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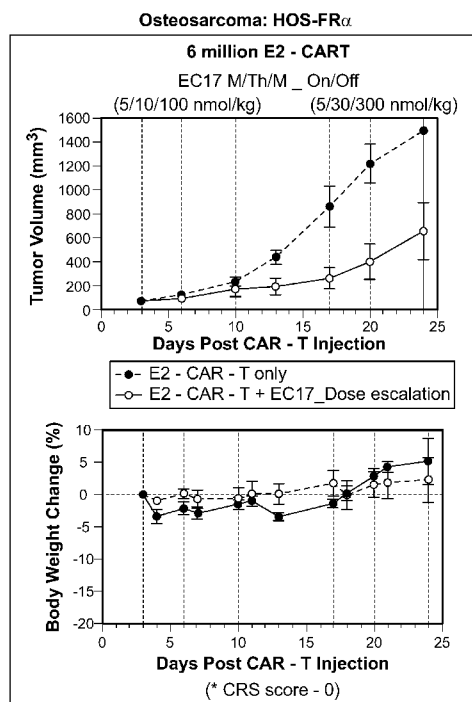
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(54) Title: SEQUENCING METHOD FOR CAR T CELL THERAPY

FIG. 4



(57) Abstract: The present disclosure relates to methods of treating a patient with a cancer by administering to the patient a composition comprising CAR T cells and administering to the patient a small molecule linked to a targeting moiety by a linker. The disclosure also relates to compositions for use in such methods.

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SEQUENCING METHOD FOR CAR T CELL THERAPY

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Patent Application No. 62/634,573 filed on February 23, 2018, U.S. Provisional Patent Application No. 62/656,265 filed on April 11, 2018, U.S. Provisional Patent Application No. 62/724,345 filed on August 29, 2018, and U.S. Provisional Patent Application No. 62/736,730 filed on September 26, 2018, the disclosures of all of which are hereby expressly incorporated by reference in their entirety.

TECHNICAL FIELD

The present disclosure relates to methods of treating a patient with a cancer by administering to the patient a composition comprising CAR T cells and administering to the patient a small molecule linked to a targeting moiety by a linker. The disclosure also relates to compositions for use in such methods.

BACKGROUND

Immunotherapy based on adoptive transfer of lymphocytes (e.g., T cells) into a patient is a valuable therapy in the treatment of cancer and other diseases. Important advancements have been made in the development of immunotherapies based on adoptive transfer of lymphocytes. Among the many different types of immunotherapeutic agents, one of the most promising of the immunotherapeutic agents being developed is T cells expressing chimeric antigen receptors (CAR T cells). The chimeric antigen receptor (CAR) is a genetically engineered receptor that is designed to target a specific antigen, for example, a tumor antigen. This targeting can result in cytotoxicity against the tumor, for example, such that CAR T cells expressing CARs can target and kill tumors via the specific tumor antigens.

First generation CARs are composed of a recognition region, e.g., a single chain fragment variable (scFv) region derived from an antibody for recognition and binding to the antigen expressed by the tumor, and an activation signaling domain, e.g., the CD3 ζ chain of T cells can serve as a T cell activation signal in CARs. Although CAR T cells have shown positive results *in vitro*, they have had limited success in eliminating disease (e.g., cancer) in clinical trials. One problem has been the inability to prolong activation and expand the CAR T cell population *in vivo*.

To address this problem, a co-stimulation domain (e.g., CD137, CD28 or CD134) has been included in second generation CARs to achieve prolonged activation of T cells *in vivo*. Addition of a co-stimulation domain enhances the *in vivo* proliferation and survival of T cells containing CARs, and initial clinical data have shown that such constructs
5 are promising therapeutic agents in the treatment of diseases, such as cancer.

Although improvements have been made in CAR T cell therapies, several problems remain. First, ‘off-target’ toxicity may occur due to normal cells that express the antigen targeted by the CAR T cells (e.g., a tumor-associated antigen). Second, unregulated CAR T cell activation may be found where the rapid and uncontrolled elimination of diseased
10 cells (e.g., cancer cells) by CAR T cells induces a constellation of metabolic disturbances, called tumor lysis syndrome, or cytokine release syndrome (CRS), which can be fatal to patients. Tumor lysis syndrome and CRS can result due to administered CAR T cells that cannot be easily regulated, and are activated uncontrollably. Accordingly, although CAR T cells show great promise as a tool in the treatment of diseases, such as cancer, additional CAR
15 T cell therapeutic protocols are needed that provide reduced off-target toxicity, and more precise control of CAR T cell activation.

SUMMARY OF THE INVENTION

In the various embodiments described herein, a small molecule ligand linked to a
20 targeting moiety by a linker is used as a bridge between the cancer and the CAR T cells directing the CAR T cells to the cancer for amelioration of the cancer. In one embodiment, the “small molecule ligand” can be, for example, a folate, DUPA, an NK-1R ligand, a CAIX ligand, a ligand of gamma glutamyl transpeptidase, an NKG2D ligand, or a CCK2R ligand, each of which is a small molecule ligand that binds specifically to cancer cells (i.e., the receptor
25 for these ligands is overexpressed on cancers compared to normal tissues).

In one embodiment, the “small molecule ligand” is linked to a “targeting moiety” that binds to the CAR expressed by CAR T cells. In various embodiments, the “targeting moiety” can be selected, for example, from 2,4-dinitrophenol (DNP), 2,4,6-trinitrophenol (TNP), biotin, digoxigenin, fluorescein, fluorescein isothiocyanate (FITC), NHS-fluorescein, pentafluorophenyl ester (PFP), tetrafluorophenyl ester (TFP), a knottin, a centyrin,
30 and a DARPin.

The “targeting moiety” binds to the recognition region of the genetically engineered CAR expressed by CAR T cells. Accordingly, the recognition region of the CAR (e.g., a single chain fragment variable region (scFv) of an antibody, an Fab, Fv, Fc, (Fab')₂ fragment, and the like) is directed to the “targeting moiety.” Thus, the small molecule ligand
5 linked to a targeting moiety by a linker acts as a bridge between the cancer and the CAR T cells, directing the CAR T cells to the cancer for amelioration of the cancer.

In one embodiment, a method of treatment of a cancer is provided. The method comprises i) administering to a patient at least one dose of a CAR T cell composition comprising CAR T cells wherein the CAR T cells comprise a CAR directed to a targeting
10 moiety; ii) administering to the patient a compound, or a pharmaceutically acceptable salt thereof, wherein the compound comprises a small molecule ligand linked to a targeting moiety by a linker and wherein the compound, or the pharmaceutically acceptable salt thereof, is administered in at least a first dose escalation sequence and a second dose escalation sequence.

In another embodiment, a method of treatment of a cancer is provided. The
15 method comprises i) administering to a patient at least one dose of a CAR T cell composition comprising CAR T cells wherein the CAR T cells comprise a CAR directed to a targeting moiety; ii) administering to the patient a compound, or a pharmaceutically acceptable salt thereof, wherein the compound comprises a small molecule ligand linked to a targeting moiety by a linker and wherein the compound, or the pharmaceutically acceptable salt thereof, is
20 administered in a first dose escalation sequence wherein, if serious CRS occurs in the first dose escalation sequence, the compound, or the pharmaceutically acceptable salt thereof, is administered using a lower dose escalation sequence wherein the first dose of the compound, or the pharmaceutically acceptable salt thereof, in the lower dose escalation sequence is lower than the first dose of the compound, or the pharmaceutically acceptable salt thereof,
25 administered in the first dose escalation sequence. In one embodiment, in the lower dose escalation sequence, the compound, or the pharmaceutically acceptable salt thereof, can be administered at about 0.5 percent, about 5 percent, and about 50 percent of a full dose of the compound, or the pharmaceutically acceptable salt thereof, on three separate days.

Additional embodiments are also described by the following enumerated clauses.
30 Any of the following embodiments in combination with any applicable embodiments described in the Summary section, the Detailed Description of the Illustrative Embodiments section, the Examples section, or the claims of this patent application, are also contemplated.

1. A method of treatment of a cancer, the method comprising
 - i) administering to a patient at least one dose of a CAR T cell composition comprising CAR T cells wherein the CAR T cells comprise a CAR directed to a targeting moiety;
 - 5 ii) administering to the patient a compound, or a pharmaceutically acceptable salt thereof, wherein the compound comprises a small molecule ligand linked to a targeting moiety by a linker and wherein the compound, or the pharmaceutically acceptable salt thereof, is administered in at least a first dose escalation sequence and a second dose escalation sequence.
- 10 2. The method of clause 1 wherein the compound, or the pharmaceutically acceptable salt thereof, is administered in at least a first dose escalation sequence, a second dose escalation sequence, and a third dose escalation sequence.
3. The method of clause 1 wherein the compound, or the pharmaceutically acceptable salt thereof, is administered in at least a first dose escalation sequence, a second dose
15 escalation sequence, a third dose escalation sequence, and a fourth dose escalation sequence.
4. The method of clause 1 wherein the compound, or the pharmaceutically acceptable salt thereof, is administered is administered in at least a first dose escalation sequence, a second dose escalation sequence, a third dose escalation sequence, a fourth dose escalation sequence, and a fifth dose escalation sequence.
- 20 5. The method of clause 1 wherein the compound, or the pharmaceutically acceptable salt thereof, is administered in at least a first dose escalation sequence, a second dose escalation sequence, a third dose escalation sequence, a fourth dose escalation sequence, a fifth dose escalation sequence, and a sixth dose escalation sequence.
6. The method of any one of clauses 1 to 5 wherein a first dose of the CAR
25 T cells and a second dose of the CAR T cells are administered to the patient.
7. The method of clause 6 wherein the first dose of the CAR T cells is a test dose to monitor the patient for tolerability to the CAR T cells.
8. The method of clause 6 wherein the second dose of the CAR T cells comprises a higher dose of the CAR T cells than the first dose of the CAR T cells.
- 30 9. The method of any one of clauses 6 to 8 wherein the first dose of the CAR T cells comprises about 0.5×10^5 of the CAR T cells per kg of patient body weight to about 1.5×10^6 of the CAR T cells per kg of patient body weight.

10. The method of any one of clauses 6 to 9 wherein the second dose of the CAR T cells comprises about 0.8×10^6 of the CAR T cells per kg of patient body weight to about 2×10^7 of the CAR T cells per kg of patient body weight.

5 11. The method of any one of clauses 1 to 10 wherein the first dose escalation sequence is followed by a period of time during which the compound, or the pharmaceutically acceptable salt thereof, is not administered.

12. The method of any one of clauses 1 to 11 wherein the second dose escalation sequence is followed by a period of time during which the compound, or the pharmaceutically acceptable salt thereof, is not administered.

10 13. The method of any one of clauses 2 to 12 wherein the third dose escalation sequence is followed by a period of time during which the compound, or the pharmaceutically acceptable salt thereof, is not administered.

14. The method of any one of clauses 3 to 13 wherein the fourth dose escalation sequence is followed by a period of time during which the compound, or the pharmaceutically acceptable salt thereof, is not administered.

15 15. The method of any one of clauses 4 to 14 wherein the fifth dose escalation sequence is followed by a period of time during which the compound, or the pharmaceutically acceptable salt thereof, is not administered.

16. The method of any one of clauses 5 to 15 wherein the sixth dose escalation sequence is followed by a period of time during which the compound, or the pharmaceutically acceptable salt thereof, is not administered.

17. The method of any one of clauses 11 to 16 wherein the period of time is about 7 days.

18. The method of any one of clauses 1 to 17 wherein the first dose escalation sequence comprises administering to the patient about 1 percent, about 10 percent, and about 100 percent of a full dose of the compound, or the pharmaceutically acceptable salt thereof, on three separate days.

19. The method of any one of clauses 1 to 18 wherein the second dose escalation sequence comprises administering to the patient about 1 percent, about 30 percent, and about 300 percent of a full dose of the compound, or the pharmaceutically acceptable salt thereof, on three separate days.

20. The method of any one of clauses 2 to 19 wherein the third dose escalation sequence comprises administering to the patient about 1 percent, about 50 percent, and about 500 percent of a full dose of the compound, or the pharmaceutically acceptable salt thereof, on three separate days.

5 21. The method of any one of clauses 3 to 20 wherein the fourth dose escalation sequence comprises administering to the patient about 1 percent, about 10 percent, and about 100 percent of a full dose of the compound, or the pharmaceutically acceptable salt thereof, on three separate days.

10 22. The method of any one of clauses 4 to 21 wherein the fifth dose escalation sequence comprises administering to the patient about 1 percent, about 30 percent, and about 300 percent of a full dose of the compound, or the pharmaceutically acceptable salt thereof, on three separate days.

15 23. The method of any one of clauses 5 to 22 wherein the sixth dose escalation sequence comprises administering to the patient about 1 percent, about 50 percent, and about 500 percent of a full dose of the compound, or the pharmaceutically acceptable salt thereof, on three separate days.

24. The method of any one of clauses 18 to 23 wherein the full dose of the compound, or the pharmaceutically acceptable salt thereof, is about 10 $\mu\text{g/kg}$ to about 50 $\mu\text{g/kg}$ of the compound, or the pharmaceutically acceptable salt thereof.

20 25. The method of any one of clauses 18 to 24 wherein the full dose of the compound, or the pharmaceutically acceptable salt thereof, is about 20 $\mu\text{g/kg}$ to about 40 $\mu\text{g/kg}$ of the compound, or the pharmaceutically acceptable salt thereof.

25 26. The method of any one of clauses 18 to 25 wherein the full dose of the compound, or the pharmaceutically acceptable salt thereof, is about 25 $\mu\text{g/kg}$ to about 35 $\mu\text{g/kg}$ of the compound, or the pharmaceutically acceptable salt thereof.

27. The method of any one of clauses 18 to 26 wherein the full dose of the compound, or the pharmaceutically acceptable salt thereof, is about 30 $\mu\text{g/kg}$ of the compound, or the pharmaceutically acceptable salt thereof.

30 28. The method of any one of clauses 6 to 27 wherein the first dose of the CAR T cells and the second dose of the CAR T cells are administered to the patient during week 1.

29. The method of any one of clauses 6 to 28 wherein the first dose of the CAR T cells and the second dose of the CAR T cells are administered to the patient during week 1 on Monday and Thursday.

5 30. The method of any one of clauses 1 to 29 wherein the first dose escalation sequence for the compound, or the pharmaceutically acceptable salt thereof, occurs during weeks 2 and 3.

31. The method of any one of clauses 1 to 30 wherein the second dose escalation sequence for the compound, or the pharmaceutically acceptable salt thereof, occurs during weeks 4 and 5.

10 32. The method of any one of clauses 2 to 31 wherein the third dose escalation sequence for the compound, or the pharmaceutically acceptable salt thereof, occurs during weeks 6 and 7.

33. The method of clause 30 wherein the compound, or the pharmaceutically acceptable salt thereof, is administered on three separate days and the three separate days are
15 Monday and Thursday of week 2 and Monday of week 3.

34. The method of clause 31 wherein the compound, or the pharmaceutically acceptable salt thereof, is administered on three separate days and the three separate days are Monday and Thursday of week 4 and Monday of week 5.

20 35. The method of clause 32 wherein the compound, or the pharmaceutically acceptable salt thereof, is administered on three separate days and the three separate days are Monday and Thursday of week 6 and Monday of week 7.

36. The method of any one of clauses 1 to 35 wherein lymphocytes are depleted in the patient before administration of the CAR T cell composition to the patient.

25 37. The method of any one of clauses 1 to 36 further comprising administering platelets to the patient, administering packed red blood cells to the patient, administering cryoprecipitate to the patient, administering intravenous immunoglobulin to the patient, and/or providing antimicrobial therapy to the patient.

30 38. The method of any one of clauses 1 to 37 wherein, if no CRS or neurotoxicity is observed in the patient during the first dose escalation sequence, the method is advanced to the second dose escalation sequence.

39. The method of any one of clauses 2 to 38 wherein, if no CRS or neurotoxicity is observed in the patient during the second dose escalation sequence, the method is advanced to the third dose escalation sequence.

40. The method of any one of clauses 3 to 39 wherein, if no CRS or
5 neurotoxicity is observed in the patient during the third dose escalation sequence, the method is advanced to the fourth dose escalation sequence.

41. The method of any one of clauses 4 to 40 wherein, if no CRS or neurotoxicity is observed in the patient during the fourth dose escalation sequence, the method is advanced to the fifth dose escalation sequence.

10 42. The method of any one of clauses 5 to 41 wherein, if no CRS or neurotoxicity is observed in the patient during the fifth dose escalation sequence, the method is advanced to the sixth dose escalation sequence.

43. The method of any one of clauses 1 to 42 wherein if fever without hypotension is observed in the patient and no neurotoxicity is observed in the patient during
15 one of the dose escalation sequences, all subsequent doses of the compound, or the pharmaceutically acceptable salt thereof, are administered to the patient at the dose escalation sequence level that caused the fever without hypotension.

44. The method of any one of clauses 1 to 43 wherein, if serious CRS or neurotoxicity occurs in the patient in any dose escalation sequence, all subsequent doses of the
20 compound, or the pharmaceutically acceptable salt thereof, are administered to the patient at the dose escalation sequence level below the dose escalation sequence level that caused the serious CRS or neurotoxicity in the patient.

45. A method of treatment of a cancer, the method comprising
i) administering to a patient at least one dose of a CAR T cell
25 composition comprising CAR T cells wherein the CAR T cells comprise a CAR directed to a targeting moiety;
ii) administering to the patient a compound, or a pharmaceutically acceptable salt thereof, wherein the compound comprises a small molecule ligand linked to a
targeting moiety by a linker and wherein the compound, or the pharmaceutically acceptable salt
30 thereof, is administered in a first dose escalation sequence wherein, if serious CRS occurs in the first dose escalation sequence, the compound, or the pharmaceutically acceptable salt thereof, is administered using a lower dose escalation sequence wherein the first dose of the compound, or

the pharmaceutically acceptable salt thereof, in the lower dose escalation sequence is lower than the first dose of the compound, or the pharmaceutically acceptable salt thereof, administered in the first dose escalation sequence.

46. The method of clause 45 wherein in the lower dose escalation sequence,
5 the compound, or the pharmaceutically acceptable salt thereof, is administered at about 0.5 percent, about 5 percent, and about 50 percent of a full dose of the compound, or the pharmaceutically acceptable salt thereof, on three separate days.

47. The method of clause 46 wherein the full dose of the compound, or the
10 pharmaceutically acceptable salt thereof, is about the full dose of the compound, or the pharmaceutically acceptable salt thereof, is about 10 µg/kg to about 50 µg/kg of the compound, or the pharmaceutically acceptable salt thereof.

48. The method of any one of clauses 46 to 47 wherein the full dose of the
compound, or the pharmaceutically acceptable salt thereof, is about 25 µg/kg to about 35 µg/kg of the compound, or the pharmaceutically acceptable salt thereof.

15 49. The method of any one of clauses 46 to 48 wherein the full dose of the compound, or the pharmaceutically acceptable salt thereof, is about 30 µg/kg of the compound, or the pharmaceutically acceptable salt thereof.

50. The method of any one of clauses 1 to 49 wherein the ligand is selected
20 from the group consisting of a folate, DUPA, an NK-1R ligand, a CAIX ligand, a ligand of gamma glutamyl transpeptidase, an NKG2D ligand, and a CCK2R ligand.

51. The method of any one of clauses 1 to 50 wherein the ligand is a folate.

52. The method of any one of clauses 1 to 50 wherein the ligand is an NK-1R
ligand.

53. The method of any one of clauses 1 to 50 wherein the ligand is DUPA.

25 54. The method of any one of clauses 1 to 50 wherein the ligand is a CCK2R
ligand.

55. The method of any one of clauses 1 to 50 wherein the ligand is a ligand
of gamma glutamyl transpeptidase.

56. The method of any one of clauses 1 to 55 wherein the targeting moiety is
30 selected from the group consisting of 2,4-dinitrophenol (DNP), 2,4,6-trinitrophenol (TNP), biotin, digoxigenin, fluorescein, fluorescein isothiocyanate (FITC), NHS-fluorescein, pentafluorophenyl ester, tetrafluorophenyl ester, a knottin, a centyrin, and a DARPIn.

57. The method of any one of clauses 1 to 56 wherein the targeting moiety is FITC.

58. The method of any one of clauses 1 to 56 wherein the targeting moiety is DNP.

5 59. The method of any one of clauses 1 to 56 wherein the targeting moiety is TNP.

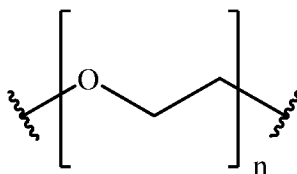
60. The method of any one of clauses 1 to 59 wherein the linker comprises polyethylene glycol (PEG), polyproline, a hydrophilic amino acid, a sugar, an unnatural peptidoglycan, a polyvinylpyrrolidone, pluronic F-127, or a combination thereof.

10 61. The method of any one of clauses 1 to 60 wherein the linker comprises PEG.

62. The method of any one of clauses 1 to 61 wherein the compound, or the pharmaceutically acceptable salt thereof, has the formula

15
$$B-L-T,$$

wherein B represents the small molecule ligand, L represents the linker, and T represents the targeting moiety, and wherein L comprises a structure having the formula



wherein n is an integer from 0 to 200.

20 63. The method of clause 62 wherein n is an integer from 0 to 150.

64. The method of clause 62 wherein n is an integer from 0 to 110.

65. The method of clause 62 wherein n is an integer from 0 to 20.

66. The method of clause 62 wherein n is an integer from 15 to 20.

67. The method of clause 62 wherein n is an integer from 15 to 110.

25 68. The method of any one of clauses 1 to 67 wherein the cancer is selected from the group consisting of lung cancer, bone cancer, pancreatic cancer, skin cancer, cancer of the head, cancer of the neck, cutaneous melanoma, intraocular melanoma, uterine cancer, ovarian cancer, endometrial cancer, rectal cancer, stomach cancer, colon cancer, breast cancer, triple negative breast cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium,

carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, Hodgkin's Disease, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, non-small cell lung cancer, cancer of the adrenal gland, sarcoma of soft tissue, osteosarcoma, including pediatric or non-pediatric
5 osteosarcoma, cancer of the urethra, prostate cancer, chronic leukemia, acute leukemia, acute myelocytic leukemia, lymphocytic lymphoma, myeloid leukemia, myelomonocytic leukemia, hairy cell leukemia, pleural mesothelioma, cancer of the bladder, Burkitt's lymphoma, cancer of the ureter, cancer of the kidney, renal cell carcinoma, carcinoma of the renal pelvis, neoplasms of the central nervous system (CNS), primary CNS lymphoma, spinal axis tumors,
10 brain stem glioma, pituitary adenoma, and adenocarcinoma of the gastroesophageal junction.

69. The method of any one of clauses 1 to 51 or 56 to 68 wherein the cancer is a folate receptor expressing cancer.

70. The method of any one of clauses 1 to 69 wherein the cancer is an osteosarcoma.

15 71. The method of any one of clauses 1 to 70 wherein the CAR has a recognition region and the recognition region is a single chain fragment variable (scFv) region of an antibody.

72. The method of any one of clauses 1 to 57 or 60 to 71 wherein the CAR has a recognition region and the recognition region of the CAR is a single chain fragment
20 variable (scFv) region of an anti-FITC antibody.

73. The method of any one of clauses 1 to 72 wherein the CAR has a co-stimulation domain and the co-stimulation domain is selected from the group consisting of CD28, CD137 (4-1BB), CD134 (OX40), and CD278 (ICOS).

74. The method of any one of clauses 1 to 73 wherein the CAR has an
25 activation signaling domain and the activation signaling domain is a T cell CD3 ζ chain or an Fc receptor γ .

75. The method of any one of clauses 1 to 57 or 60 to 74 wherein the CAR has a recognition region and the recognition region is a single chain fragment variable (scFv) region of an anti-FITC antibody, wherein the CAR has a co-stimulation domain and the co-
30 stimulation domain is CD137 (4-1BB), and wherein the CAR has an activation signaling domain and the activation signaling domain is a T cell CD3 ζ chain.

76. The method of any one of clauses 1 to 75 wherein the patient is imaged prior to administration of the compound, or the pharmaceutically acceptable salt thereof, or prior to administration of the CAR T cell composition.

77. The method of any one of clauses 1 to 76 wherein the compound,
5 or the pharmaceutically acceptable salt thereof, is not an antibody, and does not comprise a fragment of an antibody.

78. The method of any one of clauses 1 to 77 wherein the targeting moiety does not comprise a peptide epitope.

79. The method of any one of clauses 1 to 78 wherein cytokine
10 release resulting in off-target toxicity in the patient does not occur and wherein CAR T cell toxicity to the cancer occurs.

80. The method of any one of clauses 1 to 78 wherein off-target tissue toxicity does not occur in the patient and wherein CAR T cell toxicity to the cancer occurs.

81. The method of any one of clauses 1 to 78 wherein the cancer
15 comprises a tumor, wherein tumor size is reduced in the patient, and wherein off-target toxicity does not occur.

82. The method of any one of clauses 1 to 81 wherein the CAR T cells comprise a nucleic acid comprising SEQ ID NO:1.

83. The method of any one of clauses 1 to 82 wherein the CAR T
20 cells comprise a polypeptide comprising SEQ ID NO:2.

84. The method of clause 82 wherein the nucleic acid encodes a chimeric antigen receptor.

85. The method of any one of clauses 1 to 81 wherein the CAR T
25 cells comprise a nucleic acid comprising SEQ ID NO:4.

86. The method of any one of clauses 1 to 81 or 85 wherein the CAR T cells comprise a polypeptide comprising SEQ ID NO:5.

87. The method of clause 85 wherein the nucleic acid encodes a chimeric antigen receptor.

88. The method of any one of clauses 1 to 87 wherein the CAR
30 comprises humanized amino acid sequences.

89. The method of any one of clauses 1 to 87 wherein the CAR consists of humanized amino acid sequences.

90. The method of any one of clauses 1 to 89 further comprising administering to the patient a folate, a conjugate comprising a folate wherein the conjugate comprising a folate does not comprise a targeting moiety, or an agent that inhibits activation of the CAR T cells.

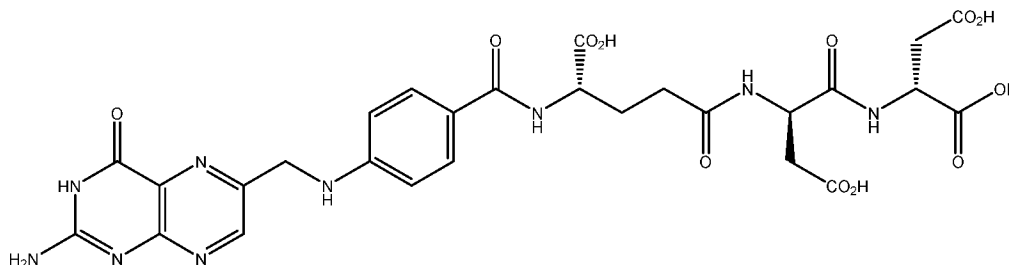
91. The method of clause 90 wherein a folate is administered.

92. The method of clause 90 wherein folic acid or leucovorin is administered.

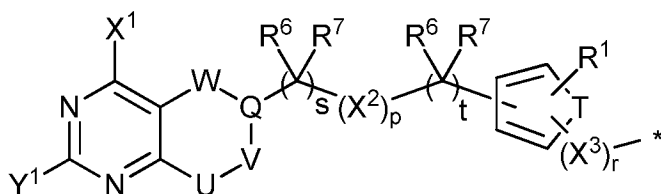
93. The method of clause 90 wherein the conjugate comprising a folate is administered.

94. The method of clause 93 wherein the conjugate comprising a folate comprises a folate linked to one or more amino acids.

95. The method of clause 93 wherein the conjugate comprising a folate has the formula



96. The method of clause 91 wherein the folate has the formula



wherein X^1 and Y^1 are each-independently selected from the group consisting of halo, R^2 , OR^2 , SR^3 , and NR^4R^5 ;

U, V, and W represent divalent moieties each independently selected from the group consisting of $-(R^{6a})C=$, $-N=$, $-(R^{6a})C(R^{7a})-$, and $-N(R^{4a})-$; Q is selected from the group consisting of C and CH; T is selected from the group consisting of S, O, N, and $-C=C-$;

X² and X³ are each independently selected from the group consisting of
 5 oxygen, sulfur, $-C(Z)-$, $-C(Z)O-$, $-OC(Z)-$, $-N(R^{4b})-$, $-C(Z)N(R^{4b})-$, $-N(R^{4b})C(Z)-$,
 $-OC(Z)N(R^{4b})-$, $-N(R^{4b})C(Z)O-$, $-N(R^{4b})C(Z)N(R^{5b})-$, $-S(O)-$, $-S(O)_2-$, $-N(R^{4a})S(O)_2-$, $-C(R^{6b})(R^{7b})-$, $-N(C\equiv CH)-$, $-N(CH_2C\equiv CH)-$, C₁-C₁₂ alkylene, and C₁-C₁₂ alkyneoxy, where Z is oxygen or sulfur;

R¹ is selected from the group consisting of hydrogen, halo, C₁-C₁₂ alkyl, and C₁-
 10 C₁₂ alkoxy;

R², R³, R⁴, R^{4a}, R^{4b}, R⁵, R^{5b}, R^{6b}, and R^{7b} are each independently selected from the group consisting of hydrogen, halo, C₁-C₁₂ alkyl, C₁-C₁₂ alkoxy, C₁-C₁₂ alkanoyl, C₁-C₁₂ alkenyl, C₁-C₁₂ alkynyl, (C₁-C₁₂ alkoxy)carbonyl, and (C₁-C₁₂ alkylamino)carbonyl;

R⁶ and R⁷ are each independently selected from the group consisting of
 15 hydrogen, halo, C₁-C₁₂ alkyl, and C₁-C₁₂ alkoxy; or, R⁶ and R⁷ are taken together to form a carbonyl group;

R^{6a} and R^{7a} are each independently selected from the group consisting of hydrogen, halo, C₁-C₁₂ alkyl, and C₁-C₁₂ alkoxy; or R^{6a} and R^{7a} are taken together to form a carbonyl group;

20 p, r, s, and t are each independently either 0 or 1; and

* represents an optional covalent bond to the rest of the conjugate, if any additional chemical moieties are part of the folate.

97. The method of clause 90 wherein the agent that inhibits activation of the CAR T cells is selected from the group consisting of a lymphocyte-specific
 25 protein tyrosine kinase inhibitor, a PI3 kinase inhibitor, an inhibitor of an IL-2 inducible T cell kinase, a JAK inhibitor, a BTK inhibitor, EC2319, and an agent that blocks CAR T cell binding to the compound, or the pharmaceutically acceptable salt thereof, but does not bind to the cancer.

98. The method of clause 90 wherein the agent that inhibits activation
 30 of the CAR T cells is administered and the agent is a lymphocyte-specific protein tyrosine kinase inhibitor.

99. The method of clause 98 wherein the lymphocyte-specific protein tyrosine kinase inhibitor is Dasatinib.

100. The method of clause 90 wherein the agent that inhibits activation of the CAR T cells is administered and the agent is a PI3 kinase inhibitor.

5 101. The method of clause 100 wherein the PI3 kinase inhibitor is GDC0980.

102. The method of clause 90 wherein the agent that inhibits activation of the CAR T cells is administered and the agent is an IL-2 inducible T cell kinase inhibitor.

10 103. The method of clause 102 wherein the IL-2 inducible T cell kinase inhibitor is BMS-509744.

104. The method of clause 90 wherein the agent that inhibits activation of the CAR T cells is administered and is an agent that blocks CAR T cell binding to the compound, or the pharmaceutically acceptable salt thereof, but does not bind to the
15 cancer.

105. The method of clause 104 wherein the agent is fluoresceinamine, FITC, or sodium fluorescein.

106. The method of clause 105 wherein the agent is sodium fluorescein.

20 107. The method of any one of clauses 90 to 106 wherein administration of the folate, the conjugate comprising a folate wherein the conjugate comprising a folate does not comprise a targeting moiety, or the agent that inhibits activation of the CAR T cells causes reduction in cytokine levels in the patient.

25 108. The method of clause 107 wherein the reduction in cytokine levels is a reduction to about the cytokine levels in an untreated patient.

109. The method of any one of clauses 90 to 108 wherein the cancer comprises a tumor and tumor size in the patient is not increased when the folate, the conjugate comprising a folate wherein the conjugate comprising a folate does not comprise a targeting moiety, or the agent that inhibits activation of the CAR T cells is administered to the patient.

30 110. The method of any one of clauses 104 to 106 wherein the agent that inhibits activation of the CAR T cells is administered to the patient when the CRS grade reaches 1, 2, 3, or 4.

111. The method of clause 110 wherein the agent that inhibits activation of the CAR T cells is administered to the patient when the CRS grade reaches 3 or 4.

112. The method of any one of clauses 104 to 106 wherein the agent that inhibits activation of the CAR T cells is administered at a dose of about .01 to about 300
5 umoles/kg of body weight of the patient.

113. The method of any one of clauses 1 to 112 wherein CRS is reduced or prevented in the patient and the method results in a decrease in tumor volume in the patient.

114. The method of any one of clauses 1 to 113 wherein body weight loss due to CRS is reduced or prevented.

10 115. The method of any one of clauses 90 to 114 further comprising re-administering the compound, or the pharmaceutically acceptable salt thereof, to the patient.

116. The method of clause 115 wherein the subsequent administration of the compound, or the pharmaceutically acceptable salt thereof, causes CAR T cell activation and an increase in cytokine levels in the patient.

15 117. The method of any one of clauses 1 to 116 wherein the CAR T cell composition is administered before the compound, or the pharmaceutically acceptable salt thereof.

118. The method of any one of clauses 1 to 117 wherein the CAR T cells are autologous.

20 119. The ;method of any one of clauses 2 to 17 wherein the first dose escalation sequence comprises administering to the patient about 1 percent, about 2 percent, and about 20 percent of a full dose of the compound, or the pharmaceutically acceptable salt thereof, on three separate days, wherein the second dose escalation sequence comprises administering to the patient about 1 percent, about 6 percent, and about 60 percent of a full dose
25 of the compound, or the pharmaceutically acceptable salt thereof, on three separate days, and wherein the third dose escalation sequence comprises administering to the patient about 1 percent, about 10 percent, and about 100 percent of a full dose of the compound, or the pharmaceutically acceptable salt thereof, on three separate days.

30 120. The ;method of any one of clauses 2 to 17 wherein the first dose escalation sequence comprises administering to the patient about 1 percent, about 10 percent, and about 100 percent of a full dose of the compound, or the pharmaceutically acceptable salt thereof, on three separate days, and wherein the second dose escalation sequence comprises

administering to the patient about 1 percent, about 20 percent, and about 200 percent of a full dose of the compound, or the pharmaceutically acceptable salt thereof, on three separate days.

121. The method of any one of clauses 2 to 17 wherein the first dose escalation sequence comprises administering to the patient about 1 percent, about 10 percent, and about 100 percent of a full dose of the compound, or the pharmaceutically acceptable salt thereof, on three separate days, and wherein the second dose escalation sequence comprises administering to the patient about 1 percent, about 10 percent, and about 100 percent of a full dose of the compound, or the pharmaceutically acceptable salt thereof, on three separate days.

122. The method of any one of clauses 2 to 17 wherein the first dose escalation sequence comprises administering to the patient about 1 percent, about 20 percent, and about 200 percent of a full dose of the compound, or the pharmaceutically acceptable salt thereof, on three separate days, and wherein the second dose escalation sequence comprises administering to the patient about 1 percent, about 20 percent, and about 200 percent of a full dose of the compound, or the pharmaceutically acceptable salt thereof, on three separate days.

123. The method of any one of clauses 119 to 122 wherein the full dose of the compound, or the pharmaceutically acceptable salt thereof, is about 500 nmoles/kg.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows a diagram of an exemplary dosing scheme according to the claimed method. In this exemplary scheme, E2 CAR T cell (CAR T cell expressing SEQ ID NO:4) administration occurs in week 1 on Monday and Thursday prior to administration of the small molecule ligand linked to the targeting moiety by a linker (e.g., EC17) beginning in week 2.

In week 2, the small molecule ligand linked to the targeting moiety by a linker (e.g., EC17) is administered on Monday and Thursday, and then in week 3 on Monday using a dose escalation sequence (i.e., Sequence 1). There is no administration of the small molecule ligand linked to the targeting moiety by a linker (e.g., EC17) during week 3 on Tuesday to Sunday. Weeks 2 to 3 are referred to as "Cycle 1".

In week 4, the small molecule ligand linked to the targeting moiety by a linker (e.g., EC17) is administered on Monday and Thursday, and then in week 5 on Monday using a dose escalation sequence (i.e., Sequence 2). There is no administration of the small molecule

ligand linked to the targeting moiety by a linker (e.g., EC17) during week 5 on Tuesday to Sunday. Weeks 4 to 5 are referred to as “Cycle 2”.

In week 6, the small molecule ligand linked to the targeting moiety by a linker (e.g., EC17) is administered on Monday and Thursday, and then in week 7 on Monday using a
5 dose escalation sequence (i.e., Sequence 3). There is no administration of the small molecule ligand linked to the targeting moiety by a linker (e.g., EC17) during week 7 on Tuesday to Sunday. Weeks 6 to 7 are referred to as “Cycle 3”. Weeks 2 to 7 are referred to as “Course 1”.

During Sequence 1, the small molecule ligand linked to the targeting moiety by a linker (e.g., EC17) is administered in week 2 on Monday and Thursday, and then in week 3 on
10 Monday with doses of the small molecule ligand linked to the targeting moiety by a linker (e.g., EC17) that are 1, 10, and 100 percent of a full dose (e.g., 31 µg/kg), respectively, of the small molecule ligand linked to the targeting moiety (e.g., EC17).

During Sequence 2, the small molecule ligand linked to the targeting moiety by a linker (e.g., EC17) is administered in week 4 on Monday and Thursday, and then in week 5 on
15 Monday with doses of the small molecule ligand linked to the targeting moiety by a linker (e.g., EC17) that are 1, 30, and 300 percent of a full dose (e.g., 31 µg/kg), respectively, of the small molecule ligand linked to the targeting moiety (e.g., EC17).

During Sequence 3, the small molecule ligand linked to the targeting moiety by a linker (e.g., EC17) is administered in week 6 on Monday and Thursday, and then in week 7 on
20 Monday with doses of the small molecule ligand linked to the targeting moiety by a linker (e.g., EC17) that are 1, 50, and 500 percent of a full dose (e.g., 31 µg/kg), respectively, of the small molecule ligand linked to the targeting moiety (e.g., EC17). Course 1 is then repeated assuming clinical benefit and tolerable toxicity.

FIG. 2 shows a general diagram of a construct used for CAR T transduction.

25 FIG. 3 shows an E2 construct vs. a 4M5.3 construct diagrammatically and shows a map of the E2 construct.

FIG. 4 (top panel) is a chart showing tumor volumes of HOS-FRα tumors when treated with E2 Car-T cells only (●) and E2 Car-T cells + EC-17 (○). FIG. 4 (bottom panel) is a chart showing the body weight change in mice bearing HOS-FRα tumors when treated with E2
30 Car-T cells only (●) and E2 Car-T cells + EC-17 (○).

FIG. 5 is a chart showing the body weight change in mice bearing THP-1-FRβ AML when treated with no Car-T (●); no EC-17 (■); EC-17 SIW 500 nmol/kg (▲); EC-17

TIW (5, 50, 500 nmol/kg on/off); (♦) EC-17 dose escalation (M/Th/M on/off).

FIG. 6 (left panel) is a chart showing liver metastatic tumor burden. FIG. 6 (right panel) is a chart showing non-liver metastatic tumor burden.

FIG. 7 is a chart showing counts of circulating THP1-FR β cells per 100 μ L blood at day 39 on a logarithmic scale.

FIG. 8 is a chart showing percentage of the total E2 CAR T cells isolated from the solid liver tumors (y axis) at different EC-17 dosing regimens. For each dosing regimen, far left bar is PD1+ LAG3+ TIM3+, second left bar is PD1+ LAG3+ TIM3-, middle bar is PD1+ LAG3- TIM3+; second right bar is PD1+ LAG3- TIM3-; far right bar is PD1- LAG3- TIM3-.

FIG. 9 shows a fully human CAR construct including anti-FITC scFv (clone E2), a full-length IgG4 spacer (Fc derived hinge-CH2(L235D, N297Q)-CH3), CD28tm transmembrane domain, 4-1BB/CD3 ζ cytoplasmic activation domains, and a non-functional truncated cell surface polypeptide of epidermal growth factor receptor (EGFRt). Bottom: Examples of CD4/CD8 T cell phenotyping performed by flow cytometry on an EGFRt-sorted (left pie charts) CAR-T cell preparation and an unsorted “clinical facsimile” (right pie charts). The color keys are as shown.

FIG. 10, Panel A: Kd values of ^{3} H-EC17 uptake by FR+ target cells after a 2 hour incubation at 37°C (calculated from the numbers of molecules bound per cell). Panel B: Kd value of ^{3} H-EC17 uptake by E2-CAR-T cells (~24% EGFRt+, ~95:5 CD8/CD4 ratio) after a 2 hour incubation at room temperature (calculated from total cell-associated radioactivity, DPM).

FIG. 11 shows functional FR levels on tumor cells measured by a ^{3} H-FA-based binding assay (100 nM, 1 hour at 37°C).

FIG. 12 shows EC17 dose finding and CRS assessment in tumor versus tumor-free mice. Panel A: Schematic diagram to show dose scheduling of CAR-T cells (~10 million “clinical facsimile”) plus EC17 dosed 3 different ways in NSG mice without or with pre-established MDA-MB-231 xenografts. Tumor-free mice received EC17 SIW 500 nmol/kg (2 doses on days 2 and 10). Tumor-bearing mice received EC17 as follows: EC17 SIW 500 nmol/kg (5 doses on days 2, 10, 17, 24, and 31), EC17 M/Th/M_Escalation-1 (repeats of 5/50/500 nmol/kg on Monday/Thursday/Monday followed by 1-week break, i.e. on days 2, 6, 10, 17, 20, 24, and 31), or EC17 M/Th/M_Escalation-2 (repeats of 5/100/1000 nmol/kg on Monday/Thursday/Monday followed by 1-week break, i.e. days 2, 6, 10, 17, 20, 24, and 31). Panel B: Systemic levels of human IFN γ on a log2 scale detected in mouse plasma on days 11

and 12 after CAR-T cell injection in tumor-bearing versus tumor-free mice (i.e., ~20 and 42 hours after the previous EC17 dose in all treated cohorts). Panel C: Circulating CAR-T cells in mouse blood identified as human CD3 ϵ + EGFRt+ events by flow cytometry and enumerated per 100 μ L of whole blood. Panel D: Measurements of change in body weight and tumor volume in tumor-bearing mice that received CAR-T cells only or CAR-T cells plus EC17 dosed 3 different ways (dashed lines indicated each EC17 dose). n = 5 mice per group. All data represent mean $\pm$ s.e.m. * $p < 0.05$ by one-way ANOVA test.

FIG. 13 shows EC17 dose escalation in safety and anti-leukemic activity *in-vivo*.

Panel A: Schematic diagrams to show dose scheduling of EC17 plus unsorted EGFRt CAR-T cells (~6 million, day 0) in NSG mice with 1-day-old intravenous THP1-FR β xenografts. Starting 3 days after CAR-T cell injection, EC17 was dosed in 3 different ways: EC17 SIW 500 nmol/kg (5 doses on days 3, 10, 17, 24, 31, and 38), EC17 TIW 5/50/500 nmol/kg (3 repeats of 5/50/500 nmol/kg on Monday/Wednesday/Friday followed by a 9-day break, i.e. on days 3/5/7, 17/19/21 and 31/33/35), or EC17 M/Th/M dose escalation on Monday/Thursday/Monday (3 escalation cycles at 5/10/100 nmol/kg in Cycle 1, 5/30/300 nmol/kg in Cycle 2, and 5/50/500 nmol/kg in Cycle 3, i.e. on days 3/6/10, 17/20/24, and 31/34/38). Panel B: Measurements of change in body weight (n = 5). Panel C: Circulating CAR-T cells as human CD3 ϵ + EGFRt+ events found per 100 μ L of mouse whole blood on a logarithmic scale on day 31. Panel D: Left bar graph: circulating tumor cells (GFP+) per 100 μ L of whole blood in all cohorts measured at the end of study (day 39); Middle bar graph: THP1-FR β infiltrated liver weights representing liver metastatic burden; Right bar graph: total tumor weights of all non-liver macrometastases. Panel E: Flow cytometric analysis of T-cell exhaustion markers, PD1, LAG3, TIM3, on preinfusion CAR-T cell product (triple-negative) and tumor-infiltrating CAR-T cells isolated from liver metastases. A cardinal feature of fully exhausted T cells is co-expression of multiple inhibitory receptor markers (i.e. triple-positive).

FIG. 14 shows antitumor activity and CRS rescue in an aggressive model of pediatric osteosarcoma. Panel A: Schematic diagram to show dose scheduling of CAR-T cells (~6 million, day 0) plus EC17 in NSG mice with 3-day-old subcutaneous HOS-FR α xenografts (n = 5). Starting 3 days after CAR-T cell injection (6 days after tumor implant), 3 cycles of EC17 M/Th/M “intra-patient” dose escalation on Monday/Thursday/Monday at 5/10/100 nmol/kg in Cycle 1, 5/30/300 nmol/kg in Cycle 2, and 5/50/500 nmol/kg in Cycle 3, i.e. on days 3/6/10, 17/20/24, and 31/34/38. Panel B: Measurements of tumor volumes and change in body

weights. Due to tumor progression, five mice received CAR-T cells only (no EC17) were euthanized on days 23-31, and five EC17 treated mice were euthanized on days 33 (2 mice) and 47 (3 mice), respectively. Panel C: Flow cytometric analysis of CAR-T cells (human CD3 ϵ +EGFRt+) per 100 μ L of whole blood plotted on a logarithmic scale (left) and tumor-infiltrating CAR-T cells at the time of euthanasia (right).

DEFINITIONS

As used herein, “a” or “an” may mean one or more. As used herein, “about” in reference to a numeric value, including, for example, whole numbers, fractions, and percentages, generally refers to a range of numerical values (e.g., +/- 5 % to 10% of the recited value) that one of ordinary skill in the art would consider equivalent to the recited value (e.g., having the same function or result).

As used herein, the terms “treat,” “treating,” “treated,” or “treatment” refer to both therapeutic treatment and prophylactic or preventative treatment.

As used herein, the terms “ameliorate,” “ameliorating,” “amelioration,” or “ameliorated” in reference to cancer can mean reducing the symptoms of the cancer, reducing the size of a tumor, completely or partially removing the tumor (e.g., a complete or partial response), causing stable disease, preventing progression of the cancer (e.g., progression free survival), or any other effect on the cancer that would be considered by a physician to be a therapeutic, prophylactic, or preventative treatment of the cancer.

As used herein, the terms “administer,” “administering,” or “administered” mean all means of introducing the compound, or pharmaceutically acceptable salt thereof, or CAR T cell composition described herein to the patient, including, but not limited to, oral, intravenous, intramuscular, subcutaneous, and transdermal.

As used herein, the term “off-target toxicity” means organ damage or a reduction in the patient’s weight that is unacceptable to the physician treating the patient, or any other effect on the patient that is unacceptable to the physician treating the patient, for example, B cell aplasia, a fever, a drop in blood pressure, or pulmonary edema.

As used herein, the terms “transduction” and “transfection” are used equivalently and the terms mean introducing a nucleic acid into a cell by any artificial method, including viral and non-viral methods.

