

Claims

1. Use of a composition comprising T cells for the manufacture of a medicament for treating multiple myeloma in a subject,
wherein the T cells comprise a chimeric antigen receptor (CAR) comprising:
 - a) an extracellular antigen binding domain comprising a first anti-BCMA binding moiety and a second BCMA binding moiety;
 - b) a transmembrane domain; and
 - c) an intracellular signaling domain,wherein the first BCMA binding moiety comprises the amino acid sequence set forth in SEQ ID NO:2 and the second BCMA binding moiety comprises the amino acid sequence set forth in SEQ ID NO:4, and
wherein the composition is administered to the subject via a single intravenous infusion to deliver to the subject a dose of CAR expressing T cells (CAR-T cells).
2. The use of claim 1, wherein the dose comprises 1.0×10^5 to 5.0×10^6 of said CAR-T cells per kilogram of the mass of the subject.
3. The use of claim 2, wherein the dose comprises 5.0×10^5 to 1.0×10^6 of said CAR-T cells per kilogram of the mass of the subject.
4. The use of claim 3, wherein the dose comprises approximately 0.75×10^6 of said CAR-T cells per kilogram of the mass of the subject.
5. The use of claim 4, wherein the dose comprises less than 1.0×10^8 of said CAR-T cells per subject.
6. The use of claim 5, wherein said single intravenous infusion is administered using a single bag of said CAR-T cells.

7. The use of claim 6, wherein said administration of said single bag of said CAR-T cells is completed no later than three hours following the thawing of said single bag of CAR-T cells.

8. The use of claim 5, wherein said single intravenous infusion is administered using two bags of said CAR-T cells.

9. The use of claim 8, wherein said administration of each of said two bags of said CAR-T cells is completed no later than three hours following the thawing of said each of said two bags of CAR-T cells.

10. The use of any one of claims 1 to 9, wherein said use is effective in obtaining minimal residual disease (MRD) negative status in said subject assessed in the bone marrow after said infusion of said CAR-T cells.

11. The use of claim 10, wherein said MRD negative status is obtained at a first follow-up time of between approximately 28 days and approximately 179 days after said infusion of said CAR-T cells.

12. The use of any one of claims 1 to 9, wherein a lymphodepleting regimen precedes said infusion of CAR-T cells.

13. The use of claim 12, wherein said lymphodepleting regimen comprises:

- (a) administration of cyclophosphamide; or
- (b) administration of fludarabine.

14. The use of claim 12, wherein the lymphodepleting regimen is administered intravenously.

15. The use of claim 12, wherein said lymphodepleting regimen precedes said infusion of CAR-T cells by 5 to 7 days.

16. The use of claim 12, wherein said lymphodepleting regimen comprises intravenous administration of cyclophosphamide and fludarabine 5 to 7 days prior to said infusion of CAR-T cells.
17. The use of claim 13, wherein said cyclophosphamide is administered intravenously at 300 mg/m².
18. The use of claim 13, wherein said fludarabine is administered intravenously at 30 mg/m².
19. The use of any one of claims 1 to 9, further comprising treating said subject for cytokine release syndrome (CRS) more than 3 days following the infusion without significantly reducing CAR-T cell expansion *in vivo*.
20. The use of claim 19, wherein said treatment of CRS comprises administering to the subject an IL-6R inhibitor.
21. The use of claim 20, wherein said IL-6R inhibitor is an antibody.
22. The use of claim 21, wherein said antibody inhibits IL-6R by binding its extracellular domain.
23. The use of claim 20, wherein said IL-6R inhibitor prevents the binding of IL-6 to IL-6R.
24. The use of claim 20, wherein the IL-6R inhibitor is tocilizumab.
25. The use of any one of claims 1 to 9, wherein the subject is treated with pre-infusion medication comprising an antipyretic and an antihistamine up to 1 hour prior to the infusion comprising CAR-T cells.
26. The use of claim 25, wherein said antipyretic comprises either paracetamol or acetaminophen.

