antigens are antigens expressed at high-level on tumor cells and at low-level on some normal cells. When no tumor-specific antigens are available to construct a CAR, affinity of CAR-T need to be fine-tuned in order to reduce off-tumor, on-target effects. A widely used antigen recognition domain is an scFv, which comprises a heavy chain variable domain and a light chain variable domain from an antibody. The scFvs recognize an epitope on the antigen. The epitope location may also play important roles in the functions of a CAR-T.

[11] In a clinical report (NCT01626495), a patient relapsed 9 months after CD19-targeted CAR T cell (CTL019) infusion due to $CD19^{-}$ leukemia that aberrantly expressed the anti-CD19 CAR. The CAR gene was unintentionally introduced into a single leukemic B cell during T cell manufacture, and its progeny cells bound in cis to the CD19 epitope on the surface of leukemic cells, masking such cells from recognition by CTL019 and leading to resistance to CTL019.

[12] The disclosures of all publications, patents, patent applications and published patent applications referred to herein are hereby incorporated herein by reference in their entirety.

SUMMARY

[13] The present application in one example provides recognition molecules (*e.g.*, transmembrane receptor), engineered immune cells, compositions and methods of treatment.

[14] One example of the present application provides an engineered immune cell ("anti-distal portion engineered immune cell") comprising on its surface a recognition molecule that comprises a binding moiety specifically binding to a target molecule on the surface of a target cell, wherein the target molecule comprises an extracellular domain, wherein the binding moiety specifically binds to a distal portion of the extracellular domain, wherein the immune cell is capable of killing a target cell that comprises on its surface the target molecule, and wherein the immune cell is capable of killing a target cell that comprises on its surface both the target molecule and the recognition molecule. In some examples, the recognition molecule comprises the binding moiety, a transmembrane domain, and an intracellular signaling domain. In some examples, the binding moiety is a single domain antibody (sdAb), an scFv, a Fab', a $(Fab')_2$, an Fv, or a peptide ligand.

[14a] In one aspect, the present disclosure refers to an engineered immune cell comprising on its surface a recognition molecule that comprises a binding moiety specifically binding to a target molecule on the surface of a target cell, wherein the target molecule comprises an extracellular domain, wherein the binding moiety specifically binds to a distal portion of the extracellular domain, wherein the immune cell is capable of killing a target cell that comprises on its surface the target molecule, and wherein the immune cell is capable of killing a target cell that comprises on its surface both the target molecule and the recognition molecule.

[15] In some examples according to one or more of the above examples of the anti-distal portion engineered immune cells, the distance from the distal portion of the extracellular domain to the membrane of the target cell is more than about 0.5 times of the distance from the binding moiety to the membrane of engineered immune cell. In some examples, the distance from the distal portion of the extracellular domain to the membrane of the target cell is more

than about 1 time of the distance from the binding moiety to the membrane of engineered immune cell. In some examples, the distance from the distal portion of the extracellular domain to the membrane of the target cell is more than about 1.5 times of the distance from the binding moiety to the membrane of engineered immune cell. In some examples, the distance from the distal portion of the extracellular domain to the membrane of the target cell is more than about 2 times of the distance from the binding moiety to the membrane of engineered immune cell.

[16] In some examples according to one or more of the above examples of the anti-distal portion engineered immune cells, the extracellular domain of the target molecule is at least about 175 amino acids long. In some examples, the binding moiety binds to a region in the extracellular domain that is about 50 amino acids or more away from the C-terminus of the extracellular domain. In some examples, the binding moiety binds to a region in the extracellular domain that is about 80 amino acids or more away from the C-terminus of the extracellular domain. In some examples, the binding moiety binds to a region that is within about 120 amino acids from the N-terminus of the extracellular domain. In some examples, the binding moiety binds to a region that is within about 80 amino acids from the N-terminus of the extracellular domain.

[17] In some examples according to one or more of the above examples of the anti-distal portion engineered immune cells, the distal portion of the extracellular domain is at least about 30 Å away from the membrane of the target cell. In some examples, the distal portion of the extracellular domain is at least about 40 Å away from the membrane of the target cell. In some examples, the distal portion of the extracellular domain is at least about 60 Å away from the membrane of the target cell. In some examples, the distal portion of the extracellular domain is at least about 90 Å away from the membrane of the target cell. In some examples, the distal portion of the extracellular domain is at least about 120 Å away from the membrane of the target cell.

