

WHAT IS CLAIMED IS:

1. A polynucleotide 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),
wherein the peptide that binds native ligands of NKG2D is a fragment of NKG2D,
wherein the fragment of NKG2D is encoded by a polynucleotide comprising SEQ ID NO. 2; and
 - (b) an effector domain comprising a transmembrane region and an intracellular signaling domain,
wherein the intracellular signaling domain comprises CD3zeta, and
wherein the CD3zeta is encoded by a polynucleotide comprising SEQ ID NO. 13.
2. The polynucleotide of Claim 1, wherein the transmembrane region of the effector domain comprises a CD8a transmembrane domain.
3. The polynucleotide of Claim 1, wherein the transmembrane region of the effector domain further comprises a CD8a hinge region.
4. The polynucleotide of Claim 3, wherein the CD8a hinge region is encoded by a polynucleotide comprising SEQ ID NO: 5.
5. The polynucleotide of Claim 1, wherein the intracellular signaling domain further comprises 4-1BB.
6. The polynucleotide of Claim 5, wherein the 4-1BB is encoded by a polynucleotide comprising SEQ ID NO. 12.
7. The polynucleotide of Claim 1, wherein the chimeric receptor comprises the fragment of NKG2D coupled to CD8a, 4-1BB and CD3z.

8. The polynucleotide of Claim 7, wherein the chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO. 18.

9. The polynucleotide of Claim 7, wherein the chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO. 108.

10. The polynucleotide of Claim 7, wherein the chimeric receptor comprises the amino acid sequence of SEQ ID NO. 19.

11. A composition comprising a Natural Killer (NK) cell expressing the chimeric receptor encoded by the polynucleotide of any one of Claims 1 to 10 for use in treating cancer or an infectious disease.

12. The composition of claim 11, wherein said NK cells are autologous cells isolated from a patient having a cancer or an infectious disease.

13. The composition of claim 11, wherein said NK cells are allogenic cells isolated from a donor.

14. Use of a polynucleotide according to any one of Claims 1-10 in the manufacture of a medicament for enhancing NK cell cytotoxicity in a mammal in need thereof.

15. Use of a polynucleotide according to any one of Claims 1-10 in the manufacture of a medicament for treating or preventing cancer or an infectious disease in a mammal in need thereof.

16. A polynucleotide 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),
wherein the peptide that binds native ligands of NKG2D is a fragment of NKG2D,

wherein the fragment of NKG2D is encoded by a polynucleotide comprising SEQ ID NO. 2, and

(b) an effector domain comprising a transmembrane region and an intracellular signaling domain,

wherein the intracellular signaling domain comprises CD3zeta,

wherein the CD3zeta is encoded by a polynucleotide comprising SEQ ID NO. 13 and

wherein the polynucleotide is co-expressed with a membrane-bound interleukin 15 (mbIL15).

17. The polynucleotide of Claim 16, wherein the transmembrane region of the effector domain comprises a CD8a transmembrane domain.

18. The polynucleotide of Claim 16, wherein the transmembrane region of the effector domain further comprises a CD8a hinge region.

19. The polynucleotide of Claim 18, wherein the CD8a hinge region is encoded by a polynucleotide comprising SEQ ID NO: 5.

20. The polynucleotide of Claim 16, wherein the mbIL15 is encoded by a polynucleotide comprising SEQ ID NO. 16.

21. The polynucleotide of Claim 20, wherein the mbIL15 is bicistronically expressed on the same polynucleotide as the chimeric receptor.

22. The polynucleotide of Claim 20, wherein mbIL15 comprises an amino acid sequence of SEQ ID NO: 17

23. The polynucleotide of Claim 20, wherein the effector domain further comprises an OX-40 domain.

24. The polynucleotide of Claim 23, wherein the chimeric receptor comprises the fragment of NKG2D coupled to a CD8a hinge, a CD8a transmembrane domain, the OX-40 domain, the CD3zeta.

25. The polynucleotide of Claim 24, wherein the chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO: 90 coupled to the mbIL15 encoded by SEQ ID NO. 16.

26. The polynucleotide of Claim 25, wherein the chimeric receptor comprises the amino acid sequence of SEQ ID NO: 91 coupled to the mbIL15 comprising the amino acid sequence of SEQ ID NO. 17.