27. The use of claim 25, wherein said antipyretic is administered to the subject either orally or intravenously.
28. The use of claim 25, wherein said antipyretic is administered to the subject at a dosage of between 650 mg and 1000 mg.
29. The use of claim 25, wherein said antihistamine comprises diphenhydramine.
30. The use of claim 25, wherein said antihistamine is administered to the subject either orally or intravenously.
31. The use of claim 25, wherein said antihistamine is administered at a dosage of between 25 mg and 50 mg, or its equivalent.
32. The use of claims 1 to 9, wherein the infusion comprising CAR-T cells further comprises an excipient selected from dimethylsulfoxide or dextran-40.
33. The use of any one of claims 1 to 9, wherein the subject received prior treatment with at least three prior lines of treatment.
34. The use of claim 33, wherein said at least three prior lines of treatment comprises treatment with at least one medicament, said at least one medicament comprising of at least one of:
- (a) a PI;
 - (b) an IMiD; or
 - (c) an anti-CD38 antibody.
35. The use of claim 33, wherein the subject has relapsed after said at least three prior lines of treatment.

36. The use of claim 33, wherein the multiple myeloma is refractory to at least two medicaments following said at least three prior lines of treatment.

37. The use of claim 36, wherein said at least two medicaments to which the subject is refractory comprise a PI and an IMiD.

38. The use of claim 36, wherein the subject is refractory to at least three medicaments.

39. The use of claim 38, wherein the subject is refractory to at least four medicaments.

40. The use of claim 39, wherein the subject is refractory to at least five medicaments.

41. The use of any one of claims 1 to 9, wherein said use is effective in obtaining an overall response rate of greater than 91%.

42. The use of claim 41, wherein said use is effective in obtaining an overall response rate of greater than 93%.

43. The use of claim 42, wherein said use is effective in obtaining an overall response rate of greater than 95%.

44. The use of claim 43, wherein said use is effective in obtaining an overall response rate of greater than 97%.

45. The use of claim 44, wherein said use is effective in obtaining an overall response rate of greater than 99%.

46. The use of claim 40, wherein the overall response rate is assessed at a median follow-up time of at least 12 months following said infusion of said CAR-T cells.

47. The use of any one of claims 1 to 9, wherein said use is effective in obtaining a median time to first response of less than 1.15 months.

48. The use of claim 47, wherein said use is effective in obtaining a median time to first response of less than 1.10 months.

49. The use of claim 48, wherein said use is effective in obtaining a median time to first response of less than 1.05 months.

50. The use of claim 49, wherein said use is effective in obtaining a median time to first response of less than 1.00 months.

51. The use of claim 50, wherein said use is effective in obtaining a median time to first response of less than 0.95 months.

52. The use of any of claims 1 to 9, wherein said use is effective in obtaining a median time to best response of less than 2.96 months.

53. The use of claim 52, wherein said use is effective in obtaining a median time to best response of less than 2.86 months.

54. The use of claim 53, wherein said use is effective in obtaining a median time to best response of less than 2.76 months.

55. The use of claim 54, wherein said use is effective in obtaining a median time to best response of less than 2.66 months.

56. The use of claim 55, wherein said use is effective in obtaining a median time to best response of less than 2.56 months.

57. The use of any one of claims 1 to 9, wherein said use is effective in obtaining minimal residual disease (MRD) negative status in said subject assessed in the bone marrow at a follow-up time of approximately 28 days or greater following said infusion of said CAR-T cells.

58. The use of claim 57, wherein said use is effective in maintaining said MRD negative status in said subject assessed in the bone marrow at a follow-up time of approximately 12 months or greater following said infusion of said CAR-T cells.

59. The use of any one of claims 1 to 9, wherein the first BCMA binding moiety and/or the second BCMA binding moiety is an anti-BCMA VHH.

60. The use of claim 59, wherein the first BCMA binding moiety is a first anti-BCMA VHH and the second BCMA binding moiety is a second anti-BCMA VHH.

61. The use of any one of claims 1 to 9, wherein the first BCMA binding moiety comprises a polypeptide encoded by the nucleic acid sequence of SEQ ID NO:10.

62. The use of any one of claims 1 to 9, wherein the second BCMA binding moiety comprises a polypeptide encoded by the nucleic acid sequence of SEQ ID NO:12.

63. The use of any one of claims 1 to 9, wherein the first BCMA binding moiety and the second BCMA binding moiety are connected to each other via a peptide linker.

