

VECTORS AND METHODS FOR *IN VIVO* TRANSDUCTION

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority under 35 U.S.C. §119 from U.S. Provisional Application Serial No. 63/023,191, filed May 11, 2020 and U.S. Provisional Application Serial No. 63/133,224, filed December 31, 2020, the disclosures of which are incorporated herein by reference.

TECHNICAL FIELD

[0002] The disclosure provides compositions and methods for cellular therapies of cancer, infection, allergic, degenerative and immune disorders.

INCORPORATION BY REFERENCE OF SEQUENCE LISTING

[0003] Accompanying this filing is a Sequence Listing entitled "Sequence-Listing_ST25.txt", created on May 11, 2021 and having 239,881 bytes of data, machine formatted on IBM-PC, MS-Windows operating system. The sequence listing is hereby incorporated herein by reference in its entirety for all purposes.

BACKGROUND

[0004] Adoptive immunotherapy has risen to the forefront of treatment approaches for cancer. T cells can be engineered to express the genes of chimeric antigen receptors (CARs) that recognize tumor associated antigens. CARs are engineered immune-receptors, which can redirect T cells to selectively kill tumor cells. The general premise for their use in cancer immunotherapy is to rapidly generate tumor-targeted T cells.

SUMMARY

[0005] The ideal gene delivery system would be easy to produce, easy to administer, and non-toxic to normal cells; it would deliver the genetic information efficiently and specifically via the bloodstream to the targeted tissues and integrate the genetic material into the host cell so that the transgene would be stably expressed. The novel systems described here fit this prescription.

[0005a] In one aspect, the present disclosure refers to a recombinant vector comprising: (a) a polynucleotide encoding a chimeric antigen receptor (CAR); and (b) a polynucleotide comprising at least one miRNA targeting sequence, wherein (a) and (b) are linked on the same polynucleotide.

[0005b] In another aspect, the present disclosure refers to a recombinant retroviral particle comprising: a gag polypeptide; a pol polypeptide; an env polypeptide; and a retroviral polynucleotide contained within a capsid of the retroviral vector, wherein the retroviral polynucleotide comprises from 5' to 3': (R-U5 domain)-(optional signal

peptide coding sequence domain)-(Binding domain coding sequence domain)-
(optional hinge/linker coding sequence domain)-(transmembrane (TM) coding
sequence domain)-(miRNA target domain(s))-(U3-R domain).

[0005c] In another aspect, the present disclosure refers to a
pharmaceutical composition comprising the recombinant retroviral particle
as disclosed herein in a pharmaceutically acceptable carrier.

[0005d] In another aspect, the present disclosure refers to a
composition comprising: (i) a retroviral vector comprising a polynucleotide
encoding an antigen binding receptor construct targeting an antigen; and
(ii) an miR-targeting sequence or miR-targeting cassette; (iii) an optional
kills switch coding domain; and (iv) a pharmaceutically acceptable carrier.

[0005e] In another aspect, the present disclosure refers to the use of
the composition as disclosed herein in the manufacture of a medicament for
providing anti-disease immunity in a subject, wherein the composition
transduces cells in vivo to give rise to immune effector cells that express
an antigen receptor binding construct.

[0005f] In another aspect, the present disclosure refers to an ex vivo
or in vitro method of producing a retroviral vector comprising transforming
a cell line expressing murine leukemia virus (MLV) gag-pol genes with a
vector as disclosed herein.

[0005g] In another aspect, the present disclosure refers to a
retrovirus produced by the method as disclosed herein.

[0005h] In another aspect, the present disclosure refers to an ex vivo
or in vitro method for identifying off-target miRNA expression profiles
comprising isolating total RNA from a cell or tissue; generating a sequence
library from the isolate RNA; sequencing the sequence library; processing
sequence reads to identify miRNA sequences; order ranking the miRNA
sequences using differential and ranked analysis; and identifying the top 1
to 5 miRNA sequences that are present in both the differential and ranked
analysis.

[0005i] In another aspect, the present disclosure refers to the use of
an agent that mobilizes hematopoietic stem cells from the bone marrow in
the manufacture of a medicament for mobilizing stem cells for in vivo gene
transfer therapy in a subject.

