

WHAT IS CLAIMED IS:

1. An activating chimeric receptor that binds HLA class I histocompatibility antigen, alpha chain E (HLA-E)/peptide complex, wherein the activating chimeric receptor transduces activation and/or costimulatory signals following binding to the HLA-E/peptide complex, wherein the activating chimeric receptor comprises:

an extracellular receptor domain, comprising an engineered variant of Natural Killer Group 2 member C (NKG2C),

wherein non-engineered NKG2C has low binding affinity for HLA-E/peptide complex, and

wherein the engineered NKG2C variant has enhanced binding affinity for HLA-E/peptide complex relative to native NKG2C, and

an effector domain comprising a transmembrane region and an intracellular signaling domain.

2. The activating chimeric receptor of Claim 1, wherein the extracellular receptor domain comprises an engineered NKG2C variant coupled to a native NKG2C transmembrane region and a native intracellular signaling domain.

3. The activating chimeric receptor of Claim 1 or Claim 2, wherein the activating chimeric receptor further comprises a DNAX-activating protein 12 (DAP12) domain.

4. An activating chimeric receptor according to any one of Claims 1 to 3, wherein the activating chimeric receptor further comprises an additional extracellular domain comprising native CD94 or chimeric CD94, wherein the chimeric CD94 comprises an extracellular domain comprising a fragment of CD94 and an effector domain comprising a transmembrane region and an intracellular region.

5. The activating chimeric receptor of Claim 4, wherein the effector domain comprises one or more of CD16, NCR1, NCR2, NCR3, 4-1BB, NKp80, DAP 10, CD3zeta, 2B4, and fragments thereof.

6. The activating chimeric receptor of Claim 4 or 5, wherein the additional extracellular domain comprises a chimeric CD94 receptor which comprises a fragment of CD94 coupled to a CD8a transmembrane domain and an effector domain comprising 4-1BB and CD3zeta.

7. The activating chimeric receptor of Claim 6, wherein the chimeric CD94 is encoded by the nucleic acid sequence of SEQ ID NO: 59, or a nucleic acid sequence having at least 95% homology to the sequence of SEQ ID NO. 59.

8. The activating chimeric receptor of Claim 6 or 7, wherein the chimeric CD94 comprises the amino acid sequence of SEQ ID NO: 60, or an amino acid sequence having at least 95% homology to the sequence of SEQ ID NO. 60.

9. An activating chimeric receptor according to any one of Claims 1 to 8, wherein the engineered NKG2C variant comprises the amino acid sequence of SEQ ID NO: 58, or an amino acid sequence having at least 95% homology to the sequence of SEQ ID NO. 58.

10. An activating chimeric receptor according to any one of Claims 1 to 9, wherein the engineered NKG2C variant comprises the amino acid sequence of SEQ ID NO: 64, or an amino acid sequence having at least 95% homology to the sequence of SEQ ID NO. 64

11. An activating chimeric receptor that binds HLA class I histocompatibility antigen, alpha chain E (HLA-E)/peptide complex, wherein the activating chimeric receptor transduces activation and/or costimulatory signals following binding to the HLA-E/peptide complex, wherein the activating chimeric receptor comprises:

an extracellular receptor domain comprising a fragment of Natural Killer Group 2 member A (NKG2A); and

an effector domain comprising a transmembrane region and an intracellular signaling domain

wherein the fragment of NKG2A is coupled to the effector domain,

wherein native NKG2A transduces inhibitory signals following binding to HLA-E/peptide complex, and

wherein the fragment of NKG2A transduces activation and/or costimulatory signals following binding to HLA-E/peptide complex.

12. The activating chimeric receptor of Claim 11, wherein the transmembrane domain comprises a CD8a transmembrane domain and the intracellular signaling domain comprises 4-1BB and CD3zeta.

13. The activating chimeric receptor of Claim 11 or 12, wherein the fragment of NKG2A comprises the amino acid sequence of SEQ ID NO: 66, or an amino acid sequence having at least 95% homology to the sequence of SEQ ID NO. 66.

14. An activating chimeric receptor according to Claim 11 or 12, wherein the activating chimeric receptor comprises the amino acid sequence of SEQ ID NO: 62, or an amino acid sequence having at least 95% homology to the sequence of SEQ ID NO. 62.

15. An activating chimeric receptor according to any one of the preceding Claims, wherein the activating chimeric receptor further comprises a GS linker.

16. An activating chimeric receptor according to any one of the preceding Claims, wherein the activating chimeric receptor further comprises a hinge region.17. The activating chimeric receptor of Claim 16, wherein the hinge region comprises a glycine serine repeating motif comprising the amino acid sequence of SEQ ID NO: 31,

wherein the hinge region comprises the amino acid sequence of SEQ ID NO: 32,

wherein the hinge region comprises the amino acid sequence of SEQ ID NO: 33,

wherein the hinge region comprises the amino acid sequence of SEQ ID NO: 34, or

wherein the hinge region comprises an amino acid sequence having at least 95% homology to the sequence of any of the foregoing.