64. The use of claim 63, wherein the peptide linker comprises the amino acid sequence of SEQ ID NO:3.

65. The use of claim 64, wherein the peptide linker comprises a polypeptide encoded by the nucleic acid sequence of SEQ ID NO:11.

66. The use of any one of claims 1 to 9, wherein the CAR polypeptide further comprises a signal peptide located at the N-terminus of the polypeptide.

67. The use of claim 66, wherein the signal peptide is derived from CD8-alpha.
68. The use of claim 67, wherein the signal peptide comprises the amino acid sequence of SEQ ID NO:1.
69. The use of claim 68, wherein the signal peptide comprises a polypeptide encoded by the nucleic acid sequence of SEQ ID NO:9.
70. The use of any one of claims 1 to 9, wherein the transmembrane domain comprises the amino acid sequence of SEQ ID NO:6.
71. The use of any one of claims 1 to 9, wherein the transmembrane domain comprises a polypeptide encoded by the nucleic acid sequence of SEQ ID NO:14.
72. The use of any one of claims 1 to 9, wherein the intracellular signaling domain comprises a primary intracellular signaling domain of an immune effector cell.
73. The use of any one of claims 1 to 9, wherein the intracellular signaling domain is derived from CD3ζ.
74. The use of any one of claims 1 to 9, wherein the intracellular signaling domain comprises one or more co-stimulatory signaling domains.
75. The use of claim 74, wherein the intracellular signaling domain comprises the amino acid sequence of SEQ ID NO:8.
76. The use of claim 74, wherein the intracellular signaling domain comprises a polypeptide encoded by the nucleic acid sequence of SEQ ID NO:16.

77. The use of claim 74, wherein the intracellular signaling domain comprises the amino acid sequence of SEQ ID NO:7.

78. The use of claim 74, wherein the intracellular signaling domain comprises a polypeptide encoded by the nucleic acid sequence of SEQ ID NO:15.

79. The use of any one of claims 1 to 9, wherein the CAR polypeptide further comprises a hinge domain located between the C-terminus of the extracellular antigen binding domain and the N-terminus of the transmembrane domain.

80. The use of claim 79, wherein the hinge domain comprises the amino acid sequence of SEQ ID NO:5.

81. The use of claim 79, wherein the hinge domain comprises a polypeptide encoded by the nucleic acid sequence of SEQ ID NO:13.

82. The use of any one of claims 1 to 9, wherein the T cells are autologous T cells.

83. The use of any one of claims 1 to 9, wherein the T cells are allogeneic T cells.

84. The use of any one of claims 1 to 9, wherein the subject is human.

85. Use of a composition comprising T cells for the manufacture of a medicament for treating multiple myeloma in a subject who has received at least three prior lines of treatment,

wherein the T cells comprise a chimeric antigen receptor (CAR) comprising the amino acid sequence of SEQ ID NO:17,

wherein the composition is administered to the subject via a single intravenous infusion to deliver to the subject a dose of approximately 0.75×10^6 CAR expressing T cells (CAR-T cells) per kilogram of the mass of the subject, and is effective in obtaining minimal residual disease (MRD) negative status in said subject assessed in the bone marrow at a follow-up time of greater than or equal to 28 days following said infusion of said CAR-T cells.

86. The use of claim 10, wherein said method is effective in obtaining said MRD negative status at:

- (i) a rate of between approximately 44% and approximately 65% at a sensitivity threshold level of 10^{-5} at a follow-up time of approximately 12 months after said infusion of said CAR-T cells;
- (ii) a rate of between approximately 57% and approximately 76% at a sensitivity threshold level of 10^{-4} at a follow-up time of approximately 18 months after said infusion of said CAR-T cells;
- (iii) a rate of between approximately 47% and approximately 68% at a sensitivity threshold level of 10^{-5} at a follow-up time of approximately 18 months after said infusion of said CAR-T cells; or
- (iv) a rate of between approximately 29% and approximately 50% at a sensitivity threshold level of 10^{-6} at a follow-up time of approximately 18 months after said infusion of said CAR-T cells.

