

CLAIMS

1. A composition comprising:
 - a) a lentiviral vector; and
 - b) an aqueous composition comprising:
 - i) N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES); and
 - ii) one or both of a free positively charged amino acid and a free nonpolar amino acid.
2. The composition of claim 1, wherein the aqueous composition is substantially free of:
 - (a) inorganic salts;
 - (b) one or both of NaCl and MgCl₂;
 - (c) HSA; and/or
 - (d) one, two, or all of PEG lipid, F108, and cholesterol.
3. The composition of claim 1, wherein the aqueous composition comprises less than 20 mM total of inorganic salts.
4. The composition of any one of claims 1-3, wherein HEPES is at a concentration of:
 - (a) 10-200 mM; or
 - (b) about 20 mM.
5. The composition of any one of claims 1-4, which comprises:
 - (a) the positively charged amino acid, wherein the positively charged amino acid is arginine, lysine, or histidine; and/or
 - (b) the nonpolar amino acid, wherein the nonpolar amino acid is proline, methionine, or tryptophan.
6. The composition of claim 5, wherein the positively charged amino acid is arginine.
7. The composition of claim 5, wherein the nonpolar amino acid is proline.

8. The composition of claim 6 or 7, wherein:
 - (a) the arginine is L-arginine;
 - (b) the arginine is at a concentration of at least 50 mM;
 - (c) the arginine is at a concentration of 25-50, 50-75, 75-100, or 100-200 mM;
 - (d) the arginine is at a concentration of about 150 mM;
 - (e) the proline is L-proline;
 - (f) the proline is at a concentration of 25-200 mM; and/or
 - (g) the proline is at a concentration of about 150 mM.
9. The composition of any one of claims 1-8, which further comprises a cryoprotectant agent.
10. The composition of claim 9, wherein the cryoprotectant agent is a carbohydrate, and wherein the carbohydrate:
 - (a) is a non-reducing carbohydrate; or
 - (b) is sucrose.
11. The composition of any one of claims 1-8, which further comprises sucrose, and wherein the sucrose is at a concentration of:
 - (a) 25-200 mM; or
 - (b) about 150 mM.
12. The composition of any one of claims 1-11, wherein the aqueous composition further comprises a stabilizing agent.
13. The composition of any one of claims 1-11, wherein the aqueous composition comprises HSA.
14. The composition of claim 13, wherein the HSA:
 - (a) comprises recombinant HSA (rHSA) or human-derived HSA;
 - (b) is present at 0.5-3% w/v; and/or
 - (c) is present at about 2% w/v.

15. The composition of any one of claims 1-14, which has a pH of:
 - (a) 6.0-7.5; or
 - (b) about 6.5.
16. The composition of any one of claims 1-15, wherein:
 - (i) the aqueous composition comprises the positively charged amino acid, wherein the positively charged amino acid comprises L-arginine;
 - (ii) the aqueous composition comprises the nonpolar amino acid, wherein the nonpolar amino acid comprises L-proline;
 - (iii) the aqueous composition further comprises sucrose; and
 - (iv) the aqueous composition is substantially free of inorganic salts.
17. The composition of claim 16, wherein:
 - (i) the L-arginine is at a concentration of 100-200 mM;
 - (ii) the L-proline is at a concentration of 25-200 mM; and
 - (iii) the sucrose is at a concentration of 25-200 mM.
18. The composition of any one of claims 1-17, wherein the osmolality of the aqueous composition is:
 - (a) from about 400 mOsm/kg to about 700 mOsm/kg; or
 - (b) about 415 mOsm/kg to about 689 mOsm/kg.
19. The composition of any one of claims 1-18, which comprises at least 1×10^6 transducing units per milliliter (TU/mL) of the lentiviral vector.
20. The composition of any one of claims 1-19, wherein the lentiviral vector comprises a transgene.
21. The composition of claim 20, wherein the transgene encodes a chimeric antigen receptor (CAR).

22. The composition of claim 21, wherein the CAR comprises an antigen binding domain, a transmembrane domain, one or more primary signaling domains, and/or one or more costimulatory signaling domains.