[18] In some examples according to one or more of the above examples of the anti-distal portion engineered immune cells, the extracellular domain of the target molecule comprises three or more Ig-like domains. In some examples, the binding moiety binds to a region outside the first two Ig-like domains from the C-terminal end of the extracellular domain. In some examples, the binding moiety binds to a region outside the first four Ig-like domains from the C-terminal end of the extracellular domain. In some examples, the binding moiety

binds to a region within the first three (*e.g.*, within the first) Ig-like domain at the N-terminal end of the extracellular domain. In some examples, the binding moiety binds to a region within the first Ig-like domain at the N-terminal end of the extracellular domain.

[19] In some examples according to one or more of the above examples of the anti-distal portion engineered immune cells, the target molecule is a transmembrane receptor. In some examples, the target molecule is selected from the group consisting of CD22, CD4, CD21 (CR2), CD30, ROR1, CD5, and CD20.

[20] In some examples according to one or more of the above examples of the anti-distal portion engineered immune cells, the target molecule is CD22. In some examples, the binding moiety competes for binding with a reference antibody that specifically binds to an epitope within Domains 1-4 of CD22 ("anti-CD22 D1-4 antibody"). In some examples, the binding moiety binds to an epitope in Domains 1-4 of CD22 that overlaps with the binding epitope of a reference anti-CD22 D1-4 antibody. In some examples, the binding moiety comprises the same heavy chain and light chain CDR sequences as those of a reference anti-CD22 D1-4 antibody. In some examples, the binding moiety comprises the same heavy chain variable domain (VH) and light chain variable domain (VL) sequences as those of a reference anti-CD22 D1-4 antibody. In some examples, the reference anti-CD22 D1-4 antibody comprises a heavy chain CDR1 (HC-CDR1) comprising the amino acid sequence of SEQ ID NO: 67, a heavy chain CDR2 (HC-CDR2) comprising the amino acid sequence of SEQ ID NO: 68, a heavy chain CDR3 (HC-CDR3) comprising the amino acid sequence of SEQ ID NO: 69, a light chain CDR1 (LC-CDR1) comprising the amino acid sequence of SEQ ID NO: 70, a light chain CDR2 (LC-CDR2) comprising the amino acid sequence of SEQ ID NO: 71, and a light chain CDR3 (LC-CDR3) comprising the amino acid sequence of SEQ ID NO: 72. In some examples, the reference anti-CD22 D1-4 antibody comprises a VH comprising the amino acid sequence of SEQ ID NO: 73 and a VL comprising the amino acid sequence of SEQ ID NO: 74.

[21] In some examples according to one or more of the above examples of the anti-distal portion engineered immune cells, the engineered immune cell is capable of killing a target cell that comprises on its surface both the target molecule and the recognition molecule by at least 3 fold as compared to an engineered immune cell comprising on its surface a recognition molecule comprising a binding moiety that binds to a proximal portion of the extracellular domain of the target molecule.

[22] One example of the present application provides an engineered immune cell ("anti-proximal portion engineered immune cell") comprising on its surface a recognition molecule that comprises a binding moiety specifically binding to a target molecule on the surface of a target cell, wherein the target molecule comprises an extracellular domain, wherein the binding moiety specifically binds to a proximal portion of the extracellular domain, wherein the engineered immune cell is capable of killing a target cell that comprises on its surface the target molecule, and wherein the engineered immune cell has no or reduced capability of killing a target cell comprising on its surface both the target molecule and the recognition molecule. In some examples, the recognition molecule comprises the binding moiety, a transmembrane domain, and an intracellular signaling domain. In some examples, the binding moiety is an sdAb, an scFv, a Fab', a (Fab')2, an Fv, or a peptide ligand.

