

that is fused to a heterologous GPI anchor attachment sequence. In some embodiments, the membrane-bound polypeptide capable of binding to CD28 is CD80, CD86, or a functional fragment thereof that is capable of inducing CD28-mediated activation of Akt, such as the extracellular domain of CD80.

[35] In illustrative embodiments of any of the methods and compositions provided herein that include a recombinant retrovirus, the membrane-bound polypeptide capable of binding CD3 can be an anti-CD3 scFv bound to a CD14 GPI anchor attachment sequence, and the membrane-bound polypeptide capable of binding to CD28 can be CD80, or the extracellular domain thereof, bound to a CD16B GPI anchor attachment sequence. In illustrative embodiments of any of the methods and compositions provided herein that include a recombinant retrovirus, the recombinant retroviruses can comprise on their surface, an anti-CD3 scFv bound to a CD14 GPI anchor attachment sequence, CD80, or the extracellular domain thereof, bound to a CD16B GPI anchor attachment sequence, and a fusion polypeptide of IL-7, or an active fragment thereof, and DAF comprising a GPI anchor attachment sequence. In illustrative embodiments of any of the methods and compositions provided herein that include a recombinant retrovirus, the IL-7, or an active fragment thereof, and DAF fusion, the anti-CD3 scFV, and the CD80, or extracellular domain thereof each comprises a DAF signal sequence.

[36] In illustrative embodiments of any of the methods and compositions provided herein that include a recombinant retrovirus or retroviruses, if not explicitly recited in the broadest aspect, the recombinant retroviruses can comprise on their surface a membrane-bound cytokine. The membrane-bound cytokine can be IL-7, IL-15, or an active fragment thereof. In other embodiments, the membrane-bound cytokine is a fusion polypeptide of IL-7, or an active fragment thereof, and DAF. For example, the fusion polypeptide can comprise the DAF signal sequence (nucleotides 1-31 of SEQ ID NO:107), IL-7 without its signal sequence (nucleotides 32-187 of SEQ ID NO:107), and a fragment of DAF that includes its GPI anchor attachment sequence (nucleotides 188-527 of SEQ ID NO:107).

[37] Illustrative embodiments of any of the method and composition aspects provided herein the pseudotyping element can comprise one or more heterologous envelope proteins. In other examples, the pseudotyping element can include one or more viral polypeptides recognized by T cells. The one or more pseudotyping elements can comprise a Measles Virus F polypeptide, a Measles Virus H polypeptide, and/or a fragment thereof. The one or more pseudotyping elements can be cytoplasmic domain deletion variants of a measles virus F polypeptide and/or a measles virus H polypeptide.

[38] In illustrative embodiments of any of the methods and compositions provided herein that include the *in vivo* control element the *in vivo* control element is the lymphoproliferative element, wherein the lymphoproliferative is inactive or less active at promoting proliferation of the T cells and/or NK cells in the absence of the compound, and wherein the compound is a molecular chaperone that binds the lymphoproliferative element and induces the activity of the lymphoproliferative element.

a.) contacting isolated quiescent T cell and/or NK cells obtained from said subject with the recombinant retrovirus of any of the embodiments disclosed herein;

b.) introducing the modified T cell and/or NK cells into the subject;

c.) administering an effective amount of acyclovir, an acyclovir prodrug, penciclovir, or a penciclovir prodrug to said subject to expand the modified T cell and/or NK cells in the subject, wherein said modified T cell and/or NK cells proliferate in said subject upon administration of acyclovir, an acyclovir prodrug, penciclovir, or a penciclovir prodrug, thereby expanding the modified PBLs in the subject; and

d.) stopping administration of acyclovir, an acyclovir prodrug, penciclovir, or a penciclovir prodrug, wherein said modified T cell and/or NK cells stop proliferating in said subject upon stopping administration of acyclovir, an acyclovir prodrug, penciclovir, or a penciclovir prodrug, thereby controlling the expansion, engraftment, and/or persistence of the modified T cell and/or NK cells in the subject.

