Claims

1. An EphA3 binding agent, optionally isolated, comprising at least one complementarity determining region (CDR) having an amino acid sequence set forth in SEQ ID NOs: 13-72 and/or Tables 4-7 or an amino acid sequence at least 70% identical thereto, preferably wherein the EphA3 binding agent comprises

    a. a heavy chain immunoglobulin variable region (VH) polypeptide comprising a CDR 1 having an amino acid sequence at least 70% identical to any one of SEQ ID NOs: 13-17 or 43-47; a CDR 2 having an amino acid sequence at least 70% identical to any one of SEQ ID NOs: 18-22 or 48-52; and a CDR 3 having an amino acid sequence at least 70% identical to any one of SEQ ID NO: 23-27 or 53-57; and/or

    ~~a.~~b. a light chain immunoglobin variable region (VL) polypeptide comprising a CDR 1 having an amino acid sequence at least 70% identical to any one of SEQ ID NOs: 28-32 or 58-62; a CDR 2 having an amino acid sequence at least 70% identical to any one of SEQ ID NOs: 33-37 or 63-67; and a CDR 3 having an amino acid sequence at least 70% identical to any one of SEQ ID NO: 38-42 or 68-72.

~~2. The EphA3 binding agent of Claim 1, comprising~~

~~a. a heavy chain immunoglobulin variable region (VH) polypeptide comprising a CDR 1 having an amino acid sequence at least 70% identical to any one of SEQ ID NOs: 13-17; a CDR having an amino acid sequence at least 70% identical to any one of SEQ ID NOs: 18-22; and a CDR 3 having an amino acid sequence at least 70% identical to any one of SEQ ID NO: 23-27; and/or~~

~~b. a light chain immunoglobin variable region (VL) polypeptide comprising a CDR 1 having an amino acid sequence at least 70% identical to any one of SEQ ID NOs: 28-32; a CDR having an amino acid sequence at least 70% identical to any one of SEQ ID NOs: 33-37; and a CDR 3 having an amino acid sequence at least 70% identical to any one of SEQ ID NO: 38-42.~~

~~3.~~2. The EphA3 binding agent of Claim 1~~2~~, wherein:

a. the VH polypeptide comprises an amino acid sequence set forth in SEQ ID NO: 153 or 155 or an amino acid sequence at least 70% identical thereto; and/or

b. the VL polypeptide comprises an amino acid sequence set forth in SEQ ID NO: 154 or 156 or an amino acid sequence at least 70% identical thereto.

~~4. The EphA3 binding agent of Claim 1, comprising~~

~~a. a VH polypeptide comprising a CDR 1 having an amino acid sequence at least 70% identical to any one of SEQ ID NOs: 43-47; a CDR 2 having a amino acid sequence at least 70% identical to any one of SEQ ID NOs: 48-52; and a CDR 3 having an amino acid sequence at least 70% identical to any one of SEQ ID NOs: 53-57; and/or~~

~~b. a VL polypeptide comprising a CDR -1 having an amino acid sequence at least 70% identical to any one of SEQ ID NOs: 58-62; a CDR 2 having a amino acid sequence at least~~

~~70% identical to any one of SEQ ID NOs: 63-67; and a CDR 3 having an amino acid sequence at least 70% identical to any one of SEQ ID NOs: 68-72.~~

~~5. The EphA3 binding agent of Claim 4, wherein:~~

~~a. the VH polypeptide comprises an amino acid sequence set forth in SEQ ID NO: 155 or an amino acid sequence at least 70% identical thereto; and/or~~

~~the VL polypeptide comprises an amino acid sequence set forth in SEQ ID NO: 156 or an amino acid sequence at least 70% identical thereto.~~

3. The EphA3 binding agent of any one of the preceding claims, wherein the EphA3 binding agent is an antibody or antibody fragment, preferably wherein the antibody or antibody fragment is a 3C3-1 or 2D4-1 monoclonal antibody or fragment thereof, preferably wherein the EphA3 binding agent is a recombinant, human or humanized antibody or antibody fragment.