18. An activating chimeric receptor according to any one of the preceding Claims, wherein the effector domain comprises one or more hemi-ITAM sequences, wherein the hemi-ITAM comprises the amino acid sequence of SEQ ID NO: 14 or comprises the amino acid sequence of SEQ ID NO: 37, or an amino acid sequence having at least 95% homology to the sequence of any of the foregoing, or wherein the effector domain comprises one or more hemi-ITSM sequences, wherein the hemi-ITSM comprises the amino acid sequence of SEQ ID NO: 15 or comprises the amino acid sequence of SEQ ID NO: 35 or an amino acid sequence having at least 95% homology to the sequence of any of the foregoing.

19. A polynucleotide encoding an activating chimeric receptor that binds HLA class I histocompatibility antigen, alpha chain E (HLA-E)/peptide complex, wherein the activating chimeric receptor transduces activation and/or costimulatory signals following binding to the HLA-E/peptide complex, wherein the activating chimeric receptor comprises:

a) an extracellular receptor domain, wherein said extracellular receptor domain comprises an engineered variant of Natural Killer Group 2 member C (NKG2C),

wherein non-engineered NKG2C has low binding affinity for HLA-E/peptide complex,

wherein the engineered NKG2C variant has enhanced binding affinity for HLA-E/peptide complex relative to native NKG2C, and

wherein the engineered NKG2C variant comprises the amino acid sequence of SEQ ID NO. 58, and

an effector domain comprising a transmembrane region and an intracellular signaling domain; or

b) an extracellular receptor domain, wherein said extracellular receptor domain comprises a fragment of Natural Killer Group 2 member A (NKG2A),

wherein the fragment of NKG2A is coupled to an effector domain comprising a transmembrane region and an intracellular signaling domain,

wherein native NKG2A transduces inhibitory signals following binding to HLA-E/peptide complex, and

wherein the fragment of NKG2A transduces activation and/or costimulatory signals following binding to HLA-E/peptide complex, and

an effector domain comprising a transmembrane region and an intracellular signaling domain.

20. The polynucleotide of Claim 19, wherein the activating chimeric receptor comprises an engineered NKG2C variant, wherein the engineered NKG2C variant is encoded by the nucleic acid sequence of SEQ ID NO: 63, or a nucleic acid sequence having at least 95% homology to the sequence of SEQ ID NO. 63.

21. The polynucleotide of Claim 19 or 20, wherein the polynucleotide further encodes a chimeric CD94 extracellular domain, wherein the chimeric CD94 comprises an extracellular receptor domain comprising a fragment of CD94 couple to a CD8a transmembrane domain and an effector domain comprising 4-1BB and CD3zeta, wherein the chimeric CD94 is encoded by the nucleic acid sequence of SEQ ID NO: 59, or a nucleic acid sequence having at least 95% homology to the sequence of SEQ ID NO. 59.

22. The polynucleotide of Claim 19, wherein the activating chimeric receptor comprises a fragment of Natural Killer Group 2 member A (NKG2A), wherein the fragment of NKG2A is encoded by a nucleic acid having the sequence of SEQ ID NO: 65, or a nucleic acid sequence having at least 95% homology to the sequence of SEQ ID NO. 65.

23. The polynucleotide of Claim 22, wherein the transmembrane domain comprises a CD8a transmembrane domain and the intracellular signaling domain comprising 4-1BB and CD3zeta.

24. The polynucleotide of claim 22 or 23, wherein the activating chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO: 61, or a nucleic acid sequence having at least 95% homology to the sequence of SEQ ID NO. 61.

25. The polynucleotide of any one of claims 19 to 24, wherein the polynucleotide further encodes a short hairpin RNA (shRNA) that specifically inhibits transcription or translation of native NKG2A,

wherein the shRNA comprises a nucleotide sequence that hybridizes under stringent conditions to the native NKG2A gene, wherein the shRNA comprises a sense fragment, which comprises a nucleotide sequence substantially identical to a target sequence in the NKG2A gene that is absent in the NKG2A fragment, and an antisense fragment, wherein the sense and antisense fragments are separated by a loop fragment.

26. The polynucleotide of any one of claims 19 to 25, wherein the polynucleotide further encodes a chimeric receptor comprising: (a) an extracellular receptor domain, wherein said extracellular receptor domain comprises a peptide that binds native ligands of Natural Killer Group 2 member D (NKG2D); and (b) an effector domain comprising a transmembrane region and an intracellular signaling domain.

27. A vector comprising a polynucleotide according to any one of Claims 19 to 26, wherein the polynucleotide is operatively linked to at least one regulatory element for expression of the activating chimeric receptor.

28. The vector of claim 27, wherein the vector is a retrovirus.

29. A genetically engineered immune cell expressing the activating receptor according to any one of Claims 1 to 18, comprising the polynucleotide according to any one of Claims 19 to 26, or the vector according to any one of Claims 27 to 28.

30. The genetically engineered immune cell of Claim 29, wherein the genetically engineered immune cell comprises a natural killer (NK) cell.

31. The genetically engineered immune cell of Claim 29 or 30, wherein the genetically engineered immune cell is an allogeneic cell isolated from a donor.