[22a] In one aspect, the present disclosure refers to an engineered immune cell comprising on its surface a recognition molecule that comprises a binding moiety specifically binding to a target molecule on the surface of a target cell, wherein the target molecule comprises an extracellular domain, wherein the binding moiety specifically binds to a proximal portion of the extracellular domain, wherein the engineered immune cell is capable of killing a target cell that comprises on its surface the target molecule, and wherein the engineered immune cell has no or reduced capability of killing a target cell comprising on its surface both the target molecule and the recognition molecule.

[23] In some examples according to one or more of the above examples of the anti-proximal portion engineered immune cells, the distance from the proximal portion of the extracellular domain to the membrane of the target cell is no more than about 2 times of the distance from the binding moiety to the membrane of engineered immune cell. In some examples, the distance from the proximal portion of the extracellular domain to the membrane of the target cell is no more than about 1.5 times of the distance from the binding moiety to the membrane of engineered immune cell. In some examples, the distance from the proximal portion of the extracellular domain to the membrane of the target cell is no more than about 1 time of the distance from the binding moiety to the membrane of engineered immune cell.

[24] In some examples according to one or more of the above examples of the anti-proximal portion engineered immune cells, the extracellular domain of the target molecule is at least about 175 amino acids long. In some examples, the binding moiety binds outside of a region that is about 80 amino acids or more away from the N-terminus of the extracellular domain. In some examples, the binding moiety binds to a region in the extracellular domain that is within about 120 amino acids from the C-terminus of the extracellular domain. In some examples, the binding moiety binds to a region in the extracellular domain that is within about 102 amino acids from the C-terminus of the extracellular domain. In some examples, the binding moiety binds to a region in the extracellular domain that is within about 50 amino acids from the C-terminus of the extracellular domain.

[25] In some examples according to one or more of the above examples of the anti-proximal portion engineered immune cells, the proximal portion of the extracellular domain is no more than about 120 Å away from the membrane of the target cell. In some examples, the proximal portion of the extracellular domain is no more than about 90 Å away from the membrane of the target cell. In some examples, the proximal portion of the extracellular domain is no more than about 60 Å away from the membrane of the target cell.

[26] In some examples according to one or more of the above examples of the anti-proximal portion engineered immune cells, the extracellular domain of the target molecule comprises two or more Ig-like domains. In some examples, the binding moiety binds to a region outside the first Ig-like domain at the N-terminal end of the extracellular domain. In some examples, the binding moiety binds to a region outside the first three Ig-like domain at the N-terminal end of the extracellular domain. In some examples, the binding moiety binds to a region within the first four Ig-like domains from the C-terminal end of the extracellular domain. In some examples, the binding moiety binds to a region within the first two Ig-like domains from the C-terminal end of the extracellular domain.

[27] In some examples according to one or more of the above examples of the anti-proximal portion engineered immune cells, the target molecule is a transmembrane receptor. In some examples, the target molecule is selected from the group consisting of CD22, CD4, CD21 (CR2), CD30, ROR1, CD5, and CD20.

[28] In some examples according to one or more of the above examples of the anti-proximal portion engineered immune cells, the target molecule is CD22. In some examples, the binding moiety competes for binding with a reference antibody that specifically binds to an epitope within Domains 5-7 of CD22 ("anti-CD22 D5-7 antibody"). In some examples, the binding moiety binds to an epitope in Domains 5-7 of CD22 that overlaps with the binding epitope of a reference anti-CD22 D5-7 antibody. In some examples, the binding moiety comprises the same heavy chain and light chain CDR sequences as those of a reference anti-CD22 D5-7 antibody. In some examples, the binding moiety comprises the same VH and VL sequences as those of a reference anti-CD22 D5-7 antibody. In some examples, the reference anti-CD22 D5-7 antibody comprises a HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 76, a HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 77, a HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 78, a LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 79, a LC-CDR2 comprising the amino acid sequence of

SEQ ID NO: 80, and a LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 81. In some examples, the reference anti-CD22 D5-7 antibody comprises a VH comprising the amino acid sequence of SEQ ID NO: 82 and a VL comprising the amino acid sequence of SEQ ID NO: 83.

[29] In some examples according to one or more of the above examples of the anti-proximal portion engineered immune cells, the engineered immune cell kills a target cell that comprises on its surface both the target molecule and the recognition molecule by no more than about 20% as compared to an engineered immune cell comprising on its surface a recognition molecule comprising a binding moiety that binds to a distal end of the extracellular domain of the target molecule.