27. The polynucleotide of Claim 23, wherein the chimeric receptor comprises the fragment of NKG2D coupled to a IgG4 hinge, a CD8a transmembrane domain, the OX-40 domain, the CD3zeta.

28. The polynucleotide of Claim 27, wherein the chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO: 100 coupled to the mbIL15 encoded by SEQ ID NO. 16.

29. The polynucleotide of Claim 28, wherein the chimeric receptor comprises the amino acid sequence of SEQ ID NO: 101 coupled to the mbIL15 comprising the amino acid sequence of SEQ ID NO. 17.

30. A composition comprising a Natural Killer (NK) cell expressing the chimeric receptor encoded by the polynucleotide of any one of Claims 15 to 29 for the treatment of cancer or an infectious disease.

31. The composition of claim 30, wherein said NK cells are autologous cells isolated from a patient having a cancer.

32. The composition of claim 30, wherein said NK cells are allogenic cells isolated from a donor.

33. Use of a polynucleotide according to any one of Claims 15 to 29 in the manufacture of a medicament for enhancing NK cell cytotoxicity in a mammal in need thereof.

34. Use of a polynucleotide according to any one of Claims 15 to 29 in the manufacture of a medicament for treating or preventing cancer or an infectious disease in a mammal in need thereof.

35. A polynucleotide 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),

wherein the peptide that binds native ligands of NKG2D is a fragment of NKG2D,

wherein the fragment of NKG2D is encoded by a polynucleotide comprising: (i) a fragment of SEQ ID NO: 1, (ii) SEQ ID NO. 2, or (iii) SEQ ID NO. 3; and

(b) an effector domain comprising a transmembrane region and an intracellular signaling domain.

36. The polynucleotide of claim 35, wherein the effector domain comprises CD16.

37. The polynucleotide of claim 35, wherein the effector domain comprises Natural Cytotoxicity Triggering Receptor 1 (NCR1).

38. The polynucleotide of claim 35, wherein the effector domain comprises Natural Cytotoxicity Triggering Receptor 2 (NCR2) or Natural Cytotoxicity Triggering Receptor 3 (NCR3).

39. The polynucleotide according to any one of Claims 35-38, wherein the effector domain further comprises 4-1BB.

40. The polynucleotide of claim 36, wherein the chimeric receptor comprises the fragment of NKG2D coupled to CD16.

41. The polynucleotide of claim 40, wherein the chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO: 23.

42. The polynucleotide of claim 40, wherein the chimeric receptor comprises the amino acid sequence of SEQ ID NO: 24.

43. The polynucleotide of claim 40, wherein the chimeric receptor comprises the fragment of NKG2D coupled to NCR1.

44. The polynucleotide of claim 43, wherein the chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO: 27.

45. The polynucleotide of claim 43, wherein the chimeric receptor comprises the amino acid sequence of SEQ ID NO: 28.

46. The polynucleotide of claim 38, wherein the chimeric receptor comprises at least a portion of the amino acid sequence of SEQ ID NO: 21.

47. The polynucleotide of claim 36, wherein the chimeric receptor comprises the fragment of NKG2D coupled to NCR3.

48. The polynucleotide of claim 47, wherein the chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO. 29.

49. The polynucleotide of claim 47, wherein the chimeric receptor comprises the amino acid sequence of SEQ ID NO. 30.

50. The polynucleotide of claim 39, wherein the chimeric receptor comprises the fragment of NKG2D coupled to a CD16 transmembrane/intracellular domain and 4-1BB.

51. The polynucleotide of claim 39, wherein the chimeric receptor comprises the fragment of NKG2D coupled to a CD8a hinge, a CD16 transmembrane/intracellular domain and 4-1BB.

52. The polynucleotide of claim 51, wherein the chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO: 25.

53. The polynucleotide of claim 51, wherein the chimeric receptor comprises the amino acid sequence of SEQ ID NO: 26.

54. The polynucleotide of claim 39, wherein the chimeric receptor comprises the fragment of NKG2D coupled to NCR1 and 4-1BB.

55. The polynucleotide of claim 54, wherein the chimeric receptor comprises the NCR1 amino acid sequence of SEQ ID NO: 20.

56. The polynucleotide of claim 39, wherein the chimeric receptor comprises the fragment of NKG2D coupled to CD8a, 4-1BB and CD3z.