[0001] The disclosure provides a recombinant vector comprising (a) a
polynucleotide encoding a chimeric antigen receptor (CAR); and (b)
polynucleotide comprising at least one miRNA targeting sequence, wherein
(a) and (b) are linked on the same polynucleotide. In one

[embodimentexample](#), the vector further comprising (c) a polynucleotide encoding a cytotoxic polypeptide that converts a prodrug to a cytotoxic drug. In another [embodimentexample](#), (a) comprises a first polynucleotide domain encoding one or more antigen binding domain(s); an optional polynucleotide domain encoding a linker; and a second polynucleotide domain operably linked to the first polynucleotide domain, wherein the second polynucleotide domain encodes a transmembrane; and a third polynucleotide domain encoding an intracellular signaling domain. In a further [embodimentexample](#), the first polynucleotide domain encodes an antibody fragment, single domain antibody, single chain variable fragment, single domain antibody, camelid VHH domain, a non-immunoglobulin antigen binding scaffold, a receptor or receptor fragment, or a bispecific antibody. In another [embodimentexample](#), the optional polynucleotide encoding a linker encodes a Gly3 sequence. In still another [embodimentexample](#), the transmembrane domain is from a member selected from the group consisting alpha, beta or zeta chain of a T-cell receptor, CD3 γ , CD3 ϵ , CD3 δ , CD28, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, CD154, KIRDS2, OX40, CD2, CD27, LFA-1 (CD11a, CD18), ICOS (CD278), 4-1BB (CD137), GITR, CD40, BAFFR, HVEM (LIGHTR), SLAMF7, NKp80 (KLRF1), CD160, CD19, IL2R beta, IL2R gamma, IL7R α , ITGA1, 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, TNFR2, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRTAM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), SLAMF6 (NTB-A, Lyl08), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, PAG/Cbp, NKp44, NKp30, NKp46, NKG2D, and/or NKG2C. In yet another [embodimentexample](#), the third polynucleotide domain encodes an intracellular signaling domain selected from the group consisting of CD3 zeta, common γ (FCER1G), γ RIIa, γ beta (Fe Epsilon R1b), CD3 γ , CD3 δ , CD3 epsilon, CD79a, CD79b, DAP10, and DAP12. In one [embodimentexample](#), the cytotoxic polypeptide that converts a prodrug to a cytotoxic drug is selected from the group consisting of a polypeptide having cytosine deaminase activity, a polypeptide having thymidine kinase activity and a combination thereof. In any of the foregoing [embodimentexamples](#), the vector is an

integrating vector. In a further [embodimentexample](#), the vector is a retroviral vector. In still a further [embodimentexample](#), the retroviral vector is a non-replicating gammaretroviral vector. In another [embodimentexample](#), the at least one miRNA targeting sequence is bound by an miRNA selected from the group consisting of hsa-miR-223-3p, hsa-miR143-3p, hsa-miR182-5p, hsa-miR-10bp, hsa-miR141-3p, has-miR486-5p and any combination of the foregoing.

[0002] The disclosure also provides a recombinant retroviral particle comprising a gag polypeptide; a pol polypeptide; an env polypeptide; and a retroviral polynucleotide contained within a capsid of the retroviral vector, wherein the retroviral polynucleotide comprises from 5' to 3': (R-U5 domain)-(optional signal peptide coding sequence domain)-(Binding domain coding sequence domain)-(optional hinge/linker coding sequence domain)-(transmembrane (TM) coding sequence domain)-(miRNA target domain(s))-(U3-R domain). In one [embodimentexample](#), the R-U5 domain has a sequence that is at least 80% identical to SEQ ID NO:25 from nucleotide 1 to about nucleotide 145. In another or further [embodimentexample](#), the binding domain coding sequence is preceded by a signal sequence. In still another or further [embodimentexample](#), the binding domain coding sequence is followed by an optional linker/spacer domain sequence. In still another or further [embodimentexample](#), the retroviral polynucleotide further comprises a kill switch domain coding sequence. In a further [embodimentexample](#), the kill switch coding domain comprises an IRES operably linked to a coding sequence for a polypeptide that converts a prodrug to a cytotoxic drug. In a further [embodimentexample](#), the polypeptide has thymidine kinase (TKO) activity or cytosine deaminase (CD) activity. In another [embodimentexample](#), the retroviral polynucleotide comprises at least one miRNA targeting sequence. In a further [embodimentexample](#), the at least one miRNA targeting sequence comprises a plurality of miRNA targeting sequences. In still a further [embodimentexample](#), the plurality of miRNA targeting sequences are the same. In yet another [embodimentexample](#), at least two of the plurality of miRNA targeting sequences are different. In another [embodimentexample](#), the U3-R domain comprises a sequence that is at least 80% identical to SEQ ID NO:25 from about nucleotide 5537 to about 6051.

