

CLAIMS

WHAT IS CLAIMED:

1. A method of treating indolent follicular lymphoma (FL) Grade 1, 2 or 3A, the method comprising administering to a subject having or suspected of having a disease that is relapsed/refractory (r/r) follicular lymphoma (FL) Grade 1, 2 or 3A a dose of CD4⁺ and CD8⁺ T cells, wherein T cells of the dose comprises a chimeric antigen receptor (CAR) that specifically binds to CD19, wherein:

the subject has relapsed or is refractory to treatment after at least one prior line of therapy for treating FL Grade 1, 2 or 3A, wherein at least one of the at least one prior lines of therapy includes treatment with an anti-CD20 antibody and an alkylating agent;

the dose of T cells comprises between at or about 5×10^7 CAR-expressing T cells and at or about 1.5×10^8 CAR-expressing T cells, inclusive;

the dose of T cells comprises a ratio of approximately 1:1 CD4⁺ T cells expressing the CAR to CD8⁺ T cells expressing the CAR; and

the administration comprises administering a plurality of separate compositions, wherein the plurality of separate compositions comprises a first composition comprising the CD8⁺ CAR-expressing T cells and a second composition comprising the CD4⁺ CAR-expressing T cells.

2. The method of claim 1, wherein the at least one prior line of therapy is one prior line of therapy that includes an anti-CD20 antibody and an alkylating agent.

3. The method of claim 2, wherein the subject has relapsed or is refractory to treatment after one prior line of therapy for treating FL Grade 1, 2 or 3A, and had progression of the disease within 24 months of initiation of the one prior line of therapy that includes an anti-CD20 antibody and an alkylating agent (POD24).

4. The method of claim 2 or claim 3, wherein the subject has relapsed or is refractory to treatment after one prior line of therapy for treating FL Grade 1, 2 or 3A, and had progression of the disease within 24 months of diagnosis after completing the one prior line of therapy that includes an anti-CD20 antibody and an alkylating agent(POD24).

5. The method of any of claims 1-4, wherein the at least one prior line of therapy that includes an anti-CD20 antibody and an alkylating agent is a chemoimmunotherapeutic combination therapy that includes rituximab, cyclophosphamide, vincristine, doxorubicin, and prednisolone (R-CHOP).

6. The method of any of claims 2-5, wherein the subject received the one prior line of therapy within six months of the original FL diagnosis.

7. The method of any of claims 1-6, wherein the subject has relapsed or is refractory to treatment after one prior line of therapy for treating FL Grade 1, 2 or 3A, and has at least one of the following: symptoms attributable to FL; threatened end-organ function, cytopenia secondary to lymphoma, or bulky disease; splenomegaly; and steady progression of disease over the preceding six months or more.

8. The method of any of claims 1-7, wherein the at least one prior line of therapy is two prior lines of therapy.

9. The method of claim 8, wherein the other of the two prior lines of therapy is selected from treatment with rituximab; obinutuzumab; bendamustine plus rituximab (BR); bendamustine plus obinutuzumab (BO); R-CHOP; rituximab, cyclophosphamide, vincristine, and prednisone (R-CVP); HSCT (optionally autologous or allogeneic HSCT); lenalidomide in combination with rituximab; or a PI3K inhibitor (PI3Ki), optionally wherein the PI3Ki is idelalisib, copanlisib, or duvelisib.

10. The method of any of claims 1-9, wherein the subject is refractory to treatment or has relapsed within 12 months of completion of a prior line of therapy, optionally wherein a prior line of therapy is a combination therapy or a monotherapy comprising a PI3Ki.
11. The method of any of claims 1-10, wherein the subject has relapsed after HSCT.
12. The method of any of claims 1-11, wherein the at least one prior line of therapy is three prior lines of therapy.
13. The method of claim 12, wherein the other two of the three prior lines of therapy are each independently selected from treatment with rituximab; obinutuzumab; bendamustine plus rituximab (BR); bendamustine plus obinutuzumab (BO); R-CHOP; rituximab, cyclophosphamide, vincristine, and prednisone (R-CVP); HSCT (optionally autologous or allogeneic HSCT); lenalidomide in combination with rituximab; or a PI3K inhibitor (PI3Ki), optionally wherein the PI3Ki is idelalisib, copanlisib, or duvelisib.
14. The method of any of claims 1-13, wherein the subject is refractory to treatment with an anti-CD20 antibody.
15. The method of any of claims 1-14, wherein the subject is refractory to a prior line of therapy that includes an anti-CD20 antibody and an alkylating agent.
16. The method of any of claims 1-15, wherein the subject relapsed within six months of completing treatment with an anti-CD20 antibody.
17. The method of any of claims 1-16, wherein the subject relapsed within six months of completing a prior line of therapy that includes an anti-CD20 antibody and an alkylating agent.