57. The polynucleotide of claim 56, wherein the chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO. 18.

58. The polynucleotide of claim 56, wherein the chimeric receptor comprises the amino acid sequence of SEQ ID NO. 19.

59. The polynucleotide of claim 39, wherein the chimeric receptor comprises the fragment of NKG2D coupled to NCR3 and 4-1BB, and wherein the NCR3 comprises the amino acid sequence of SEQ ID NO: 22.

60. The polynucleotide of claim 39, wherein the chimeric receptor comprises one or more of the NCR1 transmembrane/intracellular domain of SEQ ID NO: 20 or the NCR3 transmembrane/intracellular domain of SEQ ID NO: 22.

61. The polynucleotide of claim 39, wherein the effector domain comprises a GS linker between 4-1BB and one of CD16, NCR1, NCR3, 2B4 or NKp80.

62. The polynucleotide of any one of claims 35-61, wherein the chimeric receptor domain comprises a hinge region.

63. The polynucleotide of claim 62, wherein the hinge region is encoded by the nucleic acid sequence of SEQ ID NO: 5.

64. The polynucleotide of claim 62, wherein the hinge region is encoded by a fragment of the nucleic acid sequence of SEQ ID NO: 5.

65. The polynucleotide of claim 62, wherein the hinge region is comprises a glycine-serine repeating motif having the amino acid sequence of SEQ ID NO: 31.

66. The polynucleotide of claim 62, wherein the hinge region comprises the amino acid sequence of SEQ ID NO: 32.

67. The polynucleotide of claim 62, wherein the hinge region comprises the amino acid sequence of SEQ ID NO: 33.

68. The polynucleotide of claim 62, wherein the hinge region is encoded by the nucleic acid sequence of SEQ ID NO: 34.

69. The polynucleotide of claim 62, wherein the hinge region comprises a portion of the beta-adrenergic receptor.

70. The polynucleotide of claim 69, wherein the hinge region is encoded by the nucleic acid sequence of SEQ ID NO: 40.

71. The polynucleotide of claim 69, wherein the hinge region is encoded by the nucleic acid sequence of SEQ ID NO: 42.

72. The polynucleotide of any one claims 35-71, wherein the extracellular receptor domain further comprises a CD8a signal peptide, wherein the signal peptide comprises the nucleic acid sequence of SEQ ID NO. 4.

73. The polynucleotide of any one of claims 35-72, wherein the effector domain comprises one or more hemi-ITAM sequences.

74. The polynucleotide of claim 72, wherein the hemi-ITAM comprises the amino acid sequence of SEQ ID NO. 14.

75. The polynucleotide of claim 72, wherein the hemi-ITAM comprises the amino acid sequence of SEQ ID NO. 37.

76. The polynucleotide of any one of claims 35-75, wherein the effector domain comprises one or more ITSM sequences.

77. The polynucleotide of claim 76, wherein the ITSM comprises the amino acid sequence of SEQ ID NO. 15.

78. The polynucleotide of claim 76, wherein the ITSM comprises the amino acid sequence of SEQ ID NO. 35

79. The polynucleotide of Claim 76, wherein the effector domain comprises a 2B4 domain.

80. The polynucleotide of claim 35, wherein the chimeric receptor comprises the fragment of NKG2D coupled to a GS3 linker, a CD8a hinge, a CD16 transmembrane/intracellular domain and 4-1BB.

81. The polynucleotide of claim 80, wherein the chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO: 43.

82. The polynucleotide of claim 35, wherein the chimeric receptor comprises the fragment of NKG2D coupled to a GS3 linker, a CD16 transmembrane/intracellular domain and 4-1BB.

83. The polynucleotide of claim 82, wherein the chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO: 44.

84. The polynucleotide of claim 35, wherein the chimeric receptor comprises the fragment of NKG2D coupled to a CD16 transmembrane/intracellular domain and 4-1BB.

85. The polynucleotide of claim 84, wherein the chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO: 45.

86. The polynucleotide of claim 35, wherein the chimeric receptor comprises the fragment of NKG2D coupled to a CD8a hinge, a CD8a transmembrane domain, 4-1BB, and 2B4.

87. The polynucleotide of claim 86, wherein the chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO: 46.

88. The polynucleotide of claim 35, wherein the chimeric receptor comprises the fragment of NKG2D coupled to a beta-adrenergic extracellular domain, a beta-adrenergic transmembrane domain, 4-1BB, and 2B4.