~~6. An antigen-binding molecule that is capable of binding EphA3, wherein the antigen-binding molecule comprises:~~

~~(i) a heavy chain variable (VH) region incorporating the following CDRs: HC-CDR1 having the amino acid sequence of SEQ ID NO: 16; HC-CDR2 having the amino acid sequence of SEQ ID NO: 22 HC-CDR3 having the amino acid sequence of SEQ ID NO: 27; and~~

~~(ii) a light chain variable (VL) region incorporating the following CDRs: LC-CDR1 having the amino acid sequence of SEQ ID NO: 32; LC-CDR2 having the amino acid sequence of SEQ ID NO: 37; LC-CDR3 having the amino acid sequence of SEQ ID NO: 42;~~

~~7. An antigen-binding molecule that is capable of binding EphA3, wherein the antigen-binding molecule comprises:~~

~~(i) a heavy chain variable (VH) region incorporating the following CDRs: HC-CDR1 having the amino acid sequence of SEQ ID NO: 47 HC-CDR2 having the amino acid sequence of SEQ ID NO: 52 HC-CDR3 having the amino acid sequence of SEQ ID NO: 57~~

~~(ii) a light chain variable (VL) region incorporating the following CDRs: LC-CDR1 having the amino acid sequence of SEQ ID NO: 62; LC-CDR2 having the amino acid sequence of SEQ ID NO: 67; LC-CDR3 having the amino acid sequence of SEQ ID NO: 72;~~

~~8. The antigen-binding molecule according to any one of claim 9, wherein the antigen-binding molecule comprises: a VH region comprising an amino acid sequence having at least 70% sequence identity to the amino acid sequence of SEQ ID NO: 153; and a VL region comprising an amino acid sequence having at least 70% sequence identity to the amino acid sequence of SEQ ID NO: 154.~~

~~9. The antigen-binding molecule according to any one of claim 10, wherein the antigen-binding molecule comprises: a VH region comprising an amino acid sequence having at least 70% sequence identity to the amino acid sequence of SEQ ID NO: 155; and a VL region comprising an amino acid sequence having at least 70% sequence identity to the amino acid sequence of SEQ ID NO: 156.~~

~~10. An antigen-binding molecule, comprising (i) an antigen-binding molecule according to any one of claims 9 to 12, and (ii) an antigen-binding molecule capable of binding to an antigen other than EphA3.~~

~~11.~~4. A chimeric antigen receptor (CAR) comprising an antigen-binding ~~molecule~~ agent according to any one of claims ~~9-13~~1-3.

~~12.~~5. A chimeric antigen receptor (CAR) comprising an antigen binding domain including at least one CDR having an amino acid sequence set forth in SEQ ID NOs:1-3~~12~~ or an amino acid sequence at least 70% identical thereto, a transmembrane domain, preferably wherein the transmembrane domain comprises a CD8 transmembrane domain, preferably wherein the CD8 transmembrane domain comprises an amino acid sequence set forth in SEQ ID NO:159 or an amino acid sequence at least 70% identical thereto, and an intracellular T-cell signalling domain, preferably wherein the intracellular signalling domain comprises a CD3 zeta intracellular signalling domain, preferably wherein the intracellular signalling domain comprises a CD3 zeta amino acid sequence set forth in SEQ ID NO:162 or an amino acid sequence at least 70% identical thereto.

~~13.~~6. The CAR of Claim ~~15~~5, wherein the antigen binding domain comprises, consists or consists essentially of:

(a) a heavy chain immunoglobulin variable region (VH) polypeptide comprising a CDR 1 having an amino acid sequence at least 70% identical to any one of SEQ ID NOs:13~~-~~ to 17 or 43 to 47; a CDR 2 having an amino acid sequence at least 70% identical to any one of SEQ ID NOs: 18 to 22 or 48 to 52; and a CDR 3 having an amino acid sequence at least 70% identical to any one of SEQ ID NO: 23 to 27 or 53 to 57; and/or

(b) a light chain immunoglobulin variable region (VL) polypeptide comprising a CDR 1 having an amino acid sequence at least 70% identical to any one of SEQ ID NOs: 28 to ~~-~~32 or 58 to 62; a CDR 2 having an amino acid sequence at least 70% identical to any one of SEQ ID NOs: 33 to ~~-~~37 or 63 to 67; and a CDR 3 having an amino acid sequence at least 70% identical to any one of SEQ ID NOs: 38 to ~~-~~42 or 68 to 72.