As used herein, the term “dose escalation sequence” means that increasing doses of the compound, or the pharmaceutically acceptable salt thereof, are administered over time. As used herein, references to “first dose escalation sequence” for use in combination with a “second dose escalation sequence”, a “third dose escalation sequence”, a “fourth dose escalation sequence”, a “fifth dose escalation sequence”, and a “sixth dose escalation sequence”, etc. mean that multiple dose escalation sequences occur, and that for each separate dose escalation sequence, after the first dose escalation sequence, the first dose of the compound, or the pharmaceutically acceptable salt thereof, in the subsequent dose escalation sequence, is lower than the last dose of the compound, or the pharmaceutically acceptable salt thereof, in the prior dose escalation sequence (see, for example, Fig. 1 and the explanation of Fig. 1 in the Brief Description of the Drawings).

DETAILED DESCRIPTION OF THE ILLUSTRATIVE EMBODIMENTS

In the various embodiments described herein, a small molecule ligand linked to a targeting moiety by a linker is used as a bridge between a cancer and CAR T cells (i.e., T cells expressing a chimeric antigen receptor). The bridge directs the CAR T cells to the cancer for amelioration of the cancer. In one embodiment, the “small molecule ligand” can be a folate, a CAIX ligand, DUPA, an NK-1R ligand, a ligand of gamma glutamyl transpeptidase, an NKG2D ligand, or a CCK2R ligand, each of which is a small molecule ligand that binds specifically to a cancer cell type (i.e., the receptor for each of these ligands is overexpressed on cancers compared to normal tissues).

The “targeting moiety” linked to the small molecule ligand binds to the recognition region of the genetically engineered CAR expressed by the CAR T cells. Accordingly, the recognition region of the CAR (e.g., a single chain fragment variable region (scFv) of an antibody, an Fab, Fv, Fc, or (Fab')₂ fragment, and the like) is directed to the “targeting moiety.” Thus, the small molecule ligand linked to a targeting moiety by a linker acts as a bridge between the cancer and the CAR T cells directing the CAR T cells to the cancer for amelioration of the cancer. In various embodiments, the bridge between the cancer and the CAR T cells can be any of the applicable conjugates shown in the Examples.

The bridge is a small organic molecule so clearance from the bloodstream can be rapidly achieved (e.g., about 20 minutes or less). In one aspect, the CAR T cell response can be targeted to only those cancer cells expressing a receptor for the small molecule ligand portion of

the ‘bridge,’ thereby reducing off-target toxicity to normal tissues. Additionally, this system can be ‘universal’ because one type of CAR T cell construct can be used to target a wide variety of cancers using different ‘bridges’. Illustratively, the targeting moiety recognized by the CAR T cells may remain constant so that one type of CAR T cell construct can be used, while the
5 small molecule ligand that binds to the cancer can be altered to allow targeting of a wide variety of cancers.

In one embodiment, a method of treatment of a cancer is provided. The method comprises i) administering to a patient at least one dose of a CAR T cell composition comprising CAR T cells wherein the CAR T cells comprise a CAR directed to a targeting
10 moiety; ii) administering to the patient a compound, or a pharmaceutically acceptable salt thereof, wherein the compound comprises a small molecule ligand linked to a targeting moiety by a linker and wherein the compound, or the pharmaceutically acceptable salt thereof, is administered in at least a first dose escalation sequence and a second dose escalation sequence.

In another embodiment, a method of treatment of a cancer is provided. The
15 method comprises i) administering to a patient at least one dose of a CAR T cell composition comprising CAR T cells wherein the CAR T cells comprise a CAR directed to a targeting moiety; ii) administering to the patient a compound, or a pharmaceutically acceptable salt thereof, wherein the compound comprises a small molecule ligand linked to a targeting moiety by a linker and wherein the compound, or the pharmaceutically acceptable salt thereof, is
20 administered in a first dose escalation sequence wherein, if serious CRS occurs in the first dose escalation sequence, the compound, or the pharmaceutically acceptable salt thereof, is administered using a lower dose escalation sequence wherein the first dose of the compound, or the pharmaceutically acceptable salt thereof, in the lower dose escalation sequence is lower than the first dose of the compound, or the pharmaceutically acceptable salt thereof,
25 administered in the first dose escalation sequence. In another embodiment, in the lower dose escalation sequence, the compound, or the pharmaceutically acceptable salt thereof, can be administered at about 0.5 percent, about 5 percent, and about 50 percent of a full dose of the compound, or the pharmaceutically acceptable salt thereof, on three separate days.

In various embodiments described in the clause list below and in the claims and
30 throughout the application, the small molecule ligand linked to a targeting moiety by a linker is referred to as a “compound.”

Several embodiments are described by the following enumerated clauses. Any of the following embodiments in combination with any applicable embodiments described in the Summary section of this patent application, in the Detailed Description of the Illustrative Embodiments section, the Examples section, or the claims of this patent application, are also contemplated.

1. A method of treatment of a cancer, the method comprising
 - i) administering to a patient at least one dose of a CAR T cell composition comprising CAR T cells wherein the CAR T cells comprise a CAR directed to a targeting moiety;
 - ii) administering to the patient a compound, or a pharmaceutically acceptable salt thereof, wherein the compound comprises a small molecule ligand linked to a targeting moiety by a linker and wherein the compound, or the pharmaceutically acceptable salt thereof, is administered in at least a first dose escalation sequence and a second dose escalation sequence.
2. The method of clause 1 wherein the compound, or the pharmaceutically acceptable salt thereof, is administered in at least a first dose escalation sequence, a second dose escalation sequence, and a third dose escalation sequence.
3. The method of clause 1 wherein the compound, or the pharmaceutically acceptable salt thereof, is administered in at least a first dose escalation sequence, a second dose escalation sequence, a third dose escalation sequence, and a fourth dose escalation sequence.
4. The method of clause 1 wherein the compound, or the pharmaceutically acceptable salt thereof, is administered is administered in at least a first dose escalation sequence, a second dose escalation sequence, a third dose escalation sequence, a fourth dose escalation sequence, and a fifth dose escalation sequence.
5. The method of clause 1 wherein the compound, or the pharmaceutically acceptable salt thereof, is administered in at least a first dose escalation sequence, a second dose escalation sequence, a third dose escalation sequence, a fourth dose escalation sequence, a fifth dose escalation sequence, and a sixth dose escalation sequence.
6. The method of any one of clauses 1 to 5 wherein a first dose of the CAR T cells and a second dose of the CAR T cells are administered to the patient.
7. The method of clause 6 wherein the first dose of the CAR T cells is a test dose to monitor the patient for tolerability to the CAR T cells.

8. The method of clause 6 wherein the second dose of the CAR T cells comprises a higher dose of the CAR T cells than the first dose of the CAR T cells.

9. The method of any one of clauses 6 to 8 wherein the first dose of the CAR T cells comprises about 0.5×10^5 of the CAR T cells per kg of patient body weight to
5 about 1.5×10^6 of the CAR T cells per kg of patient body weight.

10. The method of any one of clauses 6 to 9 wherein the second dose of the CAR T cells comprises about 0.8×10^6 of the CAR T cells per kg of patient body weight to about 2×10^7 of the CAR T cells per kg of patient body weight.

11. The method of any one of clauses 1 to 10 wherein the first dose
10 escalation sequence is followed by a period of time during which the compound, or the pharmaceutically acceptable salt thereof, is not administered.

12. The method of any one of clauses 1 to 11 wherein the second dose escalation sequence is followed by a period of time during which the compound, or the pharmaceutically acceptable salt thereof, is not administered.

13. The method of any one of clauses 2 to 12 wherein the third dose
15 escalation sequence is followed by a period of time during which the compound, or the pharmaceutically acceptable salt thereof, is not administered.

14. The method of any one of clauses 3 to 13 wherein the fourth dose escalation sequence is followed by a period of time during which the compound, or the
20 pharmaceutically acceptable salt thereof, is not administered.

15. The method of any one of clauses 4 to 14 wherein the fifth dose escalation sequence is followed by a period of time during which the compound, or the pharmaceutically acceptable salt thereof, is not administered.

16. The method of any one of clauses 5 to 15 wherein the sixth dose
25 escalation sequence is followed by a period of time during which the compound, or the pharmaceutically acceptable salt thereof, is not administered.

17. The method of any one of clauses 11 to 16 wherein the period of time is about 7 days.

18. The method of any one of clauses 1 to 17 wherein the first dose
30 escalation sequence comprises administering to the patient about 1 percent, about 10 percent, and about 100 percent of a full dose of the compound, or the pharmaceutically acceptable salt thereof, on three separate days.

19. The method of any one of clauses 1 to 18 wherein the second dose escalation sequence comprises administering to the patient about 1 percent, about 30 percent, and about 300 percent of a full dose of the compound, or the pharmaceutically acceptable salt thereof, on three separate days.

5 20. The method of any one of clauses 2 to 19 wherein the third dose escalation sequence comprises administering to the patient about 1 percent, about 50 percent, and about 500 percent of a full dose of the compound, or the pharmaceutically acceptable salt thereof, on three separate days.

10 21. The method of any one of clauses 3 to 20 wherein the fourth dose escalation sequence comprises administering to the patient about 1 percent, about 10 percent, and about 100 percent of a full dose of the compound, or the pharmaceutically acceptable salt thereof, on three separate days.

15 22. The method of any one of clauses 4 to 21 wherein the fifth dose escalation sequence comprises administering to the patient about 1 percent, about 30 percent, and about 300 percent of a full dose of the compound, or the pharmaceutically acceptable salt thereof, on three separate days.

20 23. The method of any one of clauses 5 to 22 wherein the sixth dose escalation sequence comprises administering to the patient about 1 percent, about 50 percent, and about 500 percent of a full dose of the compound, or the pharmaceutically acceptable salt thereof, on three separate days.

24. The method of any one of clauses 18 to 23 wherein the full dose of the compound, or the pharmaceutically acceptable salt thereof, is about 10 µg/kg to about 50 µg/kg of the compound, or the pharmaceutically acceptable salt thereof.

25 25. The method of any one of clauses 18 to 24 wherein the full dose of the compound, or the pharmaceutically acceptable salt thereof, is about 20 µg/kg to about 40 µg/kg of the compound, or the pharmaceutically acceptable salt thereof.

26. The method of any one of clauses 18 to 25 wherein the full dose of the compound, or the pharmaceutically acceptable salt thereof, is about 25 µg/kg to about 35 µg/kg of the compound, or the pharmaceutically acceptable salt thereof.

30 27. The method of any one of clauses 18 to 26 wherein the full dose of the compound, or the pharmaceutically acceptable salt thereof, is about 30 µg/kg of the compound, or the pharmaceutically acceptable salt thereof.

28. The method of any one of clauses 6 to 27 wherein the first dose of the CAR T cells and the second dose of the CAR T cells are administered to the patient during week 1.

5 29. The method of any one of clauses 6 to 28 wherein the first dose of the CAR T cells and the second dose of the CAR T cells are administered to the patient during week 1 on Monday and Thursday.

30. The method of any one of clauses 1 to 29 wherein the first dose escalation sequence for the compound, or the pharmaceutically acceptable salt thereof, occurs during weeks 2 and 3.

10 31. The method of any one of clauses 1 to 30 wherein the second dose escalation sequence for the compound, or the pharmaceutically acceptable salt thereof, occurs during weeks 4 and 5.

15 32. The method of any one of clauses 2 to 31 wherein the third dose escalation sequence for the compound, or the pharmaceutically acceptable salt thereof, occurs during weeks 6 and 7.

33. The method of clause 30 wherein the compound, or the pharmaceutically acceptable salt thereof, is administered on three separate days and the three separate days are Monday and Thursday of week 2 and Monday of week 3.

20 34. The method of clause 31 wherein the compound, or the pharmaceutically acceptable salt thereof, is administered on three separate days and the three separate days are Monday and Thursday of week 4 and Monday of week 5.

35. The method of clause 32 wherein the compound, or the pharmaceutically acceptable salt thereof, is administered on three separate days and the three separate days are Monday and Thursday of week 6 and Monday of week 7.

25 36. The method of any one of clauses 1 to 35 wherein lymphocytes are depleted in the patient before administration of the CAR T cell composition to the patient.

30 37. The method of any one of clauses 1 to 36 further comprising administering platelets to the patient, administering packed red blood cells to the patient, administering cryoprecipitate to the patient, administering intravenous immunoglobulin to the patient, and/or providing antimicrobial therapy to the patient.

38. The method of any one of clauses 1 to 37 wherein, if no CRS or neurotoxicity is observed in the patient during the first dose escalation sequence, the method is advanced to the second dose escalation sequence.

39. The method of any one of clauses 2 to 38 wherein, if no CRS or
5 neurotoxicity is observed in the patient during the second dose escalation sequence, the method is advanced to the third dose escalation sequence.

40. The method of any one of clauses 3 to 39 wherein, if no CRS or neurotoxicity is observed in the patient during the third dose escalation sequence, the method is advanced to the fourth dose escalation sequence.

10 41. The method of any one of clauses 4 to 40 wherein, if no CRS or neurotoxicity is observed in the patient during the fourth dose escalation sequence, the method is advanced to the fifth dose escalation sequence.

42. The method of any one of clauses 5 to 41 wherein, if no CRS or neurotoxicity is observed in the patient during the fifth dose escalation sequence, the method is
15 advanced to the sixth dose escalation sequence.

43. The method of any one of clauses 1 to 42 wherein if fever without hypotension is observed in the patient and no neurotoxicity is observed in the patient during any one of the dose escalation sequences, all subsequent doses of the compound, or the pharmaceutically acceptable salt thereof, are administered to the patient at the dose escalation
20 sequence level that caused the fever without hypotension.

44. The method of any one of clauses 1 to 43 wherein, if serious CRS or neurotoxicity occurs in the patient in any dose escalation sequence, all subsequent doses of the compound, or the pharmaceutically acceptable salt thereof, are administered to the patient at the dose escalation sequence level below the dose escalation sequence level that caused the serious
25 CRS or neurotoxicity in the patient.

45. A method of treatment of a cancer, the method comprising
i) administering to a patient at least one dose of a CAR T cell composition comprising CAR T cells wherein the CAR T cells comprise a CAR directed to a targeting moiety;
30 ii) administering to the patient a compound, or a pharmaceutically acceptable salt thereof, wherein the compound comprises a small molecule ligand linked to a targeting moiety by a linker and wherein the compound, or the pharmaceutically acceptable salt

thereof, is administered in a first dose escalation sequence wherein, if serious CRS occurs in the first dose escalation sequence, the compound, or the pharmaceutically acceptable salt thereof, is administered using a lower dose escalation sequence wherein the first dose of the compound, or the pharmaceutically acceptable salt thereof, in the lower dose escalation sequence is lower
5 than the first dose of the compound, or the pharmaceutically acceptable salt thereof, administered in the first dose escalation sequence.

46. The method of clause 45 wherein in the lower dose escalation sequence, the compound, or the pharmaceutically acceptable salt thereof, is administered at about 0.5 percent, about 5 percent, and about 50 percent of a full dose of the compound, or the
10 pharmaceutically acceptable salt thereof, on three separate days.

47. The method of clause 46 wherein the full dose of the compound, or the pharmaceutically acceptable salt thereof, is about the full dose of the compound, or the pharmaceutically acceptable salt thereof, is about 10 µg/kg to about 50 µg/kg of the compound, or the pharmaceutically acceptable salt thereof.

48. The method of any one of clauses 46 to 47 wherein the full dose of the compound, or the pharmaceutically acceptable salt thereof, is about 25 µg/kg to about 35 µg/kg of the compound, or the pharmaceutically acceptable salt thereof.

49. The method of any one of clauses 46 to 48 wherein the full dose of the compound, or the pharmaceutically acceptable salt thereof, is about 30 µg/kg of the compound, or the pharmaceutically acceptable salt thereof.
20

50. The method of any one of clauses 1 to 49 wherein the ligand is selected from the group consisting of a folate, DUPA, an NK-1R ligand, a CAIX ligand, a ligand of gamma glutamyl transpeptidase, an NKG2D ligand, and a CCK2R ligand.

51. The method of any one of clauses 1 to 50 wherein the ligand is a folate.

52. The method of any one of clauses 1 to 50 wherein the ligand is an NK-1R ligand.
25

53. The method of any one of clauses 1 to 50 wherein the ligand is DUPA.

54. The method of any one of clauses 1 to 50 wherein the ligand is a CCK2R ligand.
30

55. The method of any one of clauses 1 to 50 wherein the ligand is a ligand of gamma glutamyl transpeptidase.

56. The method of any one of clauses 1 to 55 wherein the targeting moiety is selected from the group consisting of 2,4-dinitrophenol (DNP), 2,4,6-trinitrophenol (TNP), biotin, digoxigenin, fluorescein, fluorescein isothiocyanate (FITC), NHS-fluorescein, pentafluorophenyl ester, tetrafluorophenyl ester, a knottin, a centyrin, and a DARPin.

57. The method of any one of clauses 1 to 56 wherein the targeting moiety is FITC.

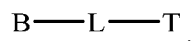
58. The method of any one of clauses 1 to 56 wherein the targeting moiety is DNP.

59. The method of any one of clauses 1 to 56 wherein the targeting moiety is TNP.

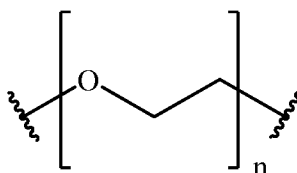
60. The method of any one of clauses 1 to 59 wherein the linker comprises polyethylene glycol (PEG), polyproline, a hydrophilic amino acid, a sugar, an unnatural peptidoglycan, a polyvinylpyrrolidone, pluronic F-127, or a combination thereof.

61. The method of any one of clauses 1 to 60 wherein the linker comprises PEG.

62. The method of any one of clauses 1 to 61 wherein the compound, or the pharmaceutically acceptable salt thereof, has the formula



wherein B represents the small molecule ligand, L represents the linker, and T represents the targeting moiety, and wherein L comprises a structure having the formula



wherein n is an integer from 0 to 200.

63. The method of clause 62 wherein n is an integer from 0 to 150.

64. The method of clause 62 wherein n is an integer from 0 to 110.

65. The method of clause 62 wherein n is an integer from 0 to 20.

66. The method of clause 62 wherein n is an integer from 15 to 20.

67. The method of clause 62 wherein n is an integer from 15 to 110.

68. The method of any one of clauses 1 to 67 wherein the cancer is selected from the group consisting of lung cancer, bone cancer, pancreatic cancer, skin cancer, cancer of the head, cancer of the neck, cutaneous melanoma, intraocular melanoma, uterine cancer, ovarian cancer, endometrial cancer, rectal cancer, stomach cancer, colon cancer, breast cancer, triple negative breast cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, Hodgkin's Disease, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, non-small cell lung cancer, cancer of the adrenal gland, sarcoma of soft tissue, osteosarcoma, including pediatric or non-pediatric osteosarcoma, cancer of the urethra, prostate cancer, chronic leukemia, acute leukemia, acute myelocytic leukemia, lymphocytic lymphoma, myeloid leukemia, myelomonocytic leukemia, hairy cell leukemia, pleural mesothelioma, cancer of the bladder, Burkitt's lymphoma, cancer of the ureter, cancer of the kidney, renal cell carcinoma, carcinoma of the renal pelvis, neoplasms of the central nervous system (CNS), primary CNS lymphoma, spinal axis tumors, brain stem glioma, pituitary adenoma, and adenocarcinoma of the gastroesophageal junction.

69. The method of any one of clauses 1 to 51 or 56 to 68 wherein the cancer is a folate receptor expressing cancer.

70. The method of any one of clauses 1 to 69 wherein the cancer is an osteosarcoma.

71. The method of any one of clauses 1 to 70 wherein the CAR has a recognition region and the recognition region is a single chain fragment variable (scFv) region of an antibody.

72. The method of any one of clauses 1 to 57 or 60 to 71 wherein the CAR has a recognition region and the recognition region of the CAR is a single chain fragment variable (scFv) region of an anti-FITC antibody.

73. The method of any one of clauses 1 to 72 wherein the CAR has a co-stimulation domain and the co-stimulation domain is selected from the group consisting of CD28, CD137 (4-1BB), CD134 (OX40), and CD278 (ICOS).

74. The method of any one of clauses 1 to 73 wherein the CAR has an activation signaling domain and the activation signaling domain is a T cell CD3 ζ chain or an Fc receptor γ .

75. The method of any one of clauses 1 to 57 or 60 to 74 wherein the CAR has a recognition region and the recognition region is a single chain fragment variable (scFv) region of an anti-FITC antibody, wherein the CAR has a co-stimulation domain and the co-stimulation domain is CD137 (4-1BB), and wherein the CAR has an activation signaling domain and the activation signaling domain is a T cell CD3 ζ chain.

76. The method of any one of clauses 1 to 75 wherein the patient is imaged prior to administration of the compound, or the pharmaceutically acceptable salt thereof, or prior to administration of the CAR T cell composition.

77. The method of any one of clauses 1 to 76 wherein the compound, or the pharmaceutically acceptable salt thereof, is not an antibody, and does not comprise a fragment of an antibody.

78. The method of any one of clauses 1 to 77 wherein the targeting moiety does not comprise a peptide epitope.

79. The method of any one of clauses 1 to 78 wherein cytokine release resulting in off-target toxicity in the patient does not occur and wherein CAR T cell toxicity to the cancer occurs.

80. The method of any one of clauses 1 to 78 wherein off-target tissue toxicity does not occur in the patient and wherein CAR T cell toxicity to the cancer occurs.

81. The method of any one of clauses 1 to 78 wherein the cancer comprises a tumor, wherein tumor size is reduced in the patient, and wherein off-target toxicity does not occur.

82. The method of any one of clauses 1 to 81 wherein the CAR T cells comprise a nucleic acid comprising SEQ ID NO:1.

83. The method of any one of clauses 1 to 82 wherein the CAR T cells comprise a polypeptide comprising SEQ ID NO:2.

84. The method of clause 82 wherein the nucleic acid encodes a chimeric antigen receptor.

85. The method of any one of clauses 1 to 81 wherein the CAR T cells comprise a nucleic acid comprising SEQ ID NO:4.

86. The method of any one of clauses 1 to 81 or 85 wherein the CAR T cells comprise a polypeptide comprising SEQ ID NO:5.

87. The method of clause 85 wherein the nucleic acid encodes a chimeric antigen receptor.

88. The method of any one of clauses 1 to 87 wherein the CAR comprises humanized amino acid sequences.

5 89. The method of any one of clauses 1 to 87 wherein the CAR consists of humanized amino acid sequences.

90. The method of any one of clauses 1 to 89 further comprising administering to the patient a folate, a conjugate comprising a folate wherein the conjugate comprising a folate does not comprise a targeting moiety, or an agent that inhibits activation of
10 the CAR T cells.

91. The method of clause 90 wherein a folate is administered.

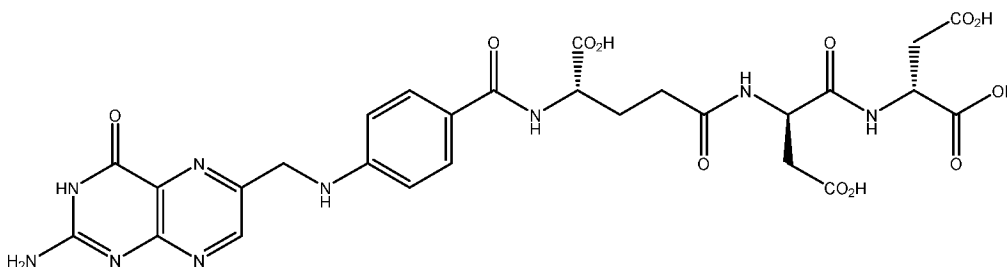
92. The method of clause 90 wherein folic acid or leucovorin is administered.

93. The method of clause 90 wherein the conjugate comprising a folate is
15 administered.

94. The method of clause 93 wherein the conjugate comprising a folate comprises a folate linked to one or more amino acids.

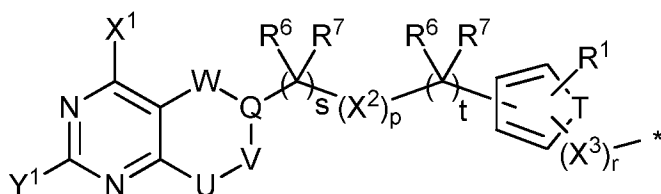
95. The method of clause 93 wherein the conjugate comprising a folate has the formula

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96. The method of clause 91 wherein the folate has the formula

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wherein X^1 and Y^1 are each-independently selected from the group consisting of halo, R^2 , OR^2 , SR^3 , and NR^4R^5 ;

- 5 U, V, and W represent divalent moieties each independently selected from the group consisting of $-(R^{6a})C=$, $-N=$, $-(R^{6a})C(R^{7a})-$, and $-N(R^{4a})-$; Q is selected from the group consisting of C and CH; T is selected from the group consisting of S, O, N, and $-C=C-$;

X^2 and X^3 are each independently selected from the group consisting of oxygen, sulfur, $-C(Z)-$, $-C(Z)O-$, $-OC(Z)-$, $-N(R^{4b})-$, $-C(Z)N(R^{4b})-$, $-N(R^{4b})C(Z)-$,

- 10 $-OC(Z)N(R^{4b})-$, $-N(R^{4b})C(Z)O-$, $-N(R^{4b})C(Z)N(R^{5b})-$, $-S(O)-$, $-S(O)_2-$, $-N(R^{4a})S(O)_2-$, $-C(R^{6b})(R^{7b})-$, $-N(C\equiv CH)-$, $-N(CH_2C\equiv CH)-$, C_1 - C_{12} alkylene, and C_1 - C_{12} alkyneoxy, where Z is oxygen or sulfur;

R^1 is selected-from the group consisting of hydrogen, halo, C_1 - C_{12} alkyl, and C_1 - C_{12} alkoxy;

- 15 R^2 , R^3 , R^4 , R^{4a} , R^{4b} , R^5 , R^{5b} , R^{6b} , and R^{7b} are each independently selected from the group consisting of hydrogen, halo, C_1 - C_{12} alkyl, C_1 - C_{12} alkoxy, C_1 - C_{12} alkanoyl, C_1 - C_{12} alkenyl, C_1 - C_{12} alkynyl, $(C_1$ - C_{12} alkoxy)carbonyl, and $(C_1$ - C_{12} alkylamino)carbonyl;

R^6 and R^7 are each independently selected from the group consisting of hydrogen, halo, C_1 - C_{12} alkyl, and C_1 - C_{12} alkoxy; or, R^6 and R^7 are taken together to form a

- 20 carbonyl group;

R^{6a} and R^{7a} are each independently selected from the group consisting of hydrogen, halo, C_1 - C_{12} alkyl, and C_1 - C_{12} alkoxy; or R^{6a} and R^{7a} are taken together to form a carbonyl group;

p, r, s, and t are each independently either 0 or 1; and

- 25 * represents an optional covalent bond to the rest of the conjugate, if any additional chemical moieties are part of the folate.

97. The method of clause 90 wherein the agent that inhibits activation of the CAR T cells is selected from the group consisting of a lymphocyte-specific protein tyrosine kinase inhibitor, a PI3 kinase inhibitor, an inhibitor of an IL-2 inducible

T cell kinase, a JAK inhibitor, a BTK inhibitor, EC2319, and an agent that blocks CAR T cell binding to the compound, or the pharmaceutically acceptable salt thereof, but does not bind to the cancer.

98. The method of clause 90 wherein the agent that inhibits activation
5 of the CAR T cells is administered and the agent is a lymphocyte-specific protein tyrosine kinase inhibitor.

99. The method of clause 98 wherein the lymphocyte-specific protein tyrosine kinase inhibitor is Dasatinib.

100. The method of clause 90 wherein the agent that inhibits activation
10 of the CAR T cells is administered and the agent is a PI3 kinase inhibitor.

101. The method of clause 100 wherein the PI3 kinase inhibitor is GDC0980.

102. The method of clause 90 wherein the agent that inhibits activation
15 of the CAR T cells is administered and the agent is an IL-2 inducible T cell kinase inhibitor.

103. The method of clause 102 wherein the IL-2 inducible T cell kinase inhibitor is BMS-509744.

104. The method of clause 90 wherein the agent that inhibits activation
20 of the CAR T cells is administered and is an agent that blocks CAR T cell binding to the compound, or the pharmaceutically acceptable salt thereof, but does not bind to the cancer.

105. The method of clause 104 wherein the agent is fluoresceinamine, FITC, or sodium fluorescein.

106. The method of clause 105 wherein the agent is sodium
25 fluorescein.

107. The method of any one of clauses 90 to 106 wherein administration of the folate, the conjugate comprising a folate wherein the conjugate comprising a folate does not comprise a targeting moiety, or the agent that inhibits activation of the CAR T cells causes reduction in cytokine levels in the patient.

30 108. The method of clause 107 wherein the reduction in cytokine levels is a reduction to about the cytokine levels in an untreated patient.

109. The method of any one of clauses 90 to 108 wherein the cancer comprises a tumor and tumor size in the patient is not increased when the folate, the conjugate comprising a folate wherein the conjugate comprising a folate does not comprise a targeting moiety, or the agent that inhibits activation of the CAR T cells is administered to the patient.

5 110. The method of any one of clauses 104 to 106 wherein the agent that inhibits activation of the CAR T cells is administered to the patient when the CRS grade reaches 1, 2, 3, or 4.

111. The method of clause 110 wherein the agent that inhibits activation of the CAR T cells is administered to the patient when the CRS grade reaches 3 or 4.

10 112. The method of any one of clauses 104 to 106 wherein the agent that inhibits activation of the CAR T cells is administered at a dose of about .01 to about 300 umoles/kg of body weight of the patient.

113. The method of any one of clauses 1 to 112 wherein CRS is reduced or prevented in the patient and the method results in a decrease in tumor volume in the patient.

15 114. The method of any one of clauses 1 to 113 wherein body weight loss due to CRS is reduced or prevented.

115. The method of any one of clauses 90 to 114 further comprising re-administering the compound, or the pharmaceutically acceptable salt thereof, to the patient.

20 116. The method of clause 115 wherein the subsequent administration of the compound, or the pharmaceutically acceptable salt thereof, causes CAR T cell activation and an increase in cytokine levels in the patient.

117. The method of any one of clauses 1 to 116 wherein the CAR T cell composition is administered before the compound, or the pharmaceutically acceptable salt thereof.

25 118. The method of any one of clauses 1 to 117 wherein the CAR T cells are autologous.

119. The method of any one of clauses 2 to 17 wherein the first dose escalation sequence comprises administering to the patient about 1 percent, about 2 percent, and about 20 percent of a full dose of the compound, or the pharmaceutically acceptable salt thereof, on three separate days, wherein the second dose escalation sequence comprises administering to the patient about 1 percent, about 6 percent, and about 60 percent of a full dose of the compound, or the pharmaceutically acceptable salt thereof, on three separate days, and

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wherein the third dose escalation sequence comprises administering to the patient about 1 percent, about 10 percent, and about 100 percent of a full dose of the compound, or the pharmaceutically acceptable salt thereof, on three separate days.

120. The ;method of any one of clauses 2 to 17 wherein the first dose
5 escalation sequence comprises administering to the patient about 1 percent, about 10 percent, and about 100 percent of a full dose of the compound, or the pharmaceutically acceptable salt thereof, on three separate days, and wherein the second dose escalation sequence comprises administering to the patient about 1 percent, about 20 percent, and about 200 percent of a full dose of the compound, or the pharmaceutically acceptable salt thereof, on three separate days.

10 121. The ;method of any one of clauses 2 to 17 wherein the first dose escalation sequence comprises administering to the patient about 1 percent, about 10 percent, and about 100 percent of a full dose of the compound, or the pharmaceutically acceptable salt thereof, on three separate days, and wherein the second dose escalation sequence comprises administering to the patient about 1 percent, about 10 percent, and about 100 percent of a full
15 dose of the compound, or the pharmaceutically acceptable salt thereof, on three separate days.

122. The ;method of any one of clauses 2 to 17 wherein the first dose escalation sequence comprises administering to the patient about 1 percent, about 20 percent, and about 200 percent of a full dose of the compound, or the pharmaceutically acceptable salt thereof, on three separate days, and wherein the second dose escalation sequence comprises
20 administering to the patient about 1 percent, about 20 percent, and about 200 percent of a full dose of the compound, or the pharmaceutically acceptable salt thereof, on three separate days.

123. The method of any one of clauses 119 to 122 wherein the full dose of the compound, or the pharmaceutically acceptable salt thereof, is about 500 nmoles/kg.

Accordingly, various embodiments are provided in the preceding
25 paragraphs and in the clause list above, and all applicable embodiments described in this “Detailed Description of Illustrative Embodiments,” the “Summary” section, the Examples, and the claims apply to the these embodiments.

As described herein, a “patient” can be a human or, in the case of veterinary applications, the patient can be a laboratory, an agricultural, a domestic, or a wild animal. In
30 various aspects, the patient can be a laboratory animal such as a rodent (*e.g.*, mouse, rat, hamster, etc.), a rabbit, a monkey, a chimpanzee, a domestic animal such as a dog, a cat, or a rabbit, an agricultural animal such as a cow, a horse, a pig, a sheep, a goat, or a wild animal in

captivity such as a bear, a panda, a lion, a tiger, a leopard, an elephant, a zebra, a giraffe, a gorilla, a dolphin, or a whale.

In various embodiments, the cancer to be treated can be selected from a carcinoma, a sarcoma, a lymphoma, a melanoma, a mesothelioma, a nasopharyngeal carcinoma, a leukemia, an adenocarcinoma, or a myeloma. In other embodiments, the cancer may be lung cancer, bone cancer, pancreatic cancer, skin cancer, cancer of the head, cancer of the neck, cutaneous melanoma, intraocular melanoma, uterine cancer, ovarian cancer, endometrial cancer, rectal cancer, stomach cancer, colon cancer, breast cancer, triple negative breast cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, Hodgkin's Disease, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, non-small cell lung cancer, cancer of the adrenal gland, sarcoma of soft tissue, osteosarcoma, including pediatric or non-pediatric osteosarcoma, cancer of the urethra, prostate cancer, chronic leukemia, acute leukemia, including acute myelocytic leukemia, a lymphocytic lymphoma, myeloid leukemia, myelomonocytic leukemia, hairy cell leukemia, pleural mesothelioma, cancer of the bladder, Burkitt's lymphoma, cancer of the ureter, cancer of the kidney, renal cell carcinoma, carcinoma of the renal pelvis, a neoplasm of the central nervous system (CNS), primary CNS lymphoma, a spinal axis tumor, a brain stem glioma, a pituitary adenoma, and an adenocarcinoma of the gastroesophageal junction.

In some aspects of these embodiments, the cancer is a folate receptor expressing cancer. In another embodiment, the cancer is a folate receptor α -expressing cancer. In yet another embodiment, the cancer is a folate receptor β -expressing cancer. In some aspects of these embodiments, the cancer is an endometrial cancer, a non-small cell lung cancer, an ovarian cancer, an osteosarcoma, including pediatric or non-pediatric osteosarcoma, or a triple-negative breast cancer. In another embodiment, the cancer being treated is a tumor. In another embodiment, the cancer is malignant. In another embodiment, the cancer is an osteosarcoma including pediatric or non-pediatric osteosarcoma.

In one embodiment, the "small molecule ligand" can be a folate, DUPA (a ligand bound by PSMA-positive human prostate cancer cells and other cancer cell types), an NK-1R ligand (receptors for the NK-1R ligand are found, for example, on cancers of the colon and pancreas), a CAIX ligand (receptors for the CAIX ligand are found, for example, on renal, ovarian, vulvar, and breast cancers), a ligand of gamma glutamyl transpeptidase (the

transpeptidase is overexpressed, for example, in ovarian cancer, colon cancer, liver cancer, astrocytic gliomas, melanomas, and leukemias), an NKG2D ligand (receptors for the NKG2D ligand are found, for example, on cancers of the lung, colon, kidney, prostate, and on T and B cell lymphomas), or a CCK2R ligand (receptors for the CCK2R ligand are found on cancers of the thyroid, lung, pancreas, ovary, brain, stomach, gastrointestinal stroma, and colon, among others), each of which is a small molecule ligand that binds specifically to a cancer cell type (i.e., the receptor for each of these ligands can be overexpressed on cancers compared to normal tissues).

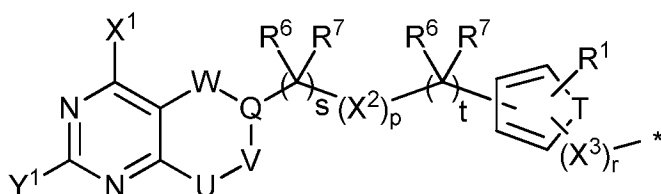
In one embodiment, the small molecule ligand may have a mass of less than about 10,000 Daltons, less than about 9000 Daltons, less than about 8,000 Daltons, less than about 7000 Daltons, less than about 6000 Daltons, less than about 5000 Daltons, less than about 4500 Daltons, less than about 4000 Daltons, less than about 3500 Daltons, less than about 3000 Daltons, less than about 2500 Daltons, less than about 2000 Daltons, less than about 1500 Daltons, less than about 1000 Daltons, or less than about 500 Daltons. In another embodiment, the small molecule ligand may have a mass of about 1 to about 10,000 Daltons, about 1 to about 9000 Daltons, about 1 to about 8,000 Daltons, about 1 to about 7000 Daltons, about 1 to about 6000 Daltons, about 1 to about 5000 Daltons, about 1 to about 4500 Daltons, about 1 to about 4000 Daltons, about 1 to about 3500 Daltons, about 1 to about 3000 Daltons, about 1 to about 2500 Daltons, about 1 to about 2000 Daltons, about 1 to about 1500 Daltons, about 1 to about 1000 Daltons, or about 1 to about 500 Daltons.

In one embodiment, a DUPA derivative can be the ligand of the small molecule ligand linked to a targeting moiety, and DUPA derivatives are described in WO 2015/057852, incorporated herein by reference.

In one embodiment, the small molecule ligand in the context of the “small molecule ligand linked to a linker” is a folate. In various embodiments, the folate can be folic acid, a folic acid analog, or another folate receptor-binding molecule. In various embodiments, analogs of folate that can be used include folinic acid (e.g., leucovorin), pteropolyglutamic acid, and folate receptor-binding pteridines such as tetrahydropterins, dihydrofolates, tetrahydrofolates, and their deaza and dideaza analogs. The terms “deaza” and “dideaza” analogs refers to the art recognized analogs having a carbon atom substituted for one or two nitrogen atoms in the naturally occurring folic acid structure. For example, the deaza analogs include the 1-deaza, 3-deaza, 5-deaza, 8-deaza, and 10-deaza analogs. The dideaza analogs

include, for example, 1,5 dideaza, 5,10-dideaza, 8,10-dideaza, and 5,8-dideaza analogs. The foregoing folic acid analogs are conventionally termed “folates,” reflecting their capacity to bind to folate receptors. Other folate receptor-binding analogs include aminopterin, amethopterin (methotrexate), N10-methylfolate, 2-deamino-hydroxyfolate, deaza analogs such as 1-deazamethopterin or 3-deazamethopterin, and 3',5'-dichloro-4-amino-4-deoxy-N10-methylpteroylglutamic acid (dichloromethotrexate).

In another embodiment, the small molecule ligand in the context of the “small molecule ligand linked to a linker” can have the formula



wherein X^1 and Y^1 are each-independently selected from the group consisting of halo, R^2 , OR^2 , SR^3 , and NR^4R^5 ;

U, V, and W represent divalent moieties each independently selected from the group consisting of $-(R^{6a})C=$, $-N=$, $-(R^{6a})C(R^{7a})-$, and $-N(R^{4a})-$; Q is selected from the group consisting of C and CH; T is selected from the group consisting of S, O, N, and $-C=C-$;

X^2 and X^3 are each independently selected from the group consisting of oxygen, sulfur, $-C(Z)-$, $-C(Z)O-$, $-OC(Z)-$, $-N(R^{4b})-$, $-C(Z)N(R^{4b})-$, $-N(R^{4b})C(Z)-$,

$-OC(Z)N(R^{4b})-$, $-N(R^{4b})C(Z)O-$, $-N(R^{4b})C(Z)N(R^{5b})-$, $-S(O)-$, $-S(O)_2-$, $-N(R^{4a})S(O)_2-$, $-C(R^{6b})(R^{7b})-$, $-N(C\equiv CH)-$, $-N(CH_2C\equiv CH)-$, C_1-C_{12} alkylene, and C_1-C_{12} alkyneoxy, where Z is oxygen or sulfur;

R^1 is selected-from the group consisting of hydrogen, halo, C_1-C_{12} alkyl, and C_1-C_{12} alkoxy;

R^2 , R^3 , R^4 , R^{4a} , R^{4b} , R^5 , R^{5b} , R^{6b} , and R^{7b} are each independently selected from the group consisting of hydrogen, halo, C_1-C_{12} alkyl, C_1-C_{12} alkoxy, C_1-C_{12} alkanoyl, C_1-C_{12} alkenyl, C_1-C_{12} alkynyl, $(C_1-C_{12}$ alkoxy)carbonyl, and $(C_1-C_{12}$ alkylamino)carbonyl;

R^6 and R^7 are each independently selected from the group consisting of hydrogen, halo, C_1 - C_{12} alkyl, and C_1 - C_{12} alkoxy; or, R^6 and R^7 are taken together to form a carbonyl group;

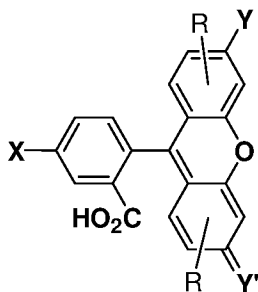
R^{6a} and R^{7a} are each independently selected from the group consisting of hydrogen, halo, C_1 - C_{12} alkyl, and C_1 - C_{12} alkoxy; or R^{6a} and R^{7a} are taken together to form a carbonyl group;

p, r, s, and t are each independently either 0 or 1; and

* represents an optional covalent bond to the rest of the conjugate, if any additional chemical moieties are part of the folate.

In one aspect, the “targeting moiety” that binds to the CAR expressed by CAR T cells can be selected, for example, from 2,4-dinitrophenol (DNP), 2,4,6-trinitrophenol (TNP), biotin, digoxigenin, fluorescein, fluorescein isothiocyanate (FITC), NHS-fluorescein, pentafluorophenyl ester, tetrafluorophenyl ester, a knottin, a centyrin, a DARPin, an affibody, an affilin, an anticalin, an atrimer, an avimer, a bicyclic peptide, an FN3 scaffold, a cys-knot, a fynomer, a Kunitz domain, or an Obody. The identity of the targeting moiety is limited only in that it should be recognized and bound by the CAR, preferably with specificity, and that it has a relatively low molecular weight. In various aspects, exemplary targeting moieties are haptens, including small molecular weight organic molecules.

In one illustrative embodiment, the targeting moiety can have the following illustrative structure:



where X is oxygen, nitrogen, or sulfur, and where X is attached to linker L; Y is OR^a , NR^{a_2} , or NR^{a_3+} ; and Y' is O, NR^a , or NR^{a_2+} ; where each R is independently selected in each instance from H, fluoro, sulfonic acid, sulfonate, and salts thereof, and the like; and R^a is hydrogen or alkyl.

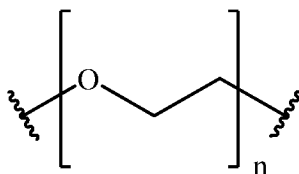
In one illustrative aspect, the linker can comprise polyethylene glycol (PEG), polyproline, a hydrophilic amino acid, a sugar, an unnatural peptidoglycan, a polyvinylpyrrolidone, pluronic F-127, or a combination thereof.

In another illustrative aspect, the linker in the compound, or pharmaceutically acceptable salt thereof, described herein can comprise a direct linkage (e.g., a reaction between the isothiocyanate group of FITC and a free amine group of a small molecule ligand) or the linkage can be through an intermediary linker. In one embodiment, if present, an intermediary
 5 linker can be any biocompatible linker known in the art, such as a divalent linker. In one illustrative embodiment, the divalent linker can comprise about 1 to about 30 carbon atoms. In another illustrative embodiment, the divalent linker can comprise about 2 to about 20 carbon atoms. In other embodiments, lower molecular weight divalent linkers (i.e., those having an approximate molecular weight of about 30 to about 300 Daltons) are employed. In another
 10 embodiment, linker lengths that are suitable include, but are not limited to, linkers having 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39 or 40, or more atoms.

In various embodiments, the small molecule ligand linked to a targeting moiety can be of the formula



wherein B represents the small molecule ligand, L represents the linker, and T represents the targeting moiety, and wherein L comprises a structure having the formula

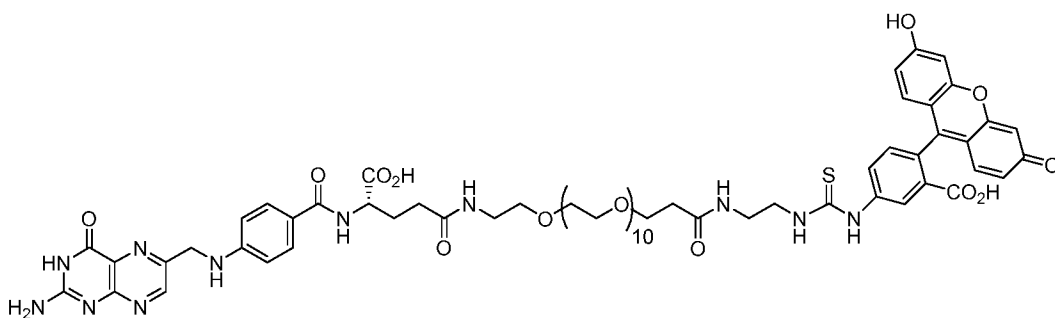
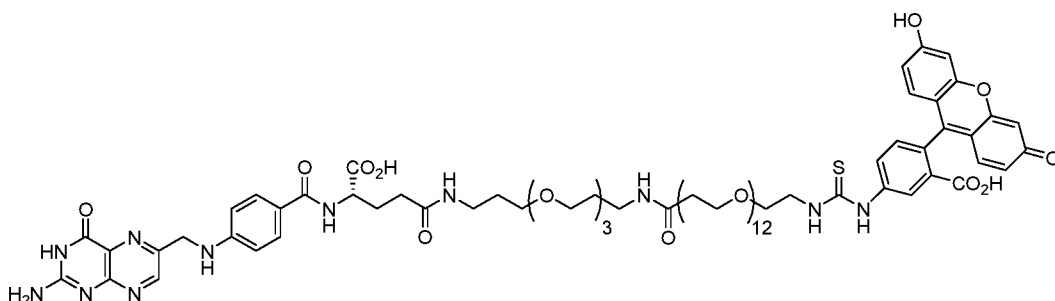
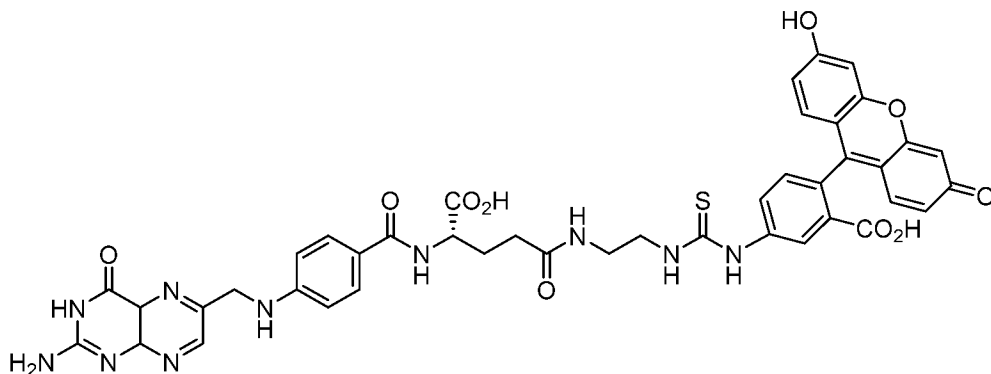


wherein n is an integer from 0 to 200. In another embodiment, n can be an integer from 0 to
 20 150, 0 to 110, 0 to 100, 0 to 90, 0 to 80, 0 to 70, 0 to 60, 0 to 50, 0 to 40, 0 to 30, 0 to 20, 0 to 15, 0 to 14, 0 to 13, 0 to 12, 0 to 11, 0 to 10, 0 to 9, 0 to 8, 0 to 7, 0 to 6, 0 to 5, 0 to 4, 0 to 3, 0 to 2, 0 to 1, 15 to 16, 15 to 17, 15 to 18, 15 to 19, 15 to 20, 15 to 21, 15 to 22, 15 to 23, 15 to 24, 15 to 25, 15 to 26, 15 to 27, 15 to 28, 15 to 29, 15 to 30, 15 to 31, 15 to 32, 15 to 33, 15 to 34, 15 to 35, 15 to 36, 15 to 37, 15 to 38, 15 to 39, 15 to 40, 15 to 50, 15 to 60, 15 to 70, 15 to
 25 80, 15 to 90, 15 to 100, 15 to 110, 15 to 120, 15 to 130, 15 to 140, 15 to 150, or n can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 50, 60, 70, 80, 90, 100, 108, 110, 120, 130, 140, or 150.