87. The use of claim 10, wherein said use is effective in obtaining said MRD negative status at:

- (i) a rate of approximately 55% at a sensitivity threshold level of 10^{-5} at a follow-up time of approximately 12 months after said administration of said CAR-T cells;
- (ii) a rate of approximately 67% at a sensitivity threshold level of 10^{-4} at a follow-up time of approximately 18 months after said infusion of said CAR-T cells;
- (iii) a rate of approximately 58% at a sensitivity threshold level of 10^{-5} at a follow-up time of approximately 18 months after said infusion of said CAR-T cells; or
- (iv) a rate of approximately 39% at a sensitivity threshold level of 10^{-6} at a follow-up time of approximately 18 months after said infusion of said CAR-T cells.

88. The use of claim 10, wherein said use is effective in obtaining said minimal residual disease (MRD) negative status at a rate of between approximately 83% and approximately 98% in subjects with evaluable samples at a sensitivity threshold level of 10^{-5} at a follow-up time of approximately 12 months after said infusion of said CAR-T cells, or at a rate of between approximately 82% and approximately 97% in subjects with evaluable samples at a sensitivity threshold level of 10^{-5} at a follow-up time of approximately 18 months after said infusion of said CAR-T cells.

89. The use of claim 10, wherein said use is effective in obtaining said minimal residual disease (MRD) negative status at a rate of approximately 93% in subjects with evaluable samples at a sensitivity threshold level of 10^{-5} at a follow-up time of approximately 12 months after said infusion of said CAR-T cells, or at a rate of approximately 92% in subjects with evaluable samples at a sensitivity threshold level of 10^{-5} at a follow-up time of approximately 18 months after said infusion of said CAR-T cells.

90. The use of claim 1, wherein said use is effective in obtaining at least one response in the subject after said infusion of said CAR-T cells, wherein said at least one response comprises, in order from better to worse:

- (i) a stringent complete response;
- (ii) a complete response;
- (iii) a very good partial response;
- (iv) a partial response; or
- (v) a minimal response.

91. The use of claim 90, wherein said use is effective in obtaining a first response before a time of between approximately 27 days and approximately 321 days after said infusion of said CAR-T cells.

92. The use of claim 91, wherein said use is effective in obtaining a first response before a time of between approximately 27 days and approximately 89 days after said infusion of said CAR-T cells.

93. The use of claim 91, wherein said use is effective in obtaining a first response before approximately 42 days after said infusion of said CAR-T cells.

94. The use of claim 91, wherein said use is effective in obtaining a first response before approximately 29 days after said infusion of said CAR-T cells.

95. The use of claim 90, wherein said use is effective in obtaining a best response of any one of minimal response, partial response, very good partial response, complete response or stringent complete response.

96. The use of claim 95, wherein said use is effective in obtaining said best response of any one of minimal response, partial response, very good partial response, complete response or stringent complete response at a rate of between approximately 91% and approximately 99% at a follow-up time of approximately 12 months after said infusion of said CAR-T cells or at a rate of between approximately 93% and approximately 100% at a follow-up time of approximately 18 months after said infusion of said CAR-T cells.

97. The use of claim 95, wherein said use is effective in obtaining said best response of any one of minimal response, partial response, very good partial response, complete response or stringent complete response at a rate of approximately 97% at a follow-up time of approximately 12 months after said infusion of said CAR-T cells or at a rate of approximately 98% at a follow-up time of approximately 18 months after said infusion of said CAR-T cells.

98. The use of claim 90, wherein said use is effective in obtaining a best response of any one of partial response, very good partial response, complete response or stringent complete response.

99. The use of claim 98, wherein said use is effective in obtaining said best response of any one of partial response, very good partial response, complete response or stringent complete response at a rate of between approximately 91% and approximately 99% at a follow-up time of approximately 12 months after said infusion of said CAR-T cells or at a rate of between approximately 93% and approximately 100% at a follow-up time of approximately 18 months after said infusion of said CAR-T cells.

100. The use of claim 98, wherein said use is effective in obtaining said best response of any one of partial response, very good partial response, complete response or stringent complete response at a rate of approximately 97% at a follow-up time of approximately 12 months after said infusion of said CAR-T cells or at a rate of approximately 97% at a follow-up time of approximately 18 months after said infusion of said CAR-T cells.

101. The use of claim 90, wherein said use is effective in obtaining a best response of any one of very good partial response, complete response or stringent complete response.