What is claimed is:

1. A recombinant vector comprising:
 - (a) a polynucleotide encoding a chimeric antigen receptor (CAR); and
 - (b) a polynucleotide comprising at least one miRNA targeting sequence,
wherein (a) and (b) are linked on the same polynucleotide.
2. The recombinant vector of claim 1, further comprising (c) a polynucleotide encoding a cytotoxic polypeptide that converts a prodrug to a cytotoxic drug.
3. The recombinant vector of claim 1, wherein (a) comprises a first polynucleotide domain encoding one or more antigen binding domain(s);
an optional polynucleotide domain encoding a linker; and
——a second polynucleotide domain operably linked to the first polynucleotide domain, wherein the second polynucleotide domain encodes a transmembrane; and
——a third polynucleotide domain encoding an intracellular signaling domain.
4. The recombinant vector of claim 3, wherein the first polynucleotide domain encodes an antibody fragment, single domain antibody, single chain variable fragment, single domain antibody, camelid VHH domain, a non-immunoglobulin antigen binding scaffold, a receptor or receptor fragment, or a bispecific antibody.
5. The recombinant vector of claim 3, wherein the optional polynucleotide encoding a linker encodes a Gly3 sequence.
6. The recombinant vector of claim 3, wherein the transmembrane domain is from a member selected from the group consisting alpha, beta or zeta chain of a T-cell receptor, CD3γ, CD3ε, CD3δ, CD28, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, CD154, KIRDS2, OX40, CD2, CD27, LFA-1 (CD11a, CD18), ICOS (CD278), 4-1BB (CD137), GITR, CD40, BAFFR, HVEM (LIGHTR), SLAMF7, NKp80 (KLRF1), CD160, CD19, IL2R beta, IL2R gamma, IL7R a, ITGA1, 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, TNFR2,

DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRT AM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, PAG/Cbp, NKp44, NKp30, NKp46, NKG2D, and/or NKG2C.

7. The recombinant vector of claim 3, wherein the third polynucleotide domain encodes an intracellular signaling domain selected from the group consisting of CD3 zeta, common FeR gamma (FCER1G), Fe gamma RIIa, FeR beta (Fe Epsilon R1b), CD3 gamma, CD3 delta, CD3 epsilon, CD79a, CD79b, DAP10, and DAP12.

8. The recombinant vector of claim 2, wherein the cytotoxic polypeptide that converts a prodrug to a cytotoxic drug is selected from the group consisting of a polypeptide having cytosine deaminase activity, a polypeptide having thymidine kinase activity and a combination thereof.

9. The recombinant vector of any one of claims 1-8, wherein the vector is an integrating vector.

10. The recombinant vector of claim 9, wherein the vector is a retroviral vector.

11. The recombinant vector of claim 10, wherein the retroviral vector is a non-replicating gammaretroviral vector.

12. The recombinant vector of claim 1, wherein the at least one miRNA targeting sequence is bound by an miRNA selected from the group consisting of hsa-miR-223-3p, hsa-miR143-3p, hsa-miR182-5p, hsa-miR-10b, hsa-miR141-3p, hsa-miR486-5p and any combination of the foregoing.

13. A recombinant retroviral particle comprising:
a gag polypeptide;
a pol polypeptide;
an env polypeptide; and
a retroviral polynucleotide contained within a capsid of the retroviral vector,

wherein the retroviral polynucleotide comprises from 5' to 3':
(R-U5 domain)-(optional signal peptide coding sequence domain)-
(Binding domain coding sequence domain)-(optional hinge/linker coding sequence domain)-(transmembrane (TM) coding sequence domain)-(miRNA target domain(s))-(U3-R domain).

14. The recombinant retroviral particle of claim 13, wherein the R-U5 domain has a sequence that is at least 80% identical to SEQ ID NO:25 from nucleotide 1 to about nucleotide 145.

15. The recombinant retroviral particle of claim 13, wherein the binding domain coding sequence is preceded by a signal sequence.

16. The recombinant retroviral particle of claim 13, wherein the binding domain coding sequence is followed by an optional linker/spacer domain sequence.