[30] In some examples according to one or more of the above examples of the engineered immune cells (including anti-distal portion and anti-proximal portion engineered immune cells), the recognition molecule is monospecific. In some examples, the recognition molecule is multispecific. In some examples, the recognition molecule comprises a second binding moiety specifically recognizing a second target molecule. In some examples, the second binding moiety is an sdAb, an scFv, a Fab', a $(Fab')_2$, an Fv, or a peptide ligand. In some examples, the binding moiety and the second binding moiety are linked in tandem. In some examples, the binding moiety is N-terminal to the second binding moiety. In some examples, the binding moiety is C-terminal to the second antigen binding moiety. In some examples, the binding moiety and the second binding moiety are linked via a linker.

[31] In some examples according to one or more of the above examples of the engineered immune cells, the binding moiety is fused to the transmembrane domain directly or indirectly. In some examples, the binding moiety is non-covalently bound to a polypeptide comprising the transmembrane domain. In some examples, the recognition molecule comprises i) a first polypeptide comprising the binding moiety and a first member of a binding pair; and ii) a second polypeptide comprising a second member of the binding pair, wherein the first member and the second member bind to each other, and wherein the second member is fused to the transmembrane domain directly or indirectly. In some examples, the binding moiety is fused to a polypeptide comprising the transmembrane domain.

[32] In some examples according to one or more of the above examples of the engineered immune cells, the recognition molecule is a chimeric antigen receptor ("CAR"). In some examples, the transmembrane domain is derived from a molecule selected from the

group consisting of CD8α, CD4, CD28, 4-1BB, CD80, CD86, CD152 and PD1. In some examples, the transmembrane domain is derived from CD8α. In some examples, the intracellular signaling domain comprises a primary intracellular signaling domain derived from CD3ζ, FcRγ, FcRβ, CD3γ, CD3δ, CD3ε, CD5, CD22, CD79a, CD79b, or CD66d. In some examples, the primary intracellular signaling domain is derived from CD3ζ. In some examples, the intracellular signaling domain comprises a co-stimulatory signaling domain. In some examples, the co-stimulatory signaling domain is derived from a co-stimulatory molecule selected from the group consisting of CD27, CD28, 4-1BB, OX40, CD40, PD-1, LFA-1, ICOS, CD2, CD7, LIGHT, NKG2C, B7-H3, TNFRSF9, TNFRSF4, TNFRSF8, CD40LG, ITGB2, KLRC2, TNFRSF18, TNFRSF14, HAVCR1, LGALS9, DAP10, DAP12, CD83, ligands of CD83 and combinations thereof. In some examples, the co-stimulatory signaling domain comprises a cytoplasmic domain of 4-1BB. In some examples, the recognition molecule further comprises a hinge domain located between the C-terminus of the binding moiety and the N-terminus of the transmembrane domain. In some examples, the hinge domain is derived from CD8α or IgG4 CH2-CH3.

[33] In some examples according to one or more of the above examples of the engineered immune cells, the recognition molecule is a chimeric T cell receptor ("cTCR"). In some examples, the transmembrane domain is derived from the transmembrane domain of a TCR subunit selected from the group consisting of TCRα, TCRβ, TCRγ, TCRδ，CD3γ, CD3ε, and CD3δ. In some examples, the transmembrane domain is derived from the transmembrane domain of CD3ε. In some examples, the intracellular signaling domain is derived from the intracellular signaling domain of a TCR subunit selected from the group consisting of TCRα, TCRβ, TCRγ, TCRδ，CD3γ, CD3ε, and CD3δ. In some examples, the intracellular signaling domain is derived from the intracellular signaling domain of CD3ε. In some examples, the transmembrane domain and intracellular signaling domain of the recognition molecule are derived from the same TCR subunit. In some examples, the recognition molecule further comprises at least a portion of an extracellular domain of a TCR subunit. In some examples, the binding moiety is fused to the N-terminus of CD3ε ("eTCR").