89. The polynucleotide of claim 88, wherein the chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO: 47

90. The polynucleotide of claim 35, wherein the chimeric receptor comprises the fragment of NKG2D coupled to a CD8a hinge, a CD8a transmembrane domain, 4-1BB, 2B4, a GS3 linker, and NKp80.

91. The polynucleotide of claim 90, wherein the chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO: 48.

92. The polynucleotide of claim 35, wherein the chimeric receptor comprises the fragment of NKG2D coupled to a CD8a hinge, a CD8a transmembrane domain, 4-1BB, a GS3 linker, and NKp80.

93. The polynucleotide of claim 92, wherein the chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO: 49.

94. The polynucleotide of claim 35, wherein the chimeric receptor comprises the fragment of NKG2D that is codon optimized coupled to a GS3 linker, an additional NKG2D fragment, a beta-adrenergic extracellular domain, a beta-adrenergic transmembrane domain, 4-1BB, an additional GS3 linker, and NKp80.

95. The polynucleotide of claim 94, wherein the chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO: 50.

96. The polynucleotide of claim 35, wherein the chimeric receptor comprises the fragment of NKG2D that is codon optimized coupled to a GS3 linker, an additional NKG2D fragment, a CD8a hinge, a CD8a transmembrane domain, 4-1BB, an additional GS3 linker, and NKp80.

97. The polynucleotide of claim 96, wherein the chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO: 51.

98. The polynucleotide of claim 35, wherein the chimeric receptor comprises the fragment of NKG2D that is codon optimized coupled to a GS3 linker, an additional NKG2D fragment, a cd8a hinge, a CD16 transmembrane/intracellular domain, and 4-1BB.

99. The polynucleotide of claim 98, wherein the chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO: 52.

100. The polynucleotide of claim 35, wherein the chimeric receptor comprises the fragment of NKG2D coupled to a CD8a hinge, a CD16 transmembrane/intracellular domain, 4-1BB, and 2B4.

101. The polynucleotide of claim 100, wherein the chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO: 53.

102. The polynucleotide of claim 35, wherein the chimeric receptor comprises the fragment of NKG2D coupled to a CD8a hinge, a CD16 transmembrane/intracellular domain, 4-1BB, a GS3 linker, and NKp80.

103. The polynucleotide of claim 102, wherein the chimeric receptor is encoded by the nucleic acid sequence of SEQ ID NO: 54.

104. The polynucleotide of any one of claims 35-103, wherein the chimeric receptor does not comprise DNAX-activating protein 10 (DAP10).

105. The polynucleotide of any one of claims 35-55 and 60-104, wherein the chimeric receptor does not comprise an ITAM motif.

106. A polynucleotide 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), wherein the peptide that binds native ligands of NKG2D is a fragment of NKG2D,

(b) a transmembrane region, wherein said transmembrane region comprises CD8a, and

(c) an effector domain, wherein said effector domain comprises 4-1BB and CD3 zeta,

wherein the polynucleotide is co-expressed with an additional construct encoding membrane-bound interleukin 15 (mbIL15).

107. A polynucleotide 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), wherein the peptide that binds native ligands of NKG2D is a fragment of NKG2D,

(b) a transmembrane region, wherein said transmembrane region comprises CD8a, and

(c) an effector domain, wherein said effector domain comprises 4-1BB and the intracellular domain of 2B4 or DAP10.

108. The polynucleotide of any of claims 106 or 107, wherein the polynucleotide is co-expressed with an additional construct encoding membrane-bound interleukin 15 (mbIL15).

109. A polynucleotide 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), wherein the peptide that binds native ligands of NKG2D is a fragment of NKG2D, wherein the fragment of NKG2D is encoded by a polynucleotide comprising: (i) a fragment of the sequence of SEQ ID NO: 1, (ii) the sequence of SEQ ID NO. 2, (iii) the sequence of SEQ ID NO. 3, or (iv) the sequence of SEQ ID NO. 68,

(b) a transmembrane region, wherein said transmembrane region comprises a CD3zeta transmembrane region, and

(c) an effector domain.

110. The polynucleotide of claim 109, wherein the polynucleotide is co-expressed with membrane-bound interleukin 15 (mbIL15).