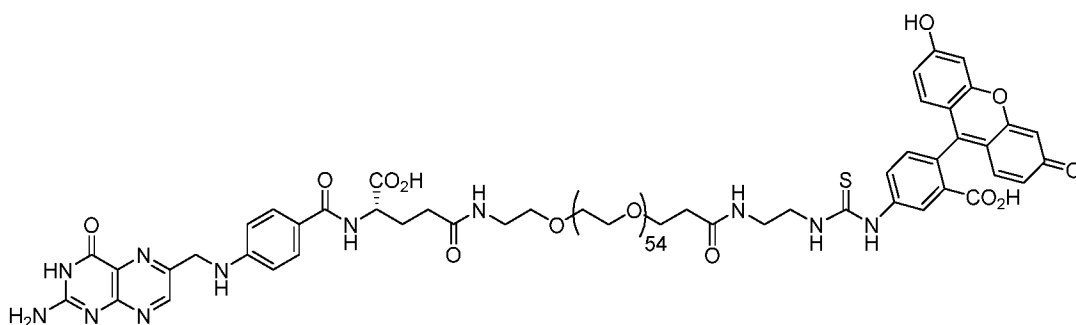
In another embodiment, the linker may be a divalent linker that may include one or more spacers. Illustrative spacers are shown in the following table. The following non-

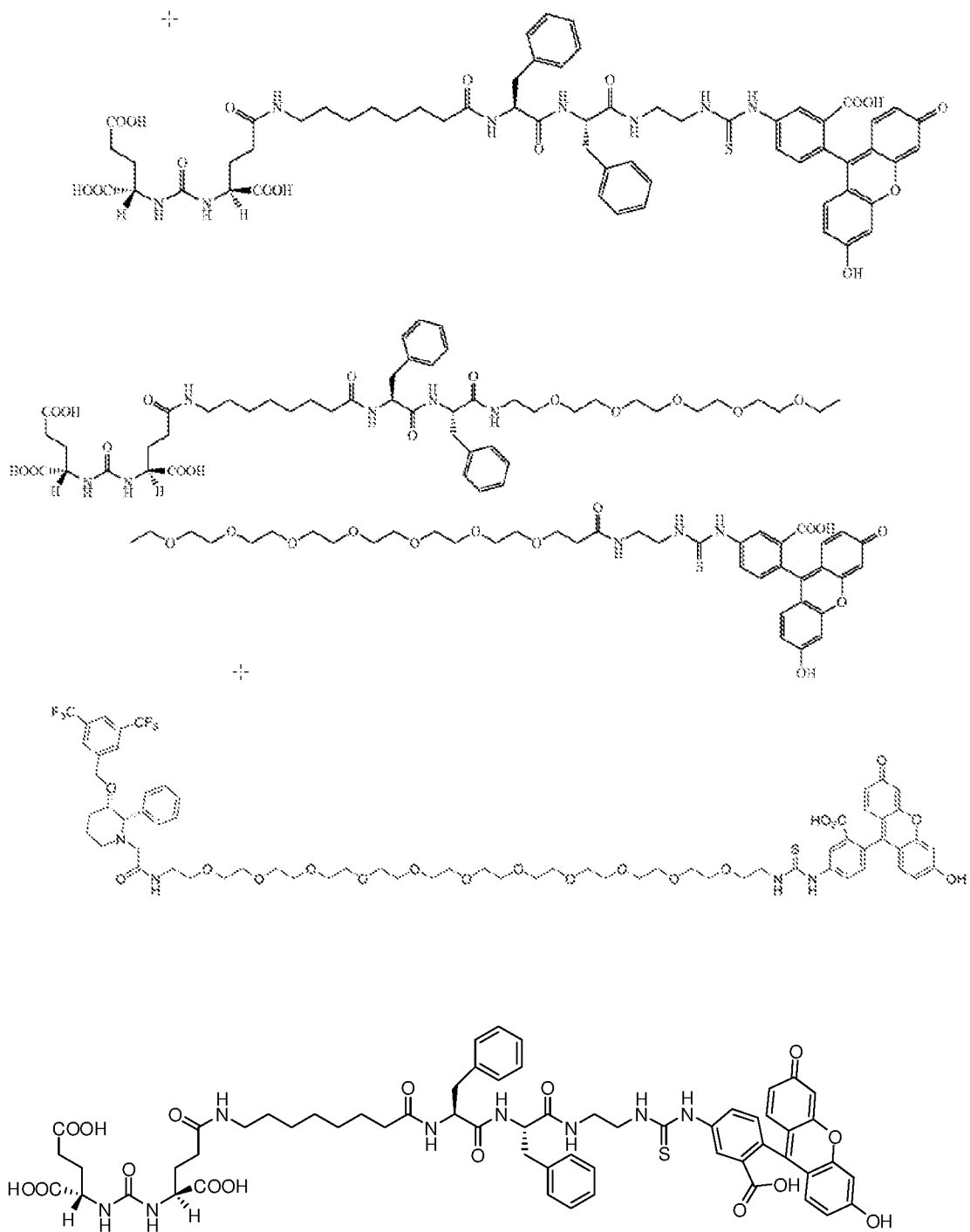
limiting, illustrative spacers are described where * indicates the point of attachment to the small molecule ligand or to the targeting moiety, or to other divalent linker portions.

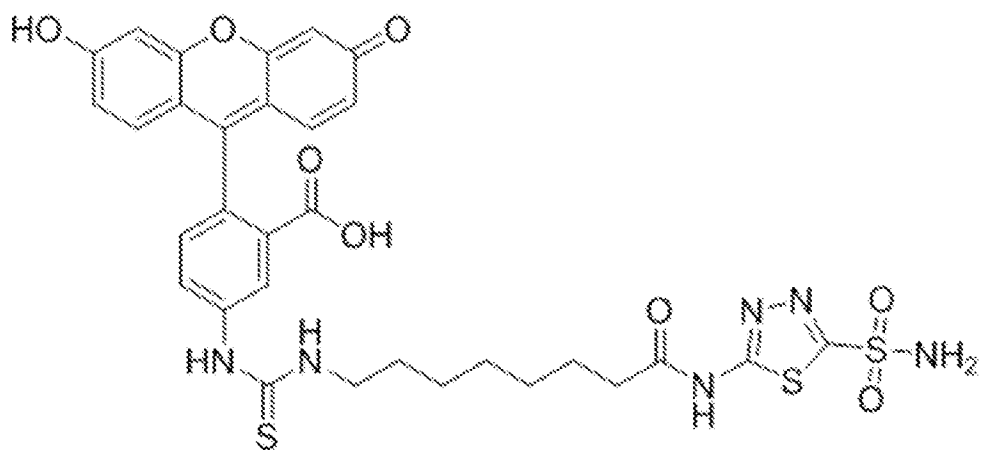
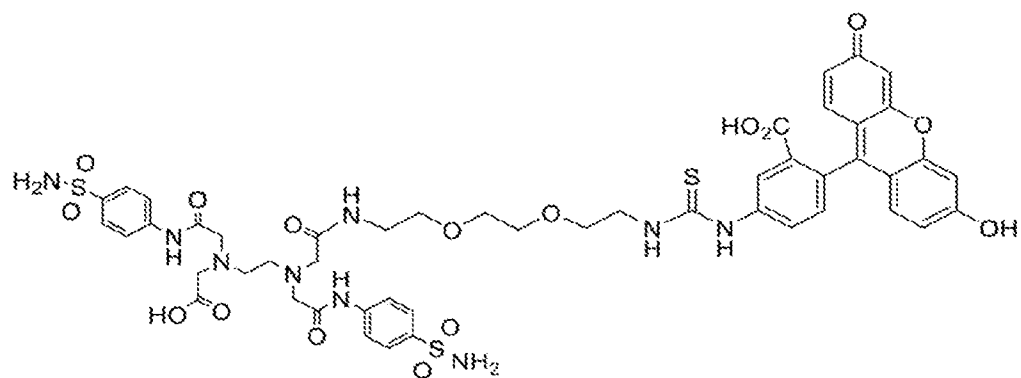
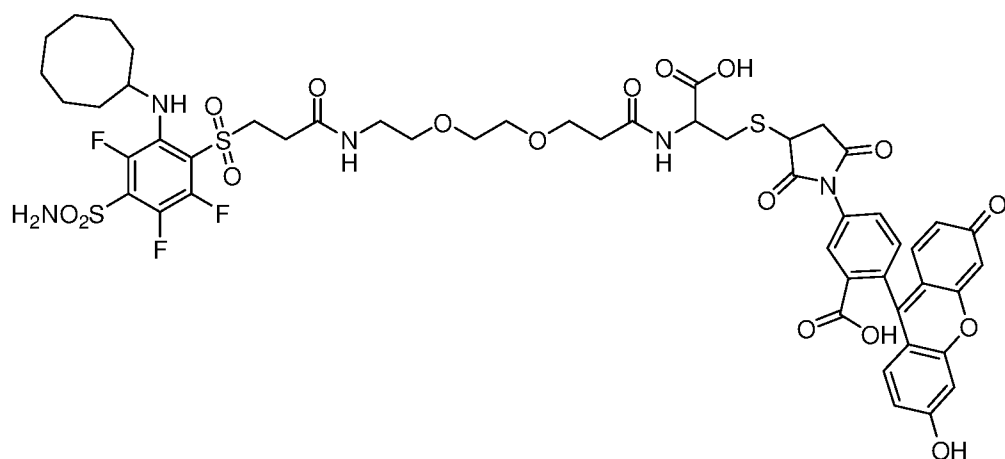
In other embodiments, the small molecule ligand linked to a targeting moiety (bridge) can have any of the following structures.

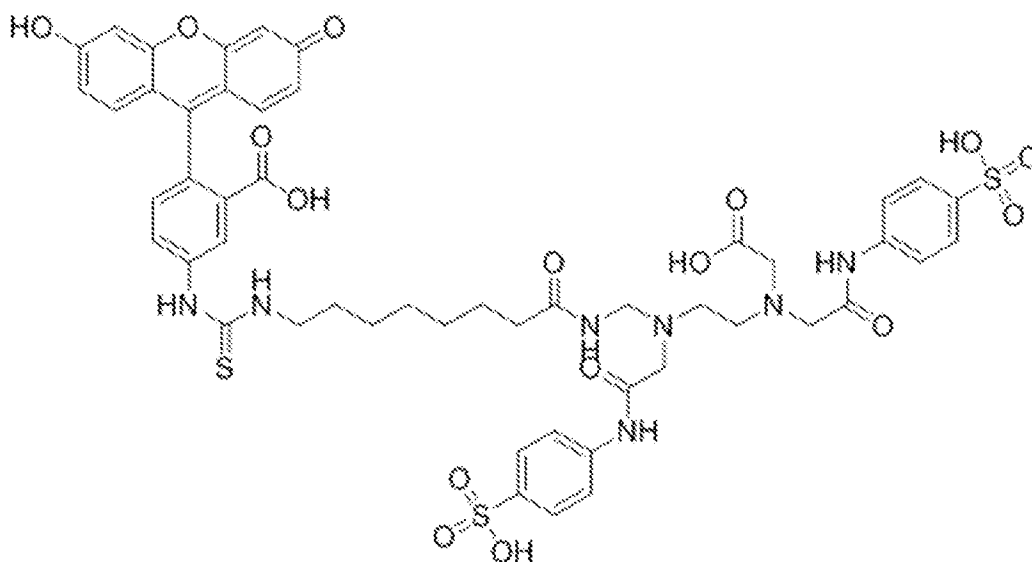


5









In other embodiments, the compound, or the pharmaceutically acceptable salt thereof, is not an antibody, and does not comprise a fragment of an antibody. In yet another embodiment, the targeting moiety does not comprise a peptide epitope.

In one illustrative embodiment, the small molecule ligand linked to a targeting moiety by a linker (the bridge) comprises fluorescein isothiocyanate (FITC) linked to the small molecule ligand. In one aspect, the cancer may overexpress a receptor for the small molecule ligand. In another aspect, for example, cytotoxic T cells, or another type of T cell, can be transformed to express a CAR that comprises anti-FITC scFv. In this aspect, the CAR may target FITC wherein the cancer is decorated with FITC molecules as a result of binding of the small molecule ligand to the cancer. Thus, toxicity to normal, non-target cells can be avoided or reduced. In this embodiment, when the anti-FITC CAR-expressing T cells bind FITC, the CAR T cells are activated and the cancer is ameliorated.

A “pharmaceutically acceptable salt” of a small molecule ligand linked to a targeting moiety by a linker is contemplated. As used herein, the term “pharmaceutically acceptable salt” refers to those salts whose counter ions may be used in pharmaceuticals. In various embodiments, such salts include, but are not limited to 1) acid addition salts, which can be obtained by reaction of the free base of the parent compound with inorganic acids such as hydrochloric acid, hydrobromic acid, nitric acid, phosphoric acid, sulfuric acid, and perchloric acid and the like, or with organic acids

such as acetic acid, oxalic acid, (D) or (L) malic acid, maleic acid, methane sulfonic acid, ethanesulfonic acid, p-toluenesulfonic acid, salicylic acid, tartaric acid, citric acid, succinic acid or malonic acid and the like; or 2) salts formed when an acidic proton present in the parent compound either is replaced by a metal ion, e.g., an alkali metal ion, an alkaline earth ion, or an aluminum ion; or coordinates with an organic base such as ethanolamine, diethanolamine, triethanolamine, trimethamine, N-methylglucamine, and the like. Pharmaceutically acceptable salts are well-known to those skilled in the art, and any such pharmaceutically acceptable salt is contemplated in connection with the embodiments described herein.

In various embodiments, suitable acid addition salts are formed from acids which form non-toxic salts. Illustrative examples include the acetate, aspartate, benzoate, besylate, bicarbonate/carbonate, bisulphate/sulphate, borate, camsylate, citrate, edisylate, esylate, formate, fumarate, gluceptate, gluconate, glucuronate, hexafluorophosphate, hibenzenate, hydrochloride/chloride, hydrobromide/bromide, hydroiodide/iodide, isethionate, lactate, malate, maleate, malonate, mesylate, methylsulphate, naphthylate, 2-napsylate, nicotinate, nitrate, orotate, oxalate, palmitate, pamoate, phosphate/hydrogen phosphate/dihydrogen phosphate, saccharate, stearate, succinate, tartrate, tosylate and trifluoroacetate salts.

In various embodiments, suitable base salts are formed from bases which form non-toxic salts. Illustrative examples include the arginine, benzathine, calcium, choline, diethylamine, diolamine, glycine, lysine, magnesium, meglumine, olamine, potassium, sodium, tromethamine and zinc salts. Hemisalts of acids and bases may also be formed, for example, hemisulphate and hemicalcium salts.

In one illustrative aspect, the compound, or a pharmaceutically salt thereof, described herein may contain one or more chiral centers, or may otherwise be capable of existing as multiple stereoisomers. Accordingly, various embodiments may include pure stereoisomers as well as mixtures of stereoisomers, such as enantiomers, diastereomers, and enantiomerically or diastereomerically enriched mixtures. In one aspect, the compound, or pharmaceutically acceptable salt thereof, described herein may be capable of existing as geometric isomers. Accordingly, various embodiments may include pure geometric isomers or mixtures of geometric isomers.

In some aspects, the compound, or pharmaceutically acceptable salt thereof, described herein may exist in unsolvated forms as well as solvated forms, including hydrated

forms. In general, the solvated forms are equivalent to unsolvated forms and are encompassed within the scope of the present invention.

The methods described herein also utilize T lymphocytes (e.g., cytotoxic T lymphocytes) engineered to express a chimeric antigen receptor (CAR) that recognizes and binds to the targeting moiety (e.g., FITC, DNP, or TNP) of the bridge. In one embodiment, the CARs described herein comprise three domains including 1) a recognition region (e.g., a single chain fragment variable (scFv) region of an antibody, a Fab fragment, and the like) which recognizes and binds to the targeting moiety with specificity, 2) a co-stimulation domain which enhances the proliferation and survival of the T lymphocytes, and 3) an activation signaling domain which generates a T lymphocyte activation signal.

In various aspects, as non-limiting examples, scFv regions of antibodies that bind 2,4-dinitrophenol (DNP), 2,4,6-trinitrophenol (TNP), biotin, digoxigenin, fluorescein, fluorescein isothiocyanate (FITC), NHS-fluorescein, pentafluorophenyl ester, tetrafluorophenyl ester, a knottin, a centyrin, a DARPin, an affibody, an affilin, an anticalin, an atrimer, an avimer, a bicyclic peptide, an FN3 scaffold, a cys-knot, a fynomer, a Kunitz domain, or an Obody can be used. In illustrative non-limiting embodiments, the scFv regions can be prepared from (i) an antibody known in the art that binds a targeting moiety, (ii) an antibody newly prepared using a selected targeting moiety, such as a hapten, and (iii) sequence variants derived from the scFv regions of such antibodies, e.g., scFv regions having at least about 80%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 99.5% sequence identity with the amino acid sequence of the scFv region from which they are derived.

In one aspect, the co-stimulation domain serves to enhance the proliferation and survival of the cytotoxic T lymphocytes upon binding of the CAR to a targeting moiety. Suitable co-stimulation domains include, but are not limited to, CD28, CD137 (4-1BB), a member of the tumor necrosis factor (TNF) receptor family, CD134 (OX40), a member of the TNFR-superfamily of receptors, CD27, CD30, CD150, DAP10, NKG2D, and CD278 (ICOS), a CD28-superfamily co-stimulatory molecule expressed on activated T cells, or combinations thereof. A skilled artisan will understand that sequence variants of these co-stimulation domains can be used without adversely impacting the invention, where the variants have the same or similar activity as the domain upon which they are modeled. In various embodiments,

such variants can have at least about 80%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 99.5% sequence identity to the amino acid sequence of the domain from which they are derived.

5 In an illustrative embodiment, the activation signaling domain serves to activate T lymphocytes (e.g., cytotoxic T lymphocytes) upon binding of the CAR to a targeting moiety. In various embodiments, suitable activation signaling domains include the T cell CD3 ζ chain, CD3 delta receptor protein, mbl receptor protein, B29 receptor protein, and the Fc receptor γ . The skilled artisan will understand that sequence variants of these activation signaling domains
10 can be used where the variants have the same or similar activity as the domain upon which they are modeled. In various embodiments, the variants have at least about 80%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or at least about 99.5% sequence identity with the amino acid sequence of the domain from which they are
15 derived.

 In one aspect, constructs encoding the CARs are prepared using genetic engineering techniques. Such techniques are described in detail in Sambrook et al., "Molecular Cloning: A Laboratory Manual", 3rd Edition, Cold Spring Harbor Laboratory Press, (2001), incorporated herein by reference, and Green and Sambrook, "Molecular Cloning: A Laboratory
20 Manual", 4th Edition, Cold Spring Harbor Laboratory Press, (2012), incorporated herein by reference.

 As examples, a plasmid or viral expression vector (e.g., a lentiviral vector, a retrovirus vector, sleeping beauty, and piggyback (transposon/transposase systems that include a non-viral mediated CAR gene delivery system)) can be prepared that encodes a fusion protein
25 comprising a recognition region, one or more co-stimulation domains, and an activation signaling domain, in frame and linked in a 5' to 3' direction. In other embodiments, other arrangements are acceptable and include a recognition region, an activation signaling domain, and one or more co-stimulation domains. In one embodiment, the placement of the recognition region in the fusion protein will generally be such that display of the region on the exterior of
30 the CAR T cell is achieved. In one embodiment, the CARs may include additional elements, such as a signal peptide (e.g., CD8 α signal peptide) to ensure proper export of the fusion protein to the cell surface, a transmembrane domain to ensure the fusion protein is maintained as an

integral membrane protein (e.g., CD8 α transmembrane domain, CD28 transmembrane domain, or CD3 ζ transmembrane domain), and a hinge domain (e.g., CD8 α hinge) that imparts flexibility to the recognition region and allows strong binding to the targeting moiety.

Diagrams of exemplary CARs are shown in Figs. 2 and 3. For Fig. 2, the fusion protein sequence can be incorporated into a lentivirus expression vector where “SP” is a signal peptide, the CAR is an anti-FITC CAR, a CD8 α hinge and a CD8 α transmembrane domain are present, the co-stimulation domain is 4-1BB, and the activation signaling domain is CD3 ζ . Exemplary nucleic acid sequences of a CAR insert are provided as SEQ ID NOS:1 and 3 and the encoded amino acid sequence is provided as SEQ ID NO:2. In yet another embodiment, SEQ ID NO:2 can comprise or consist of humanized, or human amino acid sequences.

For Fig. 3, a diagram of an exemplary CAR construct wherein the expressed CAR comprises an E2 anti-fluorescein antibody fragment is shown where the fusion protein sequence can be incorporated into an expression vector and where the CAR comprises an E2 anti-fluorescein antibody fragment, an IgG4 hinge domain, a CD28 transmembrane domain, and where the co-stimulation domain is CD137 (4-1BB), and the activation signaling domain is CD3 ζ . The CAR can comprise additional suitable domains. An exemplary nucleic acid sequence of such a CAR insert is provided as SEQ ID NO:4 and the exemplary encoded amino acid sequence is provided as SEQ ID NO:5. As used herein, “SEQ ID NO:4” means the sequence beginning at the underlined “agc” codon and ending with the underlined “ggc” codon. This portion of the longer sequence, encodes the CAR that is inserted into the T cell membrane. The other portions of the longer sequence include coding sequence for signal peptides, the EGFRt domain, etc. which are not part of the CAR that is inserted into the membrane and which functions as the chimeric antigen receptor. As used herein, “SEQ ID NO:5” means the sequence beginning at the underlined “S” and ending with the underlined “G”. This portion of the longer sequence is the amino acid sequence for the CAR that is inserted into the T cell membrane. The other portions of the longer sequence include amino acid sequences for signal peptides, the EGFRt domain, etc. which are not part of the CAR inserted into the membrane and which functions as the chimeric antigen receptor. In yet another embodiment, SEQ ID NO:5 can comprise or consist of humanized, or human amino acid sequences. SEQ ID NOS:4 and 5 are as described above and which are shown below. The start and stop codons in the longer nucleic acid sequence are underlined and the longer sequence is an exemplary sequence that can be used for transduction of T cells for use in the methods as described herein.

SEQ ID NO:4 (E2 anti-fluorescein antibody fragment CAR nucleic acid sequence (insert))

atgcttctctggtgacaagccttctgctctgtgagttaccacaccagcattctctctgatcccaagcgtgctgacacagcctagctccgtg
 5 tctgccgccctggccagaaagtgacatcagctgtagcggcagcaccagcaacatcgcaacaactacgtgtcctggtatcagcagca
 ccccggaaggccccaagctgatgatctacgacgtgtccaagcggccagcggcgtgcccgatagattttccggcagcaagagcgg
 caacagcggcagcctggatcagcggcctgcagctctgaggacgagggcgactactattgcggccctgggacgatagcctgagcga
 gttcctgtttggcaccggcaccaagctgacagtgctggcgaggcggagggatctggcgccggaggaagtggcgagggggatctca
 ggtgcagctggtggaaagcggcgcaacctggtgcagcctggcggtatctctgagactgagctgtgccgccagcggcttcacctcggc
 10 agcttcagcatgagctgggtgcgccaggctcctgggggaggactggaatgggtggcaggactgagcgccagaagcagcctgaccca
 ctacgccgatagcgtgaagggcggttcaccatcagccgggacaacgccaaagaacagcgtgtacctgcagatgaacagcctgcgggt
 ggaagataccgccgtgtactactgcgccagacggcctacgacagcagcggctactggggccacttctacagctacatggacgtgtggg
 gccagggcaccctcgtgacagtgctgagagcaagtacggaccgccctgcccccttgcctgcccccgattcgacggcggaacca
 gcgtgttctgttccccccaagcccaaggacacctgatgatcagccggacccccgaggtgacctgcgtggtgtggacgtgagcca
 15 ggaagatcccaggtgccagttcaattggtacgtggacggcgtggaagtgcacaacgccaaagaccaagcccagagaggaacagttcca
 gagcacctaccgggtggtgtctgtgctgacctgctgcaccaggactggctgaacggcgaagaatacaagtgaagggtgtccaacaag
 ggctgccagcagcatcgaagacacatcgaaggccaagggccagcctgcgagccccaggtgtacacctgctcctcccag
 gaagagatgaccaagaaccaggtgtccctgacctgcctggtgaagggttctaccccagcgacatcccggtggagtgggagagcaac
 ggccagcctgagaacaactacaagaccacctcccgtgctggacagcgacggcagcttcttctgtacagccggctgacctggaca
 20 agagccggtggcaggaaggcaacgtcttagctgcagcgtgatgcacgagggccctgcacaaccactacaccagaagagcctgagcc
 tgtccctgggcaagatgttctgggtgctggtggtggcggggtgctggcctgctacagcctgctggtgacagtggtcctcatcatctt
 tgggtgaaacggggcgagaaagaactcctgtatatattcaacaaccattatgagaccagtacaactactcaagaggaagatggctgta
 gctgccgatttcagaagaagaagaggaggtgtgaactcggggtgaagttcagcagaagcgccgacgccccctgctaccagcagg
 gccagaatcagctgtacaacgagctgaacctgggcagaagggaaggtacgacgtcctggataagcggagaggccgggacctgag
 25 atgggcggcaagcctcggcggaagaacccccaggaaggcctgtataacgaactgcagaagacaagatggccgagggcctacagcga
 gatcgccatgaaggcgagcggagggcgggcaaggggccacgacggcctgtatcagggcctgtccaccgccaccaaggatacctacg
 acgccctgcacatgcaggccctgccccaaaggctcgaggggcgcgagaggcagaggaaagtcttctaactgcggtgacgtggag
 gagaatcccggccttaggtgcttctcctggtgacaagccttctgctctgtgagttaccacaccagcattctctgatccacgaaagt
 gtgtaacggaataggtattggtgaattaaagactcactctccataaatgtacgaatattaaacacttcaaaaactgcacctcatcagtggc
 30 gatctccacatcctgcccgtggcatttaggggtgactccttcacacatactcctccttggtacacaggaactggatattctgaaaaccgta
 aaggaaatcacagggttttctgattcaggcttggcctgaaaacaggacggacctccatgccttgagaacctagaaatcatagcggca
 ggaccaagcaacatggctagtttctcttcagctgctagcctgaacataacatccttgggattacgctcccctcaaggagataagtgtgga
 gatgtgataatttcaggaaacaaaatttctgctatgcaatacaataaactggaaaaaactgttgggacctccggtcagaaaacaaaatt
 ataagcaacagaggtgaaaacagctgcaaggccacaggccaggtctgcatgcttgccttgcctccccgagggtgctggggcccgag
 35 cccagggactgcgtctcttccggaatgtcagccgagggcagggaaatcggtggacaagtgaaccttctggagggtgagccaagggagt
 ttgtggagaactctgagtgatcacagtgccacccagagtgctgcctcaggccatgaacatcacctgcacaggacggggaccagacaa
 ctgtatccagtgtgccactacattgacggccccactgcgtcaagacctgcccggcaggagtcagggagaaaacaacacctggtct
 ggaagtacgcagacgccggccatgtgtgccacctgtgccatccaaactgcacctacggatgcactgggccaggtcttgaaggctgtcca
 acgaatgggcctaagatcccgtccatgccactgggatgggtggggccctccttctgctgctggtggtggccctggggatcgccctctt
 40 atgtga

SEQ ID NO:5 (E2 anti-fluorescein antibody fragment CAR amino acid sequence (insert))

45 MLLLVTSLLCELPHPAFLIPSVLTQPSSVSAAPGQKVTISC
 GSTSNIGNNYVSWYQQHPGKAPKLMIYDVSKRPSGVDPDRFS
 SKSGNSASLDISGLQSEDEADYYCAAWDDSLSEFLFGTGTKL
 VLGGGGGSGGGGSGGGGSSQVQLVESGGNLLVQPGGSLRLSCA

ASGFTFGSFSMSWVRQAPGGGLEWVAGLSARSSLTHYADSVK
 GRFTISRDNANKNSVYLQMNSLRVEDTAVYYCARRSYDSSGY
 WGHFYSYMDVWGQGTLLVTVSESKYGPPCPPCPAPEFDGGPSV
 FLFPPKPKDTLMISRTPETCVVDVVSQEDPEVQFNWYVDGV
 5 EVHNAKTKPREEQFQSTYRVVSVLTVLHQDWLNGKEYKCKV
 SNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLT
 CLVKGFYPSDIAVEWESNGQPENNYKTTTPPVLDSDGSFFLYSR
 LTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLGLGKMFW
 VLVVVGGLACYSLLVTVAFIIFWVKRGRKKLLYIFKQPFMR
 10 PVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQ
 NQLYNELNLGRREEYDVLDRGRGRDPEMGGKPRRKNPQEG
 LYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKD
 TYDALHMQALPPRLEGGGEGRGSLLTCGDVEENPGPRMLLLV
 TSLLLCELPHPAFLIPRKVCNGIGIGEFKDSLSINATNIKHF
 15 NCTSGDLHILPVAFRGDSFTHTPPLDPQELDILKTVKEITGF
 LLIQAWPENRTDLHAFENLEIIRGRTKQHGQFSLAVVSLNITS
 LGLRSLKEISDGDVIISGNKNLCYANTINWKKLFGTSGQKTKII
 SNRGENSCKATGQVCHALCSPEGCWGPEPRDCVSCRNVSRGR
 ECVDKCNLLEGEPREFVENSECIQCHPECLPQAMNITCTGRGP
 20 DNCIQCAHYIDGPHCVKTCPAGVMGENNTLVWKYADAGHVC
 HLCHPNCTYGCTGPGLEGCP TNGPKIPSIATGMVGALLLLLVV
 ALGIGLFM

25 Another exemplary CAR construct is the 4M5.3 CAR shown diagrammatically
 in Fig. 3. As used herein, "SEQ ID NO:6" means the sequence shown below beginning at the
 underlined "gac" codon and ending with the underlined "ggc" codon. This portion of the longer
 sequence, encodes the exemplary 4M5.3 CAR. The CAR is inserted into the T cell membrane.
 The other portions of the longer sequence include coding sequence for signal peptides, the
 30 EGFRt domain, etc. which are not part of the CAR that is inserted into the membrane and
 which functions as the chimeric antigen receptor. As used herein, "SEQ ID NO:7" means the
 sequence beginning at the underlined "D" and ending with the underlined "G". This portion of
 the longer sequence is the amino acid sequence for the CAR that is inserted into the T cell
 membrane. The other portions of the longer sequence include amino acid sequences for signal
 35 peptides, the EGFRt domain, etc. which are not part of the CAR inserted into the membrane and
 which functions as the chimeric antigen receptor. In yet another embodiment, SEQ ID NO:7
 can comprise or consist of humanized, or human amino acid sequences. SEQ ID NOS:6 and 7
 are as described above and which are shown below. The start and stop codons in the longer
 nucleic acid sequence are underlined and the longer sequence is an exemplary sequence that can
 40 be used for transduction of T cells to prepare the 4M5.3 CAR.

gtgttctgttcccccaagcccaaggacacctgatgatcagccggacccccgaggtgacctgcgtggtggtggacgtgagccagg
 aagatcccaggtccagttcaattggtacgtggacggcgtggaagtgcacaacgccaagaccaagcccagagaggaacagttccaga
 gcacctaccgggtggtgtctgtgctgaccgtgctgcaccaggactggctgaacggcagaatacaagtgaaggtgtccaacaaggg
 cctgcccagcagcatcgaaaagaccatcagcaaggccaagggccagcctgcgagccccaggtgtacacctgcctccctcccagga
 5 agagatgaccaagaaccaggtgtccctgacctgcctggtgaagggcttctaccccagcgacatcgccgtggagtgggagagcaacgg
 ccagcctgagaacaactacaagaccacccctcccgtgctggacagcgacggcagcttcttctgtacagccggctgacctggacaag
 agccggtggcaggaaggaacgtcttagctgcagcgtgatgcagggccctgcacaaccactacaccagaagagcctgagcctgt
 ccctgggcaagatgttctgggtgctggtggtggtggcggtgctggcctgctacagcctgctggtgacagtggccttcacatctttg
 ggtgaaacggggcagaaagaaactcctgtatatattcaacaaccatttatgagaccagtacaaactactcaagaggaagatggctgtag
 10 ctgccgatttcagagaagaagaaggaggtgtgaactcgggtgaagttcagcagaagcgccgacgcccctgctaccagcaggg
 ccagaatcagctgtacaacgagctgaacctgggcagaaggggaaggtacgacgtcctggataagcggagaggccgggacctgagat
 gggcggaagcctcgccggaagaacccccaggaaggcctgtataacgaactgcagaaagacaagatggccgaggcctacagcgag
 atcgccatgaaggcgagcgggagggcggggcaaggccacgacggcctgtatcagggcctgtccaccgccaccaaggatacctacga
 cgccctgcacatgcaggccctgcccccaaggctcgagggcgggcgagagggcagagggaagtcttctaactgcggtgacgtggagg
 15 agaatcccggcctaggtgcttctcctggtgacaagccttctgctctgtgagttaccacaccagcattcctcctgatcccacgaaaagt
 tgtaacggaataggtattggtgaattaaagactcactctccataaatgtacgaatattaaacattcaaaaactgcacctccatcagtgccg
 atctccacatcctgcccgttgccatttaggggtgactccttcacacatactcctccttgatccacaggaactggatattctgaaaaccgtaa
 aggaaatcacaggggttttctgctgattcaggcttgccctgaaacaggacggacctccatgcccttgagaacctagaaatcatagcggcag
 gaccaagcaacatggtcagtttcttctgacgtcgcagcctgaacataacatccttgggattacgctccctcaaggagataagtgatggag
 20 atgtgataatttcaggaacaaaaatttgctatgcaatacaataaactgaaaaaactgttgggacctccggctcagaaaacaaaatta
 taagcaacagaggtgaaaacagctgcaaggccacaggccaggtctgcatgcttctgctccccgaggggtgctggggcccgagc
 ccagggactgcgtctcttccggaatgtcagccgagggcaggaatgcgtggacaagtgaaccttctggaggggtgagccaagggagtt
 tgtggagaactctgagtgacatagtgccaccagagtgcctgcctcaggccatgaacatcacctgcacaggacggggaccagacaac
 tgtatccagtgtcccactacattgacggccccactgcgtcaagacctgcccggcaggagtcattgggagaaaaacaacaccttggtctg
 25 gaagtacgcagacgcccggcatgtgtgccacctgtgccatccaaactgcacctacggatgcactgggcccaggtctgaaggctgtccaa
 cgaatgggcctaagatcccgtccatcgccactgggatggtgggggcccctccttctgctgctggtggtggccctggggatcgcccttcat
 gTGA (STOP CODON)

30 In one embodiment, the CAR has a recognition region and the recognition region
 is a single chain fragment variable (scFv) region of an anti-FITC antibody, a co-stimulation
 domain and the co-stimulation domain is CD137 (4-1BB), and an activation signaling domain
 and the activation signaling domain is a T cell CD3 ζ chain. It is well-known to the skilled
 artisan that an anti-FITC scFv and an anti-fluorescein scFv are equivalent terms.

35 In one embodiment, T lymphocytes (e.g., cytotoxic T lymphocytes) can be
 genetically engineered to express CAR constructs by transfecting a population of the T
 lymphocytes with an expression vector encoding the CAR construct. Suitable methods for
 preparing a transduced population of T lymphocytes expressing a selected CAR construct are
 well-known to the skilled artisan, and are described in Sambrook et al., "Molecular Cloning: A
 40 Laboratory Manual", 3rd Edition, Cold Spring Harbor Laboratory Press, (2001), incorporated
 herein by reference, and Green and Sambrook, "Molecular Cloning: A Laboratory Manual", 4th
 Edition, Cold Spring Harbor Laboratory Press, (2012), incorporated herein by reference.

In one embodiment, CAR T cells comprising a nucleic acid of SEQ ID NO:1, 3, 4, or 6 are provided. In another embodiment, CAR T cells comprising a polypeptide of SEQ ID NO:2, 5, or 7 are provided. In another illustrative aspect, a nucleic acid (e.g., an isolated nucleic acid) comprising SEQ ID NO:1, 3, 4, or 6 and encoding a chimeric antigen receptor is provided. In yet another embodiment, a chimeric antigen receptor polypeptide comprising SEQ ID NO:2, 5, or 7 is provided. In another embodiment, a vector is provided comprising SEQ ID NO:1, 3, 4 or 6. In another aspect, a lentiviral vector is provided comprising SEQ ID NO:1, 3, 4, or 6. In yet another embodiment, SEQ ID NO:2, 5, or 7 can comprise or consist of humanized, or human amino acid sequences.

In each of these embodiments, variant nucleic acid sequences or amino acid sequences having at least about 80%, at least about 90%, at least about 95%, at least about 97%, at least about 98%, at least about 99%, or at least about 99.5% sequence identity to SEQ ID NOS:1 to 7 are contemplated. In another embodiment, the nucleic acid sequence can be a variant nucleic acid sequence having at least about 80%, at least about 90%, at least about 95%, at least about 97%, at least about 98%, at least about 99%, or at least about 99.5% sequence identity to SEQ ID NO:1, 3, 4, or 6 as long as the variant sequence encodes a polypeptide of SEQ ID NO:2 (for SEQ ID NOS:1 and 3), 5 (for SEQ ID NO:4), or 7 (for SEQ ID NO:6). In another embodiment, the nucleic acid sequence or the amino acid sequence can be a variant nucleic acid or amino acid sequence having at least about 80%, at least about 90%, at least about 95%, at least about 97%, at least about 98%, at least about 99%, or at least about 99.5% sequence identity to SEQ ID NO:1, 3, 4, or 6 along a stretch of 200 nucleic acids or, for SEQ ID NO:2, 5, or 7 along a stretch of 200 amino acids. In one embodiment, determination of percent identity or similarity between sequences can be done, for example, by using the GAP program (Genetics Computer Group, software; now available via Accelrys on <http://www.accelrys.com>), and alignments can be done using, for example, the ClustalW algorithm (VNTI software, InforMax Inc.). A sequence database can be searched using the nucleic acid or amino acid sequence of interest. Algorithms for database searching are typically based on the BLAST software (Altschul et al., 1990). In some embodiments, the percent identity can be determined along the full-length of the nucleic acid or amino acid sequence.

Also within the scope of the invention are nucleic acids complementary to the nucleic acids represented by SEQ ID NO:1, 3, 4, or 6 and those that hybridize to the nucleic acids represented by SEQ ID NO:1, 3, 4, or 6 or those that hybridize to their complements

under highly stringent conditions. In accordance with the invention “highly stringent conditions” means hybridization at 65 °C in 5X SSPE and 50% formamide, and washing at 65 °C in 0.5X SSPE. Conditions for high stringency, low stringency and moderately stringent hybridization are described in Sambrook et al., “Molecular Cloning: A Laboratory Manual”, 3rd Edition, Cold Spring Harbor Laboratory Press, (2001), incorporated herein by reference, and
5 Green and Sambrook, “Molecular Cloning: A Laboratory Manual”, 4th Edition, Cold Spring Harbor Laboratory Press, (2012), incorporated herein by reference. In some illustrative aspects, hybridization occurs along the full-length of the nucleic acid.

In one embodiment, the T lymphocytes (e.g., cytotoxic T lymphocytes used to
10 prepare CAR T cells), used in the methods described herein, can be autologous cells, although heterologous cells can also be used, such as when the patient being treated has received high-dose chemotherapy or radiation treatment to destroy the patient’s immune system. In one embodiment, allogenic cells can be used.

In one aspect, the T lymphocytes can be obtained from a patient by means well-
15 known in the art. For example, T cells (e.g., cytotoxic T cells) can be obtained by collecting peripheral blood from the patient, subjecting the blood to Ficoll density gradient centrifugation, and then using a negative T cell isolation kit (such as EasySep™ T Cell Isolation Kit) to isolate a population of T cells from the peripheral blood. In one illustrative embodiment, the population of T lymphocytes (e.g., cytotoxic T cells) need not be pure and may contain other
20 cells such as other types of T cells (in the case of cytotoxic T cells, for example), monocytes, macrophages, natural killer cells, and B cells. In one aspect, the population being collected can comprise at least about 90% of the selected cell type, at least about 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% of the selected cell type.

In one embodiment, after the T lymphocytes (e.g., cytotoxic T cells used to
25 prepare CAR T cells) are obtained, the cells are cultured under conditions that promote the activation of the cells. In this embodiment, the culture conditions may be such that the cells can be administered to a patient without concern for reactivity against components of the culture medium. For example, the culture conditions may not include bovine serum products, such as bovine serum albumin. In one illustrative aspect, the activation can be achieved by introducing
30 known activators into the culture medium, such as anti-CD3 antibodies in the case of cytotoxic T cells. Other suitable activators include anti-CD28 antibodies. In one aspect, the population of lymphocytes can be cultured under conditions promoting activation for about 1 to about 4

days. In one embodiment, the appropriate level of activation can be determined by cell size, proliferation rate, or activation markers determined by flow cytometry.

In one illustrative embodiment, after the population of T lymphocytes (e.g., cytotoxic T lymphocytes used to prepare CAR T cells) has been cultured under conditions promoting activation, the cells can be transfected with an expression vector encoding a CAR. Suitable vectors and transfection methods for use in various embodiments are described above. In one aspect, after transfection, the cells can be immediately administered to the patient or the cells can be cultured for at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18 or more days, or between about 5 and about 12 days, between about 6 and about 13 days, between about 7 and about 14 days, or between about 8 and about 15 days, for example, to allow time for the cells to recover from the transfection. In one aspect, suitable culture conditions can be similar to the conditions under which the cells were cultured for activation either with or without the agent that was used to promote activation.

Thus, as described above, in one illustrative aspect, the methods of treatment described herein can further comprise 1) obtaining a population of autologous or heterologous T lymphocytes (e.g., cytotoxic T lymphocytes used to prepare CAR T cells), 2) culturing the T lymphocytes under conditions that promote the activation of the cells, and 3) transfecting the lymphocytes with an expression vector encoding a CAR to form CAR T cells.

In one embodiment, culture media that lacks any animal products, such as bovine serum, can be used to culture the CAR T cells. In another embodiment, tissue culture conditions typically used by the skilled artisan to avoid contamination with bacteria, fungi and mycoplasma can be used. In an exemplary embodiment, prior to being administered to a patient, the cells (e.g., CAR T cells) are pelleted, washed, and are resuspended in a pharmaceutically acceptable carrier or diluent. Exemplary compositions comprising CAR-expressing T lymphocytes (e.g., cytotoxic T lymphocytes) include compositions comprising the cells in sterile 290 mOsm saline, in infusible cryomedia (containing Plasma-Lyte A, dextrose, sodium chloride injection, human serum albumin and DMSO), in 0.9% NaCl with 2% human serum albumin, or in any other sterile 290 mOsm infusible materials. Alternatively, in another embodiment, depending on the identity of the culture medium, the CAR T cells can be administered in the culture media as the composition, or concentrated and resuspended in the culture medium before administration. In various embodiments, the CAR T cell composition can be administered to the patient via any suitable means, such as parenteral administration,

e.g., intradermally, subcutaneously, intramuscularly, intraperitoneally, intravenously, or intrathecally.

In one aspect, the total number of CAR T cells and the concentration of the cells in the composition administered to the patient will vary depending on a number of factors including the type of T lymphocytes (e.g., cytotoxic T lymphocytes) being used, the binding specificity of the CAR, the identity of the targeting moiety and the small molecule ligand, the identity of the cancer, the location of the cancer in the patient, the means used to administer the compositions to the patient, and the health, age and weight of the patient being treated. In various embodiments, suitable compositions comprising transduced CAR T cells include those having a volume of about 0.1 ml to about 200 ml and about 0.1 ml to about 125 ml.

In various embodiments, the transduced CAR T cells administered to the patient can comprise from about 1×10^5 to about 1×10^{15} or 1×10^6 to about 1×10^{15} transduced CAR T cells. In various embodiments about 1×10^5 to about 1×10^{10} , about 1×10^6 to about 1×10^{10} , about 1×10^6 to about 1×10^9 , about 1×10^6 to about 1×10^8 , about 1×10^6 to about 2×10^7 , about 1×10^6 to about 3×10^7 , about 1×10^6 to about 1.5×10^7 , about 1×10^6 to about 1×10^7 , about 1×10^6 to about 9×10^6 , about 1×10^6 to about 8×10^6 , about 1×10^6 to about 7×10^6 , about 1×10^6 to about 6×10^6 , about 1×10^6 to about 5×10^6 , about 1×10^6 to about 4×10^6 , about 1×10^6 to about 3×10^6 , about 1×10^6 to about 2×10^6 , about 2×10^6 to about 6×10^6 , about 2×10^6 to about 5×10^6 , about 3×10^6 to about 6×10^6 , about 4×10^6 to about 6×10^6 , about 4×10^6 to about 1×10^7 , about 1×10^6 to about 1×10^7 , about 1×10^6 to about 1.5×10^7 , about 1×10^6 to about 2×10^7 , about 0.2×10^6 to about 1×10^7 , about 0.2×10^6 to about 1.5×10^7 , about 0.2×10^6 to about 2×10^7 , about 0.2×10^6 to about 3×10^7 , about 0.2×10^6 to about 4×10^7 , about 0.2×10^6 to about 5×10^7 , about 0.2×10^5 to about 1.5×10^6 , about 0.5×10^5 to about 1.5×10^6 , about 0.2×10^5 to about 1.4×10^6 , about 0.2×10^5 to about 1.3×10^6 , about 0.5×10^5 to about 1.3×10^6 , about 0.8×10^6 to about 2×10^7 , about 0.8×10^6 to about 1.5×10^7 , about 0.9×10^6 to about 1.2×10^7 , or about 0.5×10^6 , 1×10^6 , 5×10^6 , 6×10^6 , 7×10^6 , 8×10^6 , 9×10^6 , 1×10^7 , 1.5×10^7 , 2×10^7 , 3×10^7 , 4×10^7 or 5×10^7 CAR T cells can be administered to the patient. These amounts can be per kg of patient body weight.

In other embodiments, the dose of the CAR T cells administered to the patient in the CAR T cell composition is selected from the group consisting of about 1 million, about 2 million, about 3 million, about 4 million, about 5 million, about 6 million, about 7 million,

about 8 million, about 9 million, about 10 million, about 11 million, about 12 million, about 12.5 million, about 13 million, about 14 million, and about 15 million of the CAR T cells. These amounts can be per kg of patient body weight.

5 In any of the embodiments described in this paragraph, the CAR T cell dose can be in numbers of CAR T cells per kg of patient body weight. In one aspect, in any embodiment described herein, a single dose or multiple doses of the CAR T cells can be administered to the patient. In one illustrative embodiment, a first dose of the CAR T cells and a second dose of the CAR T cells can be administered to the patient. In one aspect, the first dose of the CAR T cells can be a test dose to monitor the patient for tolerability to the CAR T cells, and the second dose
10 of the CAR T cells can comprise a higher dose of the CAR T cells than the first dose of the CAR T cells. In one embodiment, the first dose of the CAR T cells can comprise about 0.5×10^5 of the CAR T cells to about 1.5×10^6 of the CAR T cells. In another embodiment, the second dose of the CAR T cells can comprise about 0.8×10^6 of the CAR T cells to about 2×10^7 of the CAR T cells. In these embodiments involving a first and second dose of CAR T
15 cells, any dose of CAR T cells described herein can be administered.