17. The recombinant retroviral particle of claim 13, wherein the retroviral polynucleotide further comprises a kill switch domain coding sequence.

18. The recombinant retroviral particle of claim 17, wherein the kill switch coding domain comprises an IRES operably linked to a coding sequence for a polypeptide that converts a prodrug to a cytotoxic drug.

19. The recombinant retroviral particle of claim 18, wherein the polypeptide has thymidine kinase (TKO) activity or cytosine deaminase (CD) activity.

20. The recombinant retroviral particle of claim 13, wherein the retroviral polynucleotide comprises at least one miRNA targeting sequence.

21. The recombinant retroviral particle of claim 20, wherein the at least one miRNA targeting sequence comprises a plurality of miRNA targeting sequences.

22. The recombinant retroviral particle of claim 21, wherein the plurality of miRNA targeting sequences are the same.

23. The ~~re~~ recombinant retroviral particle of claim 21, wherein at least two of the plurality of miRNA targeting sequences are different.

24. The recombinant retroviral particle of claim 13, wherein the U3-R domain comprises a sequence that is at least 80% identical to SEQ ID NO:25 from ~~about~~ nucleotide 5537 to ~~about~~ 6051.

25. The recombinant retroviral particle of claim 13, wherein each domain can be separated from another domain by small spacer sequences of ~~about~~ 1-20 nucleotides.

26. A pharmaceutical composition comprising the recombinant retroviral particle of any one of claims 13-25 in a pharmaceutically acceptable carrier.

27. A composition comprising:

- (i) a retroviral vector comprising a polynucleotide encoding an antigen binding receptor construct targeting an antigen; and
- (ii) an miR-targeting sequence or miR-targeting cassette;
- (iii) an optional kills switch coding domain; and
- (iv) a pharmaceutically acceptable carrier.

28. The composition of claim 27, wherein the antigen binding receptor construct comprises a binding domain, an optional hinge/linker domain, a transmembrane domain, and an intracellular signaling domain.

29. The composition of claim 28, wherein the binding domain comprises an antibody, a Fv, a Fab, a (Fab')₂, a heavy chain variable region of an antibody (vH domain), a light chain variable region of an antibody (vL domain), a single domain antibody, a single chain variable fragment (scFv), a monomeric variable region of an antibody, a camelid vHH domain, a non-immunoglobulin antigen binding domain (e.g., DARPIN, an affibody, an affilin, an adnectin, an affitin, an obodies, a repebody, a fynomer, an alphabody, an avimer, an atrimer, a centyrin, a pronectin, an anticalin, a kunitz domain, an Armadillo repeat protein), a ligand or a fragment thereof having specificity to an antigen associated with a disease or disorder.

30. The composition of claim 27, wherein the retroviral vector comprises a lipid envelope.

31. The composition of claim 30, wherein the envelope is amphotrophic.

32. The composition of claim 27, wherein the antigen binding receptor construct comprises a chimeric antigen receptor.

33. The composition of claim 27, wherein the miR-targeting sequence or miR-targeting cassette comprises one or more targeting sequence for miRNA that are expressed in off-target cells.

34. The composition of claim 27, wherein the optional kills switch comprises a coding sequence from a suicide gene.

35. The composition of claim 34, wherein the suicide gene encodes a polypeptide having cytosine deaminase activity or thymidine kinase activity.

36. The composition of claim 27, wherein the polynucleotide comprises from 5' to 3': R-U5-(antigen binding receptor coding sequence)-(miR targeting sequence or cassette)-(optional suicide gene)-U3-R.