[55] In another aspect, provided herein is a method of treating cancer in a subject, said method comprising:

- a. contacting isolated quiescent T cells and/or NK cells obtained from said subject with the recombinant vector according to any of the embodiments disclosed herein;
- b. introducing the genetically modified T cells and/or NK cells into the subject; and
- c. administering an effective amount of acyclovir, an acyclovir prodrug, penciclovir, or a penciclovir prodrug to said subject to expand the modified T cell and/or NK cells in the subject, wherein said modified T cell and/or NK cells proliferate in said subject upon administration of acyclovir, an acyclovir prodrug, penciclovir, or a penciclovir prodrug, and wherein the chimeric antigen receptor in said modified T cell and/or NK cells binds cancer cells in said subject, thereby treating cancer in the subject.

[56] In another aspect, provided herein is a transduced T cell and/or NK cell, comprising a recombinant polynucleotide comprising one or more transcriptional units operatively linked to a promoter active in T cells and/or NK cells, wherein the one or more transcriptional units encode a first engineered signaling polypeptide regulated by an *in vivo* control element, wherein said first engineered signaling polypeptide comprises a constitutively active IL-7 receptor mutant, and wherein the *in vivo* control element is capable of binding, and/or designed and/or configured to bind, to a compound *in vivo*.

[57] In another aspect, provided herein is a retroviral packaging system, comprising:

a mammalian cell comprising:

- A. a first transactivator expressed from a constitutive promoter and capable of binding a first ligand and a first inducible promoter for affecting expression of a nucleic acid sequence

B. incubating the population of packaging cells comprising accumulated first transactivator in the presence of the first ligand to accumulate the second transactivator and the retroviral REV protein and an activation element typically on their surface, comprising a polypeptide capable of binding to CD3 and a polypeptide capable of binding to CD28, wherein the second transactivator is capable of binding a second ligand and a second inducible promoter for affecting expression of a nucleic acid sequence operably linked thereto in the presence versus absence of the second ligand; and

C. incubating the population of packaging cells comprising accumulated second transactivator and retroviral REV protein in the presence of the second ligand thereby inducing expression of a gag polypeptide, a pol polypeptide and a pseudotyping element capable of binding to a T cell and/or an NK cell and facilitating membrane fusion of the recombinant retrovirus thereto, wherein said pseudotyping element comprises cytoplasmic domain deletion variants of a measles virus F polypeptide and/or a measles virus H polypeptide,

wherein a packagable RNA genome is encoded by a polynucleotide operably linked to a third promoter and wherein said promoter is inducible by the second transactivator,

wherein the packagable RNA genome comprises, from 5' to 3':

- i. a 5' long terminal repeat, or active fragment thereof;
- ii. a nucleic acid sequence encoding a retroviral cis-acting RNA packaging element;
- iii. a nucleic acid sequence encoding a first engineered signaling polypeptide comprising a chimeric antigen receptor and a second engineered signaling polypeptide comprising a constitutively active IL-7 receptor mutant separated by a cleavage signal;
- iv. a fourth promoter that is active in the T cell and/or the NK cell; and
- v. a 3' long terminal repeat, or active fragment thereof, and

wherein the packagable RNA genome further comprises a riboswitch that binds a nucleoside analog antiviral drug, wherein binding of the riboswitch to the nucleoside analog antiviral drug increases expression of the IL-7 receptor mutant, thereby making the recombinant retrovirus.

[80] In an illustrative embodiment of the method, the riboswitch further controls expression of the chimeric antigen receptor in a manner regulated by binding of the riboswitch to the nucleoside analog antiviral drug. In another illustrative embodiment, the nucleoside analog antiviral drug is acyclovir and/or penciclovir. In another illustrative embodiment, the packagable RNA genome further comprises a recognition domain, wherein the recognition domain comprises a polypeptide that is recognized by an antibody that recognizes EGFR or an epitope thereof. In another illustrative embodiment, the first ligand is rapamycin and the second ligand is tetracycline or doxorubicin or the first ligand is tetracycline or doxorubicin and the second ligand is rapamycin. In another illustrative embodiment, the packaging

(0.5 mM) and elution with either acyclovir (+) or counter-targets (x). The specifically eluted aptamer fractions for each sample were subjected to PAGE for analysis.

[487] 100 pmoles of each aptamer candidate (per trial/lane) was resuspended in 1X modified selection buffer (50 mM HEPES, 100 mM KCl, 0.5 mM MgCl₂, pH 7.3) and refolded (90 °C for 5 min, then 4 °C for 5 min), then incubated at 37 °C for 30 minutes with 200 pmoles (each) of pooled counter-targets or target. Final library concentration was 0.5 μM, target/counter-targets concentration was 1 μM (incubation volume was 200 μl).