18. The method of any of claims 1-17, wherein the subject has relapsed during an anti-CD20 antibody maintenance therapy following two or more lines of therapy or within six months after completion of the anti-CD20 antibody maintenance therapy.

19. The method of any of claims 1-18, wherein the subject has at least one PET-positive lesion and at least one measurable nodal lesion or extranodal lesion, optionally wherein the a measurable nodal lesion is greater than 1.5 cm in the long axis regardless of the short axis and a measurable extranodal lesion is greater than 1.0 cm in the long and short axis.

20. A method of treating marginal zone lymphoma (MZL), the method comprising administering to a subject having or suspected of having relapsed/refractory (r/r) marginal zone lymphoma (MZL) a dose of CD4⁺ and CD8⁺ T cells, wherein T cells of the dose comprises a chimeric antigen receptor (CAR) that specifically binds to CD19, wherein:

the subject has relapsed or is refractory to treatment after at least two prior lines of therapy for treating MZL;

the dose of T cells comprises between at or about 5×10^7 CAR-expressing T cells and at or about 1.5×10^8 CAR-expressing T cells, inclusive;

the dose of T cells comprises a ratio of approximately 1:1 CD4⁺ T cells expressing the CAR to CD8⁺ T cells expressing the CAR; and

the administration comprises administering a plurality of separate compositions, wherein the plurality of separate compositions comprises a first composition comprising the CD8⁺ CAR-expressing T cells and a second composition comprising the CD4⁺ CAR-expressing T cells.

21. The method of claim 20, wherein the MZL is a subtype selected from among extra-nodal MZL (ENMZL, mostly gastric), splenic MZL (SMZL), and nodal MZL (NMZL).

22. The method of claim 20 or claim 21, wherein at least one of the at least two prior lines of therapy is a combination systemic therapy for treating the MZL or is a hematopoietic stem cell transplant (HSCT).

23. The method of any of claims 20-22, wherein at least one of the at least two prior lines of therapy is a combination systemic therapy for treating the MZL, and the combination systemic therapy is selected from rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP), or a therapy with an anti-CD20 antibody and an alkylating agent.

24. The method of any of claims 20-23, wherein at least one of the at least two prior lines of therapy is a combination systemic therapy that is an anti-CD20 antibody and an alkylating agent.

25. The method of any of claims 20-24, wherein the subject has Splenic MZL (SMZL) and at least one of the at least two prior lines of therapy is a splenectomy.

26. The method of any of claims 20-24, wherein the subject has extranodal MZL (ENMZL), and an antibiotic is not one of the at least two prior lines of therapy.

27. The method of any of claims 20-26, wherein the subject has PET non-avid disease with at least one measurable nodal lesion greater than 2.0 cm in the long axis or at least one measurable extranodal lesion.

28. The method of any of claims 1-27, wherein the subject does not have FL grade 3B (FL3B).

29. The method of any of claims 1-28, wherein the subject does not have evidence of composite DLBCL and FL, or of transformed FL (tFL).

30. The method of any of claims 1-29, wherein the subject does not have World Health Organization (WHO) subclassification of duodenal-type FL.

31. The method of any of claims 1-30, wherein the subject has relapsed to at least one prior line of therapy, and the relapse is after an initial response of complete response (CR) or partial response (PR) to the at least one prior line of therapy.

32. The method of any of claims 1-31, wherein the subject is refractory to at least one prior line of therapy, and the refractory treatment is a best response of stable disease (SD) or progressive disease (PD) to the at least one prior line of therapy.