In any embodiment described herein, the CAR T cells can be administered before or after the compound, or the pharmaceutically acceptable salt thereof. As would be understood, the designations i), ii), and iii), etc. for steps of any method described herein do not indicate an order unless otherwise stated.

20 The compound, or pharmaceutically acceptable salt thereof, or CAR T cell composition described herein can be administered to the patient using any suitable method known in the art. As described herein, the term “administering” or “administered” includes all means of introducing the compound, or pharmaceutically acceptable salt thereof, or CAR T cell composition to the patient, including, but not limited to, oral, intravenous, intramuscular,
25 subcutaneous, transdermal, and the like. In one aspect, the compound, or pharmaceutically acceptable salt thereof, described herein may be administered in a unit dosage form and/or formulations containing conventional nontoxic pharmaceutically acceptable carriers, adjuvants, and vehicles.

In one aspect, the compound, or pharmaceutically acceptable salt thereof, or
30 CAR T cell composition as described herein may be administered directly into the blood stream, into muscle, or into an internal organ. In various embodiments, suitable routes for such parenteral administration include intravenous, intraarterial, intraperitoneal, intrathecal, epidural,

intracerebroventricular, intraurethral, intrasternal, intracranial, intratumoral, intramuscular and subcutaneous delivery. In one embodiment, means for parenteral administration include needle (including microneedle) injectors, needle-free injectors and infusion techniques.

In one illustrative aspect, parenteral formulations are typically aqueous solutions
5 which may contain carriers or excipients such as salts, carbohydrates and buffering agents (preferably at a pH of from 3 to 9), but they may be more suitably formulated as a sterile non-aqueous solution or as a dried form to be used in conjunction with a suitable vehicle such as sterile, pyrogen-free water or sterile saline. In other embodiments, any of the liquid formulations described herein may be adapted for parenteral administration as described herein.
10 The preparation under sterile conditions, by lyophilization to produce a sterile lyophilized powder for a parenteral formulation, may readily be accomplished using standard pharmaceutical techniques well-known to those skilled in the art. In one embodiment, the solubility of the compound, or pharmaceutically acceptable salt thereof, used in the preparation of a parenteral formulation may be increased by the use of appropriate formulation techniques,
15 such as the incorporation of solubility-enhancing agents.

In one embodiment, the amount of the compound, or pharmaceutically acceptable salt thereof, to be administered to the patient can vary significantly depending on the cancer being treated, the route of administration of the compound, or pharmaceutically acceptable salt thereof, and the tissue distribution. In one aspect, the amount to be administered
20 to a patient can be based on body surface area, mass, and physician assessment.

In various embodiments, the compound, or the pharmaceutically acceptable salt thereof, can be administered in 1) at least a first dose escalation sequence and a second dose escalation sequence, 2) at least a first dose escalation sequence, a second dose escalation sequence, and a third dose escalation sequence, 3) at least a first dose escalation sequence, a
25 second dose escalation sequence, a third dose escalation sequence, and a fourth dose escalation sequence, 4) at least a first dose escalation sequence, a second dose escalation sequence, a third dose escalation sequence, a fourth dose escalation sequence, and a fifth dose escalation sequence, 5) at least a first dose escalation sequence, a second dose escalation sequence, a third dose escalation sequence, a fourth dose escalation sequence, a fifth dose escalation sequence,
30 and a sixth dose escalation sequence, or 6) one or more additional dose escalation sequences can be included.

In various embodiments, for the first, second, third, fourth, fifth, or sixth, etc. dose escalation sequence, the amount of the compound, or the pharmaceutically acceptable salt thereof, to be administered to the patient can be about 0.1 µg/kg to about 2000 µg/kg, 0.1 µg/kg to about 1500 µg/kg, 0.1 µg/kg to about 1000 µg/kg, 0.1 µg/kg to about 500 µg/kg, about 0.1 µg/kg to about 100 µg/kg, about 0.1 µg/kg to about 80 µg/kg, about 0.1 µg/kg to about 70 µg/kg, about 0.1 µg/kg to about 50 µg/kg, about 0.1 µg/kg to about 40 µg/kg, about 0.1 µg/kg to about 30 µg/kg, about 0.3 µg/kg to about 500 µg/kg, about 0.3 µg/kg to about 400 µg/kg, about 0.3 µg/kg to about 300 µg/kg, about 0.3 µg/kg to about 200 µg/kg, about 0.3 µg/kg to about 100 µg/kg, about 0.3 µg/kg to about 90 µg/kg, about 0.1 µg/kg to about 400 µg/kg, about 0.1 µg/kg to about 350 µg/kg, about 0.1 µg/kg to about 300 µg/kg, about 0.1 µg/kg to about 250 µg/kg, about 0.1 µg/kg to about 200 µg/kg, about 0.1 µg/kg to about 150 µg/kg, or about 0.3 µg/kg to about 150 µg/kg, or 5 µg/kg to about 2000 µg/kg, 5 µg/kg to about 1500 µg/kg, 5 µg/kg to about 1000 µg/kg, 5 µg/kg to about 500 µg/kg, about 5 µg/kg to about 100 µg/kg, about 5 µg/kg to about 80 µg/kg, about 5 µg/kg to about 70 µg/kg, about 5 µg/kg to about 50 µg/kg, about 5 µg/kg to about 40 µg/kg, or about 5 µg/kg to about 30 µg/kg. In another embodiment, for the first and fourth dose escalation sequences, the amount of the compound, or the pharmaceutically acceptable salt thereof, to be administered to the patient can be about 0.1 µg/kg to about 100 µg/kg, about 0.1 µg/kg to about 80 µg/kg, about 0.1 µg/kg to about 70 µg/kg, about 0.1 µg/kg to about 50 µg/kg, about 0.1 µg/kg to about 40 µg/kg, about 0.1 µg/kg to about 30 µg/kg, or about 0.3 µg/kg to about 30 µg/kg, about 5 µg/kg to about 100 µg/kg, about 5 µg/kg to about 80 µg/kg, about 5 µg/kg to about 70 µg/kg, about 5 µg/kg to about 50 µg/kg, about 5 µg/kg to about 40 µg/kg, about 5 µg/kg to about 30 µg/kg, or about 5 µg/kg to about 2000 µg/kg, or about 5 µg/kg to about 1500 µg/kg, or about 5 µg/kg to about 1000 µg/kg, or about 5 µg/kg to about 500 µg/kg. In another embodiment, for the second and fifth dose escalation sequences, the amount of the compound, or the pharmaceutically acceptable salt thereof, to be administered to the patient can be about 0.3 µg/kg to about 500 µg/kg, about 0.3 µg/kg to about 400 µg/kg, about 0.3 µg/kg to about 300 µg/kg, about 0.3 µg/kg to about 200 µg/kg, about 0.3 µg/kg to about 100 µg/kg, or about 0.3 µg/kg to about 90 µg/kg, or about 5 µg/kg to about 100 µg/kg, about 5 µg/kg to about 80 µg/kg, about 5 µg/kg to about 70 µg/kg, about 5 µg/kg to about 50 µg/kg, about 5 µg/kg to about 40 µg/kg, about 5 µg/kg to about 30 µg/kg, or about 5 µg/kg to about 2000 µg/kg, or about 5 µg/kg to about 1500 µg/kg, or about 5 µg/kg to about 1000 µg/kg, or about 5 µg/kg to about 500 µg/kg. In yet another embodiment,

for the third and sixth dose escalation sequences, the amount of the compound, or the pharmaceutically acceptable salt thereof, to be administered to the patient can be 0.1 $\mu\text{g/kg}$ to about 400 $\mu\text{g/kg}$, about 0.1 $\mu\text{g/kg}$ to about 350 $\mu\text{g/kg}$, about 0.1 $\mu\text{g/kg}$ to about 300 $\mu\text{g/kg}$, about 0.1 $\mu\text{g/kg}$ to about 250 $\mu\text{g/kg}$, about 0.1 $\mu\text{g/kg}$ to about 200 $\mu\text{g/kg}$, about 0.1 $\mu\text{g/kg}$ to about 150 $\mu\text{g/kg}$, or about 0.3 $\mu\text{g/kg}$ to about 150 $\mu\text{g/kg}$, or about 5 $\mu\text{g/kg}$ to about 100 $\mu\text{g/kg}$, about 5 $\mu\text{g/kg}$ to about 80 $\mu\text{g/kg}$, about 5 $\mu\text{g/kg}$ to about 70 $\mu\text{g/kg}$, about 5 $\mu\text{g/kg}$ to about 50 $\mu\text{g/kg}$, about 5 $\mu\text{g/kg}$ to about 40 $\mu\text{g/kg}$, about 5 $\mu\text{g/kg}$ to about 30 $\mu\text{g/kg}$, or about 5 $\mu\text{g/kg}$ to about 2000 $\mu\text{g/kg}$, or about 5 $\mu\text{g/kg}$ to about 1500 $\mu\text{g/kg}$, or about 5 $\mu\text{g/kg}$ to about 1000 $\mu\text{g/kg}$, or about 5 $\mu\text{g/kg}$ to about 500 $\mu\text{g/kg}$. In these embodiments, “kg” is kilograms of body weight of the patient.

In any of these embodiments, the range of amounts of the compound, or the pharmaceutically acceptable salt thereof, can be a range based on calculating percentages of a “full dose” of the compound, or the pharmaceutically acceptable salt thereof, wherein a “full dose” of the compound, or the pharmaceutically acceptable salt thereof, can be about 30 $\mu\text{g/kg}$, and wherein the percentages are about 1 percent to about 100 percent (see Sequence 1 in Fig. 1), about 1 percent to about 300 percent (see Sequence 2 in Fig. 1), and about 1 percent to about 500 percent (see Sequence 3 in Fig. 1) of the “full dose” of the compound, or the pharmaceutically acceptable salt thereof. In other embodiments, the percentages of the “full dose” of the compound, or the pharmaceutically acceptable salt thereof, administered in a dose escalation sequence can be about 1 percent, about 2 percent, about 3 percent, about 4 percent, about 5 percent, about 6 percent, about 7 percent, about 8 percent, about 9 percent, about 10 percent, about 20 percent, about 30 percent, about 40 percent, about 50 percent, about 60 percent, about 70 percent, about 80 percent, about 90 percent, about 100 percent, about 200 percent, about 300 percent, about 400 percent, or about 500 percent, and the amounts administered can be about 0.3 $\mu\text{g/kg}$, about 3 $\mu\text{g/kg}$, about 9 $\mu\text{g/kg}$, about 15 $\mu\text{g/kg}$, about 30 $\mu\text{g/kg}$, about 90 $\mu\text{g/kg}$, or about 150 $\mu\text{g/kg}$, respectively or about 17 percent, about 333 percent, about 1666 percent, or about 3333 percent, and the amounts administered can be about 5 $\mu\text{g/kg}$, about 100 $\mu\text{g/kg}$, about 500 $\mu\text{g/kg}$, or about 1000 $\mu\text{g/kg}$, respectively. In these embodiments, “kg” is kilograms of body weight of the patient.

In another embodiment, the percentages of the “full dose” of the compound, or the pharmaceutically acceptable salt thereof, administered in a dose escalation sequence can be about 1 percent, about 2 percent, and about 20 percent for the first dose escalation sequence,

about 1 percent, about 6 percent, and about 60 percent for the second dose escalation sequence, and about 1 percent, about 10 percent, and about 100 percent for the third dose escalation sequence, and the “full dose” of the compound, or the pharmaceutically acceptable salt thereof, can be 500 nmoles/kg. In this embodiment, “kg” is kilograms of body weight of the patient. In
5 this embodiment, the compound, or the pharmaceutically acceptable salt thereof, can be administered on Monday, Thursday, and Monday with about 6 days between each dose escalation cycle.

In another embodiment, the percentages of the “full dose” of the compound, or the pharmaceutically acceptable salt thereof, administered in a dose escalation sequence can be
10 about 1 percent, about 10 percent, and about 100 percent for the first dose escalation sequence, and about 1 percent, about 20 percent, and about 200 percent for the second dose escalation sequence, and the “full dose” of the compound, or the pharmaceutically acceptable salt thereof, can be 500 nmoles/kg. In this embodiment, “kg” is kilograms of body weight of the patient. In this embodiment, the compound, or the pharmaceutically acceptable salt thereof, can be
15 administered on Monday, Thursday, and Monday with about 6 days between each dose escalation cycle.

In another embodiment, the percentages of the “full dose” of the compound, or the pharmaceutically acceptable salt thereof, administered in a dose escalation sequence can be about 1 percent, about 10 percent, and about 100 percent for the first dose escalation sequence,
20 and about 1 percent, about 10 percent, and about 100 percent for the second dose escalation sequence, and the “full dose” of the compound, or the pharmaceutically acceptable salt thereof, can be 500 nmoles/kg. In this embodiment, “kg” is kilograms of body weight of the patient. In this embodiment, the compound, or the pharmaceutically acceptable salt thereof, can be administered on Monday, Thursday, and Monday with about 6 days between each dose
25 escalation cycle. In another embodiment, the compound, or the pharmaceutically acceptable salt thereof, can be administered on Monday, Thursday, and Monday with about 9 days between each dose escalation cycle. In other embodiments, the dose escalations can be repeated one, two, three, four, five, six, seven, eight, nine, or ten times or any appropriate number of times.

In yet another embodiment, the percentages of the “full dose” of the compound,
30 or the pharmaceutically acceptable salt thereof, administered in a dose escalation sequence can be about 1 percent, about 20 percent, and about 200 percent for the first dose escalation sequence, and about 1 percent, about 20 percent, and about 200 percent for the second dose

escalation sequence, and the “full dose” of the compound, or the pharmaceutically acceptable salt thereof, can be 500 nmoles/kg. In this embodiment, “kg” is kilograms of body weight of the patient. In this embodiment, the compound, or the pharmaceutically acceptable salt thereof, can be administered on Monday, Thursday, and Monday with about 6 days between each dose
5 escalation cycle. In other embodiments, the dose escalations can be repeated one, two, three, four, five, six, seven, eight, nine, or ten times or any appropriate number of times.

In another embodiment, the percentages of the “full dose” of the compound, or the pharmaceutically acceptable salt thereof, administered in the first or fourth dose escalation sequence can be about 1 percent, about 10 percent, and about 100 percent of the “full dose” of
10 the compound, or the pharmaceutically acceptable salt thereof, (about 10 to about 50, about 20 to about 40, about 25 to about 35, or about 30 $\mu\text{g/kg}$) in escalating amounts, and the amounts of the compound, or the pharmaceutically acceptable salt thereof, administered can be about 0.3 $\mu\text{g/kg}$, about 3 $\mu\text{g/kg}$, and about 30 $\mu\text{g/kg}$, respectively. In yet another embodiment, the percentages of the “full dose” of the compound, or the pharmaceutically acceptable salt thereof,
15 administered in the second or fifth dose escalation sequence can be about 1 percent, about 30 percent, and about 300 percent of the “full dose” of the compound, or the pharmaceutically acceptable salt thereof, (about 10 to about 50, about 20 to about 40, about 25 to about 35, or about 30 $\mu\text{g/kg}$) in escalating amounts, and the amounts of the compound, or the pharmaceutically acceptable salt thereof, administered can be about 0.3 $\mu\text{g/kg}$, about 9 $\mu\text{g/kg}$,
20 and about 90 $\mu\text{g/kg}$, respectively. In still another embodiment, the percentages of the “full dose” of the compound, or the pharmaceutically acceptable salt thereof, administered in the third or sixth dose escalation sequence can be about 1 percent, about 50 percent, and about 500 percent of the “full dose” of the compound, or the pharmaceutically acceptable salt thereof, (about 10 to about 50, about 20 to about 40, about 25 to about 35, or about 30 $\mu\text{g/kg}$) in
25 escalating amounts, and the amounts of the compound, or the pharmaceutically acceptable salt thereof, administered can be about 0.3 $\mu\text{g/kg}$, about 15 $\mu\text{g/kg}$, and about 150 $\mu\text{g/kg}$, respectively. In these embodiments, “kg” is kilograms of body weight of the patient.

In various other embodiments, amounts to be administered to the patient can range, for example, from about 0.05 mg to about 30 mg, 0.05 mg to about 25.0 mg, about 0.05
30 mg to about 20.0 mg, about 0.05 mg to about 15.0 mg, about 0.05 mg to about 10.0 mg, about 0.05 mg to about 9.0 mg, about 0.05 mg to about 8.0 mg, about 0.05 mg to about 7.0 mg, about 0.05 mg to about 6.0 mg, about 0.05 mg to about 5.0 mg, about 0.05 mg to about 4.0 mg, about

0.05 mg to about 3.0 mg, about 0.05 mg to about 2.0 mg, about 0.05 mg to about 1.0 mg, about 0.05 mg to about 0.5 mg, about 0.05 mg to about 0.4 mg, about 0.05 mg to about 0.3 mg, about 0.05 mg to about 0.2 mg, about 0.05 mg to about 0.1 mg, about .01 mg to about 2 mg, about 0.3 mg to about 10 mg, about 0.1 mg to about 20 mg, or about 0.8 to about 3 mg.

5 In other embodiments, the dose of the compound, or pharmaceutically acceptable salt thereof, can range, for example, from about 50 nmoles/kg to about 3000 nmoles/kg of patient body weight, about 50 nmoles/kg to about 2000 nmoles/kg, about 50 nmoles/kg to about 1000 nmoles/kg, about 50 nmoles/kg to about 900 nmoles/kg, about 50 nmoles/kg to about 800 nmoles/kg, about 50 nmoles/kg to about 700 nmoles/kg, about 50
10 nmoles/kg to about 600 nmoles/kg, about 50 nmoles/kg to about 500 nmoles/kg, about 50 nmoles/kg to about 400 nmoles/kg, about 50 nmoles/kg to about 300 nmoles/kg, about 50 nmoles/kg to about 200 nmoles/kg, about 50 nmoles/kg to about 100 nmoles/kg, about 100 nmoles/kg to about 300 nmoles/kg, about 100 nmoles/kg to about 500 nmoles/kg, about 100 nmoles/kg to about 1000 nmoles/kg, about 100 nmoles/kg to about 2000 nmoles/kg of patient
15 body weight. In other embodiments, the dose may be about 1 nmoles/kg, about 5 nmoles/kg, about 10 nmoles/kg, about 20 nmoles/kg, about 25 nmoles/kg, about 30 nmoles/kg, about 40 nmoles/kg, about 50 nmoles/kg, about 60 nmoles/kg, about 70 nmoles/kg, about 80 nmoles/kg, about 90 nmoles/kg, about 100 nmoles/kg, about 150 nmoles/kg, about 200 nmoles/kg, about 250 nmoles/kg, about 300 nmoles/kg, about 350 nmoles/kg, about 400 nmoles/kg, about 450
20 nmoles/kg, about 500 nmoles/kg, about 600 nmoles/kg, about 700 nmoles/kg, about 800 nmoles/kg, about 900 nmoles/kg, about 1000 nmoles/kg, about 2000 nmoles/kg, about 2500 nmoles/kg or about 3000 nmoles/kg of body weight of the patient. In yet other embodiments, the dose may be about 0.1 nmoles/kg, about 0.2 nmoles/kg, about 0.3 nmoles/kg, about 0.4 nmoles/kg, or about 0.5 nmoles/kg, about 0.1 nmoles/kg to about 1000 nmoles/kg, about 0.1
25 nmoles/kg to about 900 nmoles/kg, about 0.1 nmoles/kg to about 850 nmoles/kg, about 0.1 nmoles/kg to about 800 nmoles/kg, about 0.1 nmoles/kg to about 700 nmoles/kg, about 0.1 nmoles/kg to about 600 nmoles/kg, about 0.1 nmoles/kg to about 500 nmoles/kg, about 0.1 nmoles/kg to about 400 nmoles/kg, about 0.1 nmoles/kg to about 300 nmoles/kg, about 0.1 nmoles/kg to about 200 nmoles/kg, about 0.1 nmoles/kg to about 100 nmoles/kg, about 0.1
30 nmoles/kg to about 50 nmoles/kg, about 0.1 nmoles/kg to about 10 nmoles/kg, or about 0.1 nmoles/kg to about 1 nmoles/kg of body weight of the patient. In other embodiments, the dose may be about 0.3 nmoles/kg to about 1000 nmoles/kg, about 0.3 nmoles/kg to about 900

nmoles/kg, about 0.3 nmoles/kg to about 850 nmoles/kg, about 0.3 nmoles/kg to about 800 nmoles/kg, about 0.3 nmoles/kg to about 700 nmoles/kg, about 0.3 nmoles/kg to about 600 nmoles/kg, about 0.3 nmoles/kg to about 500 nmoles/kg, about 0.3 nmoles/kg to about 400 nmoles/kg, about 0.3 nmoles/kg to about 300 nmoles/kg, about 0.3 nmoles/kg to about 200 nmoles/kg, about 0.3 nmoles/kg to about 100 nmoles/kg, about 0.3 nmoles/kg to about 50 nmoles/kg, about 0.3 nmoles/kg to about 10 nmoles/kg, or about 0.3 nmoles/kg to about 1 nmoles/kg of body weight of the patient. In these embodiments, “kg” is kilograms of body weight of the patient.

In another embodiment, a first dose escalation step and a second dose escalation step with the compound (e.g., where each dose escalation step is 50 nmol/kg and then 500 nmol/kg), or a pharmaceutically acceptable salt thereof, are performed after administration of CAR-T cells (e.g., in any of the amounts described herein). In this embodiment, after the first and second dose escalation steps in, for example, weeks one and two, the level of the compound, or a pharmaceutically acceptable salt thereof, can be kept constant in week three relative to the last dose administered in week two (e.g., kept constant at 500 nmol/kg). In any embodiment described herein, the level of the compound, or a pharmaceutically acceptable salt thereof, can be kept constant in the succeeding week relative to the last dose administered in the prior week.

In various other embodiments, the dose of the compound, or the pharmaceutically acceptable salt thereof, may range from, for example, about 10 nmoles/kg to about 10000 nmoles/kg, from about 10 nmoles/kg to about 5000 nmoles/kg, from about 10 nmoles/kg to about 3000 nmoles/kg, about 10 nmoles/kg to about 2500 nmoles/kg, about 10 nmoles/kg to about 2000 nmoles/kg, about 10 nmoles/kg to about 1000 nmoles/kg, about 10 nmoles/kg to about 900 nmoles/kg, about 10 nmoles/kg to about 800 nmoles/kg, about 10 nmoles/kg to about 700 nmoles/kg, about 10 nmoles/kg to about 600 nmoles/kg, about 10 nmoles/kg to about 500 nmoles/kg, about 10 nmoles/kg to about 400 nmoles/kg, about 10 nmoles/kg to about 300 nmoles/kg, about 10 nmoles/kg to about 200 nmoles/kg, about 10 nmoles/kg to about 150 nmoles/kg, about 10 nmoles/kg to about 100 nmoles/kg, about 10 nmoles/kg to about 90 nmoles/kg, about 10 nmoles/kg to about 80 nmoles/kg, about 10 nmoles/kg to about 70 nmoles/kg, about 10 nmoles/kg to about 60 nmoles/kg, about 10 nmoles/kg to about 50 nmoles/kg, about 10 nmoles/kg to about 40 nmoles/kg, about 10 nmoles/kg to about 30 nmoles/kg, about 10 nmoles/kg to about 20 nmoles/kg, about 200

nmol/kg to about 900 nmol/kg, about 200 nmol/kg to about 800 nmol/kg, about 200 nmol/kg to about 700 nmol/kg, about 200 nmol/kg to about 600 nmol/kg, about 200 nmol/kg to about 500 nmol/kg, about 250 nmol/kg to about 600 nmol/kg, about 300 nmol/kg to about 600 nmol/kg, about 300 nmol/kg to about 500 nmol/kg, or about 400 nmol/kg to about 600 nmol/kg, of body weight of the patient. In these embodiments, “kg” is kilograms of body weight of the patient.

In all of the dose embodiments described above, the percentages of the “full dose” of the compound, or the pharmaceutically acceptable salt thereof, administered at any step in a dose escalation sequence can be about 1 percent, about 2 percent, about 3 percent, about 4 percent, about 5 percent, about 6 percent, about 7 percent, about 8 percent, about 9 percent, about 10 percent, about 20 percent, about 30 percent, about 40 percent, about 50 percent, about 60 percent, about 70 percent, about 80 percent, about 90 percent, about 100 percent, about 200 percent, about 300 percent, about 400 percent, or about 500 percent of the “full dose” of the compound, or the pharmaceutically acceptable salt thereof. The “full dose” of the compound, or the pharmaceutically acceptable salt thereof, can be any of the doses of the compound, or the pharmaceutically acceptable salt thereof, described in the preceding paragraphs as being doses administered in a dose escalation sequence.

In various other embodiments, the dose of the compound, or the pharmaceutically acceptable salt thereof, may range from, for example, about 1 nmol/kg to about 10000 nmol/kg, from about 1 nmol/kg to about 5000 nmol/kg, from about 1 nmol/kg to about 3000 nmol/kg, about 1 nmol/kg to about 2500 nmol/kg, about 1 nmol/kg to about 2000 nmol/kg, about 1 nmol/kg to about 1000 nmol/kg, about 1 nmol/kg to about 900 nmol/kg, about 1 nmol/kg to about 800 nmol/kg, about 1 nmol/kg to about 700 nmol/kg, about 1 nmol/kg to about 600 nmol/kg, about 1 nmol/kg to about 500 nmol/kg, about 1 nmol/kg to about 400 nmol/kg, about 1 nmol/kg to about 300 nmol/kg, about 1 nmol/kg to about 200 nmol/kg, about 1 nmol/kg to about 150 nmol/kg, about 1 nmol/kg to about 100 nmol/kg, about 1 nmol/kg to about 90 nmol/kg, about 1 nmol/kg to about 80 nmol/kg, about 1 nmol/kg to about 70 nmol/kg, about 1 nmol/kg to about 60 nmol/kg, about 1 nmol/kg to about 50 nmol/kg, about 1 nmol/kg to about 40 nmol/kg, about 1 nmol/kg to about 30 nmol/kg, or about 1 nmol/kg to about 20 nmol/kg. In these embodiments, “kg” is kilograms of body weight of the patient.

In another embodiment, from about 20 ug/kg of body weight of the patient to about 3 mg/kg of body weight of the patient of the compound, or the pharmaceutically acceptable salt thereof, can be administered to the patient. In another aspect, amounts can be from about 0.2 mg/kg of body weight of the patient to about 0.4 mg/kg of body weight of the patient.

In any of the above-described dose embodiments, a single dose or multiple doses of the compound, or pharmaceutically acceptable salt thereof, may be administered to the patient.

In one embodiment, the small molecule ligand linked to the targeting moiety can be administered to the patient before the CAR T cell composition. In another embodiment, the small molecule ligand linked to the targeting moiety can be administered to the patient at the same time as the CAR T cell composition, but in different formulations, or in the same formulation. In yet another embodiment, the small molecule ligand linked to the targeting moiety can be administered to the patient after the CAR T cell composition.

In one illustrative aspect, the timing between the administration of CAR T cells and the small molecule linked to the targeting moiety may vary widely depending on factors that include the type of CAR T cells being used, the binding specificity of the CAR, the identity of the targeting moiety and the small molecule ligand, the identity of the cancer, the location in the patient of the cancer, the means used to administer to the patient the CAR T cells and the small molecule ligand linked to the targeting moiety, and the health, age, and weight of the patient. In one aspect, the small molecule ligand linked to the targeting moiety can be administered before or after the CAR T cells, such as within about 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 33, 36, 39, 42, 45, 48, or 51 hours, or within about 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 9, 10 or more days.

In one embodiment, any applicable dosing schedule known in the art can be used for administration of the compound, or the pharmaceutically acceptable salt thereof, or for the CAR T cell composition. In one aspect, the dosing schedule selected for the compound, or the pharmaceutically acceptable salt thereof, and the CAR T cell composition can take into consideration the concentration of the compound, or the pharmaceutically acceptable salt thereof, and the number of CAR T cells administered, to regulate the cytotoxicity of the CAR T cell composition and to control CRS.

In one exemplary embodiment, the first dose escalation sequence, the second dose escalation sequence, the third dose escalation sequence, the fourth dose escalation sequence, the fifth dose escalation sequence, the sixth dose escalation sequence, or any additional dose escalation sequence can be followed by a period of time during which the compound, or the pharmaceutically acceptable salt thereof, is not administered. In various illustrative embodiments, the period of time that the compound, or the pharmaceutically acceptable salt thereof, is not administered can be 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 days. In another embodiment, the period of time that the compound, or the pharmaceutically acceptable salt thereof, is not administered can be 6, 7, or 8 days. In yet another embodiment, the period of time that the compound, or the pharmaceutically acceptable salt thereof, is not administered can be 7 days.

In one aspect, a first dose of the CAR T cells and a second dose of the CAR T cells are administered to the patient during week 1, for example, on Monday and Thursday. In this embodiment, the first dose escalation sequence for the compound, or the pharmaceutically acceptable salt thereof, can then occur during weeks 2 and 3. For example, the compound, or the pharmaceutically acceptable salt thereof, can be administered on three separate days and the three separate days can be Monday and Thursday of week 2 and Monday of week 3 (see Sequence 1 in Fig. 1). In this embodiment, the second dose escalation sequence for the compound, or the pharmaceutically acceptable salt thereof, can then occur during weeks 4 and 5 (see Sequence 2 in Fig. 1). For example, the compound, or the pharmaceutically acceptable salt thereof, can be administered on three separate days and the three separate days can be Monday and Thursday of week 4 and Monday of week 5. In this embodiment, the third dose escalation sequence for the compound, or the pharmaceutically acceptable salt thereof, can then occur during weeks 6 and 7 (see Sequence 3 in Fig. 1). For example, the compound, or the pharmaceutically acceptable salt thereof, can be administered on three separate days and the three separate days can be Monday and Thursday of week 6 and Monday of week 7. In other embodiments, subsequent dose escalation sequences can follow a similar sequence, or Sequence 3 can be repeated about 7 days after Sequence 3 ends as “Course 2” and no additional treatments with the compound, or the pharmaceutically acceptable salt thereof, occur until the beginning of “Course 3” (the length of “Course 2” being based on the length of time shown in Fig.1 for “Course” 1). In one embodiment, the patient can receive four Courses of therapy.

In another illustrative embodiment, a method of treatment of a cancer is provided. The method comprises i) administering to a patient at least one dose of a CAR T cell composition comprising CAR T cells wherein the CAR T cells comprise a CAR directed to a targeting moiety, ii) administering to the patient a compound, or a pharmaceutically acceptable salt thereof, wherein the compound comprises a small molecule ligand linked to a targeting moiety by a linker and wherein the compound, or the pharmaceutically acceptable salt thereof, is administered in a first dose escalation sequence wherein, if serious CRS occurs in the first dose escalation sequence, the compound, or the pharmaceutically acceptable salt thereof, is administered using a lower dose escalation sequence wherein the first dose of the compound, or the pharmaceutically acceptable salt thereof, in the lower dose escalation sequence is lower than the first dose of the compound, or the pharmaceutically acceptable salt thereof, administered in the first dose escalation sequence. In this embodiment, in the lower dose escalation sequence, the compound, or the pharmaceutically acceptable salt thereof, is administered at about 0.5 percent, about 5 percent, and about 50 percent of a full dose of the compound, or the pharmaceutically acceptable salt thereof, on three separate days. In this embodiment, the full dose of the compound, or the pharmaceutically acceptable salt thereof, can be about 10 µg/kg to about 50 µg/kg, about 20 µg/kg to about 40 µg/kg, about 25 µg/kg to about 35 µg/kg, or about 30 µg/kg. In these embodiments, "kg" is kilograms of body weight of the patient.

In various related embodiments, lymphocytes can be depleted in the patient before administration of the CAR T cell composition to the patient using, for example, cytoxan, fludarabine, and/or etoposide, and the method can further comprise additional steps including, but not limited to, administering platelets to the patient, administering packed red blood cells to the patient, administering cryoprecipitate to the patient, administering intravenous immunoglobulin to the patient, treating with calcium supplements, acid-citrate-dextrose, and/or heparin, and/or providing antimicrobial therapy to the patient. In one embodiment, lymphodepletion occurs at least about 24 hours prior to CAR T cell administration.

In another aspect, the method can comprise CRS monitoring steps. In one illustrative aspect, if no CRS or neurotoxicity is observed in the patient during the first dose escalation sequence, the method can be advanced to the second dose escalation sequence. If no CRS or neurotoxicity is observed in the patient during the second dose escalation sequence, the method can be advanced to the third dose escalation sequence. If no CRS or neurotoxicity is

observed in the patient during the third dose escalation sequence, the method can be advanced to the fourth dose escalation sequence. If no CRS or neurotoxicity is observed in the patient during the fourth dose escalation sequence, the method can be advanced to the fifth dose escalation sequence. If no CRS or neurotoxicity is observed in the patient during the fifth dose escalation sequence, the method can be advanced to the sixth dose escalation sequence, and so on.

In another illustrative embodiment, if fever without hypotension (i.e., non-serious CRS) is observed in the patient and no neurotoxicity is observed in the patient during any one of the dose escalation sequences, all subsequent doses of the compound, or the pharmaceutically acceptable salt thereof, can be administered to the patient at the dose escalation sequence level that caused the fever without hypotension. In another aspect, if CRS (i.e., serious CRS) or neurotoxicity occurs in the patient in any dose escalation sequence, all subsequent doses of the compound, or the pharmaceutically acceptable salt thereof, can be administered to the patient at the dose escalation sequence level below the dose escalation sequence level that caused the serious CRS or neurotoxicity in the patient.

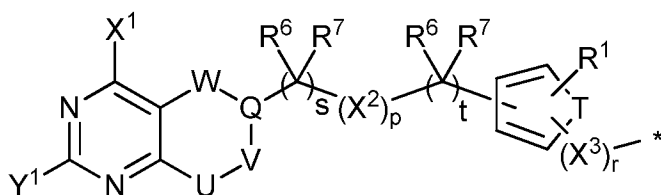
In one aspect serious CRS may include any toxicity requiring the use of cetuximab, any $\geq$ grade 3 autoimmune toxicity, any $\geq$ grade 3 toxicity that may be attributed to CAR T cell administration or administration of the compound, or the pharmaceutically acceptable salt thereof, and that occurs within 28 days following initiation of treatment except $\leq$ grade 4 fever lasting for less than 48 hours after initiation of treatment, $\leq$ grade 3 chills lasting for less than 24 hours after initiation of treatment, $\leq$ grade 3 cough lasting for less than 24 hours after initiation of treatment, $\leq$ grade 3 transaminases lasting for less than 7 days after initiation of treatment, $\leq$ grade 3 hypotension lasting for less than 48 hours after initiation of treatment, $\leq$ grade 3 CRS lasting for less than 48 hours after initiation of treatment, $\leq$ grade 3 anaphylaxis related to DMSO resolvable with Benadryl and/or epinephrine, or $\leq$ grade 3 pain controlled with oral or IV narcotic therapy, or, in addition, except for toxicities occurring about 3 weeks after the CAR T cell infusion including $\leq$ grade 3 chills lasting up to 5 days, $\leq$ grade 3 transaminases lasting up to 2 weeks, $\leq$ grade 3 CRS lasting up to 2 weeks, $\leq$ grade 4 lymphopenia, $\leq$ grade 4 leukopenia, or $\leq$ grade 3 pain lasting for up to 2 weeks controlled with oral or IV narcotic therapy.

In one embodiment, to prevent or inhibit CRS in the patient, the method can further comprise the step of administering to the patient a folate, a conjugate comprising a

folate wherein the conjugate comprising a folate does not comprise a targeting moiety, or a drug that inhibits activation of the CAR T cells. In this embodiment, any of a folate, a conjugate comprising a folate wherein the conjugate comprising a folate does not comprise a targeting moiety, or a drug that inhibits activation of the CAR T cells can be referred to herein as “a rescue agent”. In one embodiment, a folate, such as folic acid, can be administered to prevent or inhibit CRS in the patient. In this embodiment, the folate inhibits interaction of the bridge (i.e., the small molecule ligand linked to the targeting moiety by a linker) with the receptors for the bridge on the tumor inhibiting tumor lysis and preventing or inhibiting CRS in the patient.

In one embodiment, the folate administered as an inhibitor of binding of the bridge to the tumor can be, for example, folic acid, a folic acid analog, or another folate receptor-binding molecule. In various embodiments, analogs of folate that can be used include folinic acid, pteropolyglutamic acid, and folate receptor-binding pteridines such as tetrahydropterins, dihydrofolates, tetrahydrofolates, and their deaza and dideaza analogs. The terms “deaza” and “dideaza” analogs refers to the art recognized analogs having a carbon atom substituted for one or two nitrogen atoms in the naturally occurring folic acid structure. For example, the deaza analogs include the 1-deaza, 3-deaza, 5-deaza, 8-deaza, and 10-deaza analogs. The dideaza analogs include, for example, 1,5 dideaza, 5,10-dideaza, 8,10-dideaza, and 5,8-dideaza analogs. The foregoing folic acid analogs are conventionally termed “folates,” reflecting their capacity to bind to folate receptors. Other folate receptor-binding analogs include aminopterin, amethopterin (methotrexate), N10-methylfolate, 2-deamino-hydroxyfolate, deaza analogs such as 1-deazamethopterin or 3-deazamethopterin, and 3',5'-dichloro-4-amino-4-deoxy-N10-methylpteroylglutamic acid (dichloromethotrexate).

In another embodiment, the folate administered as an inhibitor of binding of the bridge to the tumor has the formula



wherein X^1 and Y^1 are each-independently selected from the group consisting of halo, R^2 , OR^2 , SR^3 , and NR^4R^5 ;

U, V, and W represent divalent moieties each independently selected from the group consisting of $-(R^{6a})C=$, $-N=$, $-(R^{6a})C(R^{7a})-$, and $-N(R^{4a})-$; Q is selected from the group consisting of C and CH; T is selected from the group consisting of S, O, N, and $-C=C-$;

5 X^2 and X^3 are each independently selected from the group consisting of oxygen, sulfur, $-C(Z)-$, $-C(Z)O-$, $-OC(Z)-$, $-N(R^{4b})-$, $-C(Z)N(R^{4b})-$, $-N(R^{4b})C(Z)-$, $-OC(Z)N(R^{4b})-$, $-N(R^{4b})C(Z)O-$, $-N(R^{4b})C(Z)N(R^{5b})-$, $-S(O)-$, $-S(O)_2-$, $-N(R^{4a})S(O)_2-$, $-C(R^{6b})(R^{7b})-$, $-N(C\equiv CH)-$, $-N(CH_2C\equiv CH)-$, C_1-C_{12} alkylene, and C_1-C_{12} alkyneoxy, where Z is oxygen or sulfur;

10 R^1 is selected from the group consisting of hydrogen, halo, C_1-C_{12} alkyl, and C_1-C_{12} alkoxy;

R^2 , R^3 , R^4 , R^{4a} , R^{4b} , R^5 , R^{5b} , R^{6b} , and R^{7b} are each independently selected from the group consisting of hydrogen, halo, C_1-C_{12} alkyl, C_1-C_{12} alkoxy, C_1-C_{12} alkanoyl, C_1-C_{12} alkenyl, C_1-C_{12} alkynyl, $(C_1-C_{12}$ alkoxy)carbonyl, and $(C_1-C_{12}$ alkylamino)carbonyl;

15 R^6 and R^7 are each independently selected from the group consisting of hydrogen, halo, C_1-C_{12} alkyl, and C_1-C_{12} alkoxy; or, R^6 and R^7 are taken together to form a carbonyl group;

R^{6a} and R^{7a} are each independently selected from the group consisting of hydrogen, halo, C_1-C_{12} alkyl, and C_1-C_{12} alkoxy; or R^{6a} and R^{7a} are taken together to form a carbonyl group;

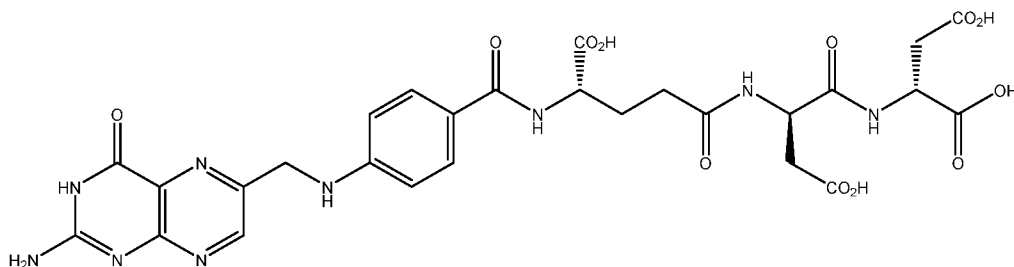
20 p, r, s, and t are each independently either 0 or 1; and

* represents an optional covalent bond to the rest of the conjugate, if any additional chemical moieties are part of the folate.

In yet another embodiment, a conjugate comprising a folate can be administered to prevent or inhibit cytokine release syndrome (CRS) in the patient. CRS can cause
25 detrimental effects to the patient, including, but not limited to weight loss, high fever, pulmonary edema, and a dangerous drop in blood pressure.

In this embodiment, the conjugate comprising a folate does not comprise a targeting moiety, and, thus, the conjugate inhibits interaction of the bridge with the tumor to prevent tumor lysis and reduce CRS in the patient. In this embodiment, the folate moiety in the
30 conjugate comprising a folate can comprise any of the folates described in the preceding paragraphs linked to a chemical moiety that does not comprise a targeting moiety. In one aspect, the conjugate comprising a folate can comprise a folate linked to one or more amino

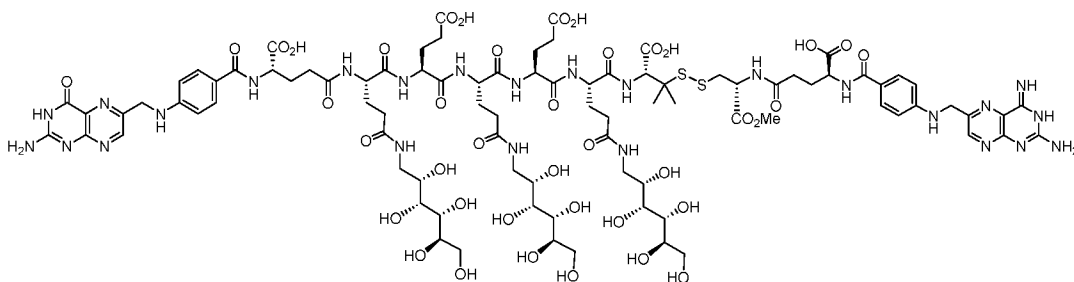
acids that do not comprise a targeting moiety. Illustratively, the conjugate comprising a folate can have the formula



5

This compound can also be referred to as “EC923”. In these embodiments, the folate or the conjugate comprising a folate can be administered to the patient in molar excess relative to the bridge (i.e., the small molecule ligand linked to a targeting moiety by a linker), such as a 10-fold excess, a 100-fold excess, a 200-fold excess a 300-fold excess a 400-fold excess a 500-fold excess a 600-fold excess a 700-fold excess a 800-fold excess a 900-fold excess, a 1000-fold excess, or a 10,000-fold excess of the folate or the conjugate comprising a folate relative to the small molecule ligand linked to a targeting moiety by a linker. The amount of the folate or the conjugate comprising a folate relative to the amount of the small molecule ligand linked to a targeting moiety by a linker needed to inhibit interaction of the bridge with the tumor can be determined by the skilled artisan.

In another embodiment, an agent that inhibits activation of the CAR T cells can be administered to the patient to inhibit CAR T cell activation and to inhibit or prevent CRS in the patient. In one aspect the agent can be selected from the group consisting of a lymphocyte-specific protein tyrosine kinase inhibitor (e.g., Dasatinib), a PI3 kinase inhibitor (e.g., GDC0980), Tocilizumab, an inhibitor of an IL-2 inducible T cell kinase (e.g., BMS-509744), JAK inhibitors, BTK inhibitors, SIP agonists (e.g. Siponimod and Ozanimod), and an agent that blocks CAR T cell binding to the bridge, but does not bind to the cancer (e.g., fluoresceinamine, FITC, or sodium fluorescein). It is understood by the skilled artisan that FITC (i.e., fluorescein) can be in the form of a salt (e.g., sodium fluorescein), or in its unsalted form, under physiological conditions or, for example, in a buffer at physiological pH. Accordingly, in one embodiment, when fluorescein is administered to a patient it may be in equilibrium between its salted form (e.g., sodium fluorescein) and its unsalted form. In another embodiment, a rescue agent that inhibits activation of CAR T cells can be a compound of the formula



This compound can also be referred to as “EC2319”.

5 In various embodiments, the rescue agent can be administered at a concentration of from about .001 nM to about 100 mM, about .01 nM to about 100 mM, about 1 nM to about 100 mM, about 10 nM to about 100 mM, about 50 nM to about 100 mM, or from about 100 nM to about 100 mM in any appropriate volume, including, for example, 0.1 ml, 0.2 ml, 0.3 ml, 0.4 ml, 0.5 ml, 0.6 ml, 0.7 ml, 0.8 ml, 0.9 ml, 1 ml, 2 ml, 3 ml, 4 ml, 5 ml, 10 ml, 100 ml, or 1000 ml. In other embodiments, the rescue agent can be administered at a dose of about .01 to about 300 umoles/kg of body weight of the patient, about .06 to about 100 umoles/kg of body weight of the patient, about .06 to about 90 umoles/kg of body weight of the patient, about .06 to about 80 umoles/kg of body weight of the patient, about .06 to about 70 umoles/kg of body weight of the patient, about .06 to about 60 umoles/kg of body weight of the patient, about .06 to about 50 umoles/kg of body weight of the patient, about .06 to about 40 umoles/kg of body weight of the patient, about .06 to about 30 umoles/kg of body weight of the patient, about .06 to about 20 umoles/kg of body weight of the patient, about .06 to about 10 umoles/kg of body weight of the patient, about .06 to about 8 umoles/kg of body weight of the patient, or about .06 to about 6 umoles/kg of body weight of the patient.

20 In these embodiments, the rescue agent can be administered to the patient in molar excess relative to the compound, or its pharmaceutically acceptable salt (i.e., the small molecule ligand linked to a targeting moiety by a linker), such as about a 10-fold excess, about a 20-fold excess, about a 30-fold excess, about a 40-fold excess, about a 50-fold excess, about a 60-fold excess, about a 70-fold excess, about a 80-fold excess, about a 90-fold excess, about a 100-fold excess, about a 200-fold excess, about a 300-fold excess, about a 400-fold excess, about a 500-fold excess, about a 600-fold excess, about a 700-fold excess, about a 800-fold excess, about a 900-fold excess, about a 1000-fold excess, or about a 10,000-fold excess of the rescue agent relative to the small molecule ligand linked to a targeting moiety by a linker. The

amount of the rescue agent relative to the amount of the small molecule ligand linked to a targeting moiety by a linker needed to inhibit interaction of the compound, or its pharmaceutically acceptable salt, with the tumor and/or the CAR T cells can be determined by the skilled artisan.

5 In another embodiment, more than one dose can be administered to the patient of the folate, the conjugate comprising a folate wherein the conjugate comprising a folate does not comprise a targeting moiety, or the agent that inhibits activation of the CAR T cells.