[488] After target/counter-target incubation, 250 μg of GrO (Angstrom Materials (Dayton, OH)) was added to adsorb unbound candidate (10-minute incubation at 37 °C).

[489] Samples were centrifuged for 5 minutes at 7,000 x g. Supernatant was recovered, denatured using 2X Formamide with 40 mM EDTA, and run on 10% denaturing PAGE with 8 M urea (supplier: American Bioanalytical; catalog #'s AB13021-01000. AB13022-01000). Running buffer was 1X TBE (supplier: Amresco/VWR; catalog # 0658-20L, diluted using DI water). DNA ladder was 20/100 DNA ladder (IDT). Gels stained with Gel Star (Lonza, 50535) and imaged on a blue light transilluminator.

[490] Candidates F1A-769, F1A-795, F1A-946, and F1A-996 appear to exhibit selective positive response in this qualitative PAGE assessment (good elution of the Aptamer from GrO with Acyclovir target and relatively lower or minimal elution with counter-targets).

Conclusion

[491] Strong candidates for acyclovir were identified after twelve rounds of iterative screening and parallel assessment; reasonable candidates for penciclovir were identified after two rounds of screening and parallel assessment.

Example 2: Isolation of conditional scFv's.

[492] Potential splice site liabilities are removed and tumor antigen specific scFv's are synthesized by overlapping oligo synthesis and cloned into the CAR shuttle construct containing the acyclovir responsive element and the primate CD3ζ promoter. As an initial prototype, anti-ECD of EPCAM or ERBB2scFv with a CD8-alpha signal peptide, stalk, and transmembrane domain is utilized. Solid tumor microenvironment restricted CAR products are generated either using methods as described in US Patent No. 8,709,755 and PCT Publication No. WO/2016/033331A1 or by direct selection from human phage libraries under permissive and non-permissive conditions. Briefly, a human V_H x V_L library from Creative Biolabs (Shirley, NY) is panned in the following tumor permissive conditions: 100 μg/ml hyaluronan, 100 kDa fraction (Lifecore Biomedical, Chaska, MN), 20 mg/ml recombinant HSA (Cyagen, Santa Clara, CA), 200 ng/ml recombinant human VEGF in 25 mM sodium bicarbonate buffer, 2 μM adenosine, 10

applications. Herein, a stable retroviral packaging cell line is generated by the simultaneous introduction of multiple constructs encoding inducible promoters and their regulators into HEK293 suspension-adapted cells (HEK293S) to stably produce the viral components, CAR genes, and their regulatory components. Two distinct inducible systems can be used to temporally control the expression of genes. One system is based on rapamycin- or rapalog-induced dimerization of two transcription factors. One transcription factor consists of three copies of the FKBP protein fused to a ZFHD1 DNA binding domain and the other transcription factor consists of a FRB protein fused to a p65 activation domain. Rapamycin or a rapalog dimerizes the transcription factors to form ZFHD1/p65 AD and can activate gene transcription at 12xZFHD1 binding sites.