[34] In some examples according to one or more of the above examples of the engineered immune cells, the engineered immune cell is a T cell. In some examples, the immune cell is selected from the group consisting of a cytotoxic T cell, a helper T cell, a natural

killer (NK) cell, a natural killer T (NK-T) cell, and a γδT cell. In some examples, the engineered immune cell further comprises a co-receptor. In some examples, the co-receptor is a chemokine receptor.

[35] In some examples according to one or more of the above examples of the engineered immune cells, the target cell is an immune cell. In some examples, the target cell is a tumor cell.

[36] One example of the present application provides a pharmaceutical composition ("anti-distal portion pharmaceutical composition") comprising the engineered immune cell according to any one of the anti-distal portion engineered immune cells described above.

[37] One example of the present application provides a method of treating an individual having a cancer, comprising administering to the individual an effective amount of the pharmaceutical composition according to any one of the anti-distal portion pharmaceutical compositions described above. In some examples, the engineered immune cells are autologous to the individual. In some examples, the cancer is selected from the group consisting of T cell lymphoma, leukemia, B-cell precursor acute lymphoblastic leukemia (ALL), and B-cell lymphoma.

[38] One example of the present application provides a method of treating an individual having an infectious disease, comprising administering to the individual an effective amount of the pharmaceutical composition according to any one of the anti-distal portion pharmaceutical compositions described above. In some examples, the engineered immune cells are autologous to the individual. In some examples, the infectious disease is an infection by a virus selected from the group consisting of HIV and HTLV. In some examples, the infectious disease is HIV.

[39] One example of the present application provides a pharmaceutical composition comprising the anti-proximal portion engineered immune cell according to any one of the anti-proximal portion engineered immune cell examples described above.

[39a] In one aspect, the present disclosure refers to a pharmaceutical composition comprising the engineered immune cell disclosed herein.

[40] One example of the present application provides a method of treating an individual having a cancer, comprising administering to the individual an effective amount of the pharmaceutical composition according to any one of the anti-proximal portion pharmaceutical compositions described above. In some examples, the engineered immune cells are allogeneic to the individual. In some examples, the cancer is selected from the group

consisting of T cell lymphoma, leukemia, B-cell precursor acute lymphoblastic leukemia (ALL), and B-cell lymphoma.

[40a] In one aspect, the present disclosure refers to use of the pharmaceutical composition disclosed herein in the manufacture of a medicament for treating an individual having a cancer.

[41] One example of the present application provides a method of treating an individual having an infectious disease, comprising administering to the individual an effective amount of the pharmaceutical composition according to any one of the anti-proximal portion pharmaceutical compositions described above. In some examples, the engineered immune cells are allogeneic to the individual. In some examples, the infectious disease is an infection by a virus selected from the group consisting of HIV and HTLV. In some examples, the infectious disease is HIV.

[41a] In one aspect, the present disclosure refers to use of the pharmaceutical composition disclosed herein in the manufacture of a medicament for treating an individual having an infectious disease.

[42] Another example of the present application provides a method of making the engineered immune cell according to any one of the anti-distal or anti-proximal portion engineered immune cells described above, comprising introducing one or more nucleic acids encoding the recognition molecule into an immune cell, thereby obtaining the engineered immune cell.

[42a] In one aspect, the present disclosure refers to an *in vitro* method of making the engineered immune cell disclosed herein, comprising introducing one or more nucleic acids encoding the recognition molecule into an immune cell, thereby obtaining the engineered immune cell.

[43] Also provided are distal portion recognition molecules (*e.g.*, transmembrane receptors), anti-distal portion engineered immune cells, or compositions according to any one of the examples described above for use in treating a cancer or an infectious disease (*e.g.*, HIV), and use of proximal portion recognition molecules (*e.g.*, transmembrane receptors), anti-proximal engineered immune cells, or compositions according to any one of the examples described above for use in treating a cancer or an infectious disease (*e.g.*, HIV).

[44] Further provided are compositions, kits and articles of manufacture comprising any one of the engineered immune cells described above.

BRIEF DESCRIPTION OF THE DRAWINGS

[45] FIG. 1A shows the structure of an exemplary anti-CD4 CAR, which is composed of a CD4 binding moiety, a hinge region, a transmembrane domain, a co-stimulatory domain and a CD3ζ signaling domain. The CD4 binding moiety can specifically recognize an epitope in Domain 1 of CD4 or an epitope in Domain 2 and/or 3 of CD4.