111. A polynucleotide 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),

wherein the peptide that binds native ligands of NKG2D is a fragment of NKG2D,

wherein the fragment of NKG2D is encoded by a polynucleotide comprising: (i) a fragment of the sequence of SEQ ID NO: 1, (ii) the sequence of SEQ ID NO. 2, (iii) the sequence of SEQ ID NO. 3, (iv) or the sequence of SEQ ID NO. 68; and

(b) an effector domain comprising a transmembrane region and an intracellular signaling domain.

112. The polynucleotide of any one of claims 35-111, wherein the polynucleotide is operably linked to at least one regulatory element for the expression of the chimeric receptor.

113. A vector comprising the polynucleotide of any one of claims 35-112, wherein the polynucleotide is operatively linked to at least one regulatory element for expression of the chimeric receptor.

114. The vector of claim 113, wherein the vector is a retrovirus.

115. A genetically engineered natural killer cell comprising the polynucleotide of any one Claims 35-112.

116. The isolated genetically engineered natural killer cell of claim 115, which is an autologous cell isolated from a patient.

117. The isolated genetically engineered natural killer cell of claim 115, which is an allogeneic cell isolated from a donor.

118. A composition comprising NK cells that express a chimeric receptor encoded by a polynucleotide of any one of Claims 35-112 for enhancing NK cell cytotoxicity in a mammal in need thereof.

119. The composition of claim 118, wherein said NK cells are autologous cells isolated from a patient.

120. The composition of claim 118, wherein said NK cells are allogeneic cells isolated from a donor.

121. A composition comprising a therapeutically effective amount of NK cells, wherein said NK cells express a chimeric receptor encoded by a polynucleotide of any one of Claims 35-112 for the use in treating or preventing cancer or an infectious disease.

122. The composition of claim 121, wherein said NK cells are autologous cells isolated from a patient having a cancer or an infectious disease.

123. The composition of claim 121, wherein said NK cells are allogenic cells isolated from a donor.

124. Use of a polynucleotide according to any one of Claims 35-112 in the manufacture of a medicament for enhancing NK cell cytotoxicity in a mammal in need thereof.

125. Use of a polynucleotide according to any one of Claims 35-112 in the manufacture of a medicament for treating or preventing cancer or an infectious disease in a mammal in need thereof.

126. Use of a vector according to Claim 114 or 115 in the manufacture of a medicament for enhancing NK cell cytotoxicity in a mammal in need thereof.

127. Use of a vector according to Claim 114 or 115 in the manufacture of a medicament for treating or preventing cancer or an infectious disease in a mammal in need thereof.

128. Use of an isolated genetically engineered natural killer cell according to any one of Claims 115-117 for enhancing NK cell cytotoxicity in a mammal in need thereof.

129. Use of an isolated genetically engineered natural killer cell according to any one of Claims 116-117 for treating or preventing cancer or an infectious disease in a mammal in need thereof.

130. A polynucleotide 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),

wherein the peptide that binds native ligands of NKG2D is a fragment of NKG2D,

wherein the fragment of NKG2D is encoded by a polynucleotide comprising: (i) a fragment of SEQ ID NO: 1, (ii) SEQ ID NO. 2, (iii) SEQ ID NO. 3; or (iv) SEQ ID NO. 68; and

(b) an effector domain comprising a transmembrane region and an intracellular signaling domain.

131. A transgenic cell comprising:

a) an immune cell comprising a chimeric receptor, the chimeric receptor comprising:

(i) an extracellular receptor domain comprising a peptide that binds native ligands of Natural Killer Group 2 member D (NKG2D),

wherein the peptide that binds native ligands of NKG2D is a fragment of NKG2D; and

(ii) an effector domain comprising a transmembrane region and an intracellular signaling domain, wherein the intracellular signaling domain comprises CD3zeta;

b) a membrane-bound interleukin 15 (mbIL15).

132. A composition comprising a Natural Killer (NK) cell expressing:

a) an immune cell comprising a chimeric receptor, the chimeric receptor comprising:

- (i) an extracellular receptor domain comprising a peptide that binds native ligands of Natural Killer Group 2 member D (NKG2D), wherein the peptide that binds native ligands of NKG2D is a fragment of NKG2D; and
 - (ii) an effector domain comprising a transmembrane region and an intracellular signaling domain, wherein the intracellular signaling domain comprises CD3zeta;
- b) a membrane-bound interleukin 15 (mbIL15), for use in treating cancer.