[500] A series of vectors as shown in FIGs. 3A-3E are generated with flanking transposon sequences for integration into the HEK293S genome. Once integrated into the genome of a cell, these sequences function as regulatory components and lox and/or FRT sites for subsequent integration using Cre and/or flp recombinases, herein referred to as landing pads. The initial 5 constructs contain polynucleotide sequences encoding puromycin resistance, GFP, RFP, and an extracellular MYC tag that is targeted to the cell membrane through an N-terminal PLss (bovine prolactin signal peptide) and anchored to the cell membrane through a platelet-derived growth factor receptor (PDGFR) C-terminal transmembrane anchoring domain. The initial 5 constructs can also include constitutive minimal CMV and minimal IL-2 promoters, a rapamycin-regulated ZFHD1-based promoter, a tetracycline-responsive element (TRE) promoter, or a bidirectional TRE (BiTRE) promoter. The construct in FIG. 3A contains a polynucleotide sequence encoding FRB domain fused to the NF κ B p65 activator domain (p65 AD) and ZFHD1 DNA binding domain fused to three FKBP repeats that is constitutively expressed. The construct in FIG. 3A also includes HIV1 REV and HSV VP65 domain SrcFlagVpx under the rapamycin-inducible ZFHD1/p65 AD promoter. The construct in FIG. 3B includes a polynucleotide encoding an rtTA sequence under the control of the ZFHD1/p65 AD promoter. The construct in FIG. 3C includes a polynucleotide encoding a puromycin resistance gene flanked by loxP sites and the extracellular MYC tag flanked by lox2272 sites. Both of these selectable markers are under the control of a BiTRE promoter, which is flanked by FRT sites. The construct in FIG. 3D includes a polynucleotide encoding GFP flanked by loxP sites that is under the control of a TRE promoter. The construct in FIG. 3D also includes a single FRT site between the TRE promoter and the 5' loxP site of GFP. The construct in FIG. 3E includes a polynucleotide encoding RFP flanked by loxP sites that is under the control of the ZFHD1/p65 AD promoter. The construct in FIG. 3E also includes a single FRT site between the ZFHD1/p65 AD promoter and the 5' loxP site of RFP. The constructs in FIGs. 3C-3E function as landing pads for other polynucleotide sequences to insert into the genome of the packaging cell line. The polynucleotide sequences to be inserted can be flanked by lox sites and inserted into the genome using Cre recombinase and the loxP sites. This results in insertion and simultaneous removal of

the genomic regions encoding puromycin resistance, the extracellular MYC tag, GFP, and RFP. Alternatively, the polynucleotide sequences can be flanked by FRT sites and inserted into the genome using flp recombinase and the FRT sites followed by removal of the polynucleotide sequences encoding puromycin resistance, the extracellular MYC tag, GFP, and RFP using Cre recombinase.

[501] To generate the packaging cell line with landing pads integrated into the genome, HEK293S cells are co-transfected with equimolar concentrations of the 5 plasmids (FIGs. 3A-3E) plus 5 µg of *in vitro*-transcribed piggybac transposase mRNA or 5 µg of a plasmid with a promoter for expressing piggybac transposase in the presence of PEI at a ratio of 2:1 or 3:1 PEI to DNA (w/w) or 2-5 µg piggybac transposase protein using a cationic peptide mixture. The transfected cells are selected with puromycin in the presence of 100 nm rapamycin and 1 µg/mL doxycycline for 2-5 days followed by fluorescence-activated cell sorting to collect cells expressing GFP and RFP. The sorted cells are grown 5 days in the absence of puromycin, rapamycin, and doxycycline and cells expressing GFP and RFP are removed also myc positive cells are removed with myc beads. Individual clones from negatively sorted cells are then screened for induction of GFP and RFP by rapamycin and doxycycline and single cell cloned. The DNA from clones is harvested and sequenced for integration analysis. Clones positive for strong inducible expression of GFP and RFP in the presence of rapamycin and doxycycline with limited background expression in the absence of rapamycin and doxycycline are expanded and banked.

[502] The HEK293S cells with the constructs from FIGs. 3A-3E integrated into the genome are then transfected with a construct containing a tricistronic polynucleotide encoding a DAF signal sequence/anti-CD3 scFvFc (UCHT1)/CD14 GPI anchor attachment site (SEQ ID NO:252), a DAF signal sequence/CD80 extra-cellular domain capable of binding CD28/CD16B GPI anchor attachment site (SEQ ID NO:253), and a DAF signal sequence/IL-7 /DAF (SEQ ID NO:107) and transposon sequences flanking the polynucleotide region for integration into the HEK293S genome (FIG. 4A). After transfection, cells are expanded for 2 days in the absence of rapamycin and doxycycline and colonies that are constitutively red are selected. Positive colonies are then transiently transfected with a construct for expressing Cre recombinase to remove remaining genomic DNA, and the RFP encoding region. Another construct (FIG. 4B) containing a polynucleotide with a BiTRE promoter and a polynucleotide region encoding the gag and pol polypeptides in one direction and a polynucleotide region encoding the measles virus F and H proteins in the other direction is transfected at the same time. The Cre recombinase integrates the construct into the genome to generate the integrated sequence shown in FIG. 4B. Resultant colonies are evaluated for protein expression in the presence of doxycycline and rapamycin and analyzed by deep sequencing for genomic integration. The remaining TRE responsive GFP site is retained for the lentiviral genome insertion.

Example 8. Generation of lentivirus vector and retroviral packaging.

