

Claims

What is claimed is:

1. A chimeric receptor comprising a polypeptide chain comprising:
 - (a) an extracellular domain comprising an NKG2D domain;
 - (b) a transmembrane domain; and
 - (c) an intracellular signaling domain.
2. A multispecific chimeric receptor comprising a polypeptide chain comprising:
 - (a) an extracellular domain comprising a first NKG2D domain, a second NKG2D domain, and a second antigen binding domain;
 - (b) a transmembrane domain; and
 - (c) an intracellular signaling domain.
3. The multispecific chimeric receptor of claim 2, wherein the extracellular domain comprises from the N terminus to the C terminus: the second antigen binding domain, the first NKG2D domain and the second NKG2D domain.
4. The chimeric receptor of any one of claims 1-3, wherein the second antigen binding domain is fused to the first NKG2D domain via a peptide linker.
5. A multispecific chimeric receptor comprising a first polypeptide chain and a second polypeptide chain each comprising:
 - (a) an extracellular domain comprising an NKG2D domain and a second antigen binding domain;
 - (b) a transmembrane domain; and
 - (c) an intracellular signaling domain.
6. The multispecific chimeric receptor of claim 5, wherein each extracellular domain further comprises a dimerization motif.
7. The multispecific chimeric receptor of claim 6, wherein the dimerization motif is displaced between the NKG2D domain and the second antigen binding domain.
8. The multispecific chimeric receptor of claim 6 or 7, wherein the dimerization motif is a leucine zipper.
9. The multispecific chimeric receptor of claim 8, wherein the second antigen binding domain is fused to the NKG2D domain via a peptide linker.

10. The multispecific chimeric receptor of any one of claims 5-9, wherein each of the first polypeptide chain and the second polypeptide chain comprises from the N terminus to the C terminus: the second antigen binding domain, the NKG2D domain, the transmembrane domain, and the intracellular signaling domain.
11. A dual chimeric receptor system comprising:
- (i) a first chimeric receptor comprising:
 - (a) a first extracellular domain comprising an NKG2D domain;
 - (b) a first transmembrane domain; and
 - (c) a first intracellular signaling domain; and
 - (ii) a second chimeric receptor comprising:
 - (a) a second extracellular domain comprising a second antigen binding domain; and
 - (b) a second transmembrane domain.
12. The dual chimeric receptor system of claim 11, wherein the second chimeric receptor further comprises a second intracellular signaling domain.
13. The multispecific chimeric receptor of any one of claims 2-10, or the dual chimeric receptor system of claim 11 or 12, wherein the second antigen binding domain is an antibody fragment.
14. The multispecific chimeric receptor or the dual chimeric receptor system of claim 13, wherein the antibody fragment specifically binds to an antigen selected from the group consisting of CD19, CD20, CD22, CD33, CD38, BCMA, CS1, ROR1, GPC3, CD123, CD138, c Met, EGFR, EGFRvIII, HER2, HER3, GD-2, NY-ESO-1, MAGE-A3, and glycolipid F77.
15. The multispecific chimeric receptor of any one of claims 2-10, or the dual chimeric receptor system of claim 11 or 12, wherein the second antigen binding domain is a ligand or a ligand binding domain.
16. The multispecific chimeric receptor or the dual chimeric receptor system of claim 15, wherein the ligand or ligand binding domain is derived from a molecule selected from the group consisting of NKG2A, NKG2C, NKG2F, IL-3, IL-13, LIT1, AICL, DNAM-1, and NKp80.
17. The multispecific chimeric receptor or the dual chimeric receptor system of claim 16, wherein the second antigen binding domain is an IL-3 domain.

18. The chimeric receptor of any one of claims 1-10 and 13-17, or the dual chimeric receptor system of any one of claims 11-17, wherein the NKG2D domain (or the first NKG2D domain and/or the second NKG2D domain) comprises the amino acid sequence of SEQ ID NO: 7 or 8.
19. The chimeric receptor of any one of claims 1-10 and 13-18, or the dual chimeric receptor system of any one of claims 11-18, wherein the transmembrane domain (or the first transmembrane domain and/or the second transmembrane domain) is derived from a molecule selected from the group consisting of CD8 α , CD4, CD28, 4-1BB, CD80, CD86, CD152 and PD1.
20. The chimeric receptor of any one of claims 1-10 and 13-19, or the dual chimeric receptor system of any one of claims 11-19, wherein the intracellular signaling domain (or the first intracellular signaling domain and/or the second intracellular signaling domain) comprises a primary intracellular signaling domain of an immune effector cell.
21. The chimeric receptor of any one of claims 1-10 and 13-20, or the dual chimeric receptor system of any one of claims 11-20, wherein the intracellular signaling domain (or the first intracellular signaling domain and/or the second intracellular signaling domain) comprises a co-stimulatory signaling domain.
22. The chimeric receptor or the dual chimeric receptor system of claim 21, wherein the co-stimulatory signaling domain is derived from a co-stimulatory molecule selected from the group consisting of CD27, CD28, 4-1BB, OX40, CD30, ICOS, CD40, CD3, LFA-1, CD2, CD7, LIGHT, NKG2C, B7-H3, Ligands of CD83 and combinations thereof.
23. A chimeric receptor comprising an amino acid sequence having at least about 85% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 16-20 and 33-35.
24. A polypeptide comprising an amino acid sequence having at least about 85% sequence identity to the amino acid sequence of SEQ ID NO: 36 or 37.
25. An isolated nucleic acid comprising a nucleic acid sequence encoding the chimeric receptor of any one of claims 1-10 and 13-23.
26. An isolated nucleic acid encoding the dual chimeric receptor system of any one of claims 11-22, comprising a first nucleic acid sequence encoding the first chimeric receptor and a second nucleic acid sequence encoding the second chimeric receptor, wherein the first nucleic acid sequence is operably linked to the second nucleic acid sequence via a third nucleic acid sequence encoding a self-cleaving peptide.