33. The method of any of claims 1-32, wherein the subject has an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1.

34. The method of any of claims 1-33, wherein the anti-CD20 antibody is a monoclonal anti-CD20 antibody.

35. The method of any of claims 1-34, wherein the anti-CD20 antibody is rituximab or obinutuzumab.

36. The method of any of claims 1-35, wherein the anti-CD20 antibody is rituximab.

37. The method of any of claims 1-36, wherein the alkylating agent is bendamustine or chlorambucil.

38. The method of any of claims 1-37, further comprising administering a lymphodepleting therapy to the subject prior to administration of the dose of CD4+ and CD8+ T cells.

39. The method of claim 38, wherein the lymphodepleting therapy is completed within about 7 days prior to initiation of the administration of the dose of CD4+ and CD8+ T cells.

40. The method of claim 38 or claim 39, wherein the administration of the lymphodepleting therapy is completed within about 2 to 7 days prior to initiation of the administration of the dose of CD4+ and CD8+ T cells.

41. The method of any of claims 38-40, wherein the lymphodepleting therapy comprises the administration of fludarabine and/or cyclophosphamide.

42. The method of any of claims 38-41, wherein the lymphodepleting therapy comprises administration of cyclophosphamide at or about 200-400 mg/m², optionally at or about 300 mg/m², inclusive, and/or fludarabine at or about 20-40 mg/m², optionally 30 mg/m², daily for 2-4 days, optionally for 3 days.

43. The method of any one of claims 38-42, wherein the lymphodepleting therapy comprises administration of cyclophosphamide at or about 300 mg/m² and fludarabine at or about 30 mg/m² daily concurrently for 3 days.

44. The method of any of claims 1-43, wherein CD19 is human CD19.

45. The method of any of claims 1-44, wherein the chimeric antigen receptor (CAR) comprises an scFv comprising the variable heavy chain region and the variable light chain region of the antibody FMC63, a spacer that is 15 amino acids or less and contains an immunoglobulin hinge

region or a modified version thereof, a transmembrane domain, and an intracellular signaling domain comprising a signaling domain of a CD3-zeta (CD3 ζ) chain and a costimulatory signaling region that is a signaling domain of 4-1BB.

46. The method of claim 45, wherein the immunoglobulin hinge region or a modified version thereof comprises the formula X_1PPX_2P , wherein X_1 is glycine, cysteine or arginine and X_2 is cysteine or threonine (SEQ ID NO:58).

47. The method of claim 45 or claim 46, wherein the immunoglobulin hinge region or a modified version thereof is an IgG1 hinge or a modified version thereof.

48. The method of claim 45 or claim 46, wherein the immunoglobulin hinge region or a modified version thereof is an IgG4 hinge or a modified version thereof.

49. The method of any of claims 45-48, wherein the spacer comprises the sequence set forth in SEQ ID NO: 1, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, or SEQ ID NO: 34, or a variant of any of the foregoing having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to the sequence set forth in SEQ ID NO: 1, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, or SEQ ID NO: 34.

50. The method of any of claims 45-49, wherein the spacer consists of the sequence set forth in SEQ ID NO: 1, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, or SEQ ID NO: 34, or a variant of any of the foregoing having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to the sequence set forth in SEQ ID NO: 1, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, or SEQ ID NO: 34.

51. The method of any of claims 45-50, wherein the spacer is at or about 12 amino acids in length.

52. The method of any of claims 45-51, wherein the spacer comprises the sequence set forth in SEQ ID NO: 1.

53. The method of any of claims 45-52, wherein the spacer consists of the sequence set forth in SEQ ID NO: 1.

54. The method of any of claims 45-53, wherein the transmembrane domain is a transmembrane domain of CD28.

55. The method of any of claims 45-54, wherein the transmembrane domain comprises the sequence of amino acids set forth in SEQ ID NO: 8 or a sequence of amino acids that exhibits at least or at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO: 8.

56. The method of any of claims 45-55, wherein the costimulatory domain comprises the sequence set forth in SEQ ID NO: 12 or is a variant thereof having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to the sequence set forth in SEQ ID NO: 12.

57. The method of any of claims 45-56, wherein the signaling domain of a CD3-zeta (CD3ζ) chain comprises the sequence set forth in SEQ ID NO: 13, 14, or 15, or is a variant thereof having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to the sequence set forth in SEQ ID NO: 13, 14, or 15.