[505] The PBMCs isolated from peripheral blood are loaded onto a PALL PBMC filter, washed once through the filter with 10 ml of AIM V (Thermo Fisher Scientific) or X-VIVO 15 media followed by perfusion with 10-60 ml of lentivirus stock (as prepared in Example 8) at 37 °C at 5 ml/hr. The PBMCs are then washed again with AIM V, CTS OpTmizer T Cell Expansion SFM, or X-VIVO 15 media containing recombinant human DNase (Pulmozyme, Genentech) followed by a wash with DNase-free Lactated Ringers (Cat # L7500, Braun). The PBMCs are then reverse perfused through the filter into a syringe. The cells (target levels of cells are 5×10^5 to 1×10^6 cells/kg) are then reinfused into the subject through intravenous infusion.

[506] Depending upon the riboswitch contained within the retroviral genome, the subject is given the respective nucleoside analogue antiviral drug or nucleoside analogue antiviral prodrug (acyclovir, valaciclovir, penciclovir, or famciclovir). Subjects can be given any therapeutically effective dose, such as 500 mg of the nucleoside analogue antiviral drug or prodrug orally three times/day. Treatment with the nucleoside analogue antiviral drug or prodrug preferably begins before reinfusion, such as 2 hours before, and can also begin at the time of reinfusion or at some time after reinfusion. The treatment can continue for at least 1, 2, 3, 4, 5, 7, 10, 14, 21, 28, 30, 60, 90, 120 days or 5, 6, 9, 12, 24, 36, or 48 months or longer. The treatment can include administration of the nucleoside analogue antiviral drug or prodrug once, twice, three, or four times daily. After reinfusion and treatment is begun, the number of infected cells is determined through blood counts on days 2, 5, 7, 11, 13, 18, 28, and 56 post-reinfusion using qPCR to quantitate the amount of viral genome. A subject experiencing fever or cytokine release syndrome may have the dose or frequency of the nucleoside analogue antiviral drug or prodrug reduced or halted. If the infected T cells fail to amplify 10,000-100,000 fold by day 18, the dose or frequency of the nucleoside analogue antiviral drug or prodrug may be increased. The clinical response of the subject can be measured through FDG PET imaging and serial CT scan. Oral dosing of the nucleoside analogue antiviral drug or prodrug can be reduced or halted following prolonged remission or in the event of excessive T cell propagation beyond 30% of total peripheral T cell counts.

Example 10. Therapeutic intervention to raise vascular or tissue pH.

[507] To reduce the binding of an antigen binding domain to its cognate antigen, NaHCO_3 is administered as an IV bolus or by IV infusion. The standard dosage is 1 mg/kg of body weight as the initial dose followed by 0.5 mg/kg every 10 minutes. A 50-milliliter bolus of NaHCO_3 will raise the serum pH approximately 0.1 of a pH unit. If the pH is 7.0, it requires four 50 mEq ampules of NaHCO_3 to correct the pH to 7.40

Example 11. Testing activity of IL-7 receptor lymphoproliferative/survival elements in PBMCs

[508] To test IL-7R α variants for their ability to mediate antigen-independent survival of T cells, thirty milliliters of human blood were drawn with acid citrate dextrose (ACD) as an anticoagulant into Vacutainer tubes. The whole blood was processed using density gradient centrifugation with Ficoll-Paque™ (General Electric) following manufacturer's instruction, to obtain peripheral blood mononuclear cells (PBMCs). Aliquots of the PBMCs were transferred aseptically to wells of a 12 well tissue culture plate, along with X-Vivo™ 15 media (Lonza) to a final concentration of 0.5 million viable cells / mL in a final volume of 1mL. Recombinant human interleukin-2 (IL-2) (Novoprotein) was also added to a concentration of 100IU/ml in some samples. Activating anti-CD3 Ab (OKT3, Novoprotein) was added at a concentration of 50ng/ml, to activate the PBMC for viral transduction. The plates were incubated overnight in a standard humidified tissue culture incubator at 37 degrees C and 5% Carbon Dioxide. After overnight incubation, lentivirus particle preparations containing the desired test constructs (FIG. 19A) were added to individual wells at a multiplicity of infection (MOI) of 5. The plate was incubated overnight in a standard humidified tissue culture incubator at 37 degrees C and 5% Carbon Dioxide. Following the overnight incubation, the contents of each of the wells of the 12 well plate were collected and centrifuged to obtain a pellet. The samples were washed once with D-PBS + 2% Human Serum Albumin (HSA), resuspended in X-Vivo15™ media, and transferred to wells of G-Rex® 6-well gas permeable cell culture devices (Wilson Wolf). Additional X-Vivo™ 15 media was added to bring the final volume of each well to 30ml. Matching control samples for each of the constructs were transferred to wells of G-Rex® 6-well gas permeable cell culture devices (Wilson Wolf) and additional media was added to bring the final volume to 30ml with 100IU/ml IL-2 for some control samples. The G-Rex® device was incubated in a standard humidified tissue culture incubator at 37 degrees C and 5% Carbon Dioxide for 7 days. Fresh IL-2 was added to the control samples containing IL-2 during the culture every 2-3days. Matched test samples without IL-2 were not supplemented. Samples were removed for tracking cell numbers and viability during expansion (Countess, Thermo Fisher) at day 7.