102. The use of claim 101, wherein said use is effective in obtaining said best response of any one of very good partial response, complete response or stringent complete response at a rate of between approximately 86% and approximately 97% at a follow-up time of approximately 12 months after said infusion of said CAR-T cells or at a rate of between approximately 88% and approximately 98% at a follow-up time of approximately 18 months after said infusion of said CAR-T cells.

103. The use of claim 101, wherein said use is effective in obtaining said best response of any one of very good partial response, complete response or stringent complete response at a rate of approximately 93% at a follow-up time of approximately 12 months after said infusion of said CAR-T cells or at a rate of approximately 95% at a follow-up time of approximately 18 months after said infusion of said CAR-T cells.

104. The use of claim 90, wherein said use is effective in obtaining a best response of complete response or stringent complete response.

105. The use of claim 104, wherein said use is effective in obtaining said best response of complete response or stringent complete response at a rate of between approximately 57% and approximately 76% at a follow-up time of approximately 12 months after said infusion of said CAR-T cells or at a rate of between approximately 73% and approximately 89% at a follow-up time of approximately 18 months after said infusion of said CAR-T cells.

106. The use of claim 104, wherein said use is effective in obtaining said best response of complete response or stringent complete response at a rate of approximately 67% at a follow-up time of approximately 12 months after said infusion of said CAR-T cells or at a rate of approximately 83% at a follow-up time of approximately 18 months after said infusion of said CAR-T cells.

107. The use of claim 90, wherein said use is effective in obtaining a best response of stringent complete response.

108. The use of claim 107, wherein said use is effective in obtaining said best response of stringent complete response at a rate of between approximately 57% and approximately 76% at a follow-up time of approximately 12 months after said infusion of said CAR-T cells or at a rate of between approximately 73% and approximately 89% at a follow-up time of approximately 18 months after said infusion of said CAR-T cells.

109. The use of claim 107, wherein said use is effective in obtaining said best response of stringent complete response at a rate of approximately 67% at a follow-up time of approximately 12 months after said infusion of said CAR-T cells or at a rate of approximately 83% at a follow-up time of approximately 18 months after said infusion of said CAR-T cells.

110. The use of claim 1, wherein said use is effective in obtaining progression-free survival of the subject.

111. The use of claim 110, wherein said use is effective in obtaining said progression-free survival of the subject at a time between said infusion of said CAR-T cells and:

- (i) approximately 209 days after said infusion of said CAR-T cells;
- (ii) approximately 386 days after said infusion of said CAR-T cells;
- (iii) approximately 632 days after said infusion of said CAR-T cells; or
- (iv) approximately 684 days after said infusion of said CAR-T cells.

112. The use of claim 110, wherein said use is effective in obtaining said progression-free survival at:

- (i) a rate of between approximately 79% and approximately 93% at a follow-up time of approximately 6 months after said infusion of said CAR-T cells;
- (ii) a rate of between approximately 67% and approximately 84% at a follow-up time of approximately 12 months after said infusion of said CAR-T cells;
- (iii) a rate of between approximately 57% and approximately 75% at a follow-up time of approximately 18 months after said infusion of said CAR-T cells;
- (iv) a rate of between approximately 57% and approximately 75% at a follow-up time of approximately 21 months after said infusion of said CAR-T cells; or
- (v) a rate of between approximately 49% and approximately 70% at a follow-up time of approximately 24 months after said infusion of said CAR-T cells.

113. The use of claim 110, wherein said use is effective in obtaining said progression-free survival at:

- (i) a rate of approximately 88% at a follow-up time of approximately 6 months after said infusion of said CAR-T cells;
- (ii) a rate of approximately 76% at a follow-up time of approximately 12 months after said infusion of said CAR-T cells;
- (iii) a rate of approximately 67% at a follow-up time of approximately 18 months after said infusion of said CAR-T cells;
- (iv) a rate of approximately 67% at a follow-up time of approximately 21 months after said infusion of said CAR-T cells; or

- (v) a rate of approximately 61% at a follow-up time of approximately 24 months after said infusion of said CAR-T cells.

114. The use of claim 1, wherein said use further comprises treating said subject for cytokine release syndrome more than approximately 1 day after said infusion of said CAR-T cells.