~~14.~~7. The CAR of Claim ~~16~~6, wherein:

(a) the VH polypeptide comprises an amino acid sequence set forth in SEQ ID NO:153 or 155 or an amino acid sequence at least 70% identical thereto; and/or

(b) the VL polypeptide comprises an amino acid sequence set forth in SEQ ID NO:154 or 156 or an amino acid sequence at least 70% identical thereto.

~~15. The CAR of Claim 15, wherein the antigen binding domain comprises, consists or consists essentially of:~~

~~(a) a VH polypeptide comprising a CDR 1 having an amino acid sequence at least 70% identical to any one of SEQ ID NOs: 43-47; a CDR 2 having an amino acid sequence at least 70% identical to any one of SEQ ID NOs: 48-52; and a CDR 3 having an amino acid sequence at least 70% identical to any one of SEQ ID NOs: 53-57; and/or~~

~~(b) a VL polypeptide comprising a CDR 1 having an amino acid sequence at least 70% identical to any one of SEQ ID NOs: 58-62; a CDR 2 having an amino acid sequence at least 70% identical to any one of SEQ ID NOs: 63-67; and a CDR 3 having an amino acid sequence at least 70% identical to any one of SEQ ID NOs: 68-72.~~

~~16. The CAR of Claim 18, wherein:~~

~~(a) the VH polypeptide comprises an amino acid sequence set forth in SEQ ID NO:155 or an amino acid sequence at least 70% identical thereto; and/or~~

~~(b) the VL polypeptide comprises an amino acid sequence set forth in SEQ ID NO:156 or an amino acid sequence at least 70% identical thereto.~~

~~17.~~8. The CAR according to any one of Claims ~~15 to 19~~5 to 7, wherein the antigen binding domain comprises a linker, such as the linker having an amino acid sequence set forth in SEQ ID NO: 158 or an amino acid sequence at least 70% identical thereto, optionally further comprising a leader sequence, preferably wherein the leader sequence comprises, consists or consists essentially of an amino acid sequence set forth in SEQ ID NO: 157 or an amino acid sequence at least 70% identical thereto.

~~18. The CAR according to any one of Claims 14 to 20, further comprising a leader sequence.~~

~~19. The CAR according to Claim 21, wherein the leader sequence comprises, consists or consists essentially of an amino acid sequence set forth in SEQ ID NO: 157 or an amino acid sequence at least 70% identical thereto.~~

~~20. The CAR according to any one of Claims 14 to 22, wherein the transmembrane domain comprises a CD8 transmembrane domain.~~

~~21. The CAR according to Claim 23, wherein the CD8 transmembrane domain comprises an amino acid sequence set forth in SEQ ID NO:159 or an amino acid sequence at least 70% identical thereto.~~

~~22. The CAR according to any one of Claims 14 to 24, wherein the intracellular signalling domain comprises a CD3 zeta intracellular signalling domain.~~

~~23. The CAR according to Claim 25, wherein the intracellular signalling domain comprises a CD3 zeta amino acid sequence set forth in SEQ ID NO:162 or an amino acid sequence at least 70% identical thereto.~~

~~24.~~9. The CAR according to any one of Claims ~~14 to 26~~4 to 8, further comprising one or more co-stimulatory domains, such as a CD28 co-stimulatory domain having the amino acid sequence set forth in SEQ ID NO:161 or an amino acid sequence at least 70% identical thereto and/or a CD137 co-stimulatory domain having the amino acid sequence set forth in SEQ ID NO:160 or an amino acid sequence at least 70% identical thereto.

