

CLAIMS

What is claimed is:

1. An isolated nucleic acid sequence encoding a membrane-bound chimeric receptor comprising a CD4 extracellular domain, a transmembrane domain, and a signaling domain, wherein the CD4 extracellular domain is capable of recognizing and binding a HIV infected cell.
2. The isolated nucleic acid sequence of claim 1, optionally wherein:

the CD4 extracellular domain is encoded by a nucleic acid sequence comprising SEQ ID NO: 3 or 64;

the transmembrane domain comprises a CD8alpha hinge encoded by nucleic acid sequence SEQ ID NO: 4 or 65 and a transmembrane domain comprising at least one domain encoded by a nucleic acid sequence comprising one selected from the group consisting of SEQ ID NOs: 5 and 8;

the signaling domain comprises a CD3zeta signaling domain encoded by a nucleic acid sequence comprising SEQ ID NO: 6 or 68; and/or

the CD4 extracellular domain specifically binds to the HIV envelope (Env) glycoprotein.
3. The isolated nucleic acid sequence of claim 1, further comprising a costimulatory signaling region, wherein the costimulatory signaling region comprises an intracellular domain of a costimulatory molecule selected from the group consisting of alpha, beta or zeta chain of a TCR zeta, FcR gamma, FcR beta, CD3 gamma, CD3 delta, CD3 epsilon, CD5, CD22, CD79a, CD79b, CD66d, a costimulatory signaling region from CD27, CD28, 4-1BB (CD137), DAP12, OX9, OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, a ligand that specifically binds with CD83, and any combination thereof.
4. The isolated nucleic acid sequence of claim 3, wherein the costimulatory signaling region is CD28 and is encoded by a nucleic acid sequence comprising SEQ ID NO: 9 or 67.

5. A vector comprising the isolated nucleic acid sequence of claim 1.
6. The vector of claim 5, wherein the nucleic acid sequence comprises at least one from the group consisting of SEQ ID NOs: 1, 7, 62 and 63.
7. An isolated amino acid sequence encoding a membrane-bound chimeric receptor comprising a CD4 extracellular domain, a transmembrane domain, and a signaling domain, wherein the CD4 extracellular domain is capable of recognizing and binding a HIV infected cell.
8. The isolated amino acid sequence of claim 7, optionally wherein:

the CD4 extracellular domain comprises the amino acid sequence SEQ ID NO: 46;

the transmembrane domain comprises a CD8alpha hinge of SEQ ID NO: 47, and a transmembrane domain comprising at least one amino acid sequence selected from the group consisting of SEQ ID NOs: 48 and 49; the signaling domain comprises a CD3zeta signaling domain comprising SEQ ID NO: 51; and/or

the CD4 extracellular domain specifically binds to the HIV envelope (Env) glycoprotein.
9. The isolated amino acid sequence of claim 8, further comprising a costimulatory signaling region, wherein the costimulatory signaling region comprises an intracellular domain of a costimulatory molecule selected from the group consisting of alpha, beta or zeta chain of a TCR zeta, FcR gamma, FcR beta, CD3 gamma, CD3 delta, CD3 epsilon, CD5, CD22, CD79a, CD79b, CD66d, a costimulatory signaling region from CD27, CD28, 4-1BB (CD137), DAP12, OX9, OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, a ligand that specifically binds with CD83, and any combination thereof.
10. The isolated amino acid sequence of claim 9, wherein the costimulatory signaling region is CD28 and comprises SEQ ID NO: 49.
11. A vector comprising a membrane-bound chimeric receptor comprising a CD4 extracellular domain, a transmembrane domain, and a signaling domain, wherein the CD4

- extracellular domain is capable of recognizing and binding a HIV infected cell, wherein the vector comprises at least one amino acid sequence selected from the group consisting of SEQ ID NOs: 44 and 45, optionally wherein the vector comprises an EF1 α promoter.
12. An isolated nucleic acid sequence encoding a chimeric antigen receptor (CAR) comprising a HIV-specific binding domain, a transmembrane domain, a costimulatory signaling region, and a signaling domain, wherein the HIV binding domain comprises an anti-HIV antibody or a fragment thereof.
 13. The isolated nucleic acid sequence of claim 12, optionally wherein:

the HIV-specific binding domain comprises a heavy and light chain;

the HIV-specific binding domain is a human antibody, a humanized antibody, and a fragment thereof; and/or

the HIV-specific binding domain specifically binds to the surface of HIV infected cells, or HIV virions.
 14. The isolated nucleic acid sequence of claim 12, wherein the antibody or a fragment thereof is selected from the group consisting of a Fab fragment, a F(ab')₂ fragment, a Fv fragment, and a single chain Fv (scFv), optionally wherein:

the scFv comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 13, 17, 21, 25, 29, 57-60 and 61; and/or the scFv is encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 12, 16, 20, 24, 28, 75-78 and 79.
 15. The isolated nucleic acid sequence of claim 12, optionally wherein:

the costimulatory signaling region comprises an intracellular domain of a costimulatory molecule selected from the group consisting of TCR, CD3 zeta, CD3 gamma, CD3 delta, CD3 epsilon, CD86, common FcR gamma, FcR beta (Fc Epsilon R1b), CD79a, CD79b, Fc gamma RIIa, DAP10, DAP12, T cell receptor (TCR), CD8, CD27, CD28, 4-1BB (CD137), OX9, OX40, CD30, CD40, PD-1, ICOS, a KIR family protein, lymphocyte

function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, a ligand that specifically binds with CD83, CDS, ICAM-1, GITR, BAFFR, HVEM (LIGHTR), SLAMF7, NKp80 (KLRF1), CD127, 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, 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, NKp44, NKp30, NKp46, NKG2D, Toll-like receptor 1 (TLR1), TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, and any combination thereof; the signaling domain comprises a CD3zeta signaling domain encoded by nucleic acid sequence SEQ ID NO: 6 or 68;

the CAR is encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 10, 14, 18, 22, 26, 34, 70-73, and 74; and/or

the CAR has an amino acid sequence selected from the group consisting of SEQ ID NOs: 11, 15, 19, 23, 27, 35, 52-55, and 56.

16. A modified cell comprising the isolated nucleic acid sequence of claim 1 or claim 12.
17. The modified cell of claim 16, wherein the cell is selected from the group consisting of a T cell, a natural killer (NK) cell, a cytotoxic T lymphocyte (CTL), and a regulatory T cell, optionally wherein:

the nucleic acid sequence is selected from the group consisting of a DNA and an mRNA; and/or

the nucleic acid sequence is introduced into the cell by at least one procedure selected from the group consisting of electroporation, usage of a lentivirus, usage of a retrovirus and a chemical-based transfection.

18. A composition comprising the modified cell of claim 16, for use in therapy.
19. Use of the modified cell of claim 16 in the manufacture of a medicament for the treatment of HIV infection in a subject in need thereof, optionally wherein:

the subject is a mammal;

the modified cell is autologous to the mammal; and/or

the treatment further comprises antiretroviral therapy (HAART); and further wherein the modified cell and the HAART are to be co-administered to the mammal.
20. A pharmaceutical composition comprising the modified cell of claim 16 and a pharmaceutically acceptable carrier.
21. A modified T cell comprising a chimeric receptor comprising a CD4 extracellular domain, a transmembrane domain, and a signaling domain, wherein the CD4 extracellular domain is encoded by a nucleic acid sequence comprising SEQ ID NOs: 3 or 64 and is capable of recognizing and binding an HIV infected cell.
22. The modified T cell of claim 21, wherein the modified T cell further comprises a disrupted CCR5 allele.
23. A modified T cell comprising a chimeric receptor comprising a CD4 extracellular domain, a transmembrane domain, and a signaling domain, wherein the CD4 extracellular domain comprises the amino acid sequence of SEQ ID NO: 46 and is capable of recognizing and binding an HIV infected cell.
24. The modified T cell of claim 23, wherein the modified T cell further comprises a disrupted CCR5 allele.

25. A method of making a T cell specific for an HIV infected cell, the method comprising introducing into a T cell a nucleic acid sequence comprising SEQ ID NO: 1.
26. A method of making an HIV-resistant T cell specific for an HIV infected cell, the method comprising:
introducing into a T cell a nucleic acid sequence comprising SEQ ID NO: 1; and
introducing into the T cell a zinc finger nuclease (ZFN) specific for the CCR5 gene,
wherein the ZFN is capable of disrupting a CCR5 allele.
27. A modified T cell comprising a chimeric receptor comprising a CD4 extracellular domain, a transmembrane domain, and a signaling domain, wherein the CD4 extracellular domain comprises the amino acid sequence set forth in SEQ ID NO: 46 and is capable of recognizing and binding an HIV infected cell, and wherein the modified T cell is resistant to HIV infection.
28. Use of a modified T cell comprising a chimeric receptor comprising a CD4 extracellular domain, a transmembrane domain, and a signaling domain in the manufacture of a medicament for the treatment of an HIV infected mammal, wherein the CD4 extracellular domain comprises the amino acid sequence set forth in SEQ ID NO: 46 and is capable of recognizing and binding an HIV infected cell, and wherein the modified T cell is resistant to HIV infection.
29. The use of claim 28, wherein the treatment further comprises a highly active antiretroviral therapy (HAART).