58. The method of any of claims 45-57, wherein the the scFv comprises a CDRL1 sequence of SEQ ID NO: 35, a CDRL2 sequence of SEQ ID NO: 55, and a CDRL3 sequence of SEQ ID NO: 56; and a CDRH1 sequence of SEQ ID NO: 38, a CDRH2 sequence of SEQ ID NO: 39, and a CDRH3 sequence of SEQ ID NO: 54.

59. The method of any of claims 45-57, wherein the the scFv comprises a CDRL1 sequence of RASQDISKYLN (SEQ ID NO: 35), a CDRL2 sequence of SRLHSGV (SEQ ID NO: 36), and a CDRL3 sequence of GNTLPYTFG (SEQ ID NO: 37); and a CDRH1 sequence of DYGVVS (SEQ ID NO: 38), a CDRH2 sequence of VIWGSETTYNSALKS (SEQ ID NO: 39), and a CDRH3 sequence of YAMDYWG (SEQ ID NO: 40).

60. The method of any of claims 45-59, wherein the scFv comprises, in order from N-terminus to C-terminus, a V_L comprising the sequence set forth in SEQ ID NO: 42, and a V_H , comprising the sequence set forth in SEQ ID NO: 41.

61. The method of any of claims 45-60, wherein the scFv comprises the sequence set forth in SEQ ID NO: 43.

62. The method of any of claims 1-61, wherein the CAR contains in order from N-terminus to C-terminus: an extracellular antigen-binding domain that is the scFv set forth in SEQ ID NO: 43, the spacer set forth in SEQ ID NO: 1, the transmembrane domain set forth in SEQ ID NO: 8, the 4-1BB costimulatory signaling domain set forth in SEQ ID NO: 12, and the signaling domain of a CD3-zeta ($CD3\zeta$) chain set forth in SEQ ID NO: 13.

63. The method of any of claims 1-62, wherein the dose of CD4+ and CD8+ T cells is between about 5×10^7 CAR+ T cells and about 1.1×10^8 CAR+ T cells, inclusive of each.

64. The method of any of claims 1-63, wherein the dose of CD4+ and CD8+ T cells is 5×10^7 CAR+ T cells.

65. The method of any of claims 1-63, wherein the dose of CD4+ and CD8+ T cells is 1×10^8 CAR+ T cells.

66. The method of any of claims 1-65, wherein:
the first composition and the second composition are administered 0 to 12 hours apart, 0 to 6 hours apart, or 0 to 2 hours apart, or wherein the administration of the first composition and the administration of the second composition are carried out on the same day, between about 0 and about 12 hours apart, between about 0 and about 6 hours apart, or between about 0 and 2 hours apart; and/or

the initiation of administration of the first composition and the initiation of administration of the second composition are carried out between about 1 minute and about 1 hour apart or between about 5 minutes and about 30 minutes apart.

67. The method of any of claims 1-66, wherein the first composition and the second composition are administered no more than 2 hours, no more than 1 hour, no more than 30 minutes, no more than 15 minutes, no more than 10 minutes, or no more than 5 minutes apart.

68. The method of any of claims 1-67, wherein the first composition and the second composition are administered less than 2 hours apart.

69. The method of any of claims 1-68, wherein the first composition comprising the CD8+ CAR-expressing T cells is administered prior to the second composition comprising the CD4+ CAR-expressing T cells.

70. The method of any of claims 1-69, wherein the dose of CD4+ T cells and CD8+ T cells is administered intravenously.

71. The method of any of claims 1-70, wherein the T cells are primary T cells obtained from a sample from the subject, optionally wherein the sample is a whole blood sample, an apheresis sample, or a leukapheresis sample.

72. The method of claim 71, wherein the sample is obtained from the subject prior to administration of the lymphodepleting therapy to the subject.

73. The method of any of claims 1-72, wherein the T cells are autologous to the subject.

74. The method of any of claims 1-73, wherein the subject is human.

75. The method of any of claims 1-74, wherein the complete response rate (CRR) among a plurality of subjects treated according to the method is greater than at or about 50%, greater than at or about 60%, greater than at or about 70%, or greater than at or about 80%.

76. The method of claim 75, wherein the CRR is the percentage of subjects with a best overall response (BOR) up to 24 months of complete response (CR).