~~25. The EphA3 binding agent of any one of Claims 1 to 8, or the antigen-binding molecule of any one of Claims 9 to 13, or the CAR of any one of Claims 14 to 27 for use in the treatment or prevention of a cancer in a subject.~~

~~26.~~10. An isolated nucleic acid encoding the EphA3 binding agent according to any one of Claims 1 to ~~8 and 28~~3, or the CAR according to any one of Claims ~~15 to 27~~4 to 9.

~~27.~~11. A genetic construct comprising the isolated nucleic acid of Claim ~~29~~10.

~~28. A nucleic acid, or a plurality of nucleic acids, optionally isolated, encoding an antigen binding molecule according to any one of claims 9 to 13, or a CAR according to claim 14.~~

~~29.~~12. An expression vector, or a plurality of expression vectors, comprising a nucleic acid or a plurality of nucleic acids according to claim 10. ~~according to claim 31~~ .

~~30. A cell comprising an antigen-binding molecule according to any one of claims 9 to 13, a CAR according to claim 14, a nucleic acid or a plurality of nucleic acids according to claim 31 , or an expression vector of a plurality of expression vectors according to claim 32.~~

~~31.~~13. A host cell comprising the nucleic acid of Claim ~~29~~ 10 and/or the genetic construct of Claim ~~30~~11, wherein the host cell is or comprises a T cell.~~The host cell of Claim 34, wherein the host cell is or comprises a T cell.~~

~~32.~~14. A method of producing an isolated EphA3 binding agent or a CAR, said method comprising; (i) culturing the host cell of Claim ~~34 or Claim 35~~13; and (ii) isolating said EphA3 binding agent or CAR from said host cell cultured in step (i).

~~33. A method comprising culturing a cell comprising a nucleic acid or a plurality of nucleic acids according to claim 31 , or an expression vector or a plurality of expression vectors according to claim 32, under conditions suitable for expression of the antigen-binding molecule or CAR from the nucleic acid(s) or expression vector(s).~~

~~34.~~15. An antibody or antibody fragment which binds and/or is raised against:

(i) the EphA3 binding agent according to any one of Claims 1 to ~~8 and 28~~3; and/or (ii) the CAR according to any one of Claims ~~15 to 27~~4 to 9.

~~35.~~16. A composition comprising the EphA3 binding agent according to any one of Claim 1 to ~~8 and 28~~3, the CAR according to any one of Claims ~~15 to 27~~4 to 9, the isolated nucleic acid according to Claim ~~29~~10, the genetic construct according to Claim ~~30~~ 11 and/or the host cell according to Claim ~~34 or Claim 35~~13 and a pharmaceutically acceptable carrier diluent or excipient.

~~36.~~ Use of ~~A composition comprising an antigen-binding molecule according to any one of claims 9 to 13, a CAR according to claim 14, a nucleic acid or a plurality of nucleic acids according to claim 31 , or an expression vector or a plurality of expression vectors according to claim 32, or a cell of claim 33.~~

~~37. The composition of claim 40, additionally comprising an agent (e.g., an immunotherapy agent, such as a checkpoint inhibitor).~~

~~38.~~17. ~~A method of treating or preventing a cancer in a subject, said method including the step of administering a therapeutically effective amount of~~ the EphA3 binding agent according to any

one of Claims 1 to 8 and 283, the CAR according to any one of Claims 15 to 27 4 to 9, the isolated nucleic acid according to Claim 2910, the genetic construct according to Claim 3011, the host cell according to Claim 34 or Claim 3513, and/or the composition of Claim 39 15, in the manufacture of a medicament for the treatment or prevention of cancerto the subject to thereby treat or prevent the cancer in the subject.

39. Use of the EphA3 binding agent of any one of Claims 1 to 8 and 28, the CAR of any one of Claims 15 to 27, the isolated nucleic acid according to Claim 29, the genetic construct according to Claim 30 and/or the host cell according to Claim 34 or Claim 35 in the manufacture of a medicament for the prevention and/or treatment of a cancer in a subject.