 In the 'rescue agent' embodiments described herein, the folate, the conjugate comprising a folate wherein the conjugate comprising a folate does not comprise a targeting
10 moiety, or the agent that inhibits activation of the CAR T cells can be administered to the patient before and/or after the compound, or the pharmaceutically acceptable salt thereof. In another aspect, the compound, or the pharmaceutically acceptable salt thereof, can be administered before and subsequent to administration of the folate, the conjugate comprising a folate wherein the conjugate comprising a folate does not comprise a targeting moiety, or the
15 agent that inhibits activation of the CAR T cells. In this embodiment, the subsequent administration of the compound, or the pharmaceutically acceptable salt thereof, can cause CAR T cell activation and an increase in cytokine levels in the patient.

 In another embodiment, administration of the folate, the conjugate comprising a folate wherein the conjugate comprising a folate does not comprise a targeting moiety, or the
20 agent that inhibits activation of the CAR T cells can cause reduction in cytokine levels in the patient. In another embodiment, the reduction in cytokine levels is a reduction to about the cytokine levels in an untreated patient. In other embodiments, the agent that inhibits activation of the CAR T cells is administered to the patient when the CRS grade reaches 1, 2, 3, or 4 or when the CRS grade reaches 3 or 4.

25 In any of the embodiments described herein where a folate is the ligand linked to the targeting moiety by a linker, the patient can be put on a folate deficient diet prior to treatment with the methods described herein, or the patient can be administered a folate in the diet. In the embodiment where the patient is administered folate, the dose can range, for example, from about 50 nmol/kg to about 3000 nmol/kg of patient body weight, about 50
30 nmol/kg to about 2000 nmol/kg, about 50 nmol/kg to about 1000 nmol/kg, about 50 nmol/kg to about 900 nmol/kg, about 50 nmol/kg to about 800 nmol/kg, about 50 nmol/kg to about 700 nmol/kg, about 50 nmol/kg to about 600 nmol/kg, about 50 nmol/kg to about 500 nmol/kg,

about 50 nmol/kg to about 400 nmol/kg, about 50 nmol/kg to about 300 nmol/kg, about 50 nmol/kg to about 200 nmol/kg, about 50 nmol/kg to about 100 nmol/kg, about 100 nmol/kg to about 300 nmol/kg, about 100 nmol/kg to about 500 nmol/kg, about 100 nmol/kg to about 1000 nmol/kg, about 100 nmol/kg to about 2000 nmol/kg of patient body weight. In other
5 embodiments, the dose may be about 100 nmol/kg, about 150 nmol/kg, about 200 nmol/kg, about 250 nmol/kg, about 300 nmol/kg, about 350 nmol/kg, about 400 nmol/kg, about 450 nmol/kg, about 500 nmol/kg, about 600 nmol/kg, about 700 nmol/kg, about 800 nmol/kg, about 900 nmol/kg, about 1000 nmol/kg, about 2000 nmol/kg, or about 3000 nmol/kg of patient body weight. In these embodiments, “kg” is kilograms of patient body weight. In one aspect, the
10 folate can be administered, for example, daily, weekly, biweekly, three times a week, or using any suitable regimen for administration of the folate.

In various embodiments described herein, the CAR T cells can persist in elevated numbers of circulating CAR T cells for as long as about 10 days, as long as about 15 days, as long as about 20 days, as long as about 25 days, as long as about 30 days, as long as
15 about 35 days, as long as about 40 days, as long as about 45 days, as long as about 50 days, as long as about 55 days, as long as about 60 days, as long as about 65 days, as long as about 70 days, as long as about 75 days, or as long as about 80 days post CAR T cell administration.

In various embodiments described herein, half-maximal effective concentrations (EC₅₀) for the compound, or the pharmaceutically acceptable salt thereof, can be about 1 pM to
20 about 2 nM, about 1 pM to about 5 nM, about 1 pM to about 10 nM, about 1 pM to about 20 nM, about 1 pM to about 30 nM, about 1 pM to about 40 nM, about 1 pM to about 50 nM, about 1 pM to about 60 nM, about 1 pM to about 70 nM, about 1 pM to about 80 nM, about 1 pM to about 90 nM, about 1 pM to about 100 nM, about 1 pM to about 200 nM, about 1 pM to about 300 nM, about 1 pM to about 400 nM, about 1 pM to about 500 nM, about 1 pM to about
25 600 nM, about 1 pM to about 700 nM, about 1 pM to about 800 nM, about 1 pM to about 900 nM, about 1 pM to about 1 nM, about 1 pM to about 900 pM, about 1 pM to about 800 pM, about 1 pM to about 700 pM, about 1 pM to about 600 pM, about 1 pM to about 500 pM, about 1 pM to about 400 pM, about 1 pM to about 300 pM, about 1 pM to about 200 pM, about 1 pM to about 100 pM, about 1 pM to about 90 pM, about 1 pM to about 80 pM, about 1 pM to about
30 70 pM, about 1 pM to about 60 pM, about 1 pM to about 50 pM, about 1 pM to about 40 pM, about 1 pM to about 30 pM, about 1 pM to about 20 pM, about 1 pM to about 10 pM, or about 1 pM to about 5 pM.

In various embodiments described herein, the K_d for binding of the compound, or the pharmaceutically acceptable salt thereof, to the CAR T cells can be about 1 nM to about 100 nM, about 1 nM to about 200 nM, about 1 nM to about 300 nM, about 1 nM to about 400 nM, about 1 nM to about 500 nM, about 1 nM to about 600 nM, about 1 nM to about 700 nM, about 1 nM to about 800 nM, about 1 nM to about 900 nM, about 100 nM to about 500 nM, about 100 nM to about 400 nM, about 100 nM to about 300 nM, about 100 nM to about 200 nM, about 100 nM to about 150 nM, or about 130 nM.

In the various embodiments described herein, EGFRt-sorted or unsorted CAR T cells can be used. In another embodiment, a “clinical facsimile” batch of CAR T cells can be used with a low differentiation profile. In another embodiment, a “research batch” of CAR T cells can be used. The “clinical facsimile” batch (~39% EGFRt+) can comprise CD4+ subsets at about 66% T_{SCM} and about 32% T_{CM} and CD8 subsets at about 95% T_{SCM} and about 3% T_{CM} . The research batch (~23% EGFRt+) can comprise CD4 subsets at about 32% T_{SCM} , about 53% T_{CM} , about 11% T_{EM} and about 3.7% T_{EFF} and CD8 subsets at about 44% T_{SCM} , about 0.28% T_{CM} , about 3.4% T_{EM} and about 52% T_{EFF} .

In various illustrative embodiments described herein, the compound, or the pharmaceutically acceptable salt thereof, can be first administered to the patient about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, or about 10 days before or after the CAR T cells, or on any appropriate day before or after the CAR T cells.

In one embodiment of the methods described herein, the cancer is imaged prior to administration to the patient of the compound, or the pharmaceutically acceptable salt thereof, or prior to administration of the CAR T cell composition to the patient. In one illustrative embodiment, imaging occurs by PET imaging. In other illustrative embodiments imaging occurs by MRI imaging or SPECT/CT imaging. The imaging method can be any suitable imaging method known in the art. In one embodiment, the imaging method can involve the use of the small molecule ligand described herein, but linked to an imaging agent suitable for the types of imaging described herein to determine if the patient is positive for folate receptor expression. In another embodiment, immunohistochemical analysis can be used for this purpose.

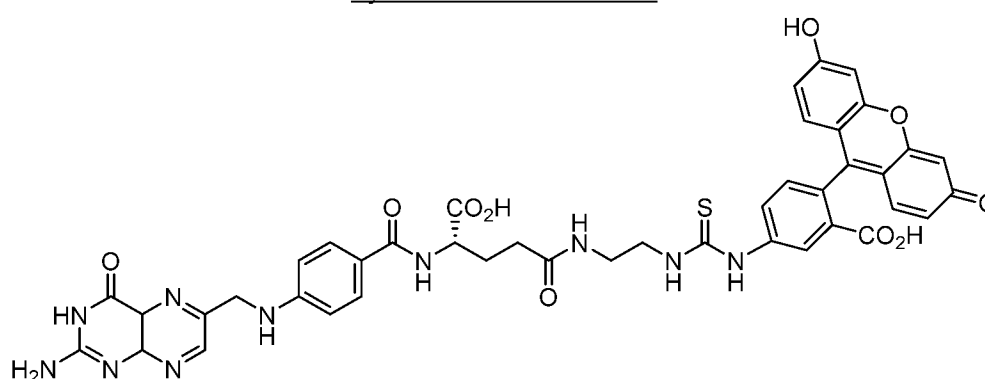
In any of the embodiments described herein, cytokine release resulting in off-target toxicity in the patient may not occur even though CAR T cell toxicity to the cancer occurs. In any embodiment described herein, off-target tissue toxicity may not occur in the

patient even though CAR T cell toxicity to the cancer occurs. In any embodiment described herein, the cancer may comprise a tumor, and tumor size may be reduced in the patient, even though off-target toxicity does not occur. In any of the embodiments described herein, CRS can be reduced or prevented and the method can result in a decrease in tumor volume in the patient.

- 5 In any embodiment described herein, body weight loss due to CRS, and CAR T cell exhaustion can be reduced or prevented. In any embodiment described herein, the cancer can comprise a tumor and a complete response for the tumor may be obtained.

EXAMPLE 1

Synthesis of FITC-Folate

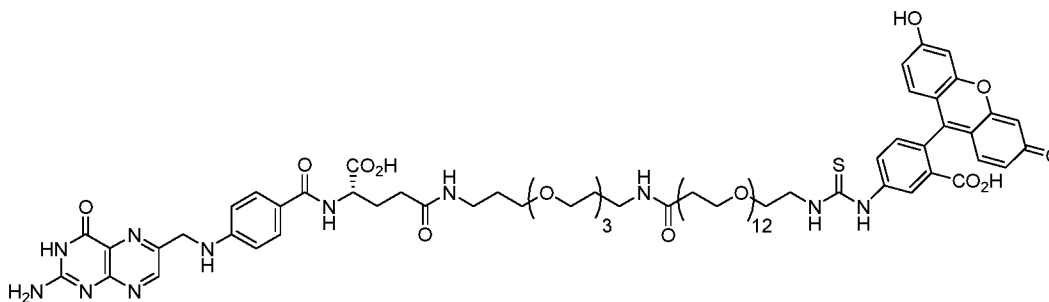


Folate- γ -ethylenediamine was coupled to fluorescein isothiocyanate (FITC) isomer I (Sigma-Aldrich) in anhydrous dimethylsulfoxide (DMF) in the presence of tetramethylguanidine and diisopropylamine. The crude product was loaded onto an Xterra

- 15 RP18 preparative HPLC column (Waters) and eluted with gradient conditions starting with 99% 5 mM sodium phosphate (mobile phase A, pH 7.4) and 1% acetonitrile (mobile phase B) and reaching 90% A and 10% B in 10 min at a flow rate of 20 mL/min. Under these conditions, the FITC-folate main peak typically eluted at 27–50 min. The quality of the FITC-folate fraction was monitored by analytical reverse-phase HPLC with a UV detector. Fractions with greater
20 than 98.0% purity (LCMS) were lyophilized to obtain the final FITC-folate product. As known in the art, the compound with this structure is also referred to as EC17.

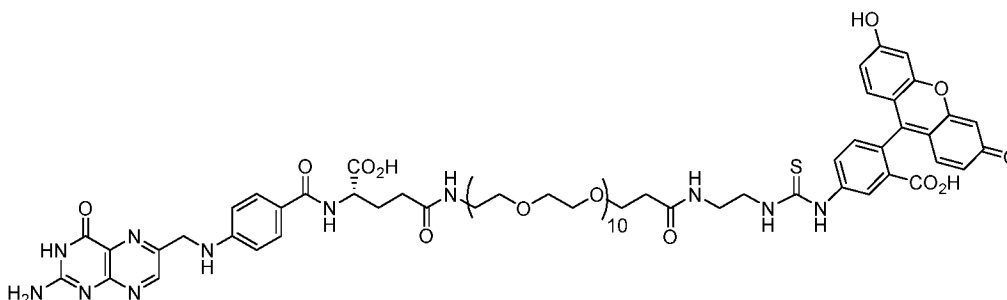
EXAMPLE 2

Synthesis of FITC-PEG12-Folate



Universal polyethylene glycol (PEG) Nova Tag™ resin (0.2 g) was loaded into a peptide synthesis vessel and washed with isopropyl alcohol (*i*-PrOH) (3 x 10 mL) and dimethylformamide (DMF, 3 x 10mL). 9-fluorenylmethoxycarbonyl (Fmoc) deprotection was carried out using 20% piperidine in DMF (3 x 10 mL). Kaiser tests were performed to assess reaction progress. To the vessel was then introduced a solution of Fmoc-L-glutamic acid 5-tert-butyl ester (Fmoc-Glu-(O-*t*-Bu)-OH) (23.5 mg) in DMF, *N,N*-diisopropylethylamine (*i*-Pr₂NEt) (4 equiv), and benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) (2 equiv). Fmoc deprotection was carried out using 20% piperidine in DMF (3 x 10 mL). To the vessel was then introduced a solution of N¹⁰-TFA-Pte-OH (22.5 mg), DMF, *i*-Pr₂NEt (4 equiv), and PyBOP (2 equiv). Argon was bubbled for 2 h, and the resin was washed with DMF (3 x 3 mL) and *i*-PrOH (3 x 3 mL). After swelling the resin in dichloromethane (DCM), a solution of 1M hydroxybenzotriazole (HOBT) in DCM/trifluoroethane (TFE) (1:1) (2 x 3 mL) was added. Argon was bubbled for 1 h, the solvent was removed, and the resin was washed with DMF (3 x 3 mL) and *i*-PrOH (3 x 3 mL). After swelling the resin in DMF, a solution of Fmoc-NH-(PEG)₁₂-COOH (46.3 mg) in DMF, *i*-Pr₂NEt (4 equiv), and PyBOP (2 equiv) was added. Argon was bubbled for 2 h, and the resin was washed with DMF (3 x 3 mL) and *i*-PrOH (3 x 3 mL). Fmoc deprotection was carried out using 20% piperidine in DMF (3 x 10 mL). Kaiser tests were performed to assess reaction progress. To the vessel was then introduced a solution of FITC (Life Technologies 21.4 mg) in DMF and *i*-Pr₂NEt (4 equiv), then Argon was bubbled for 2 h, and the resin was washed with DMF (3 x 3 mL) and *i*-PrOH (3 x 3 mL). Then to the vessel was added 2% NH₂NH₂ in DMF (2 x 2mL). The final compound was cleaved from the resin using a TFA:H₂O: triisopropylsilane (TIS) (95:2.5:2.5) (Cleavage Solution) and concentrated under vacuum. The concentrated product was precipitated in Et₂O and dried under vacuum. The crude product was purified using preparative RP-HPLC (mobile phase: A = 10 mM ammonium acetate pH = 7, B = ACN; method: 0% B to 30% B in 30 min at 13 mL/min). The pure fractions were pooled and freeze-dried, providing the FITC-PEG12-Folate.

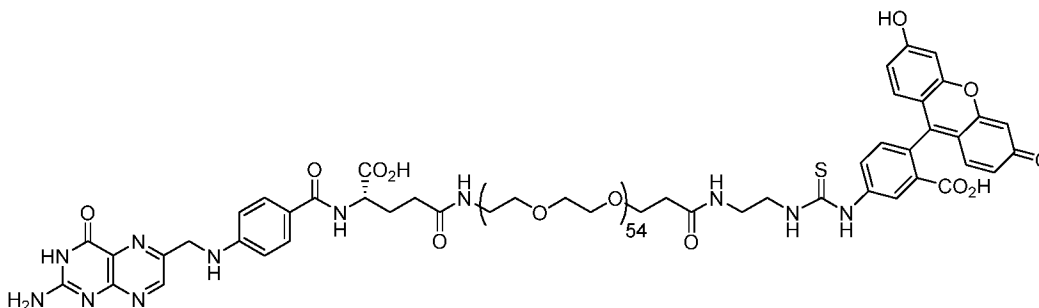
EXAMPLE 3
Synthesis of FITC-PEG20-Folate



5 Ethylenediamine, polymer-bound (200-400 mesh)-resin (50 mg) was loaded into a peptide synthesis vessel and swollen with DCM (3 mL) followed by DMF (3 mL). To the vessel was then introduced the Fmoc-PEG₂₀-COOH solution (131 mg, 1.0 equiv) in DMF, *i*-Pr₂NEt (6.0 equiv), and PyBOP (4.0 equiv). Argon was bubbled for 6 h, the coupling solution was drained, and the resin was washed with DMF (3 x 10 mL) and *i*-PrOH (3 x 10 mL). Kaiser tests were performed to assess reaction progress. Fmoc deprotection was carried out using 20% piperidine in DMF (3 x 10 mL), before each amino acid coupling. The above sequence was repeated to complete the reaction with Fmoc-Glu-OtBu (72 mg, 2.0 equiv) and Tfa.Pterioic-acid (41 mg, 1.2 equiv) coupling steps. The resin was washed with 2% hydrazine in DMF 3 x 10 mL (5 min) to cleave the trifluoro-acetyl protecting group on pterioic acid and washed with *i*-PrOH (3 x 10 mL) followed by DMF (3 x 10mL). The resin was dried under argon for 30 min. The folate-peptide was cleaved from the resin using the Cleavage Solution. 10 mL of the cleavage mixture was introduced and argon was bubbled for 1.5 h. The cleavage mixture was drained into a clean flask. The resin was washed 3 times with more cleavage mixture. The combined mixture was concentrated under reduced pressure to a smaller volume (~ 5 mL) and precipitated in ethyl ether.

20 The precipitate was collected by centrifugation, washed with ethyl ether (3 times) and dried under high vacuum. The dried Folate-PEG₂₀-EDA (1.0 equiv) was treated with FITC (50 mg, 1.5 equiv) in DMSO and DIPEA at room temperature. Progress of the reaction monitored by LCMS. After 8 h the starting material was consumed to give the product. The crude reaction mixture was purified by preparative HPLC, (mobile phase A = 10mM Ammonium Acetate, pH = 7; Organic phase B = Acetonitrile; Method: 0% B to 30% B in 35 minutes at 13 mL/min) and provided FITC-PEG₂₀-Folate in 60% yield.

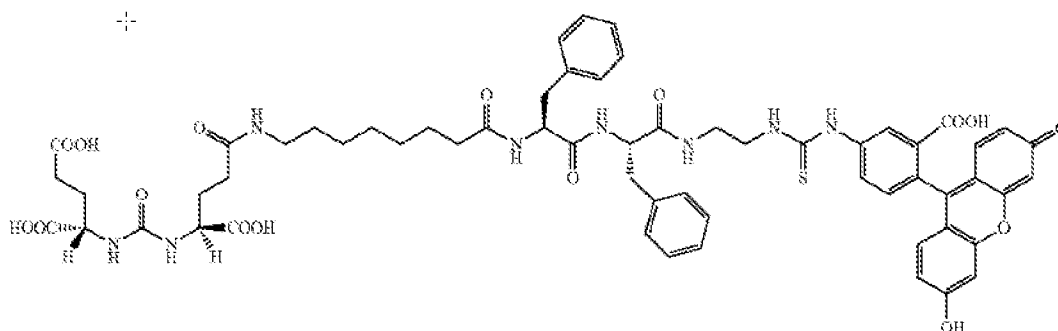
EXAMPLE 4
Synthesis of FITC-PEG108-Folate



Ethylenediamine, polymer-bound (200-400 mesh)-resin (50 mg) was loaded in a peptide synthesis vessel and swollen with DCM (3 mL) followed by DMF (3 mL). To the vessel was then introduced the Fmoc-PEG₃₆-COOH solution (161 mg, 1.0 equiv) in DMF, *i*-Pr₂NEt (6.0 equiv), and PyBOP (4.0 equiv). Argon was bubbled for 6 h, the coupling solution was drained, and the resin was washed with DMF (3 x 10 mL) and *i*-PrOH (3 x 10 mL). Kaiser tests were performed to assess reaction progress. Fmoc deprotection was carried out using 20% piperidine in DMF (3 x 10 mL), before each amino acid coupling. The above sequence was repeated to complete reaction with 2X Fmoc-PEG₃₆-COOH (161 mg, 1.0 equiv), Fmoc-Glu-OtBu (72 mg, 2.0 equiv) and Tfa.Pterioic-acid (41.0 mg, 1.2 equiv) coupling steps. At the end the resin was washed with 2% hydrazine in DMF 3 x 10mL (5 min) to cleave the trifluoroacetyl protecting group on pterioic acid and washed with *i*-PrOH (3 x 10mL) followed by DMF (3 x 10mL). The resin was dried under argon for 30 min. Folate-peptide was cleaved from the resin using the Cleavage Solution. 10mL of the cleavage mixture was introduced and argon was bubbled for 1.5 h. The cleavage mixture was drained into a clean flask. The resin was washed 3X with more Cleavage Solution. The combined mixture was concentrated under reduced pressure to a smaller volume (~ 5 mL) and precipitated in ethyl ether.

The precipitate was collected by centrifugation, washed with ethyl ether (3X) and dried under high vacuum. The dried Folate-PEG₁₀₈-EDA (1.0 equiv) was treated with FITC (50 mg, 1.5 equiv) in DMSO and DIPEA at room temperature. Reaction progress was monitored by LCMS. After 10 h starting material was consumed to give the product. The crude reaction mixture was purified by preparative HPLC, (mobile phase A = 10mM Ammonium Acetate, pH = 7; Organic phase B = Acetonitrile; Method: 0% B to 30% B in 35 minutes at 13 mL/min) and provided FITC-PEG108-Folate in 64% yield.

EXAMPLE 5
Synthesis of FITC-DUPA



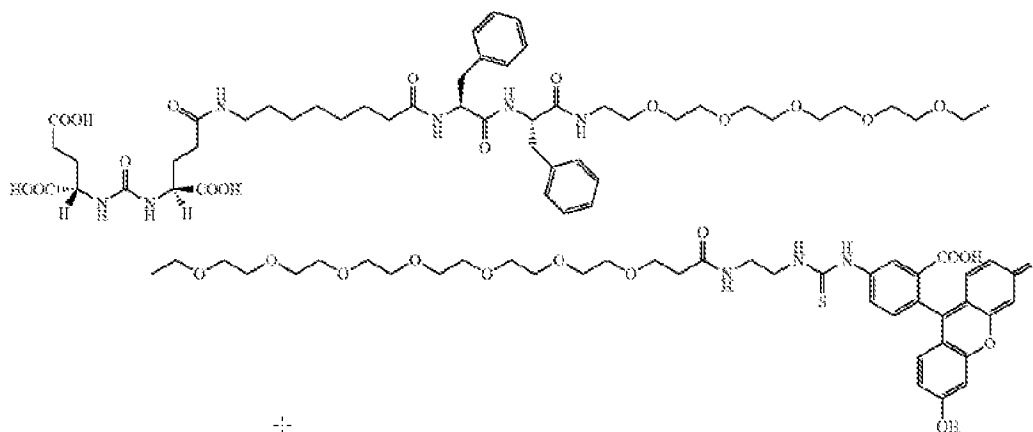
DUPA-FITC was synthesized by solid phase methodology as follows. Universal

- 5 Nova TagTM resin (50 mg, 0.53 mM) was swollen with DCM (3 mL) followed by DMF 3 mL). A solution of 20% piperidine in DMF (3 × 3 mL) was added to the resin, and argon was bubbled for 5 min. The resin was washed with DMF (3 × 3 mL) and isopropyl alcohol (*i*-PrOH, 3 × 3 mL). After swelling the resin in DMF, a solution of DUPA-(OtBu)-OH (1.5 equiv), HATU (2.5 equiv), and *i*-Pr₂NEt (4.0 equiv) in DMF was added. Argon was bubbled for 2 h,
- 10 and resin was washed with DMF (3 × 3 mL) and *i*-PrOH (3 × 3 mL). After swelling the resin in DCM, a solution of 1 M HOBt in DCM/TFE (1:1) (2 × 3 mL) was added. Argon was bubbled for 1 h, the solvent was removed and resin was washed with DMF (3 × 3 mL) and *i*-PrOH (3 × 3 mL). After swelling the resin in DMF, a solution of Fmoc-Phe-OH (2.5 equiv), HATU (2.5 equiv) and DIPEA (4.0 equiv) in DMF was added. Argon was bubbled for 2 h, and the resin
- 15 was washed with DMF (3 × 3 mL) and *i*-PrOH (3 × 3 mL). The above sequence was repeated for 2 more coupling steps for addition of 8-aminooctanoic acid and fluorescein isothiocyanate or rhodamine B isothiocyanate. The final compound was cleaved from the resin using the Cleavage Solution and concentrated under vacuum. The concentrated product was precipitated in diethyl ether and dried under vacuum. The crude product was purified using preparative RP-
- 20 HPLC [λ = 488 nm; solvent gradient: 1% B to 80% B in 25 min, 80% B wash 30 min run; A = 10 mM NH₄OAc, pH = 7; B = acetonitrile (ACN)]. ACN was removed under vacuum, and purified fractions were freeze-dried to yield FITC-DUPA as a brownish-orange solid. RP-HPLC: tR = 8.0 min (A = 10 mM NH₄OAc, pH = 7.0; B = ACN, solvent gradient: 1% B to 50% B in 10 min, 80% B wash 15 min run). ¹H NMR (DMSO-d₆/D₂O): δ 0.98–1.27 (ms, 9H); 1.45 (b, 3H); 1.68–1.85 (ms, 11H); 2.03 (m, 8H); 2.6–3.44 (ms, 12H); 3.82 (b, 2H); 4.35 (m, 1H); 6.53 (d, J = 8.1 Hz, 2H), 6.61 (dd, J = 5.3, 3.5 Hz, 2H); 6.64 (s, 2H); 7.05 (d, J = 8.2 Hz,
- 25

2H), 7.19 (m, 5H); 7.76 (d, $J = 8.0$ Hz, 1H); 8.38 (s, 1H). HRMS (ESI) (m/z): ($M + H$)⁺ calcd for C₅₁H₅₉N₇O₁₅S, 1040.3712, found, 1040.3702. UV/vis: λ max = 491 nm.

EXAMPLE 6

Synthesis of FITC-PEG12-DUPA

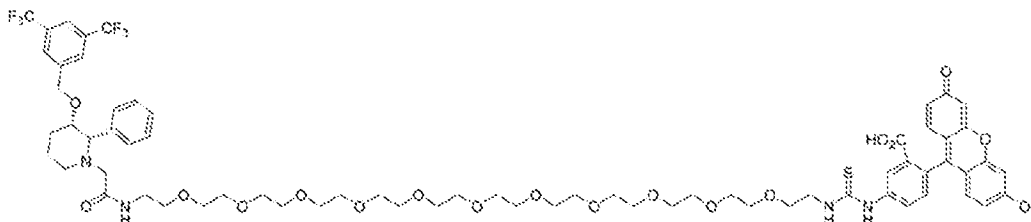


1,2-Diaminoethane trityl-resin (0.025 g) was loaded into a peptide synthesis vessel and washed with *i*-PrOH (3 x 10 mL), followed by DMF (3 x 10 mL). To the vessel was then introduced a solution of Fmoc-NH-(PEG)₁₂-COOH (42.8 mg) in DMF, *i*-Pr₂NEt (2.5 equiv), and PyBOP (2.5 equiv). The resulting solution was bubbled with Ar for 1 h, the coupling solution was drained, and the resin washed with DMF (3 x 10 mL) and *i*-PrOH (3 x 10 mL). Kaiser tests were performed to assess reaction progress. Fmoc deprotection was carried out using 20% piperidine in DMF (3 x 10 mL). This procedure was repeated to complete the all coupling steps (2 x 1.5 equiv of Fmoc-Phe-OH and 1.5 equiv of 8-aminooctanoic acid and 1.2 equiv of DUPA were used on each of their respective coupling steps). After the DUPA coupling, the resin was washed with DMF (3 x 10 mL) and *i*-PrOH (3 x 10 mL) and dried under reduced pressure. The peptide was cleaved from the resin in the peptide synthesis vessel using the Cleavage Solution. 15 mL of the Cleavage Solution was added to the peptide synthesis vessel, and the reaction was bubbled under Ar for 15 min. The resin was treated with two additional 10 mL quantities of the Cleavage Solution for 5 min each. The cleavage mixture was concentrated to about 5 mL and precipitated with ethyl ether. The precipitate was collected by centrifugation, washed with ethyl ether (3X), and dried under high vacuum, resulting in the recovery of crude material. To a stirred solution of the crude DUPA-(PEG)₁₂-EDA (10 mg) and FITC (5.6 mg) in dimethylsulfoxide (DMSO, 1 mL) was added *i*-Pr₂NEt (5 equiv) at room temperature and stirred for 6 h under argon. The reaction was monitored by LCMS and purified

by preparative HPLC (mobile phase: A = 10 mM ammonium acetate pH = 7, B = ACN; method: 0% B to 50% B in 30 min at 13 mL/min). The purified fractions were pooled and freeze-dried, providing the FITC-PEG12-DUPA.

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EXAMPLE 7
Synthesis of FITC-PEG11-NK1

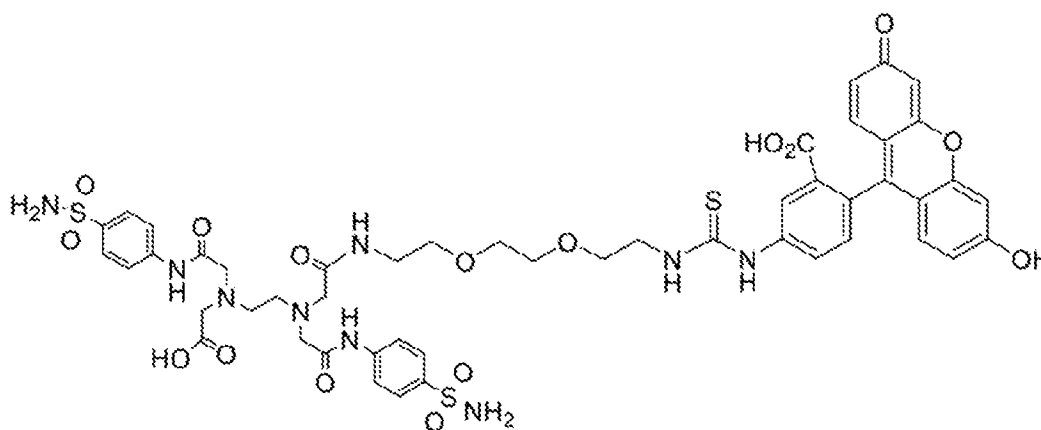


To a stirred solution of NK-1 (0.02 g, 0.0433 mmol, 1.0 eq.), *O*-(2-Aminoethyl)-*O'*-[2-(Boc-amino)ethyl]decaethylene glycol (BocNH-PEG₁₁-NH₂) (Sigma, 0.0336 g, 0.0521 mmol, 1.2 eq.), Benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) (0.027 g, 0.0521 mmol, 1.2 eq.) in dry CH₂Cl₂ was added *N,N*-Diisopropylethylamine (DIPEA) (0.076 mL, 0.4338 mmol, 10 eq.) under argon at room temperature. The reaction progress was monitored by LCMS and purified by preparative RP-HPLC (Waters, XBridge™ Prep C18, 5 μm; 19 × 100 mm column, mobile phase A = 20 mM ammonium acetate buffer, pH 7, B = acetonitrile, gradient 10–100% B in 30 min, 13 mL/min, λ = 220 nm, 254 nm). The pure fractions were collected, all organic solvents were evaporated and the sample was lyophilized for 48 h to provide the NK1-PEG₁₁-NHBoc. Yield: 40.13 mg (97%). To the NK1-PEG₁₁-NHBoc (0.0165 g, 0.015 mmol) in dry DCM was added trifluoroacetic acid (TFA, 20 eq.) and the reaction mixture was stirred for 4 h at r.t. The excess TFA was removed, and the remaining solution was diluted with water and extracted using CH₂Cl₂ (3 x 5 mL). The combined organic layers were washed with brine, dried (Na₂SO₄) and concentrated. The residue obtained was dried under vacuum and used for the next-step without further purification. A stirred solution of NK1-PEG₁₁-NH₂ (0.008 g, 0.0081 mmol, 1.0 eq.), Fluorescein isothiocyanate (FITC) (Sigma, 0.0037 g, 0.0097 mmol, 1.2 eq.) in dry dimethylsulfoxide (DMSO, 0.3 mL) was added to diisopropylethyl amine (0.0028 mL, 0.0162 mmol, 2.0 eq.) at room temperature under argon. The reaction progress was monitored by LCMS and the product was purified by preparative RP-HPLC (Waters, XBridge™ Prep C18, 5 μm; 19 × 100 mm column, mobile phase A = 20 mM ammonium acetate buffer, pH 7, B = acetonitrile, gradient 10–100% B in 30 min, 13

mL/min, $\lambda = 280$ nm). The pure fractions were collected, all organic solvents were evaporated and the sample was lyophilized for 48 h to provide the FITC-PEG11-NK1 in a yield of 8.54 mg (77%).

*Note: The NK-1 compound was synthesized by a two-step procedure starting from the base ligand, which was prepared by using a procedure in the literature. (Ref: DESIGN AND DEVELOPMENT OF NEUROKININ-1 RECEPTOR-BINDING AGENT DELIVERY CONJUGATES, Application Number: PCT/US2015/44229; incorporated herein by reference.

EXAMPLE 8 Synthesis of FITC-PEG2-CA9



CA9 ligand (53.6mg) was dissolved in DMF (2-3mL) in a 50mL round bottom flask using a Teflon magnetic stir bar. Ambient air was removed using a vacuum and replaced with nitrogen gas, this was done in three cycles. The round bottom flask was kept under constant nitrogen gas. To the flask, 28.9mg of N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC) was added followed by 21.6mg 1-Hydroxybenzotriazole hydrate (HOBt) and 18.9 μ L of Boc-PEG₂-NH₂ (Sigma Aldrich). 5.4 μ L of triethylamine (TEA) was added and the reaction was stirred overnight. The reaction mixture was purified using HPLC and confirmed with UHPLC-MS (target m/z of 831). Acetonitrile was removed using high vacuum rotary evaporation and the product lyophilized. The compound was mixed with 1:1 TFA:DCM for 30 minutes. The TFA/DCM was removed using high vacuum rotary evaporation followed by 30 minutes on high vacuum. The compound was then dissolved in DMF and combined with 5 molar equivalents of *i*-Pr₂NEt, 16 mg of

fluorescein isothiocyanate (Life Technologies) and stirred for 1 h. The reaction mixture was purified by HPLC and the target compound was confirmed with UHPLC-MS (target m/z of 1120). The samples were lyophilized and stored at -20 °C.

5

EXAMPLE 9

T Cell Preparation

Human peripheral blood mononuclear cells (PBMCs) were isolated from whole blood of healthy donors by using Ficoll density gradient centrifugation (GE Healthcare Lifesciences). T cells were then isolated from PBMCs by using an EasySep™ Human T Cell Isolation Kit (STEM CELL technologies). T cells were cultured in TexMACS medium (Miltenyi Biotech Inc) with 40-100 IU/mL human IL-2 (Miltenyi Biotech), 2% human AB type serum, and 1% penicillin/streptomycin sulfate. Dynabeads Human T-Activator CD3/CD28 (ThermoFisher Scientific) were added to T cells at 1:1 ratio to activate T cells. 12-24 hours after activation, T cells were transduced with FITC-CAR lentiviral particles in the presence of 8 µg/mL polybrene (Santa Cruz Biotech) by spinfection at 1,200 g for 90 minutes at 22-32 °C. T cell mixture containing those with CAR modification (CAR-Ts) and those without CAR modification (non-transformed Ts) was cultured in the presence of activation beads for 6 days before the removal of activation beads. Fluorescence-Activated Cell Sorting was used to sort out CAR-T cells (GFP positive) and non-transformed T cells (GFP negative) based on their GFP expression. The sorted T cells were cultured for 7-15 days before injection into mice. When a T cell mixture was used, CAR-T cells and non-transformed T cells were mixed at the desired ratio before mouse injection.

25

EXAMPLE 10

Generation of lentiviral vector encoding CAR gene

An overlap PCR method was used to generate CAR constructs comprising scFv against fluorescein. scFV against fluorescein, 4M5.3 (Kd = 270 fM, 762bp) derived from anti-fluorescein (4-4-20) antibody was synthesized. Sequence encoding the human CD8α signal peptide (SP, 63bp), the hinge, and transmembrane region (249bp), the cytoplasmic domain of 4-1BB (CD137, 141bp) and the CD3ζ chain (336bp), as shown in Figure 2, were fused with the anti-fluorescein scFV by overlapping PCR. The resulting CAR construct (1551bp) was inserted into EcoRI/NotI cleaved lentiviral expression vector pCDH-EF1-MCS-(PGK-GFP) (Figure 2,

System Biosciences). The sequence of the CAR construct in lentiviral vector was confirmed by DNA sequencing.

An exemplary CAR nucleic acid coding sequence may comprise:

5
ATGGCCTTACCAGTGACCGCCTTGCTCCTGCCGCTGGCCTTGCTGCTCCACGCCGCC
AGGCCGGATGTCGTGATGACCCAGACCCCCCTCAGCCTCCCAGTGTCCCTCGGTGA
CCAGGCTTCTATTAGTTGCAGATCCAGCCAGTCCCTCGTGCACTCTAACGGTAATAC
CTACCTGAGATGGTATCTCCAGAAGCCCCGGACAGAGCCCTAAGGTGCTGATCTACA
10 AAGTCTCCAACCGGGTGTCTGGAGTCCCTGACCGCTTCTCAGGGAGCGGTTCCGGC
ACCGACTTCACCCTGAAGATCAACCGGGTGGAGGCCGAAGACCTCGGCGTCTATTT
CTGCTCTCAGAGTACACATGTGCCCTGGACCTTCGGCGGAGGGACCAAGCTGGAGA
TCAAAAGCTCCGCAGACGATGCCAAGAAAGATGCCGCTAAGAAAGACGATGCTAA
GAAAGACGATGCAAAGAAAGACGGTGGCGTGAAGCTGGATGAAACCGGAGGAGG
15 TCTCGTCCAGCCAGGAGGAGCCATGAAGCTGAGTTGCGTGACCAGCGGATTACCT
TTGGGCACTACTGGATGAACTGGGTGCGACAGTCCCCAGAGAAGGGGCTCGAATG
GGTCGCTCAGTTCAGGAACAAACCCTACAATTATGAGACATACTATTTCAGACAGCG
TGAAGGGCAGGTTTACTATCAGTAGAGACGATTCCAAATCTAGCGTGTACCTGCAG
ATGAACAATCTCAGGGTCGAAGATACAGGCATCTACTATTGCACAGGGGCATCCTA
20 TGGTATGGAGTATCTCGGTGAGGGGACAAGCGTCACAGTCAGTTTCGTGCCGGTCT
TCCTGCCAGCGAAGCCCACCACGACGCCAGCGCCGCGACCAACACCGGCGCC
CACCATCGCGTCGCAGCCCCCTGTCCCTGCGCCCAGAGGCGTGCCGGCCAGCGGCGG
GGGGCGCAGTGCACACGAGGGGGGCTGGACTTCGCCTGTGATATCTACATCTGGGCG
CCCTTGGCCGGGACTTGTGGGGTCCTTCTCCTGTCACTGGTTATCACCTTTACTGC
25 AACCACAGGAACCGTTTCTCTGTTGTTAAACGGGGCAGAAAGAACTCCTGTATAT
ATTCAAACAACCATTTATGAGACCAGTACAACTACTCAAGAGGAAGATGGCTGTA
GCTGCCGATTTCCAGAAGAAGAAGAAGGAGGATGTGAACTGAGAGTGAAGTTCAG
CAGGAGCGCAGACGCCCCCGCGTACCAGCAGGGCCAGAACCAGCTCTATAACGAG
CTCAATCTAGGACGAAGAGAGGAGTACGATGTTTTGGACAAGAGACGTGGCCGGG
30 ACCCTGAGATGGGGGGAAAGCCGAGAAGGAAGAACCCTCAGGAAGGCCTGTACAA
TGAAGTGCAGAAAGATAAGATGGCGGAGGCCTACAGTGAGATTGGGATGAAAGGC
GAGCGCCGGAGGGGCAAGGGGCACGATGGCCTTTACCAGGGTCTCAGTACAGCCA

CCAAGGACACCTACGACGCCCTTCACATGCAGGCCCTGCCCCCTCGCTAA (SEQ ID NO:1).

In the exemplary nucleic acid sequence shown above (SEQ ID NO:1) the first ATG is the start
5 codon. An exemplary CAR amino acid sequence may comprise:

MALPVTALLLPLALLLHAARPDVVMQTPLSLPVS LGDQASISCRSSQSLVHSNGNTYL
RWYLQKPGQSPKVLIIKVSNRVSGVPDRFSGSGSGTDFTLKINRVEAEDLG VYFCSQST
HVPWTFGGGT KLEIKSSADDAKKDAKKDDAKKDDAKKDG GVKLDETGGGLVQPG
10 GAMKLSCVTSGFTFGHYWMNWVRQSPEKGLEWVAQFRNKPYNYETYYS DSVKGRFT
ISRDDSKSSVYLQMNNLRVEDTGIYYCTGASYGMEYLGQGT SVTVSFVPVFLPAKPTT
TPAPRPPTPAPTIASQPLSLRPEACRPAAGGA VHTRGLDFACDIYIWAPLAGTCGVLLLS
LVITLYCNHRNRFSVVKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELR
VKFSRSADAPAYQQGQNQLYNELNLGRREEYDVL DKRRGRDPGEMGGKPRRKNPQEG
15 LYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
(SEQ ID NO:2)

An exemplary insert may comprise:

20 GCCACCATGGCCTTACCAGTGACCGCCTTGCTCCTGCCGCTGGCCTTGCTGCTCCAC
GCCGCCAGGCCGGATGTCGTGATGACCCAGACCCCCCTCAGCCTCCCAGTGTCCCT
CGGTGACCAGGCTTCTATTAGTTGCAGATCCAGCCAGTCCCTCGTGCACTCTAACG
GTAATACCTACCTGAGATGGTATCTCCAGAAGCCCGGACAGAGCCCTAAGGTGCTG
ATCTACAAAGTCTCCAACCGGGTGTCTGGAGTCCCTGACCGCTTCTCAGGGAGCGG
25 TTCCGGCACC GACTTCACCCTGAAGATCAACCGGGTGGAGGCCGAAGACCTCGGCG
TCTATTTCTGCTCTCAGAGTACACATGTGCCCTGGACCTTCGGCGGAGGGACCAAG
CTGGAGATCAAAAGCTCCGCAGACGATGCCAAGAAAGATGCCGCTAAGAAAGACG
ATGCTAAGAAAGACGATGCAAAGAAAGACGGTGGCGTGAAGCTGGATGAAACCGG
AGGAGGTCTCGTCCAGCCAGGAGGAGCCATGAAGCTGAGTTGCGTGACCAGCGGA
30 TTCACCTTTGGGCACTACTGGATGAACTGGGTGCGACAGTCCCCAGAGAAGGGGCT
CGAATGGGTGCTCAGTTCAGGAACAAACCCTACAATTATGAGACATACTATTAG
ACAGCGTGAAGGGCAGGTTTACTATCAGTAGAGACGATTCCAAATCTAGCGTGTAC

CTGCAGATGAACAATCTCAGGGTCGAAGATACAGGCATCTACTATTGCACAGGGGC
 ATCCTATGGTATGGAGTATCTCGGTACAGGGGACAAGCGTCACAGTCAGTTTCGTGC
 CGGTCTTCCTGCCAGCGAAGCCCACCACGACGCCAGCGCCGCGACCACCAACACCG
 GCGCCCACCATCGCGTCGCAGCCCCTGTCCCTGCGCCCAGAGGCGTGCCGGCCAGC
 5 GCGGGGGGGCGCAGTGCACACGAGGGGGGCTGGACTTCGCCTGTGATATCTACATCT
 GGGCGCCCTTGGCCGGGACTTGTGGGGTCCTTCTCCTGTCACTGGTTATCACCTTT
 ACTGCAACCACAGGAACCGTTTCTCTGTTGTTAAACGGGGCAGAAAGAACTCCTG
 TATATATTCAAACAACCATTTATGAGACCAGTACAACTACTCAAGAGGAAGATGG
 CTGTAGCTGCCGATTTCCAGAAGAAGAAGAAGGAGGATGTGAACTGAGAGTGAAG
 10 TTCAGCAGGAGCGCAGACGCCCCCGCTACCAGCAGGGCCAGAACCAGCTCTATA
 ACGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGTTTTGGACAAGAGACGTGG
 CCGGGACCCTGAGATGGGGGGAAAGCCGAGAAGGAAGAACCCTCAGGAAGGCCT
 GTACAATGAACTGCAGAAAGATAAGATGGCGGAGGCCTACAGTGAGATTGGGATG
 AAAGGCGAGCGCCGGAGGGGCAAGGGGCACGATGGCCTTTACCAGGGTCTCAGTA
 15 CAGCCACCAAGGACACCTACGACGCCCTTCACATGCAGGCCCTGCCCCCTCGCTAA
 (SEQ ID NO:3)

In the exemplary insert described above (SEQ ID NO:3), the first GCCACC sequence may
 comprise a restriction enzyme cleavage site, followed by the ATG start codon. The encoded
 20 amino acid sequence may comprise:

MALPVTALLLPLALLLHAARPDVVMQTPLSLPVS LGDQASISCRSSQSLVHSNGNTYL
 RWYLQKPGQSPKVLIIKVSNRVSGVPDRFSGSGSGTDFTLKINRVEAEDLG VYFCSQST
 HVPWTFGGGTKLEIKSSADDAKKDAKKDDAKKDDAKKDDGGVKLDETGGGLVQPG
 25 GAMKLSCVTSGFTFGHYWMNWVRQSPEKGLEWVAQFRNKPYNYETYYSDSVKGRFT
 ISRDDSKSSVY LQMNNLRVEDTGIYYCTGASYGMEYLGQGT SVTVSFVPVFLPAKPTT
 TPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLS
 LVITLYCNHRNRFSVVKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELR
 VKFSRSADAPAYQQGQNQLYNELNLGRREEYDVL DKRRGRDPEMGGKPRRKNPQEG
 30 LYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
 (SEQ ID NO:2)

EXAMPLE 11

Production of lentivirus containing CAR gene for human T cell transduction

To prepare lentiviral virus containing an anti-fluorescein (i.e., anti-FITC) single chain fragment variable (scFv) CAR, a HEK-293TN packaging cell line was co-transfected with the
5 lentiviral vector encoding anti-fluorescein scFv CAR and a 2nd generation of a lentiviral packaging plasmid mix (Cellecta) or ViraPower Lentiviral Packaging Mix (ThermoFisher). After 24 and 48 hours of transfection, supernatants containing the lentivirus with the CAR gene were harvested and virus particles were concentrated by the standard polyethylene glycol virus concentration method (Clontech) for future transduction with human T cells.

10

EXAMPLE 12

Isolation of Human T cells from human PBMC

T cells were isolated from human peripheral blood mononuclear cells (PBMC) by Ficoll density gradient centrifugation (GE Healthcare Lifesciences). After washing away remaining
15 Ficoll solution, T cells were isolated by using an EasySep™ Human T Cell Isolation Kit (STEM CELL technologies). Purified T cells were cultured in TexMACS™ medium (Miltenyi Biotech Inc) with 1% penicillin and streptomycin sulfate in the presence of human IL-2 (100 IU/mL, Miltenyi Biotech Inc). T cells were cultured at density of 1×10^6 cells/mL in multi-well plates. T cells were split and re-feed every 2-3 days.