37. The composition of claim 27 or 36, wherein the polynucleotide comprises a gammaretroviral R, U3 and U5 sequence.

38. The composition of claim 29, where the antigen is selected from the group consisting of: CD5; CD19; CD123; CD20; CD22; CD24; CD30; CD33, CD34; CD38, CD72; CD97; CD171; CS1 (also referred to as CD2 subset 1, CRACC, MPL, SLAMF7, CD319, and 19A24); C-type lectin-like molecule-1 (CLL-1 or CLECL1); epidermal growth factor receptor variant III (EGFRviii); ganglioside G2 (GD2); ganglioside GD3 (aNeu5Ac(2-8)aNeu5Ac(2-3)bDGalp(1-4)bDG1cp(1-1)Cer); TNF receptor family member B cell maturation (BCMA); Tn antigen ((Tn Ag or (GalNAc α -Ser/Thr)); prostate-specific membrane antigen (PSMA); Receptor tyrosine kinase-like orphan receptor 1 (ROR1); Fms Like Tyrosine Kinase 3 (FLT3); Tumor-associated glycoprotein 72 (TAG72); CD44v6; a glycosylated CD43 epitope; Carcinoembryonic antigen (CEA); Epithelial cell adhesion molecule (EPCAM); B7H3 (CD276); KIT (CD117); Interleukin-13 receptor subunit alpha-2 (IL-13Ra2 or CD213A2); Mesothelin; Interleukin 11 receptor alpha (IL-11Ra); prostate stem cell antigen (PSCA); Protease Serine 21 (Testisin or PRSS21); vascular endothelial growth factor receptor 2 (VEGFR2); Lewis(Y) antigen; Platelet-derived growth factor receptor beta (PDGFR-beta); Stage-specific embryonic antigen-4 (SSEA-4); Folate receptor alpha (FRa or FR1); Folate receptor beta (FRb); Receptor tyrosine-protein kinase ERBB2 (Her2/neu); Mucin 1, cell surface associated (MUC1); epidermal growth factor receptor (EGFR); neural cell adhesion molecule (NCAM); Prostase; prostatic acid phosphatase (PAP); elongation factor 2 mutated (ELF2M); Ephrin B2; fibroblast activation protein alpha (FAP); insulin-like growth factor 1 receptor (IGF-I receptor), carbonic anhydrase IX (CAIX); Proteasome (Prosome, Macropain) Subunit, Beta Type, 9 (LMP2); glycoprotein 100 (gp100); oncogene fusion protein consisting of breakpoint cluster

region (BCR) and Abelson murine leukemia viral oncogene homolog 1 (Abl) (bcr-abl); tyrosinase; ephrin type-A receptor 2 (EphA2); sialyl Lewis adhesion molecule (sLe); ganglioside GM3 (aNeu5Ac(2-3)bDCIalp(1-4)bDGlcp(1-1)Cer); transglutaminase 5 (TGS5); high molecular weight-melanoma associated antigen (HMWMAA); o-acetyl-GD2 ganglioside (OAcGD2); tumor endothelial marker 1 (TEM1/CD248); tumor endothelial marker 7-related (TEM7R); claudin 6 (CLDN6); thyroid stimulating hormone receptor (TSHR); G protein coupled receptor class C group 5, member D (GPRC5D); chromosome X open reading frame 61 (CXORF61); CD179a; anaplastic lymphoma kinase (ALK); Polysialic acid; placenta-specific 1 (PLAC1); hexasaccharide portion of globoH glycosphingolipid (GloboH); mammary gland differentiation antigen (NY-BR-1); uroplakin 2 (UPK2); Hepatitis A virus cellular receptor 1 (HAVCR1); adrenoceptor beta 3 (ADRB3); pannexin 3 (PANX3); G protein-coupled receptor 20 (GPR20); lymphocyte antigen 6 complex, locus K 9 (LY6K); Olfactory receptor 51E2 (OR51E2); TCR Gamma Alternate Reading Frame Protein (TARP); Wilms tumor protein (WT1); Cancer/testis antigen 1 (NY-ESO-1); Cancer/testis antigen 2 (LAGE-1a); Melanoma-associated antigen 1 (MAGE-A1); ETS translocation-variant gene 6, located on chromosome 12p (ETV6-AML); sperm protein 17 (SPA17); X Antigen Family, Member 1A (XAGE1); angiopoietin-binding cell surface receptor 2 (Tie 2); melanoma cancer testis antigen-1 (MAD-CT-1); melanoma cancer testis antigen-2 (MAD-CT-2); Fos-related antigen 1; tumor protein p53 (p53); p53 mutant; prostein; survivin; telomerase; prostate carcinoma tumor antigen-1 (PCT A-1 or Galectin 8), melanoma antigen recognized by T cells 1 (MelanA or MART1); Rat sarcoma (Ras) mutant; human Telomerase reverse transcriptase (hTERT); sarcoma translocation breakpoints; melanoma inhibitor of apoptosis (ML-IAP); ERG (transmembrane protease, serine 2 (TMPRSS2) ETS fusion gene); N-Acetylglucosaminyl-transferase V (NA17); paired box protein Pax-3 (PAX3); Androgen receptor; Cyclin B1; v-myc avian myelocytomatosis viral oncogene neuroblastoma derived homolog (MYCN); Ras Homolog Family Member C (RhoC); Tyrosinase-related protein 2 (TRP-2); Cytochrome P450 1B 1 (CYP1B 1); CCCTC-Binding Factor (Zinc Finger Protein)-Like (BORIS or Brother of the Regulator of Imprinted Sites), Squamous Cell Carcinoma Antigen Recognized By T Cells 3 (SART3); Paired box