[46] FIG. 1B shows phenotypes of two different kinds of anti-CD4 CAR-T cells. The CAR in CAR-T No. 1 contains an scFv specifically recognizing an epitope in Domain 1 of CD4, and can kill the CD4+ cells in both CAR+ and CAR- population. The CAR in CAR-T No. 2 contains an scFv specifically recognizing an epitope in domain 2 of CD4 and was not effective in killing the CAR+ target CD4+ cells.

CLAIMS

What is claimed is:

1. An engineered immune cell comprising on its surface a recognition molecule that comprises a binding moiety specifically binding to a target molecule on the surface of a target cell, wherein the target molecule comprises an extracellular domain,
   wherein the binding moiety specifically binds to a distal portion of the extracellular domain,
   wherein the immune cell is capable of killing a target cell that comprises on its surface the target molecule, and
   wherein the immune cell is capable of killing a target cell that comprises on its surface both the target molecule and the recognition molecule.
2. The engineered immune cell of claim 1, wherein:
   (i) the distance from the distal portion of the extracellular domain to the membrane of the target cell is more than about 0.5 times of the distance from the binding moiety to the membrane of engineered immune cell;
   (ii) the extracellular domain of the target molecule is at least about 175 amino acids long; and/or
   (iii) the distal portion of the extracellular domain is at least about 30 Å away from the membrane of the target cell.
3. The engineered immune cell of claim 1 or 2, wherein:
   (i) the binding moiety binds to a region in the extracellular domain that is about 50 amino acids or more away from the C-terminus of the extracellular domain; and/or
   (ii) the binding moiety binds to a region that is within about 80 amino acids from the N-terminus of the extracellular domain.
4. The engineered immune cell of any one of claims 2-3, wherein the extracellular domain of the target molecule comprises three or more Ig-like domains, and wherein:
   (i) the binding moiety binds to a region outside the first two Ig-like domains from the C-terminal end of the extracellular domain; and/or
   (ii) the binding moiety binds to a region within the first Ig-like domain at the N-terminal end of the extracellular domain.
5. The engineered immune cell of any one of claims 1-4, wherein:
   (i) the binding moiety competes for binding with a reference antibody that specifically binds to an epitope within Domains 1-4 of CD22 (“anti-CD22 D1-4 antibody”);

(ii) the binding moiety binds to an epitope in Domains 1-4 of CD22 that overlaps with the binding epitope of a reference anti-CD22 D1-4 antibody;

(iii) the binding moiety comprises the same heavy chain and light chain CDR sequences as those of a reference anti-CD22 D1-4 antibody; and/or

(iv) the binding moiety comprises the same heavy chain variable domain (VH) and light chain variable domain (VL) sequences as those of a reference anti-CD22 D1-4 antibody.

6. The engineered immune cell of claim 5, wherein:

(i) the reference anti-CD22 D1-4 antibody comprises a heavy chain CDR1 (HC-CDR1) comprising the amino acid sequence of SEQ ID NO: 67, a heavy chain CDR2 (HC-CDR2) comprising the amino acid sequence of SEQ ID NO: 68, a heavy chain CDR3 (HC-CDR3) comprising the amino acid sequence of SEQ ID NO: 69, a light chain CDR1 (LC-CDR1) comprising the amino acid sequence of SEQ ID NO: 70, a light chain CDR2 (LC-CDR2) comprising the amino acid sequence of SEQ ID NO: 71, and a light chain CDR3 (LC-CDR3) comprising the amino acid sequence of SEQ ID NO: 72; and/or

(ii) the reference anti-CD22 D1-4 antibody comprises a VH comprising the amino acid sequence of SEQ ID NO: 73 and a VL comprising the amino acid sequence of SEQ ID NO: 74.

7. The engineered immune cell of any one of claims 1-6, wherein the engineered immune cell is capable of killing a target cell that comprises on its surface both the target molecule and the recognition molecule by at least 3 fold as compared to an engineered immune cell comprising on its surface a recognition molecule comprising a binding moiety that binds to a proximal portion of the extracellular domain of the target molecule.