20

EXAMPLE 13

Transduction of human T cells

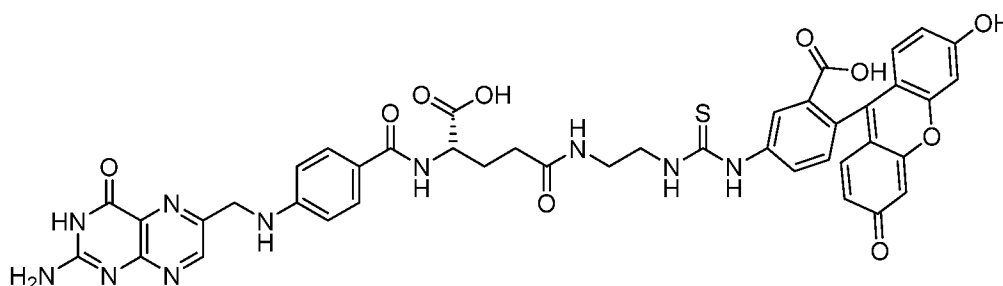
Isolated T cells were activated with Dynabeads coupled with anti-CD3/CD28 antibodies (Life Technologies) for 12-24 hours in the presence of human IL-2 (100 IU/mL), then
25 transduced with lentivirus encoding an anti-fluorescein CAR gene. Cells were harvested after 72 hours and the expression of CAR on transduced T cells was identified by measuring GFP fluorescent cells using flow cytometry.

EXAMPLE 14

SYNTHESIS OF FITC-FOLATE

30

Folate- γ -ethylenediamine was coupled to fluorescein isothiocyanate (FITC) isomer I (Sigma-Aldrich) in anhydrous dimethylsulfoxide (DMF) in the presence of tetramethylguanidine and diisopropylamine. The crude product was loaded onto an Xterra RP18 preparative HPLC column (Waters) and eluted with gradient conditions starting with 99% 5 mM sodium phosphate (mobile phase A, pH7.4) and 1% acetonitrile (mobile phase B) and reaching 90% A and 10% B in 10 min at a flow rate of 20 mL/min. Under these conditions, the FITC-folate main peak typically eluted at 27–50 min. The quality of the FITC-folate fraction was monitored by analytical reverse-phase HPLC with a UV detector. Fractions with greater than 98.0% purity (LCMS) were lyophilized to obtain the final FITC-folate product.



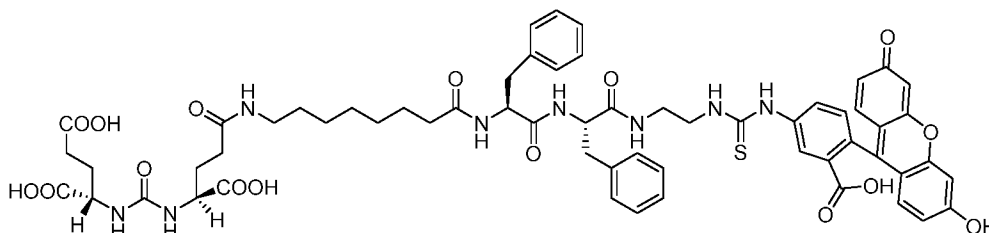
EXAMPLE 15

SYNTHESIS OF FITC-DUPA

DUPA-FITC was synthesized by solid phase methodology as follows. Universal NovaTag resin (50 mg, 0.53 mM) was swollen with dichloromethane (DCM) (3 mL) followed by dimethylformamide (DMF, 3 mL). A solution of 20% piperidine in DMF (3 $\times$ 3 mL) was added to the resin, and argon was bubbled for 5 min. The resin was washed with DMF (3 $\times$ 3 mL) and isopropyl alcohol (i-PrOH, 3 $\times$ 3 mL). After swelling the resin in DMF, a solution of DUPA-(OtBu)-OH (1.5 equiv), HATU (2.5 equiv) and DIPEA (4.0 equiv) in DMF was added. Argon was bubbled for 2 h, and resin was washed with DMF (3 $\times$ 3 mL) and i-PrOH (3 $\times$ 3 mL). After swelling the resin in DCM, a solution of 1 M HOBt in DCM/trifluoroethane (TFE) (1:1) (2 $\times$ 3 mL) was added. Argon was bubbled for 1 h, the solvent was removed and resin was washed with DMF (3 $\times$ 3 mL) and i-PrOH (3 $\times$ 3 mL). After swelling the resin in DMF, a solution of Fmoc-Phe-OH (2.5 equiv), HATU (2.5 equiv) and DIPEA (4.0 equiv) in DMF was

added. Argon was bubbled for 2 h, and resin was washed with DMF (3 × 3 mL) and i-PrOH (3 × 3 mL). The above sequence was repeated for 2 more coupling steps for addition of 8-aminooctanoic acid and fluorescein isothiocyanate or rhodamine B isothiocyanate. Final compound was cleaved from the resin using a trifluoroacetic acid (TFA):H₂O:

- 5 triisopropylsilane:cocktail (95:2.5:2.5) and concentrated under vacuum. The concentrated product was precipitated in diethyl ether and dried under vacuum. The crude product was purified using preparative RP-HPLC [λ = 488 nm; solvent gradient: 1% B to 80% B in 25 min, 80% B wash 30 min run; A = 10 mM NH₄OAc, pH = 7; B = acetonitrile (ACN)]. ACN was removed under vacuum, and pure fractions were freeze-dried to yield DUPA-FITC as a
- 10 brownish-orange solid. RP-HPLC: t_R = 8.0 min (A = 10 mM NH₄OAc, pH = 7.0; B = ACN, solvent gradient: 1% B to 50% B in 10 min, 80% B wash 15 min run). ¹H NMR (DMSO-d₆/D₂O): δ 0.98–1.27 (ms, 9H); 1.45 (b, 3H); 1.68–1.85 (ms, 11H); 2.03 (m, 8H); 2.6–3.44 (ms, 12H); 3.82 (b, 2H); 4.35 (m, 1H); 6.53 (d, J = 8.1 Hz, 2H), 6.61 (dd, J = 5.3, 3.5 Hz, 2H); 6.64 (s, 2H); 7.05 (d, J = 8.2 Hz, 2H), 7.19 (m, 5H); 7.76 (d, J = 8.0 Hz, 1H); 8.38 (s, 1H).
- 15 HRMS (ESI) (m/z): (M + H)⁺ calcd for C₅₁H₅₉N₇O₁₅S, 1040.3712, found, 1040.3702. UV/vis: λ max = 491 nm.



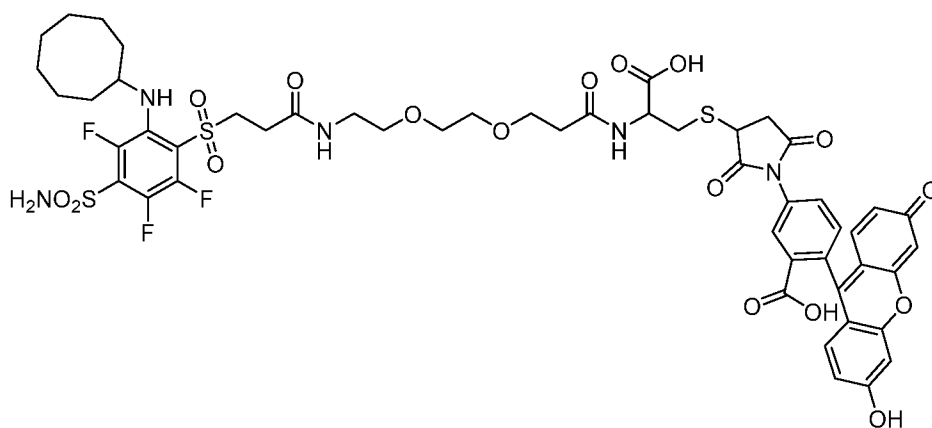
20

EXAMPLE 16

SYNTHESIS OF FITC-CA9

- In a 50mL round bottom flask CA9 ligand (53.6mg, synthesized in lab) was dissolved in a desired amount of N,N-Dimethylformamide (DMF) (2-3mL) using a Teflon
- 25 magnetic stir bar. Ambient air was removed using vacuum and replaced with nitrogen gas, this was done in three cycles. Then the round bottom was kept under constant nitrogen gas. To the flask, 28.9mg of N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC) was added followed by 21.6mg 1-Hydroxybenzotriazole hydrate (HOBt) and 18.9 μ L of Boc-PEG2-

NH₂ (purchased from Sigma Aldrich). 5.4μL of triethylamine (TEA) was added last and the reaction was allowed to stir overnight. The reaction mixture was purified using HPLC and confirm with UHPLC-MS (target m/z of 831). Acetonitrile was removed using high vacuum rotary evaporation and place on lyophilizer for 48 hours. Deprotection of Boc was done with
 5 with 1:1 TFA:DCM for 30 minutes. TFA/DCM was removed using high vacuum rotary evaporation followed by 30 minutes on high vacuum. The compound was then dissolved in DMF and combined with 5 molar equivalents of N,N-Diisopropylethylamine (DIPEA). 16mg of fluorescein isothiocyanate (purchased from Life Technologies) was added to the solution and stirred for 1 hour. Reaction mixture was purified by HPLC and target compound was confirmed
 10 with UHPLC-MS (target m/z of 1120). The samples was placed on lyophilizer for 48 hours and store compound at -20°C.



15

EXAMPLE 17

SYNTHESIS OF FITC-NK1R

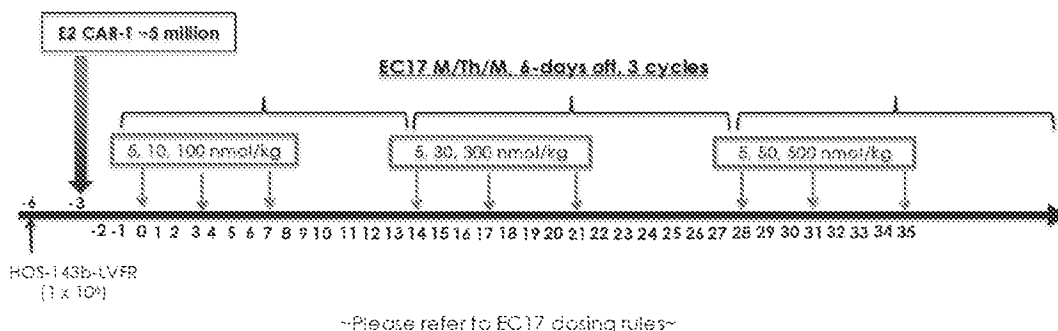
To a stirred solution of NK-1 (0.02 g, 0.0433 mmol, 1.0 eq.), O-(2-Aminoethyl)-
 20 O'-[2-(Boc-amino)ethyl]decaethylene glycol (BocNH-PEG11-NH₂) (Sigma, 0.0336 g, 0.0521 mmol, 1.2 eq.), Benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PYBOP) (0.027 g, 0.0521 mmol, 1.2 eq.) in dry CH₂Cl₂ was added N,N-Diisopropylethylamine (DIPEA) (0.076 mL, 0.4338 mmol, 10 eq.) under argon at room temperature. The reaction progress was monitored by LCMS and purified by preparative RP-
 25 HPLC (Waters, XBridge™ Prep C18, 5 μm; 19 × 100 mm column, mobile phase A = 20 mM

ammonium acetate buffer, pH 7, B = acetonitrile, gradient 10–100% B in 30 min, 13 mL/min, λ = 220 nm, 254 nm). The pure fractions were collected, evaporated all organic solvents and lyophilized the sample for 48 h to furnish the NK1-PEG11-NHBoc. Yield: 40.13 mg (97%). To the NK1-PEG11-NHBoc (0.0165 g, 0.015 mmol) in dry CH₂Cl₂ was added trifluoroacetic acid (TFA, 20 eq.) and reaction mixture was stirred for 4 h at r.t. The excess of TFA was removed, diluted with water and extracted using CH₂Cl₂ (3x5 mL). The combined organic layers were washed with brine, dried (Na₂SO₄) and concentrated. The residue obtained was dried under vacuum and used for next-step without further purification. A stirred solution of NK1-PEG11-NH₂ (0.008 g, 0.0081 mmol, 1.0 eq.), Fluorescein isothiocyanate (FITC) (Sigma, 0.0037 g, 0.0097 mmol, 1.2 eq.) in dry dimethylsulfoxide (DMSO, 0.3 mL) was added diisopropylethylamine (0.0028 mL, 0.0162 mmol, 2.0 eq.) at room temperature under argon. The reaction progress was monitored by LCMS and purified by preparative RP-HPLC (Waters, XBridgeTM Prep C18, 5 μ m; 19 $\times$ 100 mm column, mobile phase A = 20 mM ammonium acetate buffer, pH 7, B = acetonitrile, gradient 10–100% B in 30 min, 13 mL/min, λ = 280 nm). The pure fractions were collected, evaporated all organic solvents and lyophilized the sample for 48 h to furnish the NK1-PEG11-FITC (5). Yield: 8.54 mg (77%).

The NK-1 compound was synthesized by a two-step procedure starting from base ligand, which was prepared by using a literature procedure. (Ref: DESIGN AND DEVELOPMENT OF NEUROKININ-1 RECEPTOR-BINDING AGENT DELIVERY CONJUGATES, Application Number: PCT/US2015/44229, incorporated herein by reference in its entirety).

EXAMPLE 18

HOS-FR- α and AML Study Schema



Sequential escalating doses of EC17 will be administered under the following rules:

- * If no CRS or neurotoxicity, advance through EC17 dose escalation as planned.
- * If CRS grade ≤ 2 , administer all subsequent EC17 doses at the Sequence level that provoked the non-serious CRS in the same cycle.
- * If CRS grade >2 , cancel further EC17 doses in the current Cycle, and commence all subsequent Cycles at the next highest Sequence below that which provoked the ~~s~~CRS.

A. Tumor implantation

HOS-FR α (i.e. HOS-143b-LV-FR α) is a subclone of HOS-143B (ATCC CRL-8303)
5 stably transfected with human FR α . HOS-143B was originally derived from a 13-year-old Caucasian female with osteosarcoma. The tumor cells were grown in folate-deficient RPMI 1640 with 5% FBS at 37 °C in a 5% CO₂ humidified atmosphere. For the in-vivo study, tumor cells were inoculated subcutaneously at 1×10^6 cells per animal.

10 B. CAR-T cell administration

EGFRt-sorted anti-FITC E2 scFv-CAR T cells were frozen in a T-cell freezing medium. Upon arrival, vials of frozen CAR-T cells were immediately stored at -80°C. The CAR-T cells were quickly thawed at 37°C, washed twice with PBS, and used for animal injection at 6 million viable EGFRt+ E2 CAR-T cells (CD4/CD8 at ~1:1) per animal. A small aliquot was
15 taken on the day infusion for flow cytometric analysis of E2-CAR T-cell phenotypes.

C. Preparation of dosing drug solutions

EC17 dosing solutions were prepared when dosing began by weighing appropriate amounts of each compound, reconstituting in PBS, pH 7.4, sterile filtering the drug solution
20 through a 0.22 μ m PVDF syringe filter, and freezing aliquots for each day of dosing at -20°C.

D. Compound administration

All EC17 doses were given towards the end of day (3-4 PM) to allow potential CRS (cytokine release syndrome) to develop overnight. On the following morning, animals were
25 scored according to a CRS grading system (see below).

E. Evaluation

CRS grading system

CRS Grading Scale					
0	1	2	3	4	5
Normal	~pilo ~greasy still active	pilo greasy less active	pilo, greasy bunched, thin not active, unless stimulated	moribund bunched, pale lethargic	Death

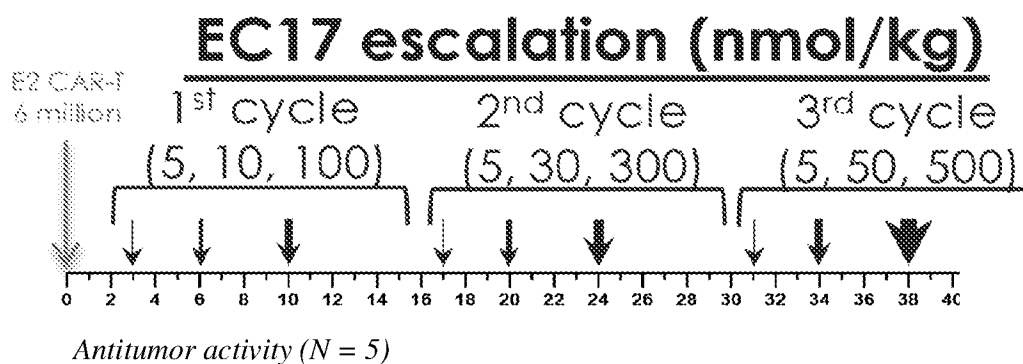
5

Tumor growth, body weight, and overall assessment: Tumor sizes were monitored 2-3 times/week and body weight measured 2-3 times/week. On the days immediately after any EC17 dose, body weight measurement was taken daily and attention was given to gross animal morphology and behavior.

10

Euthanasia: Euthanasia was performed when mice had lost $\geq 20\%$ of their weight, when tumors reached $\geq 1500 \text{ mm}^3$. Euthanasia was also performed if mice lost a lot of weight in a short duration (e.g., due to severe head-tilt), or when they approached moribund conditions per CRS grading system.

15

EC17 intra-host dose escalation treatment (3 cycles)

As seen in Figure 4:

Cohort 1 (E2-CAR T-cells only) showed HOS-FR α grew aggressively without EC17 treatment.

Cohort 2 (EC17 5/100/1000 nmol/kg, M/Th/M) showed significant tumor regression without body weight loss or CRS through this study.

The slow EC17 dose escalation (M/Th/M) regimen was safe. No body weight loss or symptoms of cytokine release syndrome (CRS) were observed in the HOS-FR α tumor-bearing mice.

One objective of this E2-CAR T-cell study was to test EC17/E2 CAR-T cell activity and related toxicity (e.g. sCRS) using multiple dosing regimens starting 3 days after CAR-T infusion. Three different EC17 dosing regimens include (a) once-a-week (SIW) at 500 nmol/kg, (b) "TIW-like" at 5, 50, and 500 nmol/kg on Monday/Wednesday/Friday with 9-day intervals, and (c) "TIW-like" at 5, 100, and 1000 nmol/kg on Monday/Thursday/Monday with 7-day intervals.

Experimental Groups (C = Cycle):

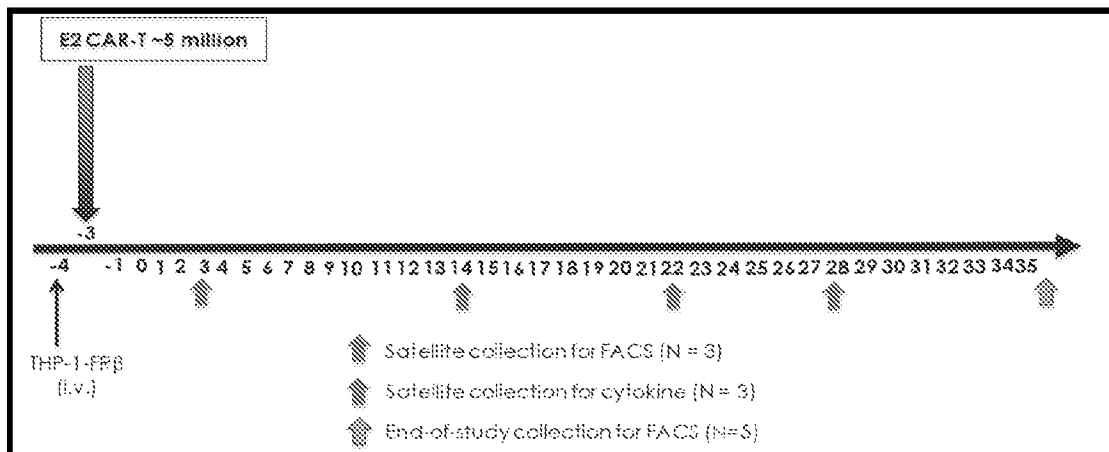
<u>Cohort</u>	<u>Groups</u>	<u>T cells[§]</u>	<u>C1 Wk1 (EC17)</u>	<u>C2 Wk1 (EC17)</u>	<u>C3 Wk1 (EC17)</u>	<u>N (Efficacy)</u>	<u>N (Satellite)</u>
<u>1</u>	<u>Tumor only</u>	<u>N/A</u>	<u>N/A</u>	<u>N/A</u>	<u>N/A</u>	<u>5</u>	<u>N/A</u>
<u>2</u>	<u>CAR-T only</u>	<u>6 million CAR-T</u>	<u>N/A</u>	<u>N/A</u>	<u>N/A</u>	<u>5</u>	<u>0/ 3/ 0/ 3/ 0/ 3/ 3</u>
<u>3</u>	<u>EC17 SIW</u>	<u>6 million CAR-T</u>	<u>500 nmol/kg</u>	<u>500 nmol/kg</u>	<u>500 nmol/kg</u>	<u>N/A</u>	<u>3/ 3/ 0/ 3/ 0/ 3/ 3</u>
<u>4*</u>	<u>EC17 TIW (On/Off)</u>	<u>6 million CAR-T</u>	<u>5 nmol/kg 50 nmol/kg 500 nmol/kg</u>	<u>5 nmol/kg 50 nmol/kg 500 nmol/kg</u>	<u>5 nmol/kg 50 nmol/kg 500 nmol/kg</u>	<u>N/A</u>	<u>0/ 0/ 0/ 3/ 3/ 0/ 3</u>
<u>5*</u>	<u>EC17 "TIW" M/Th/M</u>	<u>6 million CAR-T</u>	<u>5 nmol/kg 10 nmol/kg 100 nmol/kg</u>	<u>5 nmol/kg 30 nmol/kg 300 nmol/kg</u>	<u>5 nmol/kg 50 nmol/kg 500 nmol/kg</u>	<u>5</u>	<u>0/ 3/ 3/ 3/ 0/ 3/ 3</u>

*Groups 4-5: Skip dosing for Week 2 of each cycle.

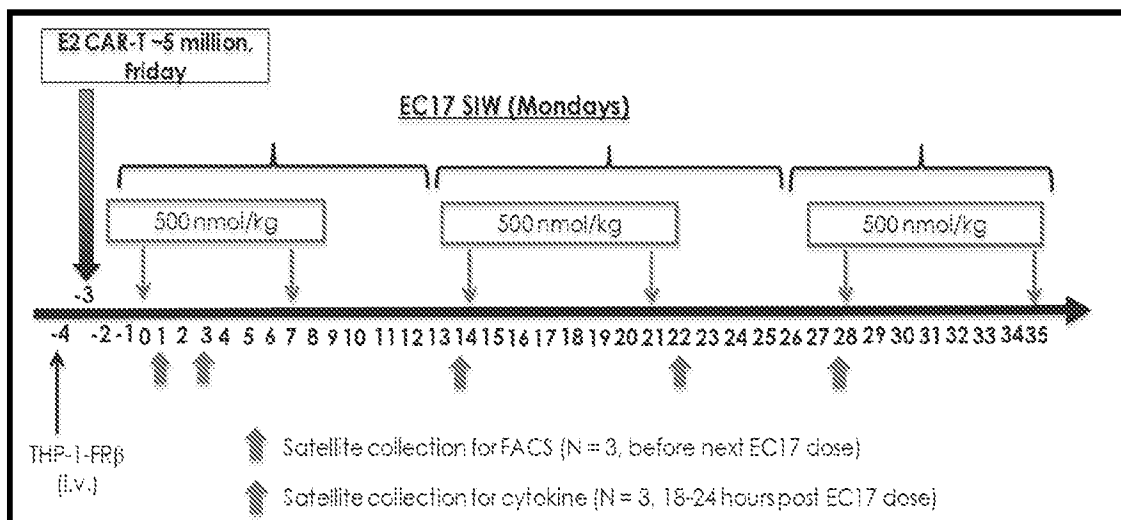
Study Schema

Group 1 (no treatment): only for efficacy comparison.

Group 2 (CAR-T only):

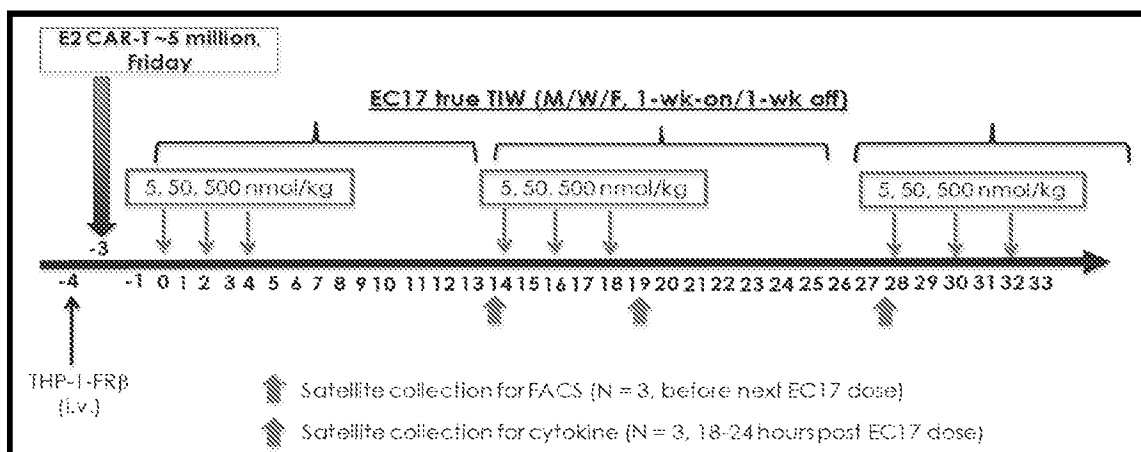


Group 3 (EC17 SIW):

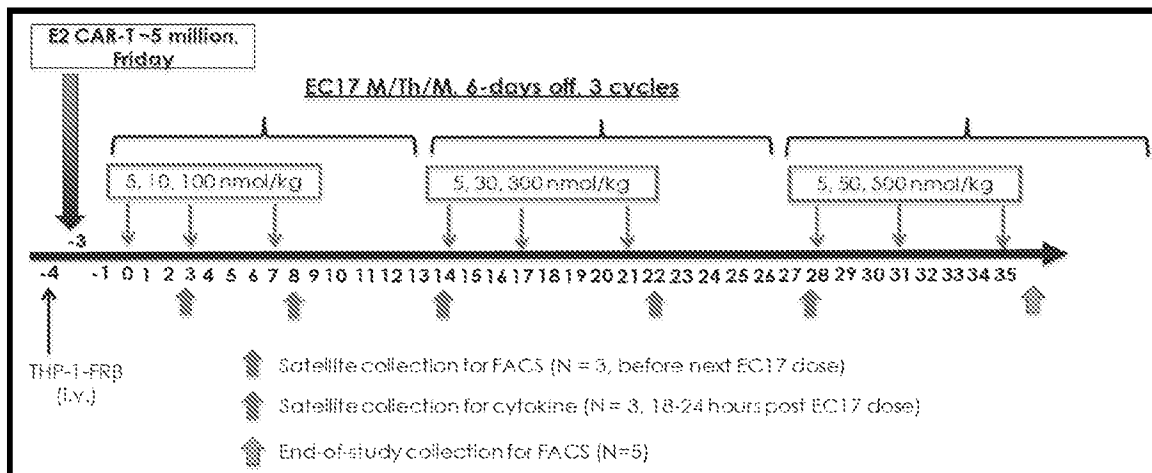


5

Group 4 (EC17 true TIW):



Groups 5 - EC17 low-dose “TIW” (M/Th/M, 6-day off) with/efficacy:



A. Tumor implantation

- 5 THP-1-FR β AML tumor cells were grown in folate-deficient RPMI 1640 with 5-10% FBS at 37 °C in a 5% CO₂ humidified atmosphere. THP-1-FR β tumor cells in serum-free folate-deficient RPMI1640 medium were i.v. injected at 5 x 10⁶ cells per animal.

B. CAR-T cell administration

- 10 Anti-FITC E2 scFv-CAR T cells were frozen in a T-cell freezing medium. Upon arrival, vials of frozen CAR-T cells were immediately stored at -80°C. The CAR-T cells were quickly thawed at 37°C, washed twice with CAR-T cell culture medium, and used for animal injection at 6 million viable EGFRt+ E2 CAR-T cells (CD4/CD8 at ~1:1) per animal. A small aliquot was taken on the day infusion for flow cytometric analysis of E2-CAR T-cell phenotypes.
- 15

C. Preparation of dosing drug solutions

- 20 EC17 and bis-EDA-FITC dosing solutions were prepared when dosing began by weighing appropriate amounts of each compound, reconstituting in PBS, pH 7.4, sterile filtering the drug solution through a 0.22 μ m PVDF syringe filter, and freezing aliquots for each day of dosing at -20°C.

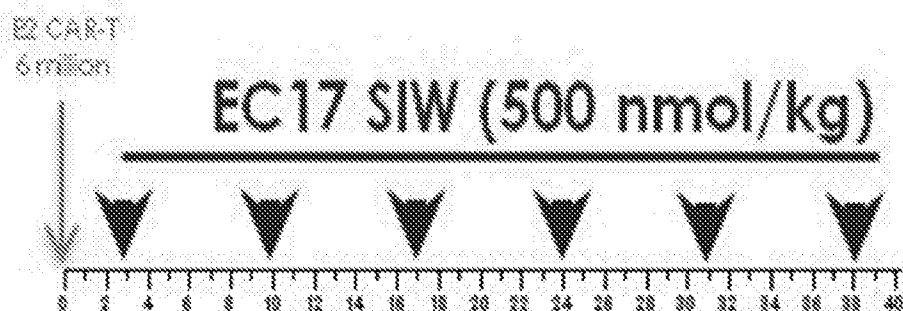
D. Compound administration

All EC17 doses were given towards the end of day (3-4 PM) to allow potential CRS (cytokine release syndrome) to develop overnight. In the following morning, animals were scored according to a CRS grading system (see below).

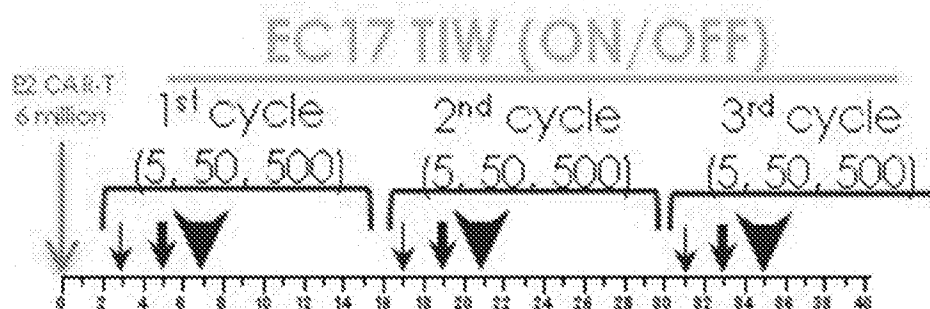
EC17 dose schedule:

Cohorts 1-2: no EC17 dose given

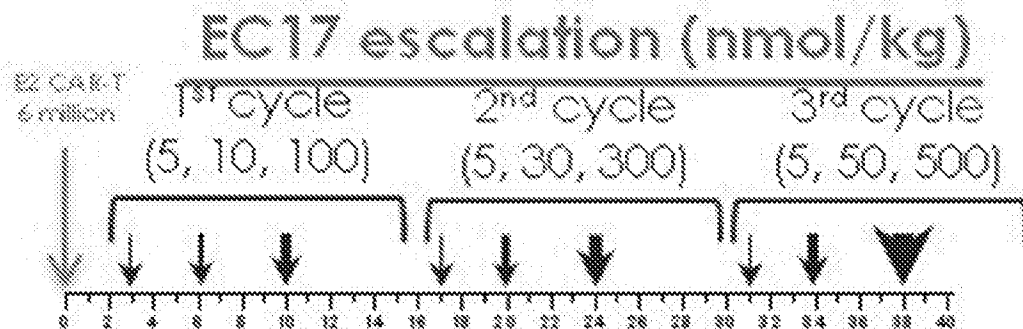
Cohort 3:



Cohort 4:



Cohort 5:



Monitoring/Efficacy:

Daily body weight measurement on the days after EC17 dose is required. Attention was given to gross animal morphology and behavior. Euthanasia was performed if mice lose a lot of weight in a short duration or when mice are approaching moribund conditions per CRS grading system or neurotoxicity.

CRS grading system

CRS Grading Scale					
0	1	2	3	4	5
Normal	~pilo ~greasy still active	pilo greasy less active	pilo, greasy bunched, thin not active, unless stimulated	moribund bunched, pale lethargic	Death

Tumor growth, body weight, and overall assessment: Body weight measured 2-3 times/week. On the days immediately after any EC17 dose, body weight measurement was taken daily and attentions were given to gross animal morphology and behavior.

Flow cytometric analysis

Whole blood cell analysis: Plasma was removed from predetermined volumes of whole EDTA treated blood and RBCs were lysed with RBC lysis solution. The leukocyte pellets were

then resuspended in flow cytometry staining solution (1% bovine serum albumin, 50mg/mL human IgG (Equitech Bio, cat#SLH56-0001), 0.9% sodium azide in a phosphate buffered saline, pH=7.4) and leukocyte surface marker staining was performed using the following antibodies: anti-human CD45 (clone HI30, eBioscience #47-0459-42 at 1:20 (v/v) dilution),
 5 anti-human CD137 (clone 4B4-1, BD Bioscience #564092 at 1:20 (v/v) dilution), anti-human CD8 α [clone RPA-T8, BD Bioscience, catalog #557746 at 1:20 (v/v) dilution], anti-human CD4 [clone SK3, eBioscience catalog #46-0047-42 at 1:20 (v/v) dilution], anti-human EGFR [R&D systems, clone Hu1, catalog #FAB9577B @ 1:10(v/v)], anti-human PD1 [BD Biosciences, clone EH12.1, catalog#562511 @ 1:20 (v/v)], anti-human LAG3 [BD Biosciences,
 10 clone T47-530, catalog #565616 @ 1:20 (v/v)], anti-human TIM3 [BD Biosciences, clone 7D3, catalog #565558 @ 1:20(v/v)], anti-human CD3 ϵ [BD Biosciences, clone SK7, catalog #557832 @ 1:20 (v/v)]. After leukocyte staining, cells were washed with PBS and resuspended in cold PBS containing 53,000 CountBright™ beads [Invitrogen catalog #C36950] and transferred to flow cytometry collection tubes. Flow cytometry data was collected on the Gallios flow
 15 cytometer (Beckman Coulter, Brea, CA). Determination of the concentration of CAR T cells in each blood sample was calculated according to Invitrogen's instructions. CAR T cells were identified as human CD3 ϵ + EGFRt+ events and easily distinguished and counted using the Kaluza™ flow cytometry software. The number of CAR T cells in the circulation of each infused mouse was then represented on the graphs as the total number of CAR T cells per 50 μ L
 20 of whole blood analyzed. Statistical significance was determined by utilizing an unpaired, two-tailed, students t-test with significance set at p<0.05.

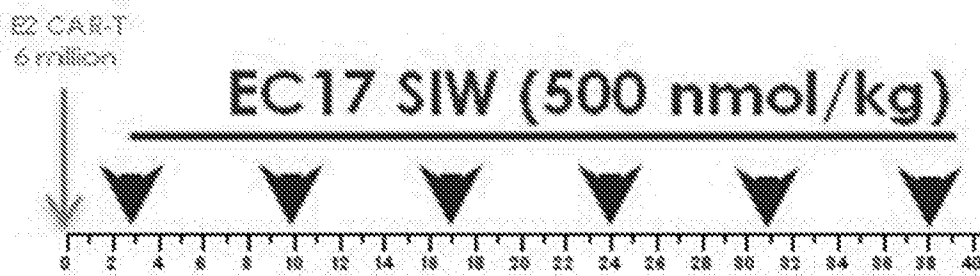
Tumor and tissue analysis: Solid tumors (100-1000 mm³) were harvested, weighed, and minced into small pieces then transferred into 50 mL tubes containing 20 mL of a tumor
 25 digestion cocktail. The enzymatic tumor digestion cocktail consisted of 0.5 mg/mL Collagenase IV (Sigma-Aldrich, Catalog# C5138), 0.5 mg/mL Hyaluronidase (Sigma-Aldrich, Catalog# H3506) and 0.1 mg/mL DNase I (Sigma-Aldrich, Catalog# DN25) in serum-free and folate-deficient RPMI1640 medium supplemented with antibiotics. The tumor fragments were digested for one hour at 37°C at 300 rpm on a horizontal shaker. Afterwards, the tumor digest
 30 was centrifuged at 400 x g for 5 minutes and tumor cell pellet underwent a red blood cell lysis step, was then washed with cold phosphate-buffered saline (PBS, pH 7.4) and finally filtered through a 40 μ m nylon cell strainer.

Results and Conclusions

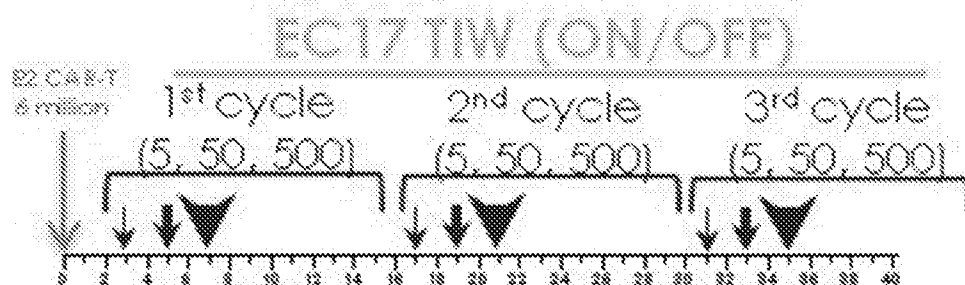
A. Three different dose schedules of EC17 administration showed different patterns of body weight loss and different levels of CRS

- 5 As seen in Figure 5, Cohort 1 (no treatment) and Cohort 2 (CAR-T only) didn't show much body weight loss and had no CRS. Cohort 3 (EC17 SIW) showed EC17 dose-dependent body weight loss after each EC17 dose, and showed grade 1-2 CRS throughout the study. Cohort 5 (EC17 dose escalation) showed little body weight loss during the first two cycles, but started to show similar body weight loss as cohort 2 after the end of second cycle of EC17 dose. Cohort 4 (EC17 TIW ON/OFF) showed mildest body weight loss among three treatment groups. Neither Cohort 4 or 5 showed any CRS greater than 1.

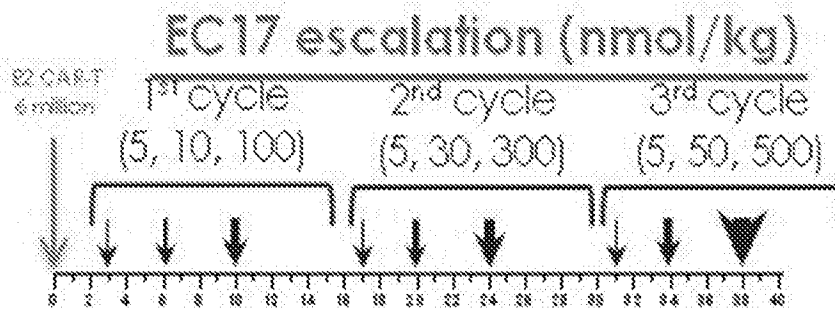
Cohort 3



Cohort 4



15

Cohort 5

Three different dose schedules of EC17 administration showed different anti-tumor activities.

- 5 As seen in Figure 6, liver and non-liver tumor burden were assessed on Day 39 across all cohorts. Cohort 2 (CAR-T only) didn't show any difference comparing to Cohort 1 (no treatment). Cohort 4 showed the best anti-tumor activity among the three different EC17 dose schedules.

Elimination of circulating AML cell line, THP1-FR β

- 10 In studies using CD19 specific anti-ALL therapy, expansion and persistence of CAR T cells in the blood has been reported to correlate with elimination of circulating CD19+ ALL cells resulting in complete responses to therapy. In this experiment we perform a similar assay in our mouse AML model, to determine the anti-tumor activity of our adaptor controlled E2 CAR T cell by looking for the disappearance of our GFP labeled human AML cell line, THP1-FR β , in response to different dosing regimens of the CAR T adaptor molecule, EC17. Figure 7 displays the circulating THP1-FR β cells with the y-axis showing the total number of GFP+ cells per 100 μ L of whole blood on a logarithmic scale. The different bars represent the different treatment cohorts. It was demonstrated that a high AML cell burden existed in the circulation 40 days post infusion of AML cells into mice receiving neither CAR T cells nor EC17. No
- 15 reduction of AML cells in the blood was seen in mice receiving an infusion of both AML cells and E2 CAR T cells, but no EC17 treatment. This demonstrates negligible anti-tumor activity by potential allogeneic reactivity of the human T cells with the allogeneic THP1 cells. Interestingly a significant reduction in circulating THP1 cell burden in all three cohorts of mice infused with E2 CAR T cells plus EC17 at different dosing regimens was seen, thus
- 20 demonstrating the effectiveness of the CAR T therapy under three different adaptor dosing regimens.
- 25

E2-CAR-T exhaustion phenotypes

Under chronic antigen stimulation, for example in instances of chronic viral infections or cancer, T cells undergo a process of exhaustion where they are no longer able to proliferate, secrete inflammatory cytokines or kill antigen presenting target cells. CAR T cells also possess the potential for exhaustion under chronic stimulation of the CAR by constant presence of scFv stimulating antigen. Because our E2 CAR T cells only react to the presence of our bridge molecule, EC17, bound to the surface of FR+ tumor cells, we have the ability to prevent chronic antigen stimulation of the E2 CAR T cells by ceasing treatment with EC17 and hence removing the presence of surface bound antigen. The rest period characterized by the absence of surface antigen prevents chronic antigen exposure of E2 CAR T cells and prevents the resulting exhaustion that could result.

To confirm that our EC17 dosing regimens do not result in E2 CAR T cell exhaustion, flow cytometry analysis was performed on single cell preparations from THP1-FR β liver metastases and surface markers which are expressed specifically on exhausted T cells. As T cells approach exhaustion, there is an increase in the co-expression of surface inhibitory receptors, PD1, LAG3 and TIM3. Figure 8 shows a bar graph where the y-axis illustrates the percentage of the total E2 CAR T cells isolated from the solid liver tumors, which express the various combinations of the three surface markers which is represented by the different bars. Of note, fully exhausted T cells which simultaneously express all three markers are represented by the left-hand bar in each group. The left group shows the pre-infusion of E2 CAR T cells to the liver tumor CAR T cells isolated from the three different EC17 treated cohorts. The inhibitory receptor expression is almost zero in the preinfusion CAR T cell product as most of the T cells are negative for all three surface markers. Importantly, all three EC17 treated cohort E2 CAR T cells possessed the exhausted triple positive cells and interestingly, cohort 4 (EC17 TIW) expresses the fewest number of double and triple positive events and further consisted of a significant number of triple negative CAR T cells. These data suggest that all three EC17 dosing regimens utilized in this mouse experiment might be useful in the clinic in that there is significant anti-tumor activity and there is very little induction of CAR T exhaustion.

EXAMPLES 19 to 25 - See FIGURES 9 to 14.

EXAMPLE 19

Cell Lines and Reagents

Unless otherwise noted, all FR+ and FR-negative cancer cell lines were maintained in RPMI-1640 medium (Gibco BRL) supplemented with 10% heat-inactivated fetal calf serum without (FFRPMI) or with (RPMI) 2.4 μ M folic acid (FA). KB (FR α -expressing human cervical carcinoma with HeLa markers) and CHO- β (Chinese hamster ovary cells transfected with human FR β) were used as the sources of FR α and FR β for radioligand binding assays, respectively. MDA-MB-231 represents a FR α subclone of human TNBC (triple negative breast cancer) cell line. For AML studies, a green fluorescent protein (GFP)-expressing isogenic pairs of FR β -positive (THP1-FR β) and FR-negative (THP1-FG12) cell lines were provided. Both were established from THP-1 (ATCC, TIB-202), a commonly used cell model for researching pediatric AML which was originally derived from a 1-year-old male infant with acute monocytic leukemia. For osteosarcoma studies, HOS-FR α was established by lentiviral transduction of FR-negative HOS-143b (ATCC, CRL8303) with FOLR1 *gene* encoding the human FR α . HOS-143b is originally established from a primary tumor of a 13-year-old Caucasian female and is highly tumorigenic in NSG mice. The GFP-expressing bioluminescent pairs of FR+ HOS-FR α^{FLuc} and FR-negative HOS-143b $^{\text{FLuc}}$ were transduced with lentiviral firefly luciferase.

LEGENDplexTM human cytokine panels were purchased from BioLegend (San Diego, CA). The *lactate dehydrogenase* (LDH) based CytoTox 96[®] non-radioactive cytotoxicity assay kit was purchased from Promega (Madison, WI). Commercially available anti-human antibodies used for multicolor flow cytometry were: CD45RA (clone HI100), CD45RO (clone UCHL1), CD4 (clone SK3), and CD69 (clone FN50) from Thermo Fisher Scientific (Waltham, MA); CD3 ϵ (clone SK7), CD8 α (clone RPA-T8), CD137/4-1BB (clone 4B4-1), CD25 (clone M-A251), PD1 (clone EH12.1), LAG3 (clone T47-530), and TIM3 (clone 7D3) from BD Bioscience (San Jose, CA); biotinylated anti-human EGFR (Cetuximab, clone Hu1) from R&D systems (Minneapolis, MN); and FR α (clone LK26) from BioLegend (San Diego, CA). A fluorophore-conjugated anti-biotin was also purchased from BioLegend. APC-conjugated anti-FITC mouse IgG2a/kappa antibody (clone NAWESLEE), CountBrightTM beads (Invitrogen), Annexin V staining buffer, and AlexaFluor-647-conjugated Annexin V were purchased from Thermo Fisher Scientific. For enzymatic digestion of tumor tissues, collagenase IV, hyaluronidase and DNase I were all purchased from Sigma-Aldrich (St. Louis, MO).

EC17 or folate-FITC [FA-(γ)-ethylenediamine-FITC] was synthesized at Endocyte. ^3H -EC17 was either purchased from Moravek biochemicals (Brea, CA) at a specific activity of ~ 0.952 Ci/mmol or prepared at Endocyte by conjugating FITC with ^3H -FA-(γ)-ethylenediamine made by ViTrax (Placentia, CA) at a specific activity of ~ 1.2 Ci/mmol. ^3H -FA was also
 5 purchased from ViTrax at a specific activity of 59 Ci/mmol. For CRS rescue, sodium fluorescein dosing solution was diluted from AK-FLUOR[®] 25% (fluorescein injection, USP) which was purchased from Purdue Pharmacy (NDC 17478-250-25).

EXAMPLE 20

Humanized CAR Construct and CAR-Modified T Cells

Previous studies used a GFP+ second-generation anti-FITC scFv (clone 4M5.3) CAR containing the hinge and transmembrane sequences of CD8 α and 4-1BB/CD3 ζ signaling domains (i.e., FITC-4M5.3-scFv-CD8 α hinge-CD8 α tm-4-1BB/CD3 ζ). For translation into first-in-human testing, the second-generation fully human FITC-specific (clone E2) CAR
 15 construct (herein referred to as E2) was developed (Figure 9, top diagram). CAR-modified T cells are shown in Figure 9, bottom pie charts.

The construct described herein is a FITC-specific CAR construct including (1) a fully human anti-FITC scFv (clone E2, Kd = 0.75 nM), (2) an IgG4 hinge-CH2(L235D, N297Q)-CH3 spacer fused to a CD28-transmembrane domain, (3) a second-generation 4-1BB/CD3 ζ -endodomain, and (4) a cell-surface human EGFRt tag (Figure 9, top diagram) (SEQ ID NOS:4 and 5 are the nucleic acid and amino acid sequences, respectively). To generate CAR-modified
 20 T cells, lentivirus was produced in 293T cells co-transfected with CAR-encoding ePHIV7 lentiviral vector. Donor CD4+ and CD8+ T cells were purified by immunomagnetic selection and transduced separately or at about a 50:50 ratio. In general, only one round of CD3/CD28 bead activation followed by one or two rounds of rapid *in vitro* expansion were carried out. For preclinical evaluations, several batches of EGFRt-sorted CD4, CD8 and unsorted CD4/CD8 CAR-T cells were used. All CAR-T cell preparations were analyzed prior to cryopreservation and after thawing to determine EGFRt expression and CD4/CD8 ratios by flow cytometry. Using combinations of surface markers, differentiation status of CD4+ and CD8+ CAR-T cell
 30 subsets on the day of infusion was analyzed and defined as T_N, CD45RA+ CD45RO- CD62L+ CD95- naïve T cells; T_{SCM}, CD45RA+ CD45RO- CD62L+ CD95+ stem cell memory T cells; T_{CM}, CD45RA- CD45RO+ CD62L+ CD95+ central memory T cells; T_{EM}, CD45RA-

CD45RO+ CD62L- CD95+ effector memory cells; and T_{EFF}, CD45RA+ CD45RO- CD62L- CD95+ effector T cells. For preclinical testing described below, studies included two batches of EGFRt-sorted pure CD4 and CD8 subsets (after mixing at about 1:1 ratios) and several batches of unsorted ~1:1 EGFRt+ CD4/CD8 admixture including a “clinical facsimile” preparation with low differentiation profiles.