- ~~27. An isolated nucleic acid comprising a nucleic acid sequence having at least about 85% sequence identity to a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 21-27 and SEQ ID NOs: 38-40.~~
- ~~28. An engineered immune effector cell, comprising the chimeric receptor or the dual chimeric receptor system of any one of claims 1-23, or the isolated nucleic acid of any one of claims 25-27.~~
- ~~29. A pharmaceutical composition, comprising the engineered immune effector cell of claim 28, and a pharmaceutically acceptable carrier.~~
- ~~30. A method of treating a cancer in an individual, comprising administering to the individual an effective amount of the pharmaceutical composition of claim 29.~~
1. A multispecific chimeric receptor comprising a polypeptide chain comprising:
- (a) an extracellular domain comprising a first NKG2D domain, a second NKG2D domain, and a second antigen binding domain;
 - (b) a transmembrane domain; and
 - (c) an intracellular signaling domain;
- wherein the first NKG2D domain is derived from extracellular domain of NKG2D,
- wherein the first NKG2D domain is a reverse NKG2D,
- wherein the second NKG2D domain is derived from extracellular domain of NKG2D,
- and wherein the second NKG2D is a forward NKG2D domain having the amino acid sequence in the same order as the wildtype NKG2D domain.
2. The multispecific chimeric receptor of claim 1, wherein the extracellular domain comprises from the N-terminus to the C-terminus: the second antigen binding domain, the first NKG2D domain and the second NKG2D domain.
3. The multispecific chimeric receptor of claim 1, wherein the second antigen binding domain is fused to the first NKG2D domain via a peptide linker.
4. The multispecific chimeric receptor of claim 1, wherein the second antigen binding domain is an antibody fragment.
5. The multispecific chimeric receptor of claim 4, wherein the antibody fragment specifically binds to an antigen selected from the group consisting of CD19, CD20, CD22, CD33, CD38, BCMA, CS1, ROR1, GPC3, CD123, CD138, c-Met, EGFR, EGFRvIII, HER2, HER3, GD-2, NY-ESO-1, MAGE A3, and glycolipid F77.
6. The multispecific chimeric receptor of claim 1, wherein the second antigen binding domain is a ligand or a ligand binding domain.
7. The multispecific chimeric receptor of claim 6, wherein the ligand or ligand binding domain is derived from a molecule selected from the group consisting of NKG2A, NKG2C, NKG2F, IL-3, IL-13, LLT1, AICL, DNAM-1, and NKp80.

8. The multispecific chimeric receptor of claim 7, wherein the second antigen binding domain is an IL-3 domain.
9. The multispecific chimeric receptor of any one of claims 1-8, wherein each of the first NKG2D domain and the second NKG2D domain comprises the amino acid sequence of SEQ ID NO: 7 or 8.
10. The multispecific chimeric receptor of claim 1, wherein the transmembrane domain is derived from a molecule selected from the group consisting of CD8 α , CD4, CD28, 4-1BB, CD80, CD86, CD152 and PD1.
11. The multispecific chimeric receptor of claim 1, wherein the intracellular signaling domain comprises a primary intracellular signaling domain of an immune effector cell.
12. The multispecific chimeric receptor of claim 1, wherein the intracellular signaling domain comprises a co-stimulatory signaling domain.
13. The multispecific chimeric receptor of claim 12, wherein the co-stimulatory signaling domain is derived from a co-stimulatory molecule selected from the group consisting of CD27, CD28, 4-1BB, OX40, CD30, ICOS, CD40, CD3, LFA-1, CD2, CD7, LIGHT, NKG2C, B7-H3, Ligands of CD83 and combinations thereof.
14. A multispecific chimeric receptor comprising an amino acid sequence comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 16-17.
15. An isolated nucleic acid comprising a nucleic acid sequence encoding the multispecific chimeric receptor of any one of claims 1-14.
16. An isolated nucleic acid comprising a nucleic acid sequence comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 21-22.
17. An engineered immune effector cell, comprising the multispecific chimeric receptor of any one of claims 1-14, or the isolated nucleic acid of claim 15 or 16.
18. A pharmaceutical composition, comprising the engineered immune effector cell of claim 17, and a pharmaceutically acceptable carrier.
19. Use of the pharmaceutical composition of claim 18 in the manufacture of a medicament for the treatment of a cancer, wherein the cancer comprises multiple myeloma, acute lymphoblastic leukemia, chronic lymphocytic leukemia.