23. The composition of claim 22, wherein:

(a) the CAR comprises an antigen-binding domain that binds a tumor antigen selected from the group consisting of: TSHR, CD19, CD123, CD22, CD30, CD171, CS-1, CLL-1, CD33, EGFRvIII, GD2, GD3, BCMA, Tn Ag, PSMA, ROR1, FLT3, FAP, TAG72, CD38, CD44v6, CEA, EPCAM, B7H3, KIT, IL-13Ra2, Mesothelin, IL-11Ra, PSCA, PRSS21, VEGFR2, LewisY, CD24, PDGFR-beta, SSEA-4, CD20, Folate receptor alpha, ERBB2 (Her2/neu), MUC1, EGFR, NCAM, Prostase, PAP, ELF2M, Ephrin B2, IGF-I receptor, CAIX, LMP2, gp100, bcr-abl, tyrosinase, EphA2, Fucosyl GM1, sLe, GM3, TGS5, HMWMAA, o-acetyl-GD2, Folate receptor beta, TEM1/CD248, TEM7R, CLDN6, GPRC5D, CXORF61, CD97, CD179a, ALK, Polysialic acid, PLAC1, GloboH, NY-BR-1, UPK2, HAVCR1, ADRB3, PANX3, GPR20, LY6K, OR51E2, TARP, WT1, NY-ESO-1, LAGE-1a, MAGE-A1, legumain, HPV E6,E7, MAGE A1, ETV6-AML, sperm protein 17, XAGE1, Tie 2, MAD-CT-1, MAD-CT-2, Fos-related antigen 1, p53, p53 mutant, prostatein, survivin and telomerase, PCTA-1/Galectin 8, MelanA/MART1, Ras mutant, hTERT, sarcoma translocation breakpoints, ML-IAP, ERG (TMPRSS2 ETS fusion gene), NA17, PAX3, Androgen receptor, Cyclin B1, MYCN, RhoC, TRP-2, CYP1B1, BORIS, SART3, PAX5, OY-TES1, LCK, AKAP-4, SSX2, RAGE-1, human telomerase reverse transcriptase, RU1, RU2, intestinal carboxyl esterase, mut hsp70-2, CD79a, CD79b, CD72, LAIR1, FCAR, LILRA2, CD300LF, CLEC12A, BST2, EMR2, LY75, GPC3, FCRL5, and IGLL1, and any combination thereof;

(b) the one or more primary signaling domains comprises a CD3-zeta stimulatory domain;

(c) the one or more costimulatory signaling domains comprises an intracellular domain selected from:

(i) a costimulatory protein selected from the group consisting of OX40, CD27, CD28, ICAM-1, LFA-1 (CD11a/CD18), ICOS(CD278), 4-1BB (CD137), ICAM-1, GITR, BAFFR, HVEM (LIGHTR), SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD160, CD19, CD4, CD8alpha, CD8beta, IL2R beta, IL2R gamma, IL7R alpha, ITGA4, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, NKG2D,

NKG2C, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRTAM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, CD19a; and

(ii) a ligand that specifically binds to CD83 selected from a 4-1 BB (CD137) costimulatory domain and a CD28 costimulatory domain; and/or

(d) the transmembrane domain:

(i) comprises a transmembrane domain of a protein selected from the alpha, beta, or zeta chain of T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, and CD154;

(ii) comprises a transmembrane domain of CD8;

(iii) comprises the amino acid sequence of SEQ ID NO: 6, or an amino acid sequence having at least about 90% sequence identity thereto; or

(iv) is encoded by a nucleic acid sequence of SEQ ID NO: 17, or a nucleic acid sequence having at least about 90% sequence identity thereto.

24. The composition of any one of claims 1-23, wherein:

(a) the composition exhibits a level of sub-visible particles of greater than or equal to 25 μm of about 50-100 particles/mL or lower;

(b) the composition exhibits a level of sub-visible particles of greater than or equal to 10 μm of about 1100-2000 particles/mL or lower; and/or

(c) the lentiviral vector within the composition retains a hydrodynamic diameter of between about 85-200 nm.

25. A kit comprising: a lentiviral vector and an aqueous composition of any one of claims 1-24.

26. A method of making a CAR-expressing cell, the method comprising:

providing a composition of any one of claims 1-24; and

contacting the composition with a population of immune effector cells under conditions that allow for transduction of one or more of the immune effector cells,

thereby making the CAR-expressing cell.

27. The method of claim 26, wherein the population of immune effector cells comprises one or both of T cells and NK cells.

28. Use of the composition of any one of claims 1-24, in the manufacture of a medicament for delivering a transgene to a subject, wherein the lentiviral vector of the composition comprises the transgene.

29. The use of claim 28, wherein the composition further comprises:

- (a) a particle; and/or
- (b) a cell activation agent.

30. The use of claim 29, wherein the particle is a silica particle.

31. The use of claim 29, wherein:

- (a) the lentiviral vector is noncovalently or covalently associated with a silica particle that is a mesoporous silica particle;
- (b) the cell activation agent is noncovalently or covalently associated with a silica particle that is a mesoporous silica particle;
- (c) the cell activation agent comprises an agent that stimulates CD3/TCR complex and/or an agent that stimulates a costimulatory molecule and/or growth factor receptor; and/or
- (d) the cell activation agent is a multispecific binding molecule comprising: (i) an anti-CD3 binding domain, and (ii) a costimulatory molecule binding domain.

32. The use of claim 31, wherein the costimulatory molecule binding domain is an anti-CD2 binding domain or an anti-CD28 binding domain.