Amid a series of different CAR constructs synthesized and evaluated, the fully human anti-FITC scFv (FITC-E2) CAR was chosen for preclinical development (Figure 9). This second-generation fully human CAR consisted of anti-FITC scFv (clone E2), an IgG4-Fc spacer/hinge with double mutations in the CH2 region (L235D and N297Q) to reduce binding to FcγR, a CD28 transmembrane domain, and 4-1BB/CD3ζ signaling domains appended to a cell-surface EGFRt tag by a T2A ribosomal skip sequence (i.e., FITC-E2-scFv-IgG4hinge-CD28tm-4-1BB/CD3ζ-T2A-EGFRt). For preclinical studies, both EGFRt-sorted and unsorted E2 CAR-T cells were prepared at ~1:1 CD4/CD8 ratios, and T cell subtype phenotyping was routinely performed by flow cytometry at the time of CAR T cell infusion (day 0) for each *in vivo* experiment. A typical expression pattern of EGFRt-sorted CAR-T cells included both CD4 and CD8 subsets at approximately 42% T_{SCM}, 10% T_{CM}, 12% T_{EM} and 34% T_{EFF} (Figure 9, pie charts on the left). Only EGFRt-sorted CAR-T cells were used for co-culture and pharmacokinetic studies. For tumor therapy, a “clinical facsimile” batch with a low differentiation profile (Figure 9, pie charts on the right) for MDA-MB-231 was used, and a research batch was used for THP1-FRβ and HOS-FRα studies. The “clinical facsimile” batch (~39% EGFRt+) included CD4+ subsets at ~66% T_{SCM} and ~32% T_{CM} and CD8 subsets at ~95% T_{SCM} and about 3% T_{CM}. The research batch (~23% EGFRt+) was more differentiated and included CD4 subsets at about 32% T_{SCM}, about 53% T_{CM}, about 11% T_{EM} and about 3.7% T_{EFF} and CD8 subsets at about 44% T_{SCM}, about 0.28% T_{CM}, about 3.4% T_{EM} and about 52% T_{EFF}.

EXAMPLE 21

EC17 CAM's Bispecific Affinity

The bispecific affinities of EC17 CAM (a CAM is equivalent to a “bridge” or the “compound” in this application) were assessed using ³H-EC17 in cell-based radioligand binding assays. For binding to FR+ targets, KB and CHO-β cells were pre-seeded overnight in 24-well tissue culture plates and incubated with 0.1, 0.5, 1, 5, 10, 20, and 40 nM of ³H-EC17 in

FFRPMI for 2 h at 37 °C. Afterwards, the cells were rinsed with phosphate-buffered saline (PBS, pH 7.4) and lysed with 1% sodium dodecylsulfate. The whole cell lysates were quantitated for the level of radioactivity and cellular protein content by standard Pierce BCA protein assay. The number of ³H-EC17 molecules bound per cell was calculated to determine the dissociation constants (K_d) for FR α (KB) and FR β (CHO- β) respectively (Figure 10).

The EC17 has already been tested in the clinic for immunotherapy and optical imaging purposes. To directly quantify its bispecific binding affinities, ³H-EC17 was synthesized and radioligand binding assays were carried out on KB and CHO- β cell lines representing FR α + and FR β + target cells, respectively, and on unsorted EGFRt CAR-T cells representing the effector cells. When binding to its targets, EC17 demonstrated similar affinities towards both FR α and FR β with low K_d values of 1.7 nM and 0.8 nM, respectively (Figure 10, Panel A). Upon binding to unsorted E2-CAR-T cells (~24% EGFRt+, ~95:5 CD8/CD4 ratio), the K_d value was estimated at ~130 nM (Figure 10, Panel B).

EXAMPLE 22

Tumor Models

All animal care and use were performed according to NIH guidelines and in compliance with protocols approved by the Purdue University Animal Use and Care Committee. Female 4 to 5-week-old NOD/SCID gamma (NSGTM) mice (stock number: 005557) were purchased from The Jackson Laboratory (Bar Harbor, ME). Unless specifically indicated, all animals were maintained on a FA-deficient diet (TestDiet, St. Louis, MO) upon arrival and throughout the study. To establish subcutaneous xenografts, MDA-MB-231 and HOS-FR α were implanted in the right flank region at 2.5 x 10⁶ and 1 x 10⁶ cells per animal, respectively. For intravenous xenografts, THP1-FR β cells were inoculated at 5 x 10⁶ cells per animal. Subcutaneous tumors were measured 2-3 times per week with a caliper and calculated using the ellipsoidal formula (length x width²)/2. Euthanasia was performed per study design or when (i) the animals had lost $\geq$ 20% of body weight or approached moribund conditions, (ii) subcutaneous tumors reached $\geq$ 1500 mm³ in size, or (iii) animals displayed signs of swollen belly and severe distress (i.e., THP1-FR β). All animal doses (CAR-T cells, EC17, sodium fluorescein) were given intravenously.

EXAMPLE 23

Tumor Therapies

In a therapeutic setting, EC17 CAM can be given before or after CAR-T cell injection. As described herein, the first dose of EC17 was administered 2-3.5 days after CAR-T cells to allow for an observation period of human T cells in tumor-bearing mice. Two batches of
5 unsorted E2-CAR-T cells (23% or 39% EGFRt+, 1:1 CD4/CD8) were used for *in vivo* studies. On the day of infusion for each experiment (day 0), frozen CAR-T cells were quickly thawed at 37°C, washed 2x with Dulbecco's 1X PBS (pH 7.4) and injected into the tail vein at desired EGFRt+ E2-CAR-T cell doses. In addition, a small aliquot of CAR-T cells was analyzed by
10 flow cytometry for CD4 to CD8 ratio and differentiation status of CAR-T cells. On the first day of EC17 dose, tumor-bearing animals were randomly assigned into groups according to their tumor sizes or the same number of days post intravenous implantation (i.e., THP1-FRβ).

For MDA-MB-231 studies, mice received a high dose (~10 million) of a "clinical facsimile" of E2-CAR-T cells (~39% EGFRt+) with a low differentiation profile (Figure 9).

15 Two days later, 3 different treatment regimens of EC17 were started at an average tumor size of $\sim 293 \pm 39 \text{ mm}^3$ (Figure 12). The EC17 dosing was given once-a-week (SIW) at 500 nmol/kg on Mondays, or as escalating doses of 5, 50 or 100, and 500 or 1000 nmol/kg (i.e., 5/50/500 or 5/100/1000) on Monday, Thursday, and Monday with a 6-day break in-between cycles. Control mice were left untreated (received CAR-T cells but no EC17). For comparison, a cohort of
20 tumor-free littermates also received the same number of CAR-T cells without or with EC17 SIW at 500 nmol/kg.

For AML studies (Figure 13), mice were intravenously infused with THP1-FRβ tumor cells one day prior to receiving a low dose of ~6 million E2-CAR-T cells (~23% EGFRt+). At ~3.5 days post CAR-T cell infusion, EC17 was dosed in 3 different ways including i) SIW at
25 500 nmol/kg, ii) thrice at 5/50/500 nmol/kg on Monday/Wednesday/Friday followed by a 9-day break in between cycles (TIW On/Off), and iii) as escalating doses of 5/10/100 nmol/kg in Cycle 1, 5/30/300 nmol/kg in Cycle 2, and 5/50/500 nmol/kg in Cycle 3, all on Monday/Thursday/Monday with a 6-day break in between cycles (M/Th/M_On/Off). On day
30 31, satellite animals were harvested for quantification of CAR-positive T cells identified in the mouse blood as human CD3ε+ EGFRt+ events and calculated as absolute numbers per 100 μL of whole blood. Upon euthanasia or at the end of study on day 38, total tumor load in THP1-FRβ tumor-bearing mice was assessed by measuring GFP+ tumor cells in the blood by flow

cytometry and collecting liver weight (with metastatic lesions) and total weight of nonliver macrometastases found in the body. Tumor fragments of liver metastases were enzymatically digested into single-cell suspensions and viable cell populations were analyzed for the status of CAR-T cell exhaustion using anti-human PD1, LAG3 and TIM3 (clones
5 EH12.1, T47-530 and 7D3, respectively).

For the osteosarcoma study (Figure 14), two cohorts of mice were subcutaneously implanted with HOS-FR α tumor cells 3 days prior to receiving ~6 million of the same CAR-T cell preparation used in the THP1-FR β study (Figure 13). At ~3.5 days post CAR-T cell infusion, one cohort of mice was given up to 3 cycles of EC17 at 5/10/100 nmol/kg in Cycle 1,
10 5/30/300 nmol/kg in Cycle 2, and 5/50/500 nmol/kg in Cycle 3, all following the Monday/Thursday/Monday regimen with a 6-day break in-between cycles. At the end of study, circulating CD3 ϵ + EGFRt+ CAR-T cells per 100 μ L of mouse blood were enumerated. HOS-FR α tumors (+/- EC17 treatment) were also harvested and digested for flow cytometric analysis of tumor-infiltrating CAR-T cells.

A Dose Escalation Trial against an Aggressive Osteosarcoma Model

For our intended purpose, HOS-FR α is a low FR-expressing but most aggressive tumor model with a functional FR level of $\sim 5.82 \pm 1.45$ pmol/mg protein. In parallel to the THP1-FR β study described above, two cohorts of mice with 3-day-old HOS-FR α tumors were given the
20 same E2-CAR-T cells at the same dose (~6 million). One cohort was treated with the same accelerated EC17 dose escalation regimen at 5/10/100 nmol/kg in Cycle 1, 5/30/300 nmol/kg in Cycle 2, and 5/50/500 nmol/kg in Cycle 3, on Monday/Thursday/Monday schedule followed by a 6-day break (Figure 14, panel A). As HOS-FR α tumors grew aggressively without EC17 treatment, accelerating EC17 dose escalation at this CAR-T cell dose was safe (no CRS or body
25 weight loss) and resulted in a significant delay in tumor growth within the first two cycles of treatment (Figure 14, panel B). Upon protocol-mandated euthanasia due only to tumor size (≥ 1500 mm³), flow cytometric analyses performed on whole blood showed an EC17-dependent CAR-T cell expansion up to day 47 but higher on day 33 (Figure 14, panel C, left bar graph).
Ex vivo tumor analysis on day 33 indicated a low but significant intratumoral CD3 ϵ + EGFRt+
30 CAR-T cell population in EC17-treated animals which amounted to ~1% total viable digested tumor-derived cells (Figure 14, panel C, right bar graph). As large HOS-FR α tumors stopped

responding to treatment in Cycle 3 (Figure 14, panel B), intratumoral CAR-T cells also diminished on day 47 (Figure 14, panel C). Notably, HOS-FR α tumors analyzed by a ^3H -FA radioligand assay upon disease progression showed similar FR α levels with and without EC17 treatment. Thus, it appeared that HOS-FR α tumors in NSG mice quickly outgrew the tumor infiltrating capability of CAR-T cells and may have decreased cytolytic activity due to T cell exhaustion as suggested by *in vitro* co-culture studies.

EC17 Dose Finding Study in Tumor-containing and Tumor-free Mice

The initial EC17 dose finding studies were conducted in MDA-MB-231 tumor bearing mice as shown by the schematic diagram of the experimental layout (Figure 12, panel A). NSG mice without or with MDA-MB-231 tumors ($\sim 211 \pm 65 \text{ mm}^3$) were engrafted on day 0 with ~ 10 million of a “clinical facsimile” batch of E2-CAR-T cells ($\sim 39\%$ EGFRt+, 51:49 CD4/CD8). This batch of CAR-T cells consisted of mostly T_{SCM} and T_{CM} phenotypes (see Figure 9). Two days after the CAR-T cell injection, one cohort of tumor-bearing mice were left untreated while three cohorts were treated with different regimens of EC17, including single injection per week (SIW) at 500 nmol/kg, or as escalating EC17 dose levels of 5/50/500 (Escalation-1) or 5/100/1000 nmol/kg (Escalation-2) given on a Monday/Thursday/Monday schedule with 1-week drug-free intervals. For comparison, two tumor-free cohorts were either untreated or treated with EC17 SIW at 500 nmol/kg.

Human T cell-derived IFN γ levels in mouse blood were measured in all cohorts using satellite animals on days 11 and 12 (~ 20 and 42 hours after the previous EC17 dose). Compared to tumor-bearing mice that received CAR-T cells only, all EC17 treated tumor cohorts had $\sim 30\times$ (day 11) and $\sim 10\times$ (day 12) higher IFN γ production in mouse plasma, and the levels of this cytokine decreased naturally from 20 hours to 42 hours later (Figure 12, panel B). In accordance with IFN γ release, EC17 also triggered CAR-T cell expansion identified as human CD3 ϵ + EGFRt+ events in mouse blood, and cells persisted up to 54 days in tumor-bearing animals (last measurement) (Figure 12, panel C). In tumor-free cohorts, no CAR-T cell expansion was detected by flow cytometry, but low levels of IFN γ were detected on days 11 and 12 in animals that received the same number of CAR-T cells plus EC17 (Figure 12, panels B-C). Moreover, no CRS symptoms (grade 0 out of a 0-5 scale) or body weight loss was observed in tumor-free mice that received two weekly doses of EC17 at 500 nmol/kg.

Moderate-to-severe CRS symptoms (grades 2-3) and significant body weight losses

(Figure 12, panel D) were observed in tumor-bearing cohorts with continued EC17 dosing independent of the regimen. While EC17 SIW at 500 nmol/kg caused the earliest onset of CRS and body weight loss, the aggressive EC17 Escalation-2 regimen (up to 1000 nmol/kg) caused persistent body weight loss with animal recovery occurring after EC17 dose cessation (Figure 12, panel D, top row). Notably, symptoms of graft-versus-host disease (GVHD) included red itching skin and hairlessness and became obvious at ~1 month after CAR-T cell engraftment. Although animals receiving only CAR-T cells showed signs of nonspecific CAR-T cell/tumor alloreactivity, only EC17-treated cohorts produced 100% cures (Figure 12, panel D, bottom row). Therefore, EC17 administration in the presence of FR+ tumors was the key to drive i) CAR-T cell activation, ii) cytokine production, and iii) *in vivo* CAR-T cell expansion and persistence. Under specific conditions, however, severe CRS (grade ≥ 3) was triggered by a high CAR-T cell dose in combination with EC17 doses equal to or greater than 500 nmol/kg.

EC17 CAM Dosing Control in Anti-leukemic Activity

Intravenously implanted GFP-expressing THP1-FR β tumor cells developed disseminated diseases in NSG mice with tumor cells in the circulation and liver/non-liver metastases throughout the body. THP1-FR β tumor cells could also localize to the mouse ovary which appeared inflamed during the early stage of tumor progression. Therefore, total tumor burden in each animal in study cohorts was assessed by quantitating circulating GFP+ tumor cells in the blood, liver weights, and all-inclusive non-liver macrometastases visible to the naked eye. Although THP1-FR β expressed a low level of FR *in vitro*, THP1-FR β tumor metastases were found to express a higher than expected functional FRs level at $\sim 8.9 \pm 2.8$ pmol/mg membrane protein. Thus, THP1-FR β tumor-bearing mice were engrafted with a research batch of EGFRt-unsorted E2-CAR-T cells ($\sim 23\%$ EGFRt+, 1:1 CD4:CD8) at ~ 6 million/animal and then treated with 3 different EC17 dosing regimens (Figure 13). Starting 3 days after CAR-T cell injection, EC17 dosing regimens began as SIW at 500 nmol/kg continuously, three times a week (TIW) at 5/50/500 nmol/kg on Monday/Wednesday/Friday followed by a 9-day break, or by an accelerated dose escalation regimen at 5/10/100 nmol/kg in Cycle 1, 5/30/300 nmol/kg in Cycle 2, and 5/50/500 nmol/kg in Cycle 3, on Monday/Thursday/Monday (M/Th/M) followed by a 6-day break (Figure 13, panel A). While some body weight loss and grade 1-2 CRS in Cycles 2 and 3 were observed in animals

receiving EC17 SIW treatment, animals that received EC17 TIW had the least body weight loss with grade 0-1 CRS only (Figure 13, panel B). Amongst the animals that received the 3 cycles of EC17 M/Th/M dose escalation, grade 0-1 CRS and very mild body weight loss were observed in Cycle 2 after the last dose of EC17 of 300 nmol/kg. Using satellite animals on day 5 31, CAR-T cells were enumerated in the blood and the data demonstrated EC17-dependent CAR-T cell expansion and persistence in all treated cohorts (Figure 13, panel C). Compared to control animals that received tumor cells only or tumor cells plus CAR-T cells without EC17, EC17 dosed with any of the 3 “intra-patient” escalation formats effectively reduced circulating THP1-FR β tumor cells in the blood and showed similar activities against liver tumor metastases 10 (Figure 13, panel D, left and middle bar graphs). Only minor allogeneic reactivity was seen against THP1-FR β liver metastases in mice that received CAR-T cells only. While EC17 SIW and TIW at 10-fold dose escalation (on/off) successfully controlled non-liver macrometastases, EC17 M/Th/M dose escalation at a slow pace per cycle (Figure 13, panel A) failed to control the non-liver macrometastases (Figure 13, panel D, far right panel). At the end of study (i.e., 39 15 days post CAR-T cell injection), CAR-T cells isolated from liver THP1-FR β tumor metastases appeared to have the least expression of double- and triple-positive T cell inhibitory receptors, PD1, LAG3 and TIM3 (Figure 13, panel E). Overall, no severe CRS (i.e., grades ≥ 3) was observed in any EC17 treated cohorts. Nevertheless, the more aggressive EC17 TIW dose escalation (on/off) schedule trended to be the best regimen for reducing overall THP1-FR β 20 tumor burden in these mice.

EXAMPLE 24

Functional FR Assessments

These functional FR assessments apply to examples described herein. Besides pediatric 25 cancer cell lines transfected with FR α (HOS-FR α) and FR β (THP1-FR β), cancer cell lines of different histology and FR expression levels (Figure 11) were included. As estimated by a radioligand binding assay (100 nM ^3H -FA, 1 h at 37°C), the ranking order of total available FRs on these cell lines was: 9×10^4 (OV90, a low-FR expressing ovarian cancer cell line), 1.9×10^5 (THP1-FR β), 2.4×10^5 (HOS-FR α^{fLuc}), 7×10^5 (HOS-FR α), 2.1×10^6 (MDA-MB-231) and 4.8×10^6 (KB) FA molecules/cell. Also included as FR-negative controls were HOS-143b $^{\text{fLuc}}$ and 30 THP1-FG12 parent cell lines. Thus, the general ranking of functional FR expression on co-

cultured FR+ cancer cell lines was: KB > MDA-MB-231 > HOS-FR α > HOS-FR α^{fluc} > THP1-FR β (AML) > OV90.

EXAMPLE 25

5

Statistics

Statistical analyses were performed using the computer program GraphPad Prism (GraphPad Software Inc., San Diego, CA). Data were analyzed using Student's t-test or one-way ANOVA. If applicable, data were further analyzed across treatment groups using appropriate multiple comparison post-test. * = $p < 0.05$ was considered statistically significant in all tests.

10

WHAT IS CLAIMED IS:

1. A method of treatment of a cancer, the method comprising
 - i) administering to a patient at least one dose of a CAR T cell composition comprising CAR T cells wherein the CAR T cells comprise a CAR directed to a targeting moiety;
 - ii) administering to the patient a compound, or a pharmaceutically acceptable salt thereof, wherein the compound comprises a small molecule ligand linked to a targeting moiety by a linker and wherein the compound, or the pharmaceutically acceptable salt thereof, is administered in at least a first dose escalation sequence and a second dose escalation sequence.
2. The method of claim 1 wherein the compound, or the pharmaceutically acceptable salt thereof, is administered in at least a first dose escalation sequence, a second dose escalation sequence, and a third dose escalation sequence.
3. The method of claim 1 wherein the compound, or the pharmaceutically acceptable salt thereof, is administered in at least a first dose escalation sequence, a second dose escalation sequence, a third dose escalation sequence, and a fourth dose escalation sequence.
4. The method of claim 1 wherein the compound, or the pharmaceutically acceptable salt thereof, is administered in at least a first dose escalation sequence, a second dose escalation sequence, a third dose escalation sequence, a fourth dose escalation sequence, and a fifth dose escalation sequence.
5. The method of claim 1 wherein the compound, or the pharmaceutically acceptable salt thereof, is administered in at least a first dose escalation sequence, a second dose escalation sequence, a third dose escalation sequence, a fourth dose escalation sequence, a fifth dose escalation sequence, and a sixth dose escalation sequence.
6. The method of any one of claims 1 to 5 wherein a first dose of the CAR T cells and a second dose of the CAR T cells are administered to the patient.
7. The method of claim 6 wherein the first dose of the CAR T cells is a test dose to monitor the patient for tolerability to the CAR T cells.
8. The method of claim 6 wherein the second dose of the CAR T cells comprises a higher dose of the CAR T cells than the first dose of the CAR T cells.

9. The method of any one of claims 1 to 8 wherein the first dose escalation sequence is followed by a period of time during which the compound, or the pharmaceutically acceptable salt thereof, is not administered.

5 10. The method of any one of claims 1 to 9 wherein the second dose escalation sequence is followed by a period of time during which the compound, or the pharmaceutically acceptable salt thereof, is not administered.

11. The method of any one of claims 2 to 10 wherein the third dose escalation sequence is followed by a period of time during which the compound, or the pharmaceutically acceptable salt thereof, is not administered.

10 12. The method of any one of claims 3 to 11 wherein the fourth dose escalation sequence is followed by a period of time during which the compound, or the pharmaceutically acceptable salt thereof, is not administered.

15 13. The method of any one of claims 4 to 12 wherein the fifth dose escalation sequence is followed by a period of time during which the compound, or the pharmaceutically acceptable salt thereof, is not administered.

14. The method of any one of claims 5 to 13 wherein the sixth dose escalation sequence is followed by a period of time during which the compound, or the pharmaceutically acceptable salt thereof, is not administered.

20 15. The method of any one of claims 9 to 14 wherein the period of time is about 7 days.

16. The method of any one of claims 1 to 15 wherein the first dose escalation sequence comprises administering to the patient about 1 percent, about 10 percent, and about 100 percent of a full dose of the compound, or the pharmaceutically acceptable salt thereof, on three separate days.

25 17. The method of any one of claims 1 to 16 wherein the second dose escalation sequence comprises administering to the patient about 1 percent, about 30 percent, and about 300 percent of a full dose of the compound, or the pharmaceutically acceptable salt thereof, on three separate days.

30 18. The method of any one of claims 2 to 17 wherein the third dose escalation sequence comprises administering to the patient about 1 percent, about 50 percent, and about 500 percent of a full dose of the compound, or the pharmaceutically acceptable salt thereof, on three separate days.

19. The method of any one of claims 3 to 18 wherein the fourth dose escalation sequence comprises administering to the patient about 1 percent, about 10 percent, and about 100 percent of a full dose of the compound, or the pharmaceutically acceptable salt thereof, on three separate days.

5 20. The method of any one of claims 4 to 19 wherein the fifth dose escalation sequence comprises administering to the patient about 1 percent, about 30 percent, and about 300 percent of a full dose of the compound, or the pharmaceutically acceptable salt thereof, on three separate days.

10 21. The method of any one of claims 5 to 20 wherein the sixth dose escalation sequence comprises administering to the patient about 1 percent, about 50 percent, and about 500 percent of a full dose of the compound, or the pharmaceutically acceptable salt thereof, on three separate days.

15 22. The method of any one of claims 16 to 21 wherein the full dose of the compound, or the pharmaceutically acceptable salt thereof, is about 10 µg/kg to about 50 µg/kg of the compound, or the pharmaceutically acceptable salt thereof.

23. The method of any one of claims 16 to 22 wherein the full dose of the compound, or the pharmaceutically acceptable salt thereof, is about 20 µg/kg to about 40 µg/kg of the compound, or the pharmaceutically acceptable salt thereof.

20 24. The method of any one of claims 16 to 23 wherein the full dose of the compound, or the pharmaceutically acceptable salt thereof, is about 25 µg/kg to about 35 µg/kg of the compound, or the pharmaceutically acceptable salt thereof.

25 25. The method of any one of claims 16 to 24 wherein the full dose of the compound, or the pharmaceutically acceptable salt thereof, is about 30 µg/kg of the compound, or the pharmaceutically acceptable salt thereof.

26. The method of any one of claims 1 to 25 wherein, if no CRS or neurotoxicity is observed in the patient during the first dose escalation sequence, the method is advanced to the second dose escalation sequence.

30 27. The method of any one of claims 2 to 26 wherein, if no CRS or neurotoxicity is observed in the patient during the second dose escalation sequence, the method is advanced to the third dose escalation sequence.

28. The method of any one of claims 3 to 27 wherein, if no CRS or neurotoxicity is observed in the patient during the third dose escalation sequence, the method is advanced to the fourth dose escalation sequence.

29. The method of any one of claims 4 to 28 wherein, if no CRS or
5 neurotoxicity is observed in the patient during the fourth dose escalation sequence, the method is advanced to the fifth dose escalation sequence.

30. The method of any one of claims 5 to 29 wherein, if no CRS or neurotoxicity is observed in the patient during the fifth dose escalation sequence, the method is advanced to the sixth dose escalation sequence.

10 31. The method of any one of claims 1 to 30 wherein if fever without hypotension is observed in the patient and no neurotoxicity is observed in the patient during any one of the dose escalation sequences, all subsequent doses of the compound, or the pharmaceutically acceptable salt thereof, are administered to the patient at the dose escalation sequence level that caused the fever without hypotension.

15 32. The method of any one of claims 1 to 30 wherein, if serious CRS or neurotoxicity occurs in the patient in any dose escalation sequence, all subsequent doses of the compound, or the pharmaceutically acceptable salt thereof, are administered to the patient at the dose escalation sequence level below the dose escalation sequence level that caused the serious CRS or neurotoxicity in the patient.

20 33. A method of treatment of a cancer, the method comprising
i) administering to a patient at least one dose of a CAR T cell composition comprising CAR T cells wherein the CAR T cells comprise a CAR directed to a targeting moiety;
ii) administering to the patient a compound, or a pharmaceutically
25 acceptable salt thereof, wherein the compound comprises a small molecule ligand linked to a targeting moiety by a linker and wherein the compound, or the pharmaceutically acceptable salt thereof, is administered in a first dose escalation sequence wherein, if serious CRS occurs in the first dose escalation sequence, the compound, or the pharmaceutically acceptable salt thereof, is administered using a lower dose escalation sequence wherein the first dose of the compound, or
30 the pharmaceutically acceptable salt thereof, in the lower dose escalation sequence is lower than the first dose of the compound, or the pharmaceutically acceptable salt thereof, administered in the first dose escalation sequence.

34. The method of claim 33 wherein in the lower dose escalation sequence, the compound, or the pharmaceutically acceptable salt thereof, is administered at about 0.5 percent, about 5 percent, and about 50 percent of a full dose of the compound, or the pharmaceutically acceptable salt thereof, on three separate days.

5 35. The method of claim 34 wherein the full dose of the compound, or the pharmaceutically acceptable salt thereof, is about the full dose of the compound, or the pharmaceutically acceptable salt thereof, is about 10 µg/kg to about 50 µg/kg of the compound, or the pharmaceutically acceptable salt thereof.

10 36. The method of any one of claims 34 to 35 wherein the full dose of the compound, or the pharmaceutically acceptable salt thereof, is about 25 µg/kg to about 35 µg/kg of the compound, or the pharmaceutically acceptable salt thereof.

37. The method of any one of claims 34 to 36 wherein the full dose of the compound, or the pharmaceutically acceptable salt thereof, is about 30 µg/kg of the compound, or the pharmaceutically acceptable salt thereof.

15 38. The method of any one of claims 1 to 37 wherein the CAR T cells comprise a nucleic acid comprising SEQ ID NO:4.

39. The method of any one of claims 1 to 38 wherein the CAR T cells comprise a polypeptide comprising SEQ ID NO:5.

20 40. The method of claim 38 wherein the nucleic acid encodes a chimeric antigen receptor.

41. The method of any one of claims 2 to 15 wherein the first dose escalation sequence comprises administering to the patient about 1 percent, about 2 percent, and about 20 percent of a full dose of the compound, or the pharmaceutically acceptable salt thereof, on three separate days, wherein the second dose escalation sequence comprises administering to the patient about 1 percent, about 6 percent, and about 60 percent of a full dose of the compound, or the pharmaceutically acceptable salt thereof, on three separate days, and wherein the third dose escalation sequence comprises administering to the patient about 1 percent, about 10 percent, and about 100 percent of a full dose of the compound, or the pharmaceutically acceptable salt thereof, on three separate days.

30 42. The method of any one of claims 2 to 15 wherein the first dose escalation sequence comprises administering to the patient about 1 percent, about 10 percent, and about 100 percent of a full dose of the compound, or the pharmaceutically acceptable salt thereof, on

three separate days, and wherein the second dose escalation sequence comprises administering to the patient about 1 percent, about 20 percent, and about 200 percent of a full dose of the compound, or the pharmaceutically acceptable salt thereof, on three separate days.

43. The method of any one of claims 2 to 15 wherein the first dose escalation
5 sequence comprises administering to the patient about 1 percent, about 10 percent, and about 100 percent of a full dose of the compound, or the pharmaceutically acceptable salt thereof, on three separate days, and wherein the second dose escalation sequence comprises administering to the patient about 1 percent, about 10 percent, and about 100 percent of a full dose of the compound, or the pharmaceutically acceptable salt thereof, on three separate days.

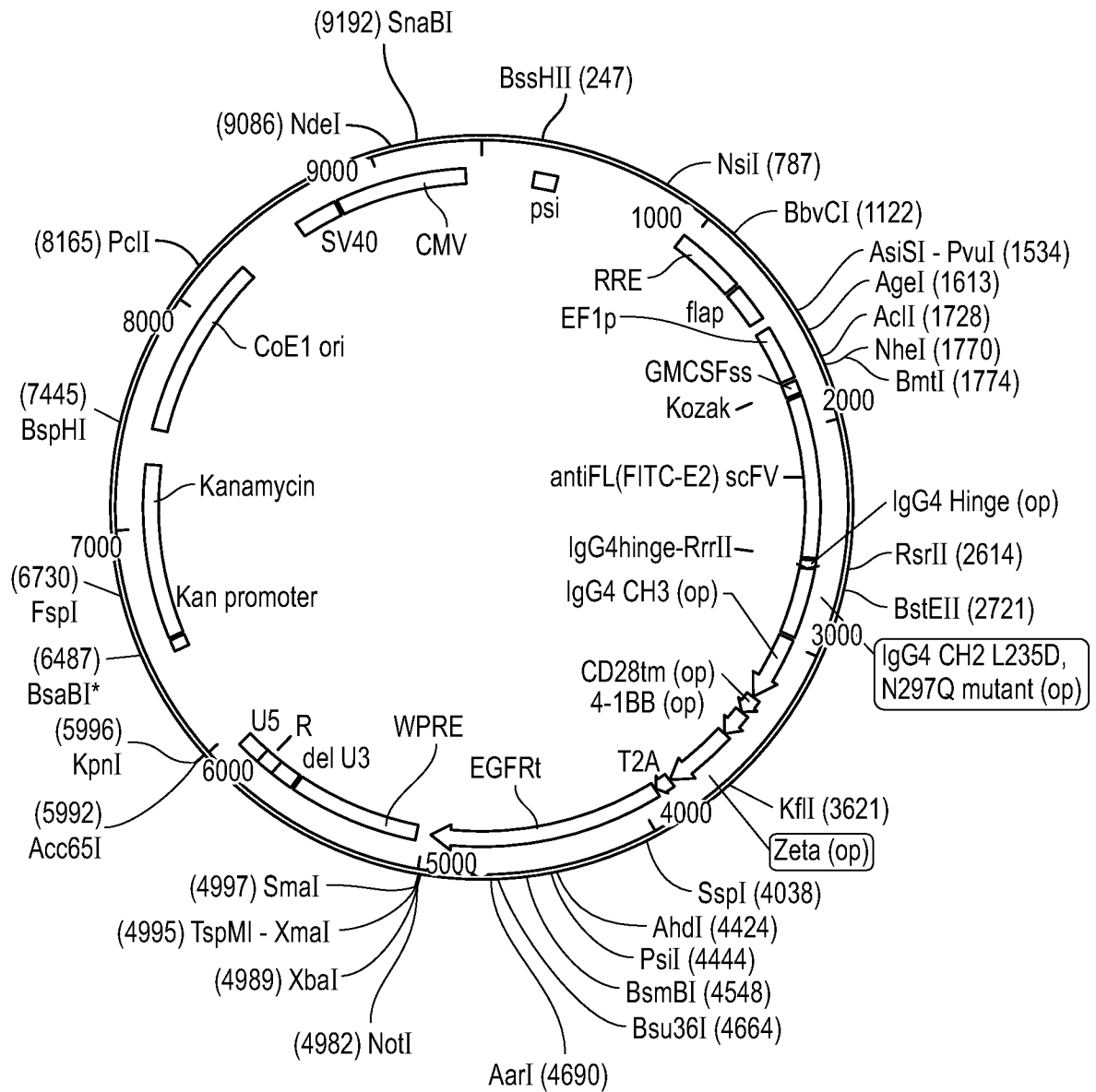
10 44. The method of any one of claims 2 to 15 wherein the first dose escalation sequence comprises administering to the patient about 1 percent, about 20 percent, and about 200 percent of a full dose of the compound, or the pharmaceutically acceptable salt thereof, on three separate days, and wherein the second dose escalation sequence comprises administering to the patient about 1 percent, about 20 percent, and about 200 percent of a full dose of the
15 compound, or the pharmaceutically acceptable salt thereof, on three separate days.

45. The method of any one of claims 41 to 44 wherein the full dose of the compound, or the pharmaceutically acceptable salt thereof, is about 500 nmoles/kg.

		Course 1								
		Cycle 1			Cycle 2		Cycle 3			
Week 1	Week 2	3	Week 3	Week 4	5	Week 5	Week 6	7	Week 7	Week 8
Part A Safety Assessment	M ² ↑	Th ↑	M ↑	Tuesday-Sunday	M ↑	Th ↑	M ↑	Tuesday-Sunday	Tuesday-Sunday	Restaging Studies. ^d
			No EC17 administration			No EC17 administration			No EC17 administration	
Mon and Thu E2-CAR T-Cell Infusions Occurs only prior to Course 1	EC17 Escalating Dose Sequence 1 ^b (1,10, 100%) ^c			EC17 Escalating Dose Sequence 2 ^b (1,30, 300%) ^c		EC17 Escalating Dose Sequence 3 ^b (1,50, 500%) ^c		Courses 2-4 ^e		
	→			→		→		→		

2/15

1. pJ03290: antiFL(FITC-E2)scFv - IgG4 hinge (CH2(L235D, N297Q)-CH3)-CD28 TM-4-1BB-CD3z-T2A-EGFRt
2. pJ03340: antiFL(4M5.3)scFv - IgG4 hinge (CH2(L235D, N297Q)-CH3)-CD28 TM-4-1BB-CD3z-T2A-EGFRt

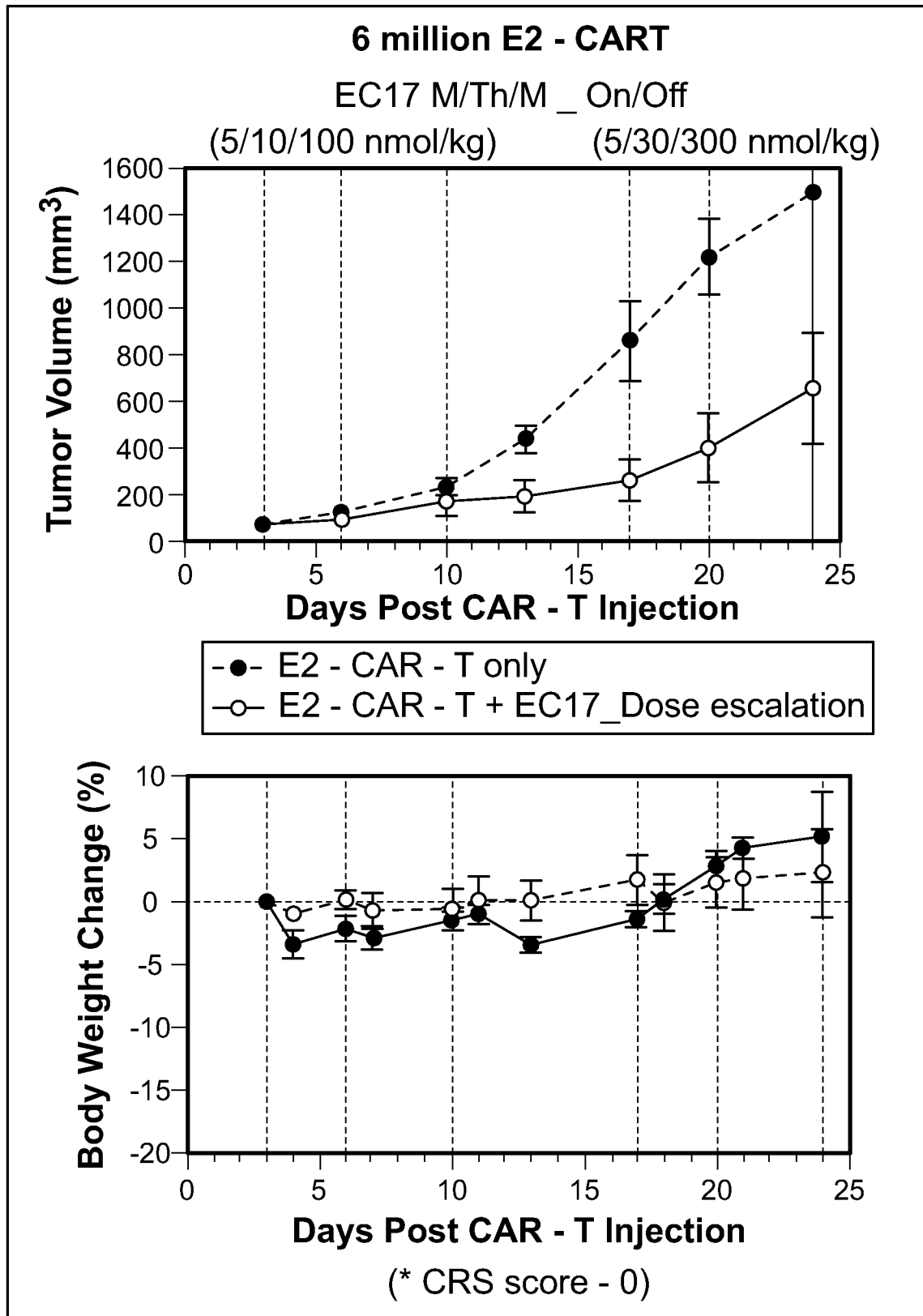


pJ03290_antiFL(FITC-E2)scFv-IgG4hinge-CH2
(L235D, N297Q)-CH3-CD28tm41BB-zeta-T2A-EGFRt-epHIV7.2
9465 bp

FIG. 3

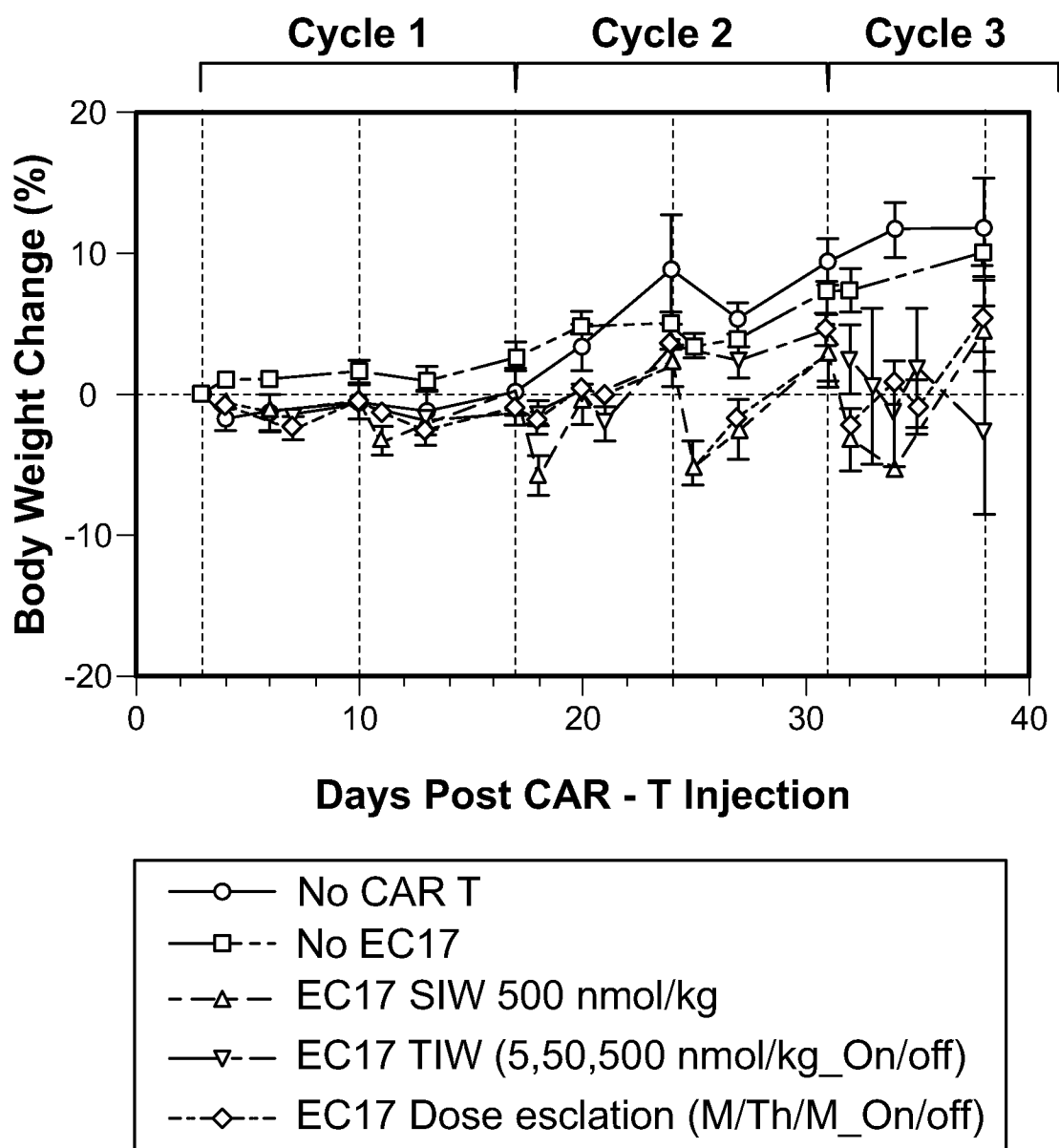
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3/15