protein Pax-5 (PAX5); proacrosin binding protein sp32 (OY-TES1); lymphocyte-specific protein tyrosine kinase (LCK); A kinase anchor protein 4 (AKAP-4); synovial sarcoma, X breakpoint 2 (SSX2); Receptor for Advanced Glycation End products (RAGE-1); renal ubiquitous 1 (RU1); renal ubiquitous 2 (RU2); legumain; human papilloma virus E6 (HPV E6); human papilloma virus E7 (HPV E7); intestinal carboxyl esterase; heat shock protein 70-2 mutated (mut hsp70-2); CD79a; CD79b; Leukocyte-associated immunoglobulin-like receptor 1 (LAIR1); Fc fragment of IgA receptor (FCAR or CD89); Leukocyte immunoglobulin-like receptor subfamily A member 2 (LILRA2); CD300 molecule-like family member f (CD300LF); C-type lectin domain family 12 member A (CLEC12A); bone marrow stromal cell antigen 2 (BST2); EGF-like module-containing mucin-like hormone receptor-like 2 (EMR2); lymphocyte antigen 75 (LY75); Glypican-3 (GPC3); Fc receptor-like 5 (FCRL5); immunoglobulin lambda-like polypeptide 1 (IGLL1); MPL; Biotin; c-MYC epitope Tag; LAMP1 TROP2; GFRalpha4; CDH17; CDH6; NYBR1; CDH19; CD200R; Slea (CA19.9; Sialyl Lewis Antigen); Fucosyl-GM1; PTK7; gpNMB; CDH1-CD324; DLL3; CD276/B7H3; IL11Ra; IL13Ra2; CD179b-IGL11; TCRgamma-delta; NKG2D; CD32 (FCGR2A) ; Tn ag; Tim1-/HVCR1; CSF2RA (GM-CSFR-alpha); TGFbetaR2; Lews Ag; TCR-beta1 chain; TCR-beta2 chain; TCR-gamma chain; TCR-delta chain; FITC; Leutenizing hormone receptor (LHR) ; Follicle stimulating hormone receptor (FSHR) ; Gonadotropin Hormone receptor (CGHR or GR) ; CCR4; GD3; SLAMF6; SLAMF4; HIV1 envelope glycoprotein; HTLV1-Tax; CMV pp65; EBV-EBNA3c; KSHV K8.1; KSHV-gH; influenza A hemagglutinin (HA); GAD; PDL1; Guanylyl cyclase C (GCC); auto antibody to desmoglein 3 (Dsg3); auto antibody to desmoglein 1 (Dsg1); HLA; HLA-A; HLA-A2; HLA-B; HLA-C; HLA-DP; HLA-DM; HLA-DOA; HLA-DOB; HLA-DQ; HLA-DR; HLA-G; IgE; CD99; Ras G12V; Tissue Factor 1 (TF1); AFP; GPRC5D; Claudin18.2 (CLD18A2 or CLDN18A.2); P-glycoprotein; STEAP1; Liv1; Nectin-4; Cripto; gpA33; BST1/CD157; low conductance chloride channel; and the antigen recognized by TNT antibody.

39. The composition of any one of claims 27-38, wherein the composition is formulated for administration to a subject.

40. Use of the composition of claim 26 in the manufacture of a medicament for ~~A method of~~ providing anti-disease immunity in a

subject ~~comprising administering to the subject an effective amount of the composition of claim 26~~, wherein the composition transduces cells *in vivo* to give rise to immune effector cells that express an antigen receptor binding construct.

41. The ~~method-use~~ of claim 40, wherein the composition comprises from 10^3 to 10^{11} vector transforming units (TU) per dose.