32. A genetically engineered NK cell comprising an activating chimeric receptor that binds HLA class I histocompatibility antigen, alpha chain E (HLA-E)/peptide complex, wherein the activating chimeric receptor transduces activation and/or costimulatory signals following binding to the HLA-E/peptide complex, wherein the activating chimeric receptor comprises:

a) an extracellular receptor domain, wherein said extracellular receptor domain comprises an engineered variant of Natural Killer Group 2 member C (NKG2C),

wherein non-engineered NKG2C has low binding affinity for HLA-E/peptide complex,

wherein the engineered NKG2C variant has enhanced binding affinity for HLA-E/peptide complex relative to native NKG2C, and

wherein the engineered NKG2C variant comprises the amino acid sequence of SEQ ID NO. 58, and

an effector domain comprising a transmembrane region and an intracellular signaling domain; or

b) an extracellular receptor domain, wherein said extracellular receptor domain comprises a fragment of Natural Killer Group 2 member A (NKG2A),

wherein the fragment of NKG2A is coupled to an effector domain comprising a transmembrane region and an intracellular signaling domain,

wherein native NKG2A transduces inhibitory signals following binding to HLA-E/peptide complex, and

wherein the fragment of NKG2A transduces activation and/or costimulatory signals following binding to HLA-E/peptide complex, and

an effector domain comprising a transmembrane region and an intracellular signaling domain.

33. The genetically engineered NK cell of Claim 32, wherein the activating chimeric receptor comprises an engineered NKG2C variant.

34. The genetically engineered NK cell of Claim 32 or 33, further comprising a chimeric CD94 extracellular domain comprising a fragment of CD94 couple to a CD8a transmembrane domain and an effector domain comprising 4-1BB and CD3zeta.

35. The genetically engineered NK cell of Claim 32, wherein the activating chimeric receptor comprises a fragment of Natural Killer Group 2 member A (NKG2A) and the transmembrane domain comprises a CD8a transmembrane domain and the intracellular signaling domain comprises 4-1BB and CD3zeta.

36. A genetically engineered NK cell according to any one of Claims 32 to 35, wherein the cell further comprises a chimeric receptor comprising: (a) an extracellular receptor domain, wherein said extracellular receptor domain comprises a peptide that binds native ligands of Natural Killer Group 2 member D (NKG2D); and (b) an effector domain comprising a transmembrane region and an intracellular signaling domain.

37. An amino acid sequence of SEQ ID NO: 64, or an amino acid sequence having at least 95% homology to the sequence of SEQ ID NO. 64

38. An amino acid sequence of SEQ ID NO: 66, or an amino acid sequence having at least 95% homology to the sequence of SEQ ID NO. 66.

39. A method for generating a genetically engineered NK cell with enhanced cytotoxicity comprising:

contacting a population of NK cells with a vector encoding an activating chimeric receptor, wherein the activating chimeric receptor comprises:

a) an extracellular receptor domain, wherein said extracellular receptor domain comprises an engineered variant of Natural Killer Group 2 member C (NKG2C),

wherein non-engineered NKG2C has low binding affinity for HLA-E/peptide complex,

wherein the engineered NKG2C variant has enhanced binding affinity for HLA-E/peptide complex relative to native NKG2C, and

wherein the engineered NKG2C variant comprises the amino acid sequence of SEQ ID NO. 58, and

an effector domain comprising a transmembrane region and an intracellular signaling domain; or

b) an extracellular receptor domain, wherein said extracellular receptor domain comprises a fragment of Natural Killer Group 2 member A (NKG2A),

wherein the fragment of NKG2A is coupled to an effector domain comprising a transmembrane region and an intracellular signaling domain,

wherein native NKG2A transduces inhibitory signals following binding to HLA-E/peptide complex, and

wherein the fragment of NKG2A transduces activation and/or costimulatory signals following binding to HLA-E/peptide complex, and

an effector domain comprising a transmembrane region and an intracellular signaling domain.

40. The method of Claim 39, further comprising contact the NK cell with a vector encoding a chimeric receptor comprising: (a) an extracellular receptor domain, wherein said extracellular receptor domain comprises a peptide that binds native ligands of Natural Killer Group 2 member D (NKG2D); and (b) an effector domain comprising a transmembrane region and an intracellular signaling domain.

41. Use of the activating chimeric receptor according to any one of Claims 1 to 18, the polynucleotide according to any one of Claims 19 to 26, the vector according to any one of Claims 27 to 28, the genetically engineered immune cell according to any one of Claims 29 to 31, the genetically engineered NK cell according to any one of Claims 32 to 36, an amino acid according to Claim 37 or 38,

or the method according to any one of Claims 39 to 40 for treating or preventing cancer in a mammal in need thereof.

42. Use of the activating chimeric receptor according to any one of Claims 1 to 18, the polynucleotide according to any one of Claims 19 to 26, the vector according to any one of Claims 27 to 28, the genetically engineered immune cell according to any one of Claims 29 to 31, the genetically engineered NK cell according to any one of Claims 32 to 36, an amino acid according to Claim 37 or 38, or the method according to any one of Claims 39 to 40 in the manufacture of a medicament for treating or preventing cancer in a mammal in need thereof.