Osteosarcoma: HOS-FR α *FIG. 4*

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4/15

*FIG. 5*

5/15

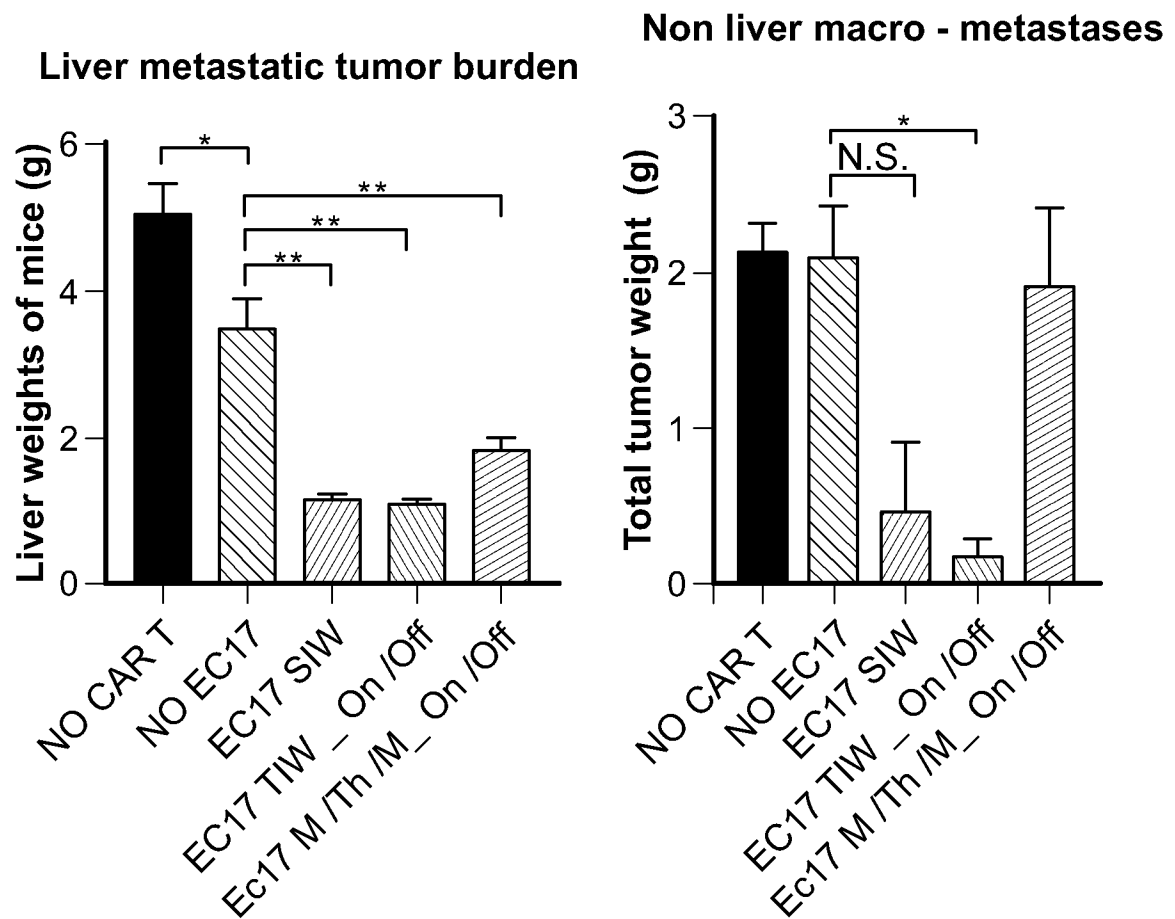
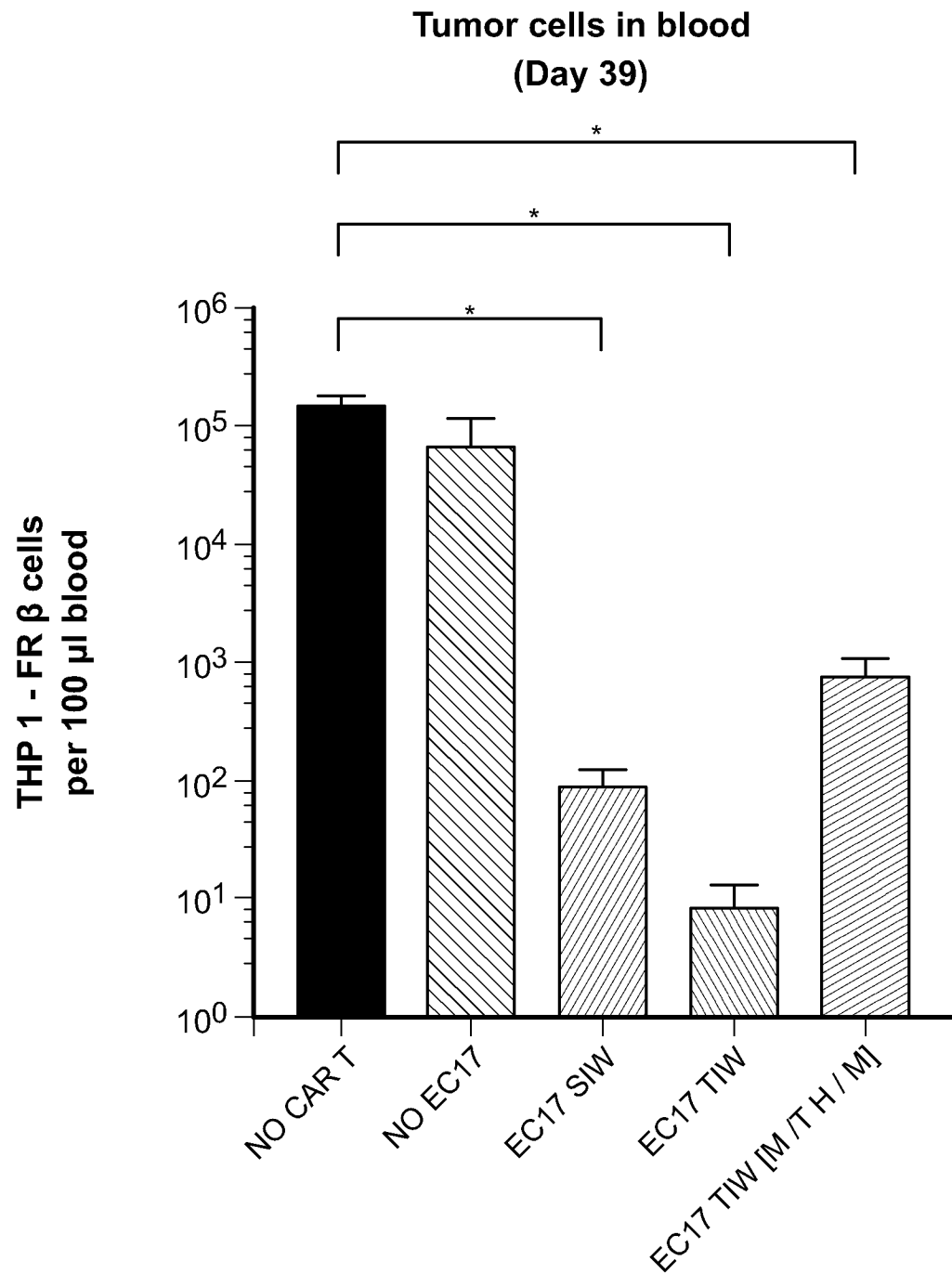
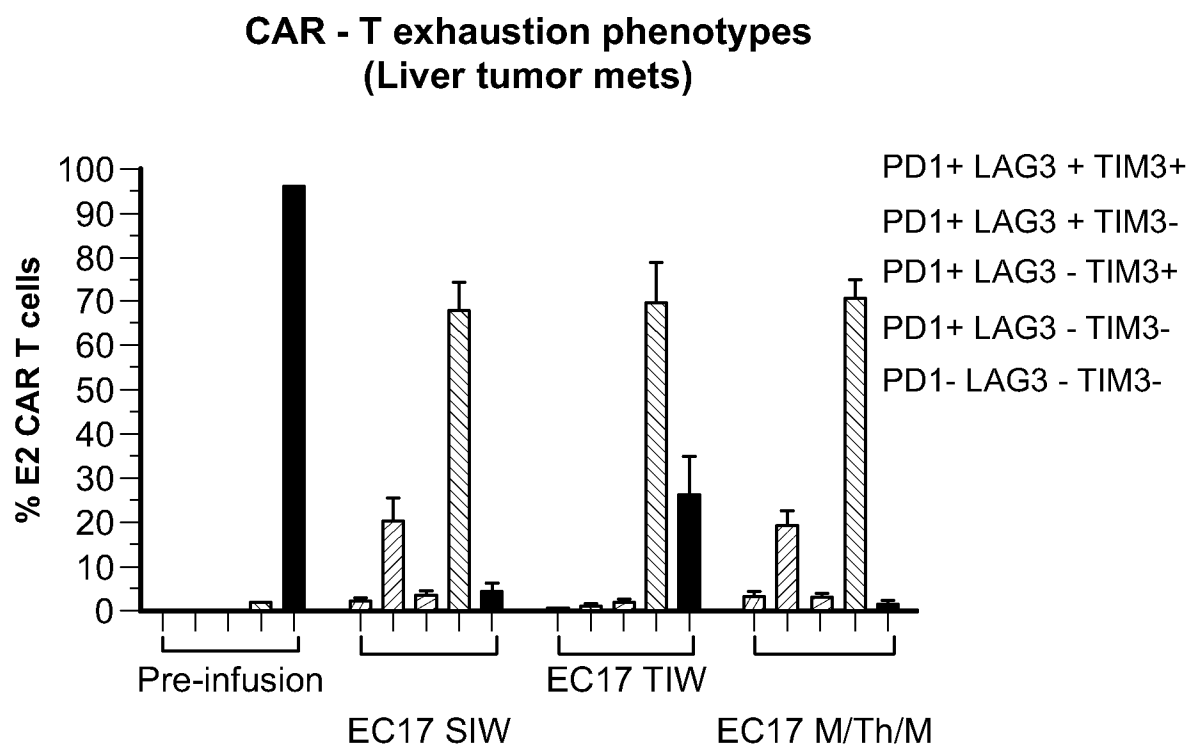


FIG. 6

6/15

*FIG. 7*

7/15

*FIG. 8*

8/15

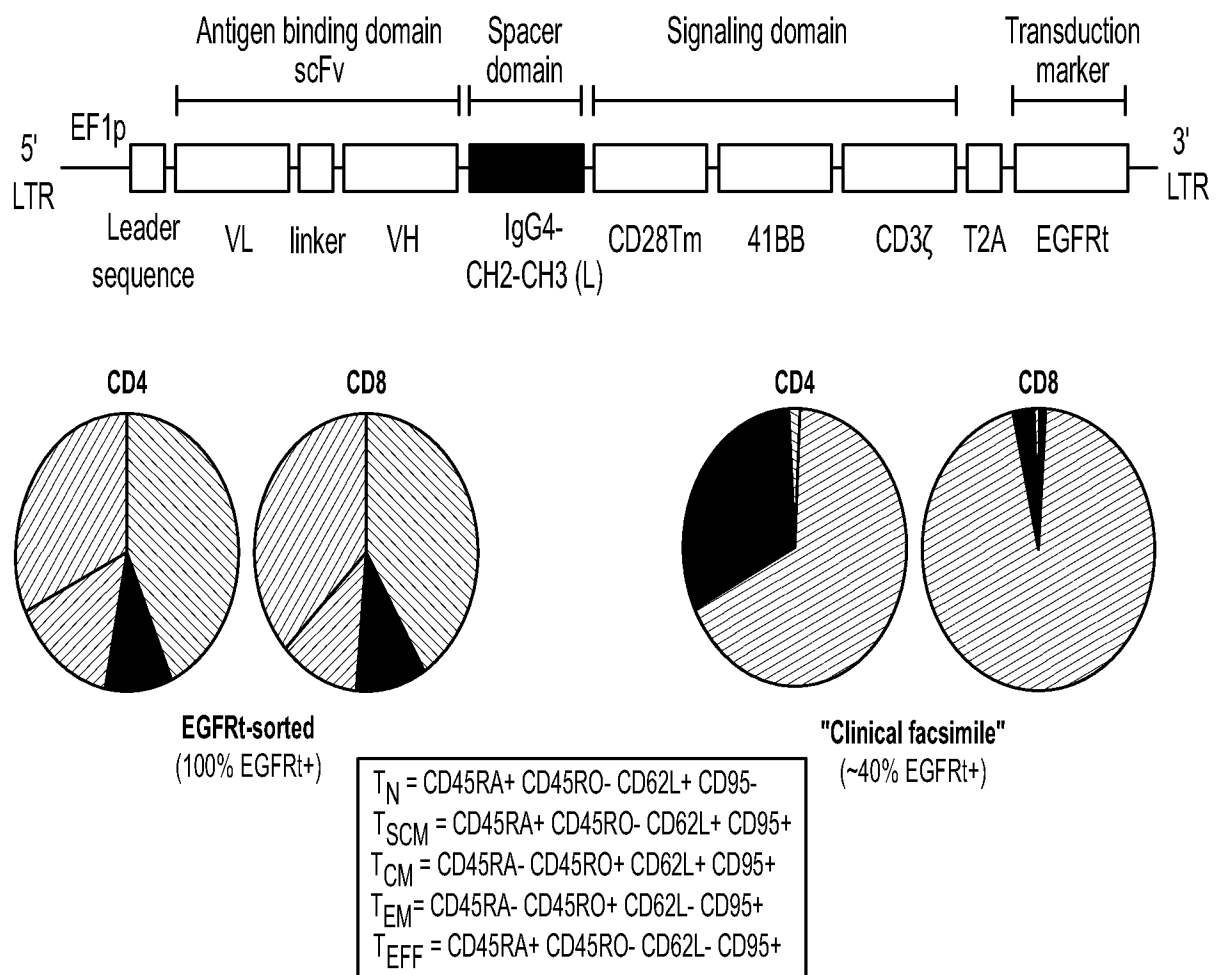


FIG. 9

9/15

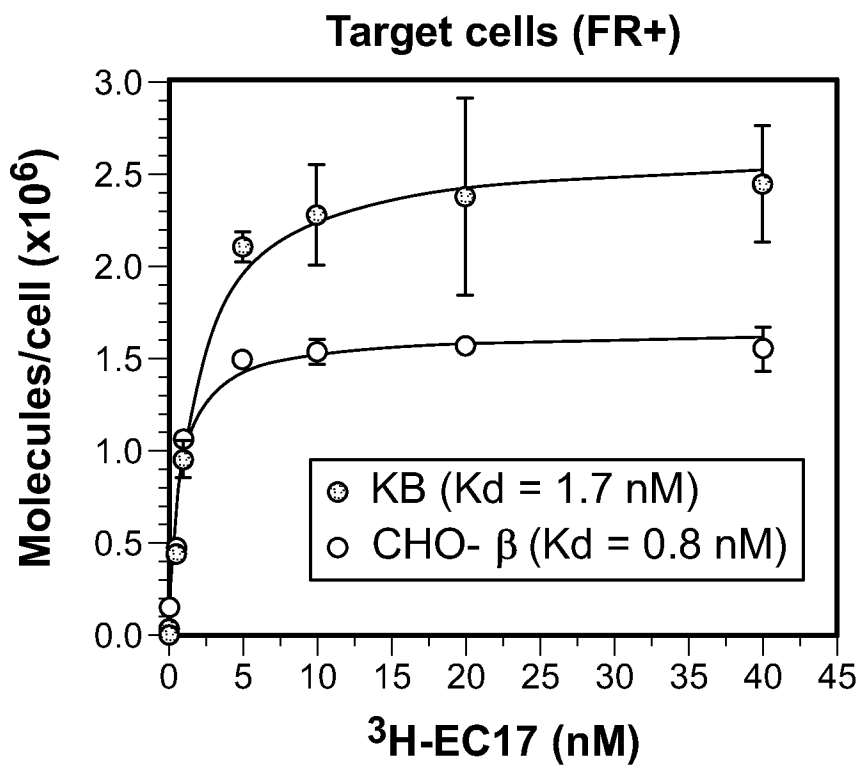


FIG. 10A

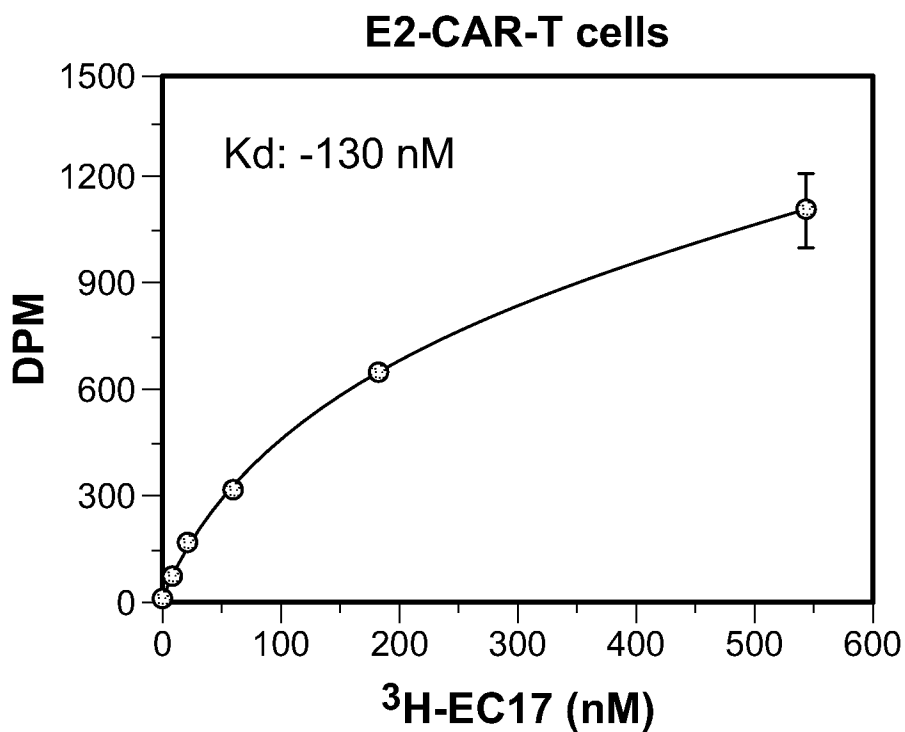
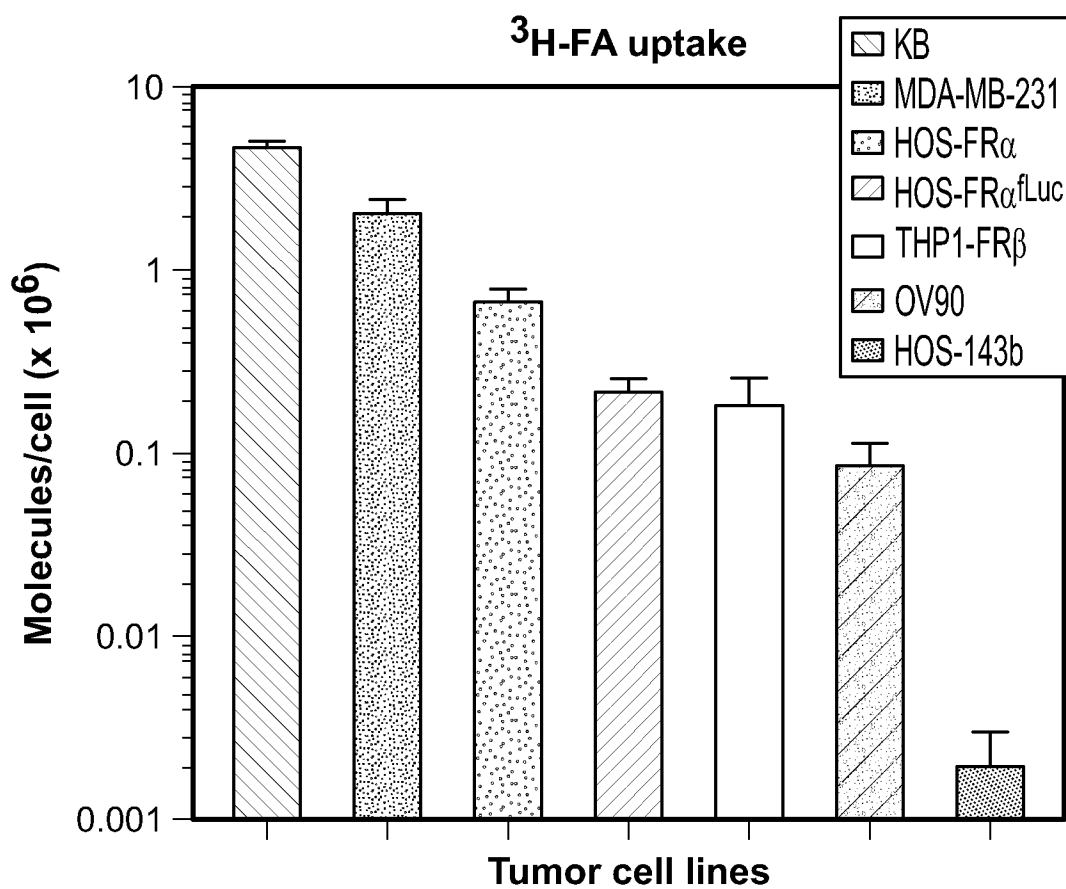
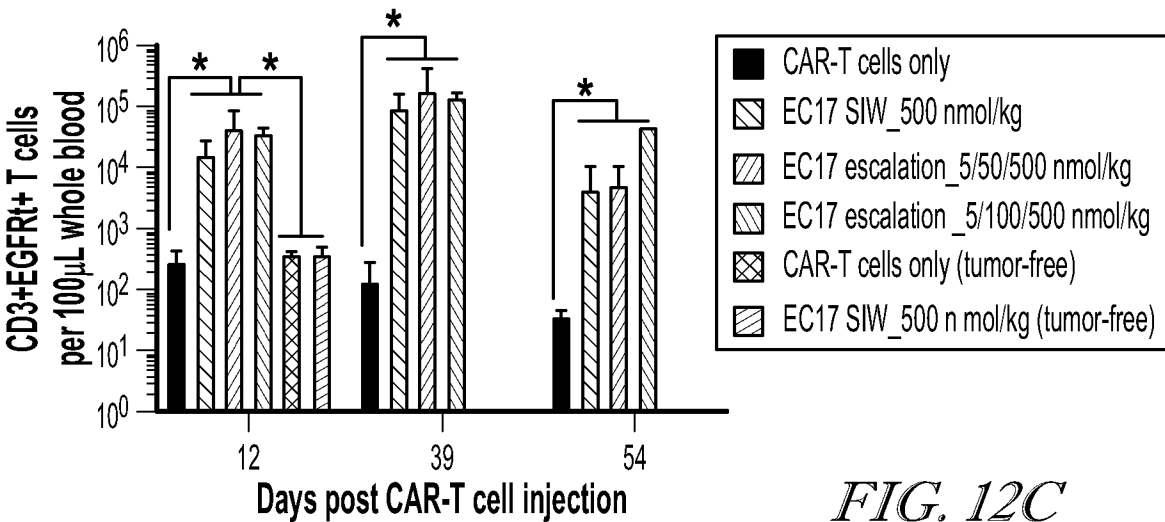
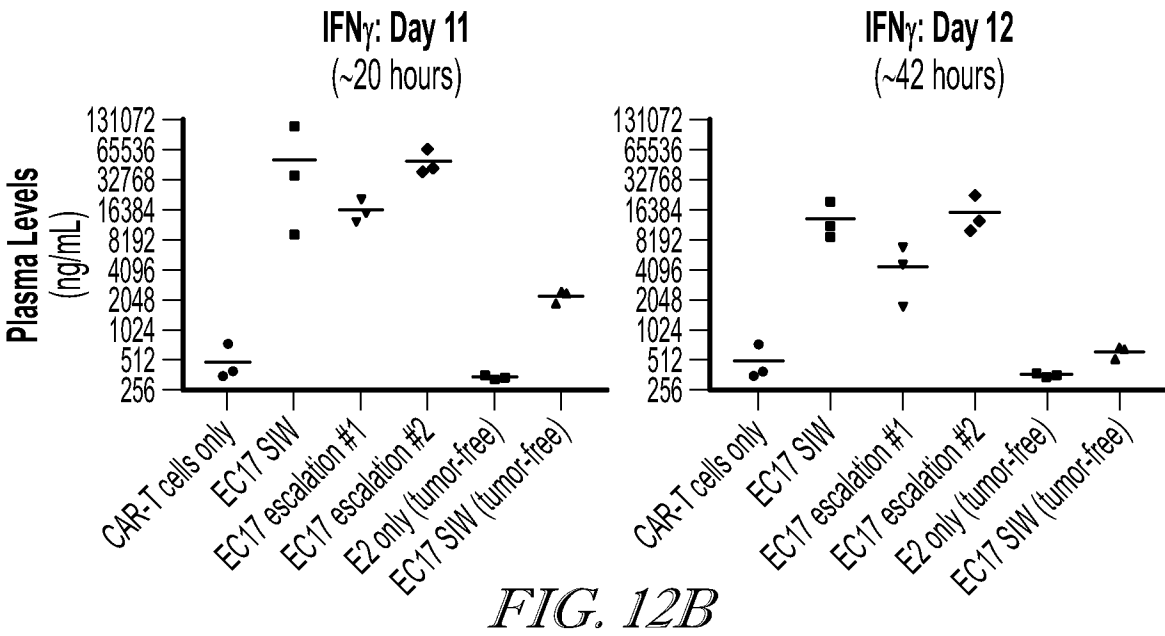
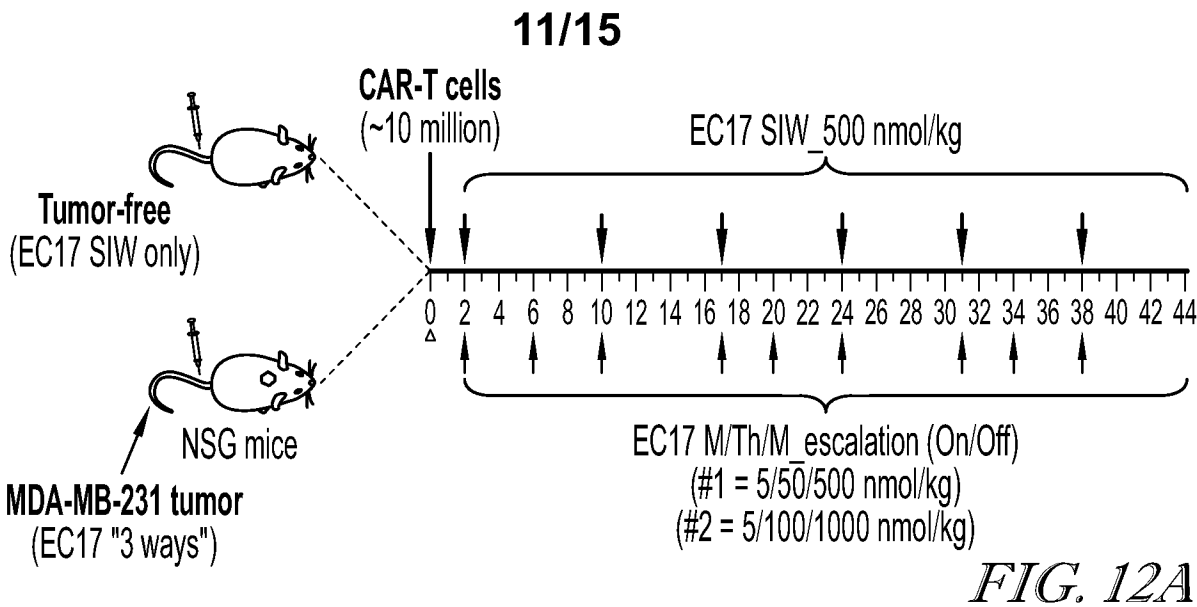


FIG. 10B

10/15

*FIG. 11*



12/15

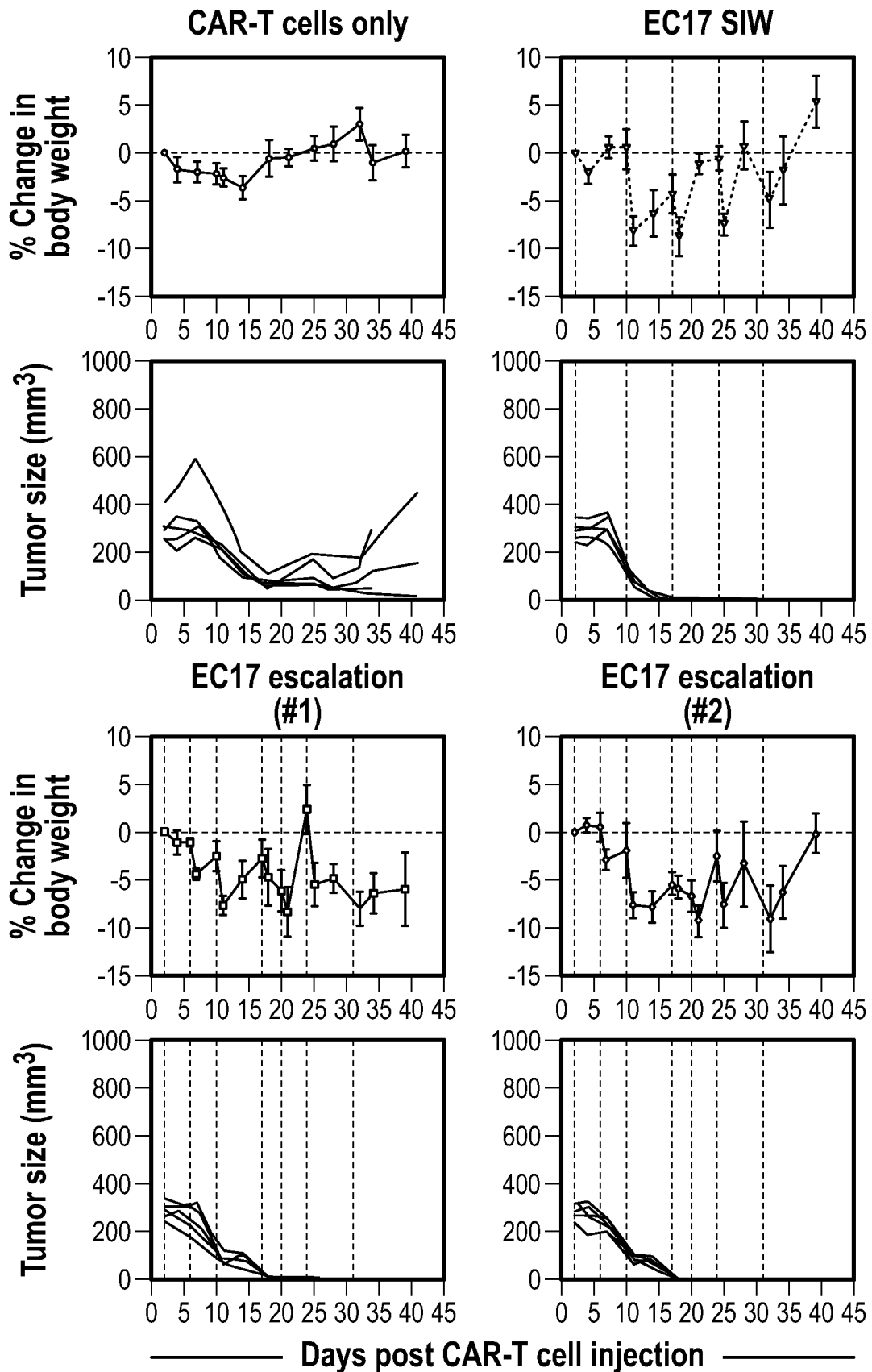


FIG. 12D

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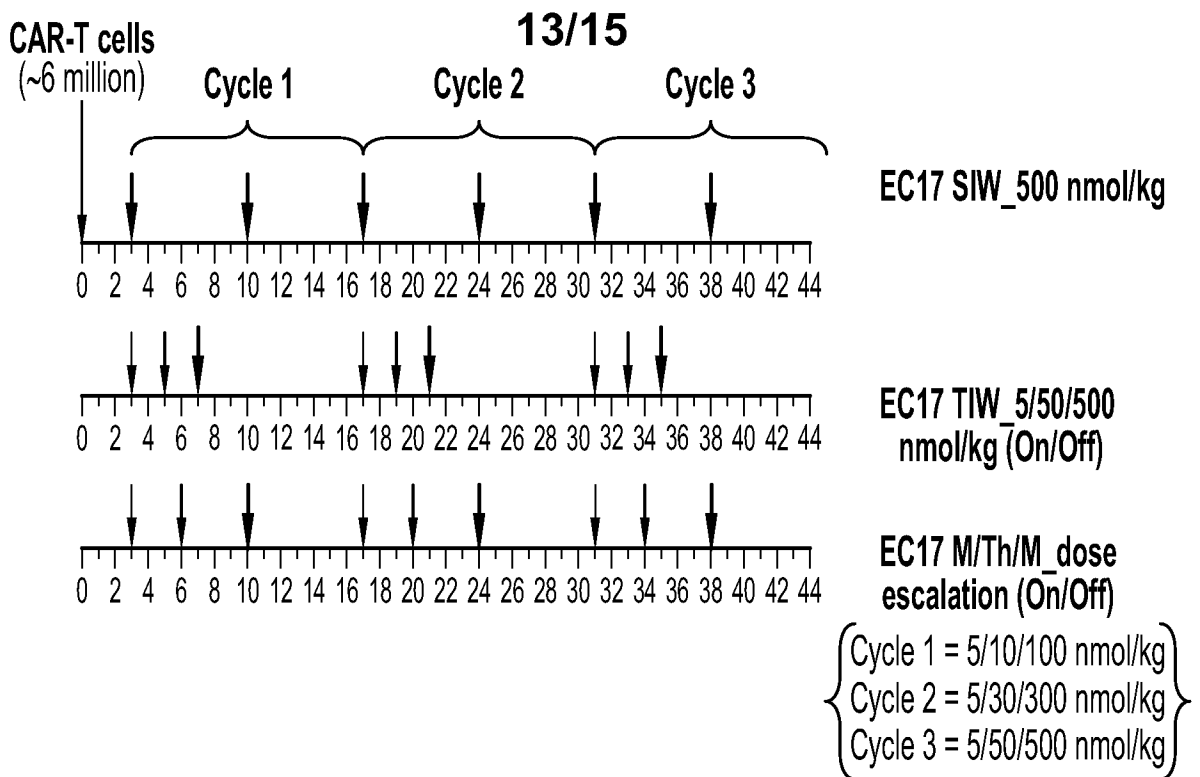


FIG. 13A

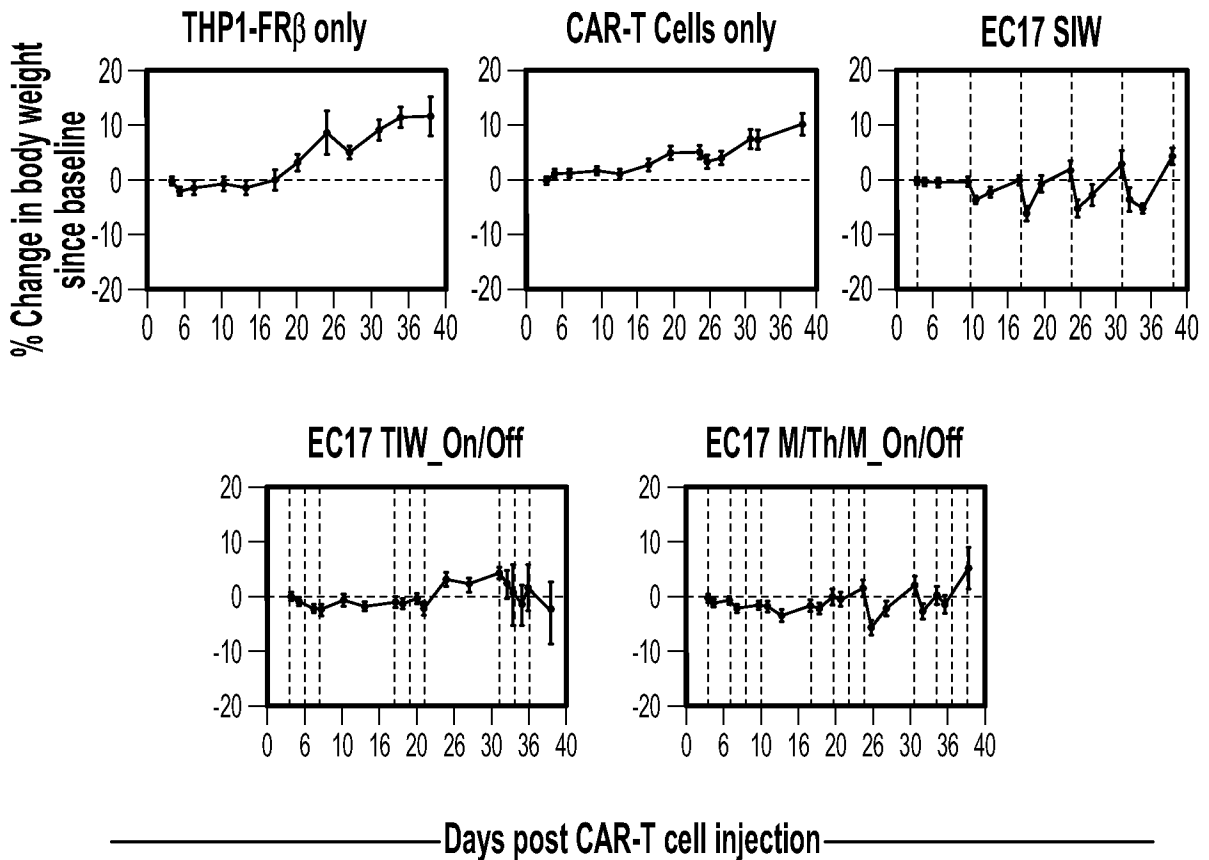


FIG. 13B

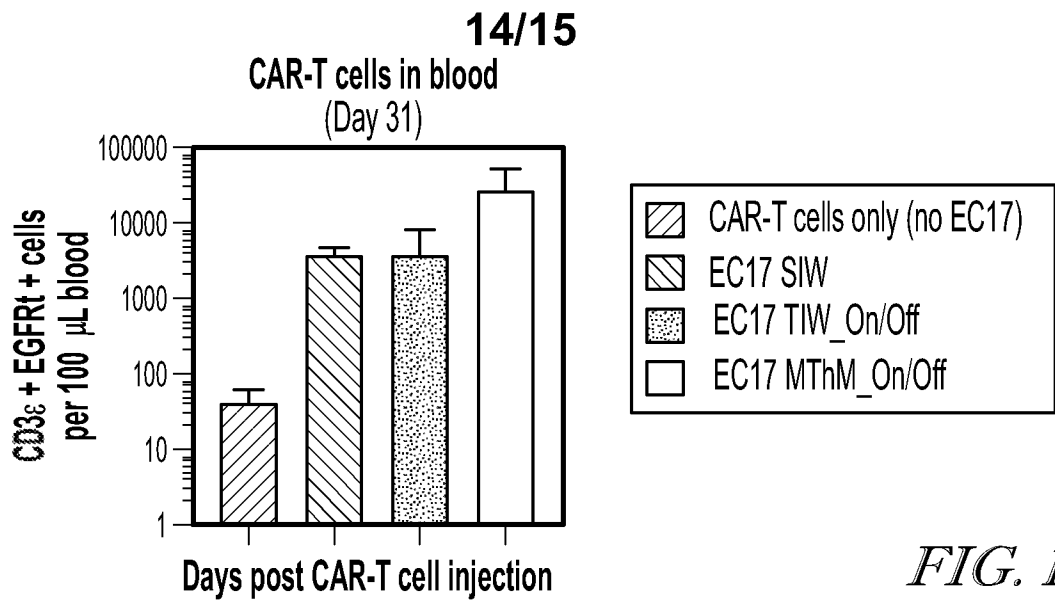


FIG. 13C

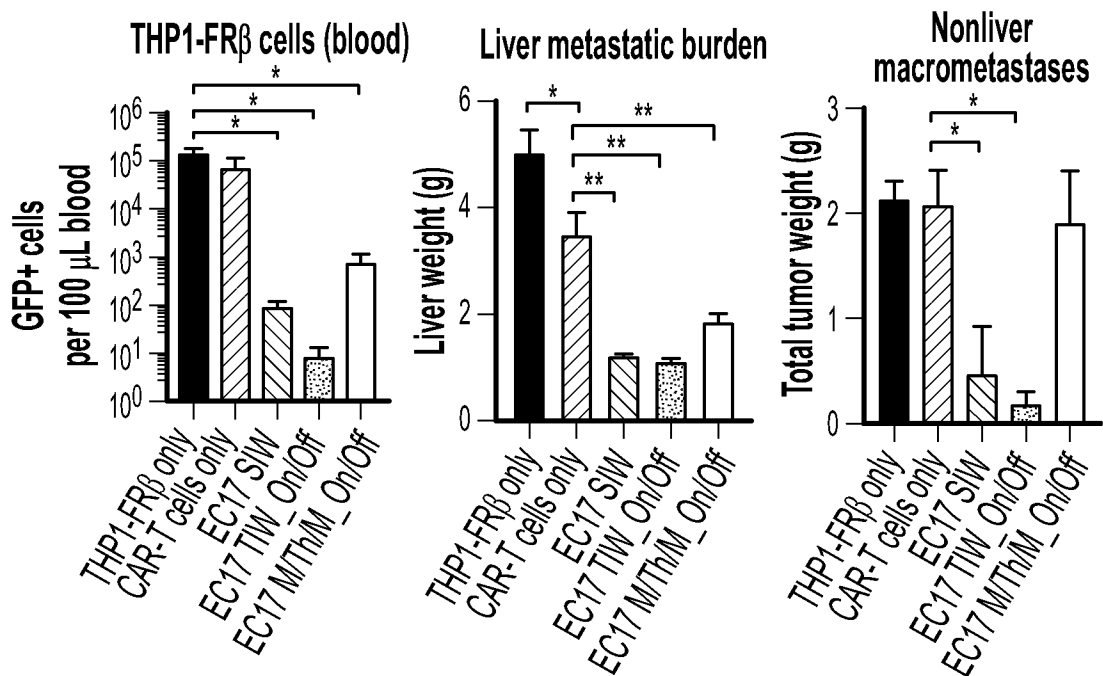


FIG. 13D

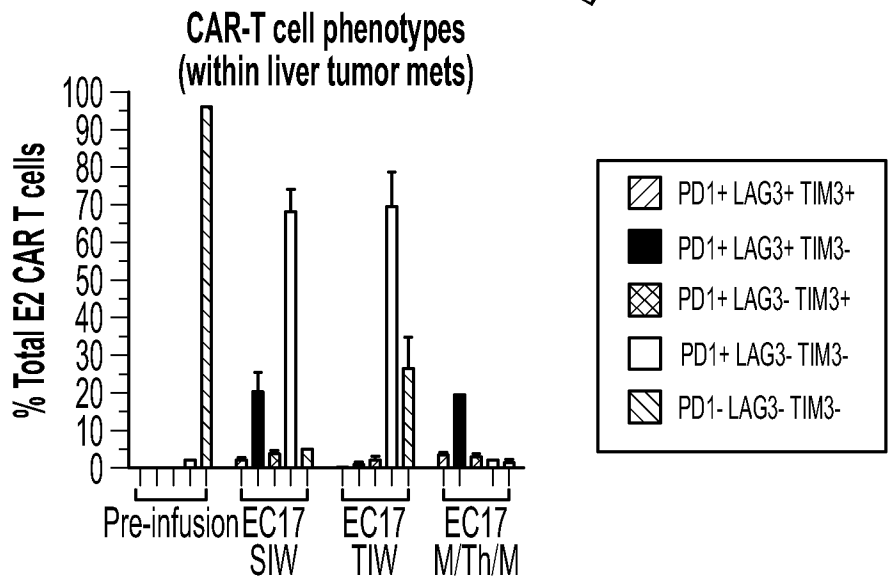
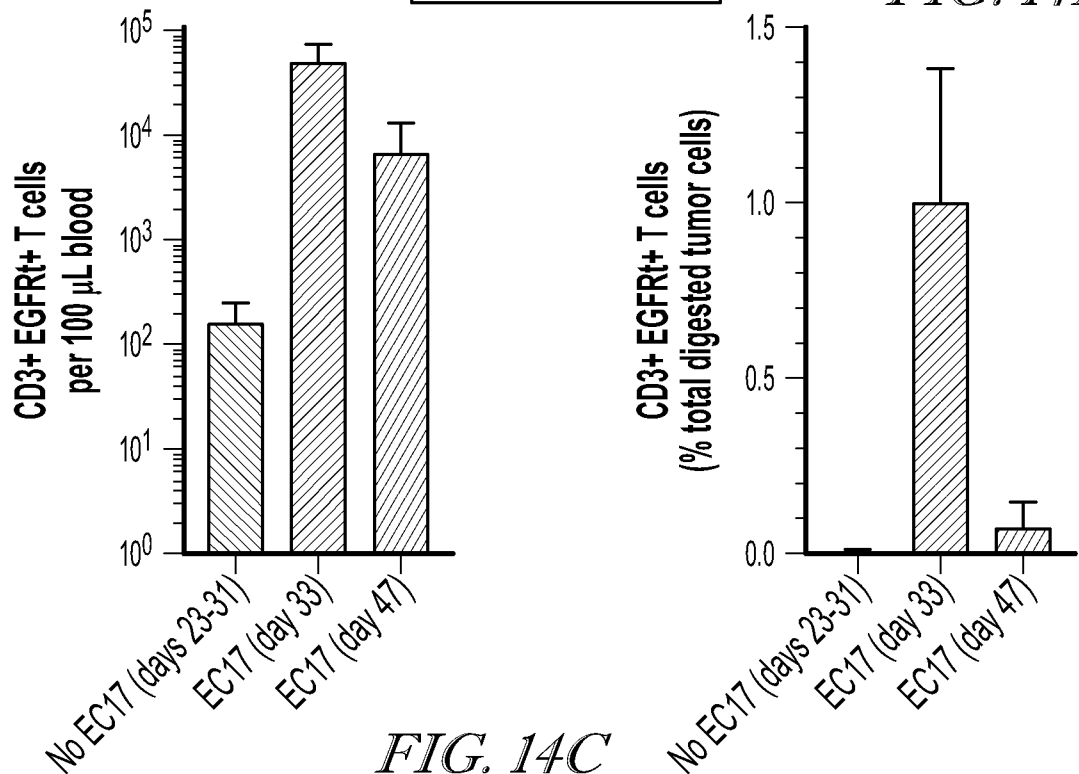
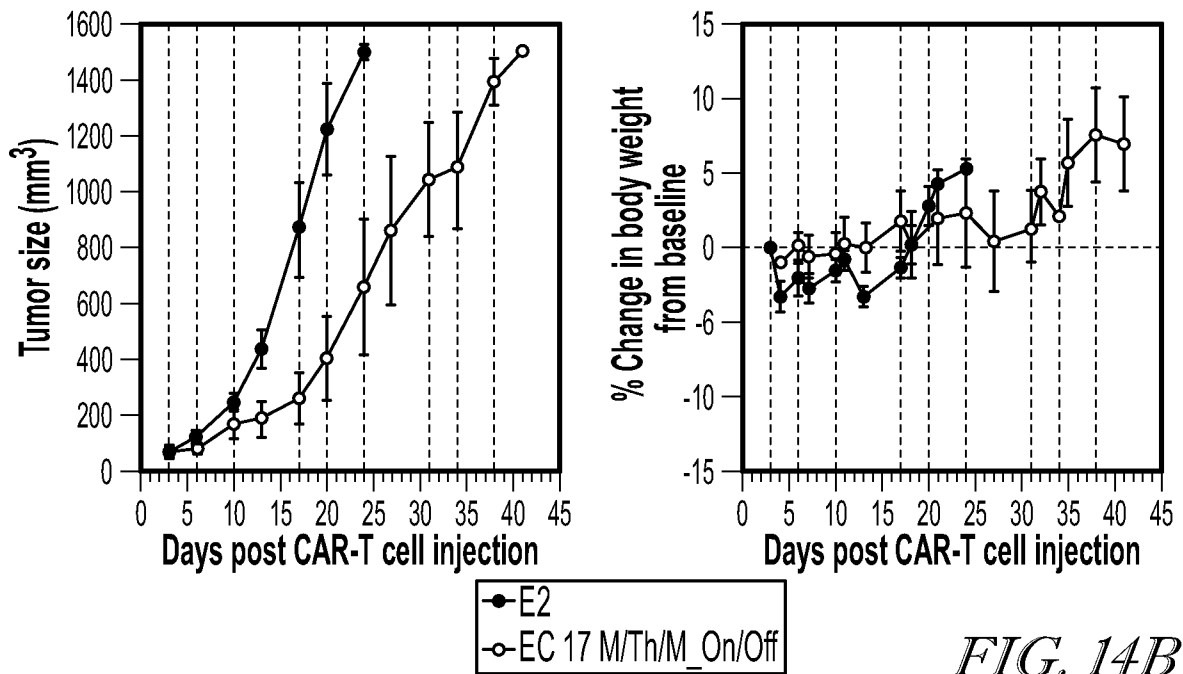
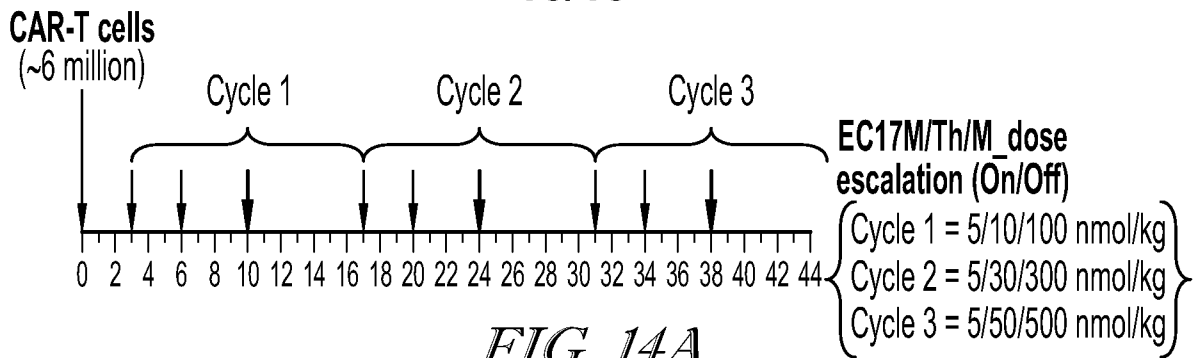


FIG. 13E

15/15



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 19/19191

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 39/00, C07K 16/44, C07K 14/705, C12N 15/86 (2019.01)

CPC - A61K 39/0011, A61K 47/551, A61K 2039/505, A61K 2039/5156, C07K 2317/73, C07K 2319/03

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History Document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2017/177149 A2 (PURDUE RESEARCH FOUNDATION) 12 October 2017 (12.10.2017); abstract; pg 3, ln 15-21; pg 3, ln 6-14; pg 41, ln 6-24; Pg 42, ln 28- pg 43, ln 1	1-8 and 33-36
Y	WO 2016/149665 A1 (BAYLOR COLLEGE OF MEDICINE) 22 September 2016 (22.09.2016); abstract; para [0197], [0238], [0239]	1-8 and 33-36

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

11 June 2019

Date of mailing of the international search report

01 JUL 2019

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Authorized officer:

Lee W. Young

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PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 19/19191

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 9-32, 37-45
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 19/19191

Box No. 1 Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

a. ☐ forming part of the international application as filed:

☐ in the form of an Annex C/ST.25 text file.

☐ on paper or in the form of an image file.

b. ☐ furnished together with the international application under PCT Rule 13ter.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

☐ in the form of an Annex C/ST.25 text file (Rule 13ter.1(a)).

☐ on paper or in the form of an image file (Rule 13ter.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

The USPTO will not be able to provide a sequence search for this application for the following reason: ISA/225 mailed on 05 March 2019. The applicant did not, within the prescribed time limit, pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rule 13ter.1(a) or (b). Accordingly, ISA/US cannot consider the sequence listing submitted on 05 April 2019.