40. The EphA3 binding agent or the CAR of Claim 28, the method of Claim 42 or the use of Claim 43, wherein the cancer is or comprises glioblastoma multiforme.

41.18. An *ex vivo* method of detecting EphA3 or a cell expressing EphA3, said method including the step of forming a complex between the EphA3 binding agent according to any one of Claims 1 to 8 and 283, or the CAR according to any one of Claims 15 to 284 to 9 and EphA3 to thereby detect EphA3 or the cell expressing EphA3, preferably wherein the cell is or comprises a cancer cell.

42. The method of Claim 45, wherein the cell is or comprises a cancer cell.

43. An isolated protein comprising, consisting essentially of or consisting of an amino acid sequence set forth in any one of SEQ ID NOS:13 to 156 and/or Tables 2-5 or an amino acid sequence at least 70% identical thereto.

44. An isolated nucleic acid comprising, consisting or consisting essentially of a nucleic acid sequence set forth in any one of SEQ ID NOS: 1 to 12 and/or Table 1 or a nucleic acid sequence at least 70% identical thereto.

45.19. A human T-cell expressing: (a) a T-cell receptor (TCR) that is activated by binding to a CMV antigen; and (b) a chimeric antigen receptor (CAR) comprising an antigen-binding domain that binds to an epitope on EphA3, preferably wherein the antigen-binding domain is a scFv comprising a heavy chain variable (VH) region and a light chain variable (VL) region.

46. The T-cell of claim 49, wherein the antigen-binding domain is a scFv comprising a heavy chain variable (VH) region and a light chain variable (VL) region.

47.20. A T-cell that comprises (a) a T-cell receptor (TCR) that expresses a TCR that is specific for a CMV antigen; and (b) an antigen-binding molecule, for example a CAR, that binds to EphA3, preferably wherein the antigen-binding molecule is a scFv comprising a heavy chain variable (VH) region, wherein the VH region has the amino acid sequence set forth in SEQ ID NO: 153 or 155 and a light chain variable (VL) region, wherein the VL region has an amino acid sequence that is set forth in SEQ ID NO: 154 or 156.

48. The T-cell of claim 52, wherein the antigen-binding molecule is a scFv comprising a heavy chain variable (VH) region and a light chain variable (VL) region.

49. The T-cell of claim 52 or claim 53, wherein the antigen-binding molecule is a CAR.

~~50. The T-cell of any one of claims 51 , 53 or 54, wherein the VH region has the amino acid sequence set forth in SEQ ID NO: 153, and wherein the VL region has an amino acid sequence that is set forth in SEQ ID NO: 154.~~

~~51. The T-cell of any one of claims 51 , 53, or 54, wherein the VH region has the amino acid sequence set forth in SEQ ID NO: 155, and wherein the VL region has an amino acid sequence that is set forth in SEQ ID NO: 156.~~

~~52.~~21. The T-cell of any one of claim~~s 49 to 56~~ 19 or claim 20, wherein the CAR comprises a transmembrane domain selected from a CD4, CD8, or CD28 transmembrane domain and a costimulatory domain selected from a 4-1BB or CD28 co-stimulatory domain.

~~53. The T-cell of any one of claims 49 to 57, wherein the CAR comprises a costimulatory domain selected from a 4-1BB or CD28 co-stimulatory domain.~~

~~54.~~22. The T-cell of any one of claims ~~49 to 58~~19 to 21, wherein the CMV antigen comprises a peptide derived from one or more of pp50, pp65, IE-I, gB and gH, preferably wherein the CMV antigen comprises a peptide selected from the amino acid sequences set forth in SEQ ID NO: 181 -211.