[509] FIG. 19A provides a schematic of the IL7R α constructs that were tested. These constructs were inserted into a recombinant lentiviral genome. The recombinant retroviruses were used to transduce PBMCs. FIG. 19A shows a schematic of wild-type IL7R α (SEQ ID NO:229), which consists of a signal sequence (SS), an extracellular domain (ECD), a transmembrane (TM), and an intracellular domain (ICD). "1" indicates the site of a fibronectin type III domain; "2" indicates the site of a WSXWS motif; "3" indicates a Box 1 site, "4" indicates the site of a protein kinase C (PKC) phosphorylation site, and "5" indicates a Box 2 site.

[510] Variant "A" is the IL-7R α with an InsPPCL at position 243 (Shochat et al 2011, *J. Exp. Med.* Vol. 208 No. 5 901-908) but without the S185C mutation, expressed on a transcript with a GFP polypeptide, a

CLAIMS

1. An isolated polynucleotide, comprising:
 - a) an aptamer RNA comprising an aptamer domain capable of binding graphene oxide in the presence of guanine, guanosine, and 2'-deoxyguanosine, and capable of eluting from graphene oxide by binding acyclovir or penciclovir;
 - b) a reverse complement of the aptamer RNA;
 - c) a coding DNA encoding the aptamer RNA; or
 - d) a reverse complement DNA encoding the reverse complement of the aptamer RNA.
2. The isolated polynucleotide according to claim 1, wherein the aptamer domain comprises a non-stem region encoded by a DNA that shares at least 80% sequence identity to any one of SEQ ID NOs:108, 147, 164, 183, 198, 220, or 221, or a reverse complement thereof.
3. An isolated polynucleotide according to claim 1 or claim 2, wherein the isolated polynucleotide comprises the reverse complement of the aptamer RNA, and wherein the isolated polynucleotide further comprises the reverse complement of a target polynucleotide encoding a target polypeptide operably linked to a promoter.
4. The isolated polynucleotide of claim 3, wherein the isolated polynucleotide further comprises the reverse complement of a function switching domain.
5. A method for genetically modifying a T cell, comprising: contacting the T cell *ex vivo* with a replication incompetent recombinant retrovirus to form a transduction reaction mixture,
wherein the replication incompetent recombinant retrovirus comprises:
 - a) a pseudotyping element on its surface that is capable of binding to the T cell and facilitating membrane fusion of the replication incompetent recombinant retrovirus thereto; and
 - b) a polynucleotide comprising one or more transcriptional units operatively linked to a promoter active in the T cell, wherein the one or more transcriptional units encode a first engineered signaling polypeptide comprising a first chimeric antigen receptor comprising an antigen-specific targeting

region, a transmembrane domain, and an intracellular activating domain,
wherein the T cell is contacted ex vivo with the replication incompetent recombinant retrovirus for between 15 minutes and 14 hours before the T cell is washed out of the transduction reaction mixture, and

wherein said contacting facilitates membrane fusion of the T cell to the replication incompetent recombinant retrovirus to produce a genetically modified T cell.

6. The method of claim 5, wherein the replication incompetent recombinant retrovirus is a lentivirus that further comprises a membrane-bound polypeptide capable of binding to CD3.

