by the antigen-binding domain is CD19.

7. The surface modified particle of claim 6, wherein the antigen-binding domain of the chimeric antigen receptor is or comprises antibody SJ25C1 or an antigen-binding fragment thereof.

8. The surface modified particle of claim 6, wherein the antigen-binding domain of the chimeric antigen receptor is or comprises antibody FMC63 or an antigen-binding fragment thereof.

9. The surface modified particle of claim 7, wherein the antigen-binding fragment is or comprises an scFv.

10. The surface modified particle of claim 8, wherein the antigen-binding fragment is or comprises an scFv.

11. The surface modified particle of claim 2, 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.

12. The surface modified particle of any of claims 1-11, 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.

13. The surface modified particle of any of claims 1-12, wherein the particle has a diameter of or of about 2.8 μm or 4.5 μm .

14. The surface modified particle of any of claims 1-13, 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.

15. The surface modified particle of any of claims 1-14, 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.

16. The surface modified particle of claim 1, 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.

17. The surface modified particle of any of claims 2-3 and 6-16, 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.

18. The surface modified particle of any of claims 1-17, 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.

19. The surface modified particle of claim 18, wherein the particle comprises one or both of an anti-CD2 antibody and an anti-CD28 antibody.

20. A composition, comprising a plurality of the surface modified particles of any of claims 1, 4-5, 12-16, and 18-19.

21. The composition of claim 20, wherein:
the concentration of the binding molecule in the composition is between 0.5 µg/mL and 500 µg/mL, 1 µg/mL and 200 µg/mL or 5 µg/mL and 100 µg/mL; or
the concentration of the binding molecule in the composition is at least or at least about 1 µg/mL, 5 µg/mL, 10 µg/mL, 25 µg/mL, 50 µg/mL, 100 µg/mL or 200 µg/mL.

22. The composition of claim 20 or claim 21, wherein:
the plurality of surface modified particles comprises a mean diameter of between 2 µm and 5 µm; or
the plurality of surface modified particles comprises a mean density of between 1 g/cm³ and 2 g/cm³.

23. A composition, comprising a plurality of the surface modified particles of any of claims 2-3 and 6-19.

24. The composition of claim 23, wherein:

the concentration of the binding molecule in the composition is between 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 $\mu\text{g/mL}$.

25. The composition of claim 23 or claim 24, wherein:

the plurality of surface modified particles comprises a mean diameter of between 2 μm and 5 μm ; or

the plurality of surface modified particles comprises a mean density of between 1 g/cm^3 and 2 g/cm^3 .

26. A kit, comprising the surface modified particle of any of claims 1-19 or the composition of any of claims 20-22 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.

27. A kit, comprising the surface modified particle of any of claims 1-19 or the composition of any of claims 23-25 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.

28. A method for expanding immune cells, comprising incubating a population of cells with the surface modified particle of any of claims 1-19 or the composition of any of claims 20-22.

29. A method for expanding immune cells, comprising incubating a population of cells with the surface modified particle of any of claims 1-19 or the composition of any of claims 23-25.

30. The method of claim 28 or claim 29, 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.

31. The method of claim 28 or claim 30, 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.

32. The method of claim 29 or claim 30, 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.

33. The method of any of claims 28-32, wherein during the incubating, the ratio of total cells in the population to the plurality of surface modified particles of the composition is from 5:1 to 1:5, 3:1 to 1:3, or 2:1 to 1:2, each inclusive.

34. The method of claim 33, wherein the ratio is or is about 1:1.

35. The method of any of claims 28-34, 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.

36. The method of any of claims 28-35, 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.

37. The method of claim 36, wherein the ratio of the CD4⁺ cells to the CD8⁺ cells is between 1:2 and 2:1, or 1:3 and 3:1, or is or is about 1:1.

38. A method of genetically engineering an immune 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 28-37.

39. The method of claim 38, wherein at least a portion of the contacting and incubating are carried out simultaneously.

40. The method of claim 38 or claim 39, wherein the contacting is carried out by transduction with a viral vector.

41. The method of claim 40, wherein the viral vector is a retroviral vector, a gamma-retroviral vector, or a lentiviral vector.

42. The method of any of claims 38-41, 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.

43. A method of selecting or enriching immune cells, comprising contacting a population of cells with the surface modified particle of any of claims 1-19 or the composition of any of claims 20-22.

44. A method of selecting or enriching immune cells, comprising contacting a population of cells with the surface modified particle of any of claims 1-19 or the composition of any of claims 23-25.