8. An engineered immune cell comprising on its surface a recognition molecule that comprises a binding moiety specifically binding to a target molecule on the surface of a target cell,

wherein the target molecule comprises an extracellular domain,

wherein the binding moiety specifically binds to a proximal portion of the extracellular domain,

wherein the engineered immune cell is capable of killing a target cell that comprises on its surface the target molecule, and

wherein the engineered immune cell has no or reduced capability of killing a target cell comprising on its surface both the target molecule and the recognition molecule.

9. The engineered immune cell of claim 8, wherein:

(i) the distance from the proximal portion of the extracellular domain to the membrane of the target cell is no more than about 2 times of the distance from the binding moiety to the membrane of engineered immune cell;

(ii) the extracellular domain of the target molecule is at least about 175 amino acids long; and/or

(iii) the proximal portion of the extracellular domain is no more than about 90 Å away from the membrane of the target cell.

10. The engineered immune cell of claim 8 or 9, wherein:

(i) the binding moiety binds to a region in the extracellular domain that is about 50 amino acids or more away from the C-terminus of the extracellular domain; and/or

(ii) the binding moiety binds to a region that is within about 80 amino acids from the N-terminus of the extracellular domain.

11. The engineered immune cell of any one of claims 8-10, wherein the extracellular domain of the target molecule comprises two or more Ig-like domains, and wherein:

(i) the binding moiety binds to a region outside the first Ig-like domain at the N-terminal end of the extracellular domain; and/or

(ii) the binding moiety binds to a region within the first two Ig-like domains from the C-terminal end of the extracellular domain.

12. The engineered immune cell of claim 11, wherein:

(i) the binding moiety competes for binding with a reference antibody that specifically binds to an epitope within Domains 5-7 of CD22 ("anti-CD22 D5-7 antibody");

(ii) the binding moiety binds to an epitope in Domains 5-7 of CD22 that overlaps with the binding epitope of a reference anti-CD22 D5-7 antibody;

(iii) the binding moiety comprises the same heavy chain and light chain CDR sequences as those of a reference anti-CD22 D5-7 antibody; and/or

(iv) the binding moiety comprises the same VH and VL sequences as those of a reference anti-CD22 D5-7 antibody.

13. The engineered immune cell of claim 12, wherein:

(i) the reference anti-CD22 D5-7 antibody comprises a HC-CDR1 comprising the amino acid sequence of SEQ ID NO: 76, a HC-CDR2 comprising the amino acid sequence of SEQ ID NO: 77, a HC-CDR3 comprising the amino acid sequence of SEQ ID NO: 78, a LC-CDR1 comprising the amino acid sequence of SEQ ID NO: 79, a LC-CDR2 comprising the amino

acid sequence of SEQ ID NO: 80, and a LC-CDR3 comprising the amino acid sequence of SEQ ID NO: 81; and/or

(ii) the reference anti-CD22 D5-7 antibody comprises a VH comprising the amino acid sequence of SEQ ID NO: 82 and a VL comprising the amino acid sequence of SEQ ID NO: 83.

14. The engineered immune cell of any one of claims 8-13, wherein the engineered immune cell kills a target cell that comprises on its surface both the target molecule and the recognition molecule by no more than about 20% as compared to an engineered immune cell comprising on its surface a recognition molecule comprising a binding moiety that binds to a distal end of the extracellular domain of the target molecule.
15. The engineered immune cell of any one of claims 1-14, wherein the binding moiety is an sdAb, an scFv, a Fab', a (Fab')2, an Fv, or a peptide ligand.
16. The engineered immune cell of any one of claims 1-15, wherein the recognition molecule comprises the binding moiety, a transmembrane domain, and an intracellular signaling domain.
17. The engineered immune cell of any one of claims 1-16, wherein the target molecule is a transmembrane receptor.
18. The engineered immune cell of claim 17, wherein the target molecule is selected from the group consisting of CD22, CD4, CD21 (CR2), CD30, ROR1, CD5, and CD20.
19. The engineered immune cell of any one of claims 1-18, wherein the recognition molecule is multispecific.
20. The engineered immune cell of any one of claims 1-19, wherein the recognition molecule is a chimeric antigen receptor ("CAR").
21. The engineered immune cell of claim 20, wherein:

(i) the transmembrane domain is derived from a molecule selected from the group consisting of CD8α, CD4, CD28, 4-1BB, CD80, CD86, CD152 and PD1, optionally wherein the transmembrane domain is derived from CD8α;

(ii) the intracellular signaling domain comprises a primary intracellular signaling domain derived from CD3ζ, FcRγ, FcRβ, CD3γ, CD3δ, CD3ε, CD5, CD22, CD79a, CD79b, or CD66d;

(iii) the intracellular signaling domain comprises a co-stimulatory signaling domain, optionally wherein the co-stimulatory signaling domain is derived from a co-stimulatory

molecule selected from the group consisting of CD27, CD28, 4-1BB, OX40, CD40, PD-1, LFA-1, ICOS, CD2, CD7, LIGHT, NKG2C, B7-H3, TNFRSF9, TNFRSF4, TNFRSF8, CD40LG, ITGB2, KLRC2, TNFRSF18, TNFRSF14, HAVCR1, LGALS9, DAP10, DAP12, CD83, ligands of CD83 and combinations thereof; and/or

(iv) the recognition molecule further comprises a hinge domain located between the C-terminus of the binding moiety and the N-terminus of the transmembrane domain, optionally wherein the hinge domain is derived from CD8α or IgG4 CH2-CH3.

22. The engineered immune cell of any one of claims 1-21, wherein the recognition molecule is a chimeric T cell receptor ("cTCR").
23. The engineered immune cell of claim 22, wherein:

(i) the transmembrane domain is derived from the transmembrane domain of a TCR subunit selected from the group consisting of TCRα, TCRβ, TCRγ, TCRδ，CD3γ, CD3ε, and CD3δ, optionally wherein the transmembrane domain is derived from the transmembrane domain of CD3ε;

(ii) the intracellular signaling domain is derived from the intracellular signaling domain of a TCR subunit selected from the group consisting of TCRα, TCRβ, TCRγ, TCRδ， CD3γ, CD3ε, and CD3δ;

(iii) the transmembrane domain and intracellular signaling domain of the recognition molecule are derived from the same TCR subunit; and/or

(iv) the recognition molecule further comprises at least a portion of an extracellular domain of a TCR subunit.

24. The engineered immune cell of claim 23(iv), wherein the binding moiety is fused to the N-terminus of CD3ε ("eTCR").
25. The engineered immune cell of any one of claims 1-24, wherein the immune cell is selected from the group consisting of a cytotoxic T cell, a helper T cell, a natural killer (NK) cell, a natural killer T (NK-T) cell, and a γδT cell.
26. The engineered immune cell of any one of claims 1-25, further comprising a co-receptor, optionally wherein the co-receptor is a chemokine receptor.
27. The engineered immune cell of any one of claims 1-26, wherein the target cell is an immune cell.
28. The engineered immune cell of any one of claims 1-26, wherein the target cell is a tumor cell.

29. A pharmaceutical composition comprising the engineered immune cell of any one of claims 1-28.
30. Use of the pharmaceutical composition of claim 29 in the manufacture of a medicament for treating an individual having a cancer.
31. The use of claim 30, wherein the binding moiety specifically binds to a distal portion of the extracellular domain, and wherein the engineered immune cells are autologous to the individual.
32. The use of claim 30 or 31, wherein the cancer is selected from the group consisting of T cell lymphoma, leukemia, B-cell precursor acute lymphoblastic leukemia (ALL), and B-cell lymphoma.
33. Use of the pharmaceutical composition of claim 29 in the manufacture of a medicament for treating an individual having an infectious disease.
34. The use of claim 33, wherein the binding moiety specifically binds to a proximal portion of the extracellular domain, and wherein the engineered immune cells are allogeneic to the individual.
35. The use of claim 33 or 34, wherein the infectious disease is an infection by a virus selected from the group consisting of HIV and HTLV.
36. An *in vitro* method of making the engineered immune cell of any one of claims 1-35, comprising introducing one or more nucleic acids encoding the recognition molecule into an immune cell, thereby obtaining the engineered immune cell.