7. The method of claim 6, wherein the membrane-bound polypeptide that is capable of binding to CD3 is an anti-CD3 antibody and wherein contacting the T cell with the replication incompetent recombinant retrovirus comprises contacting peripheral blood mononuclear cells (PBMCs) comprising the T cell with the replication incompetent recombinant retrovirus.

8. The method of claim 7, wherein the pseudotyping element comprises a vesicular stomatitis virus envelope protein, a Measles Virus F polypeptide, or a Measles Virus H polypeptide, or a functional fragment thereof, and the replication incompetent recombinant retrovirus further comprises a membrane-bound polypeptide that is capable of binding to CD28.

9. The method of claim 5, wherein the one or more transcriptional units encode a second engineered signaling polypeptide that comprises a lymphoproliferative element that promotes proliferation and/or survival of T cells, and wherein said genetically modified cell is capable of surviving in culture for at least 7 days in the absence of IL-2.

10. The method of claim 9, wherein the lymphoproliferative element comprises a constitutively active cytokine receptor polypeptide or fragment thereof and wherein expression of the constitutively active cytokine receptor polypeptide or fragment thereof is regulated.

11. The method of claim 10, wherein the lymphoproliferative element comprises an intracellular signaling domain of an IL-7 receptor, an intracellular signaling domain of an IL-12 receptor, an intracellular signaling domain of an IL-15 receptor, an intracellular signaling domain of an IL-21 receptor, or an intracellular signaling domain of a transforming growth factor β decoy receptor.

12. The method of claim 9, wherein the first engineered signaling polypeptide and the second engineered signaling polypeptide are expressed from a single transcript on the reverse strand from a viral genome expressed from a viral LTR or functional fragment thereof.

13. The method of claim 9, wherein the contacting is performed for between 15 minutes and 8 hours.

14. The method of claim 5, wherein the T cell is a resting T cell.

15. The method of claim 14, wherein the pseudotyping element comprises the membrane-bound polypeptide capable of binding to CD3.

16. A replication incompetent recombinant retrovirus comprising:

a) a pseudotyping element on its surface that is capable of binding to a T cell and facilitating membrane fusion of the replication incompetent recombinant retrovirus thereto; and

b) a polynucleotide comprising one or more transcriptional units operatively linked to a promoter active in the T cell, wherein the one or more transcriptional units encode a first engineered signaling polypeptide comprising a first chimeric antigen receptor comprising an antigen-specific targeting region, a transmembrane domain, and an intracellular activating domain; and a second engineered signaling polypeptide that comprises a lymphoproliferative element that promotes proliferation and/or survival of T cells when cultured for at least 7 days in the absence of IL-2.

17. The replication incompetent recombinant retrovirus of claim 16, wherein the replication incompetent recombinant retrovirus is a lentivirus that further comprises a membrane-bound polypeptide capable of binding to CD3.

18. The replication incompetent recombinant retrovirus of claim 17, wherein the membrane-bound polypeptide that is capable of binding to CD3 is an anti-CD3 antibody.

19. The replication incompetent recombinant retrovirus of claim 18, wherein the anti-CD3 antibody is a GPI-anchored anti-CD3 antibody.

20. The replication incompetent recombinant retrovirus of claim 16, wherein the pseudotyping element comprises a vesicular stomatitis virus envelope protein, a Measles Virus F polypeptide, or a Measles Virus H polypeptide, or a functional fragment thereof, and the replication incompetent recombinant retrovirus further comprises a membrane-bound polypeptide that is capable of binding to CD28.

21. The replication incompetent recombinant retrovirus of claim 16, wherein the lymphoproliferative element comprises a constitutively active cytokine receptor polypeptide or fragment thereof and wherein expression of the constitutively active cytokine receptor polypeptide or fragment thereof is regulated.

22. The replication incompetent recombinant retrovirus of claim 16, wherein the lymphoproliferative element comprises an intracellular signaling domain of an IL-7 receptor, an intracellular signaling domain of an IL-12 receptor, an intracellular signaling domain of an IL-15 receptor, an intracellular signaling domain of an IL-21 receptor, or an intracellular signaling domain of a transforming growth factor β decoy receptor.

23. The replication incompetent recombinant retrovirus of claim 16, wherein the first engineered signaling polypeptide and the second engineered signaling polypeptide are expressed from a single transcript on the reverse strand from a viral genome expressed from a viral LTR or functional fragment thereof.