77. The method of claim 75 or claim 76, wherein the subject has FL Grade 1, 2, or 3A, and the CRR is assessed by PET-CT.

78. The method of claim 75 or claim 76, wherein the subject has MZL, and the CRR is assessed by CT.

79. Use of a dose of CD4⁺ and CD8⁺ T cells to treat a subject having or suspected of having a disease that is relapsed/refractory (r/r) follicular lymphoma (FL) Grade 1, 2 or 3A, wherein:

T cells of the dose comprise a chimeric antigen receptor (CAR) that specifically binds to CD19;

the subject has relapsed or is refractory to treatment after at least one prior line of therapy for treating FL Grade 1, 2 or 3A, wherein the at least one of the at least one prior line of therapy includes treatment with an anti-CD20 antibody and an alkylating agent;

the dose of T cells comprises between at or about 5×10^7 CAR-expressing T cells and at or about 1.5×10^8 CAR-expressing T cells, inclusive;

the dose of T cells comprises a ratio of approximately 1:1 CD4⁺ T cells expressing the CAR to CD8⁺ T cells expressing the CAR; and

the dose is formulated for administration as a plurality of separate compositions, wherein the plurality of separate compositions comprises a first composition comprising the CD8⁺ CAR-expressing T cells and a second composition comprising the CD4⁺ CAR-expressing T cells.

80. Use of a dose of CD4⁺ and CD8⁺ T cells in the manufacture of a medicament for the treatment of a subject having or suspected of having a disease that is relapsed/refractory (r/r) follicular lymphoma (FL) Grade 1, 2 or 3A, wherein:

T cells of the dose comprise a chimeric antigen receptor (CAR) that specifically binds to CD19;

the subject has relapsed or is refractory to treatment after at least one prior line of therapy for treating FL Grade 1, 2 or 3A, wherein at least one of the at least one prior line of therapy includes treatment with an anti-CD20 antibody and an alkylating agent;

the dose of T cells comprises between at or about 5×10^7 CAR-expressing T cells and at or about 1.5×10^8 CAR-expressing T cells, inclusive;

the dose of T cells comprises a ratio of approximately 1:1 CD4⁺ T cells expressing the CAR to CD8⁺ T cells expressing the CAR; and

the dose is formulated for administration as a plurality of separate compositions, wherein the plurality of separate compositions comprises a first composition comprising the CD8⁺ CAR-expressing T cells and a second composition comprising the CD4⁺ CAR-expressing T cells.

81. Use of a dose of CD4⁺ and CD8⁺ T cells to treat a subject having or suspected of having relapsed/refractory (r/r) marginal zone lymphoma (MZL), wherein:

T cells of the dose comprises a chimeric antigen receptor (CAR) that specifically binds to CD19;

the subject has relapsed or is refractory to treatment after at least two prior lines of therapy for treating MZL;

the dose of T cells comprises between at or about 5×10^7 CAR-expressing T cells and at or about 1.5×10^8 CAR-expressing T cells, inclusive;

the dose of T cells comprises a ratio of approximately 1:1 CD4⁺ T cells expressing the CAR to CD8⁺ T cells expressing the CAR; and

the dose is formulated for administration of a plurality of separate compositions, wherein the plurality of separate compositions comprises a first composition comprising the CD8⁺ CAR-expressing T cells and a second composition comprising the CD4⁺ CAR-expressing T cells.

82. Use of a dose of CD4⁺ and CD8⁺ T cells in the manufacture of a medicament for treatment of a subject having or suspected of having relapsed/refractory (r/r) marginal zone lymphoma (MZL), wherein:

T cells of the dose comprises a chimeric antigen receptor (CAR) that specifically binds to CD19;

the subject has relapsed or is refractory to treatment after at least two prior lines of therapy for treating MZL;

the dose of T cells comprises between at or about 5×10^7 CAR-expressing T cells and at or about 1.5×10^8 CAR-expressing T cells, inclusive;

the dose of T cells comprises a ratio of approximately 1:1 CD4⁺ T cells expressing the CAR to CD8⁺ T cells expressing the CAR; and

the dose is formulated for administration of a plurality of separate compositions, wherein the plurality of separate compositions comprises a first composition comprising the CD8⁺ CAR-expressing T cells and a second composition comprising the CD4⁺ CAR-expressing T cells.