~~55. The T-cell of any one of claims 49 to 58, wherein the CMV antigen comprises a peptide selected from the amino acid sequences set forth in SEQ ID NO: 181 -211.~~

~~56.~~23. A method of preparing a bi-specific T-cell population ~~for use~~that is useful in treating cancer, the method comprising:

(a) ~~obtaining~~ providing an isolated population of cells comprising PBMC ~~from a subject~~ and treating the cells to obtain a subpopulation of T-cells that express a TCR that binds to a CMV antigen;

(b) treating the subpopulation of cells to introduce a vector encoding a CAR that binds to an epitope of EphA3, thereby creating a population of bi-specific T-cells expressing a TCR that binds to a CMV antigen and a CAR that binds to an epitope on EphA3, preferably wherein the CAR comprises an antigen-binding domain that binds to an epitope on EphA3, a spacer, a transmembrane domain, for example CD4, CD8 or CD28 transmembrane, a co-stimulatory domain, for example 41BB or CD28 co-stimulatory domain, and a CD3 zeta signalling domain ; and

(c) expanding the population of bi-specific T-cells.

~~57. The method of claim 61 , wherein the CAR comprises an antigen-binding domain that binds to an epitope on EphA3, a spacer, a transmembrane domain, a co-stimulatory domain, and a CD3 zeta signalling domain.~~

~~58.~~24. The method of claim ~~62~~ 23, wherein the antigen-binding domain is a scFv comprising a heavy chain variable region and a light chain variable region, preferably wherein the heavy chain variable (VH) region has the amino acid sequence set forth in SEQ ID NO: 153 or 155, and wherein the light chain variable (VL) region has an amino acid sequence that is set forth in SEQ ID NO: 154 or 156.

~~59. The method of claim 63, wherein the heavy chain variable (VH) region has the amino acid sequence set forth in SEQ ID NO: 153, and wherein the light chain variable (VL) region has an amino acid sequence that is set forth in SEQ ID NO: 154.~~

~~60. The method of claim 63, wherein the heavy chain variable (VH) region has the amino acid sequence set forth in SEQ ID NO: 155, and wherein the light chain variable (VL) region has an amino acid sequence that is set forth in SEQ ID NO: 156.~~

~~61. The method of any one of claims 62 to 65, wherein the transmembrane domain is a CD4, CD8, or CD28 transmembrane domain.~~

~~62. The method of any one of claims 62 to 66, wherein the co-stimulatory domain is a 4-1BB or CD28 co-stimulatory domain.~~

~~63.~~25. The method of any one of claim~~s 62 to 67~~ 23 or claim 24, wherein treating the cells in step (a) comprises exposing the T-cells to a pool of immunogenic peptides comprising HLA class I and class ll-restricted CMV peptide epitopes capable of inducing proliferation of peptide-specific T-cells, preferably wherein the T-cells express a TCR specific for a CMV antigen and are expanded in the presence of a T-cell antigen before administration to the subject, wherein the CMV antigen comprises one or more CMV peptides, or a CMV vaccine, wherein at least one CMV peptide is selected from pp50, p65, IE-I, gB and gH, preferably wherein at least one CMV peptide is selected form the amino acid sequence set forth in SEQ ID NO: 181 -211 .

~~64. The method of any one of claims 62 to 68, wherein the T-cells express a TCR specific for a CMV antigen and are expanded in the presence of a T-cell antigen before administration to the subject.~~

~~65. The method of claim 68, wherein the CMV antigen comprises one or more CMV peptides, or a CMV vaccine.~~

~~66. The method of claim 70, wherein at least one CMV peptide is selected from pp50, p65, IE-I, gB and gH.~~

~~67. The method of claim 70 or claim 71, wherein at least one CMV peptide is selected form the amino acid sequence set forth in SEQ ID NO: 181 -211.~~

~~68. The method of claim 68, wherein the peptide pool comprises at least one peptide epitope derived from each of the CMV antigens pp50, pp65, IE-I, gB, and gH.~~

~~69. The method of claim 73, wherein at least one of the CMV peptide epitope amino acid sequences in the peptide pool set forth in SEQ ID NO: 181 -211, or a combination thereof.~~

~~a. The method of claim 74, wherein the peptide pool comprises each of the CMV peptide epitope amino acid sequences set forth in SEQ ID NO: 181 -211.~~