115. The use of claim 114, wherein said use is effective in obtaining a rate of recovery from said cytokine release syndrome of between approximately 1% and approximately 99% at a time of approximately 1, 3, 4, 6, 16 or 97 days after first observance of said cytokine release syndrome.

116. The use of claim 1, wherein said use further comprises treating said subject for immune effector cell-associated neurotoxicity more than approximately 3 days after said infusion of said CAR-T cells.

117. The use of claim 116, wherein said use is effective in obtaining a rate of recovery from said immune effector cell-associated neurotoxicity of between approximately 1% and approximately 17% at a time of approximately 1, 4, 5, 8, 12 or 16 days after first observance of said immune effector cell-associated neurotoxicity.

118. The use of any one of claims 95 to 109, wherein said use is effective in obtaining said best response before a time of between approximately 27 days and approximately 534 days after said infusion of said CAR-T cells.

119. The use of any one of claims 95 to 109, wherein said use is effective in obtaining said best response before a time of between approximately 27 days and approximately 293 days after said infusion of said CAR-T cells.

120. The use of any one of claims 95 to 109, wherein said use is effective in obtaining said best response before approximately 153 days after said infusion of said CAR-T cells.

121. The use of any one of claims 95 to 109, wherein said use is effective in obtaining said best response before approximately 78 days after said infusion of said CAR-T cells.

122. The use of any one of claims 90 to 109, wherein said use is effective in maintaining a response in the subject at a follow-up time between the time of said first response and:

- (i) approximately 180 days after said infusion of said CAR-T cells;
- (ii) approximately 357 days after said infusion of said CAR-T cells;
- (iii) approximately 606 days after said infusion of said CAR-T cells; or
- (iv) approximately 654 days after said infusion of said CAR-T cells.

123. The use of any one of claims 90 to 109, wherein said use is effective in maintaining a response at:

- (i) a rate of between approximately 77% and approximately 91% at a follow-up time of approximately 6 months after said infusion of said CAR-T cells;
- (ii) a rate of between approximately 63% and approximately 81% at a follow-up time of approximately 12 months after said infusion of said CAR-T cells;
- (iii) a rate of between approximately 56% and approximately 75% at a follow-up time of approximately 18 months after said infusion of said CAR-T cells;
- (iv) a rate of between approximately 52% and approximately 72% at a follow-up time of approximately 21 months after said infusion of said CAR-T cells; or
- (v) a rate of between approximately 48% and approximately 70% at a follow-up time of approximately 24 months after said infusion of said CAR-T cells.

124. The use of any one of claims 90 to 109, wherein said use is effective in maintaining a response at:

- (i) a rate of approximately 85% at a follow-up time of approximately 6 months after said infusion of said CAR-T cells;
- (ii) a rate of approximately 74% at a follow-up time of approximately 12 months after said infusion of said CAR-T cells;
- (iii) a rate of approximately 67% at a follow-up time of approximately 18 months after said infusion of said CAR-T cells;

- (iv) a rate of approximately 63% at a follow-up time of approximately 21 months after said infusion of said CAR-T cells; or
- (v) a rate of approximately 60% at a follow-up time of approximately 24 months after said infusion of said CAR-T cells.

125. The use of any one of claims 104 to 109, further wherein said use is effective in obtaining minimal residual disease (MRD) negative status in said subject assessed in the bone marrow at a sensitivity threshold level of 10^{-5} between the time of said administration of said CAR-T cells and approximately 3 months after said administration of said CAR-T cells.

126. The use of claim 125, wherein said use is effective in obtaining either MRD negative complete response or MRD negative stringent complete response at a rate of between approximately 25% and approximately 44% at a follow-up time of approximately 12 months after said infusion of said CAR-T cells or at a rate of between approximately 33% and approximately 54% at a follow-up time of approximately 18 months after said infusion of said CAR-T cells.

127. The use of claim 125, wherein said use is effective in obtaining either minimal residual disease (MRD) negative complete response or minimal residual disease (MRD) negative stringent complete response at a rate of approximately 34% at a follow-up time of approximately 12 months after said infusion of said CAR-T cells or at a rate of approximately 43% at a follow-up time of approximately 18 months after said infusion of said CAR-T cells.