42. The ~~method-use~~ of claim 40 or 41, wherein the antigen receptor binding construct is not expressed in cells that express an miRNA that binds to the miRNA target sequence of the polynucleotide

43. The ~~method-use~~ of claim 40 or 41, wherein the anti-disease immunity treats a disease selected from the group consisting of a proliferative disease, a precancerous condition, a cancer, and a non-cancer related indication associated with expression of a disease-associated antigen to which the antigen receptor binding construct binds.

44. The ~~method-use~~ of claim 43, wherein the cancer is a hematologic cancer chosen from one or more of chronic lymphocytic leukemia (CLL), acute leukemias, acute lymphoid leukemia (ALL), B-cell acute lymphoid leukemia (B-ALL), T-cell acute lymphoid leukemia (T-ALL), chronic myelogenous leukemia (CML), B cell prolymphocytic leukemia, blastic plasmacytoid dendritic cell neoplasm, Burkitt's lymphoma, diffuse large B cell lymphoma, primary effusion lymphoma, follicular lymphoma, hairy cell leukemia, small cell- or a large cell-follicular lymphoma, malignant lymphoproliferative conditions, MALT lymphoma, mantle cell lymphoma, marginal zone lymphoma, multiple myeloma, myelodysplasia and myelodysplastic syndrome, non-Hodgkin's lymphoma, Hodgkin's lymphoma, plasmablastic lymphoma, plasmacytoid dendritic cell neoplasm, Waldenstrom macroglobulinemia, or pre-leukemia.

45. The ~~method-use~~ of claim 43, wherein the cancer is selected from the group consisting of colon cancer, rectal cancer, renal-cell carcinoma, liver cancer, non-small cell carcinoma of the lung, cancer of the small intestine, cancer of the esophagus, melanoma, bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, cutaneous or intraocular malignant melanoma, uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, testicular cancer, uterine cancer, carcinoma of the

fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, Hodgkin's Disease, non-Hodgkin's lymphoma, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, solid tumors of childhood, cancer of the bladder, cancer of the kidney or ureter, carcinoma of the renal pelvis, neoplasm of the central nervous system (CNS), primary CNS lymphoma, tumor angiogenesis, spinal axis tumor, brain stem glioma, pituitary adenoma, Kaposi's sarcoma, Merkel cell cancer, epidermoid cancer, squamous cell cancer, T-cell lymphoma, environmentally induced cancers, combinations of said cancers, and metastatic lesions of said cancers.

46. The ~~method-use~~ of claim 40, wherein the vector transduces cells selected from T-lymphocytes (T-Cells), naïve T-cells, memory T-Cells, Tregs, NK cells, hematopoietic stem cells, and any combination thereof.

47. The composition of claim 26, wherein the retroviral vector comprises the sequence of SEQ ID NO:25.

48. An ex vivo or in vitro method of producing a retroviral vector comprising transforming a cell line expressing murine leukemia virus (MLV) gag-pol genes with a vector of claim 1.

49. The method of claim 48, wherein the vector comprises a sequence as set forth in SEQ ID NO:1-23 or 24.

50. A retrovirus produced by the method of claim 48 or 49.

51. An ex vivo or in vitro method for identifying off-target miRNA expression profiles comprising isolating total RNA from a cell or tissue;

generating a sequence library from the isolate RNA;
sequencing the sequence library;
processing sequence reads to identify miRNA sequences;
order ranking the miRNA sequences using differential and ranked analysis; and

identifying the top 1 to 5 miRNA sequences that are present in both the differential and ranked analysis.

52. Use of an agent that mobilizes hematopoietic stem cells from the bone marrow in the manufacture of a medicament ~~A method of~~

for mobilizing stem cells for *in vivo* gene transfer therapy in a
subject, ~~comprising~~

~~— administering to a subject an agent that mobilizes~~
~~hematopoietic stem cells from the bone marrow.~~

53. The ~~method-use~~ of claim 52, wherein the agent is to be
administered prior to administering a vector comprising a transgene
for *in vivo* transduction of hematopoietic stem cells.

54. The ~~method-use~~ of claim 52, wherein the agent is selected
from the group consisting of plerixafor, G-CSF, Sildenafil,
filgrastim, lenograstim, GM-CSF, molgramostim, sargramostim and any
combination thereof.

55. The ~~method-use~~ of claim 53, further comprising
~~administering a composition of 27 comprising that~~ the vector
comprised in the composition of claim 27 is to be administered to
the subject.