

CLAIMS

What is claimed is:

1. A method of treating cancer in a subject, the method comprising administering to the
 5 subject an immune effector cell comprising a chimeric antigen receptor (CAR),
 wherein the CAR comprises an extracellular domain comprising chlorotoxin, a
 functional variant of chlorotoxin, a chlorotoxin-like peptide, a functional variant of
 the chlorotoxin-like peptide or a combination thereof, a transmembrane domain and
 at least one intracellular signaling domain.
- 10 2. The method of claim 1, wherein the signaling domain is selected from the group
 consisting of: CD3 zeta, MB1 chain, B29, FcRIII, FcRI, and combinations of the
 foregoing.
3. The method of claim 1, wherein the transmembrane domain is selected from the group
 consisting of: T-cell receptor alpha chain, T-cell receptor beta chain, T-cell receptor
 15 zeta chain, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33,
 CD37, CD64, CD80, CD86, CD134, CD137, CD154, KIRDS2, OX40, CD2, CD27,
 LFA-1, ICOS, 4-1BB, GITR, CD40, BAFFR, HVEM, SLAMF7, NKp80, CD160,
 CD19, IL2R β , IL2R γ , IL7R α , ITGA1, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6,
 VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM,
 20 CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, TNFR2,
 DNAM1, SLAMF4, CD84, CD96, CEACAM1, CRTAM, Ly9, CD160, PSGL1,
 CD100, SLAMF6, SLAM, BLAME, SELPLG, LTBR, PAG/Cbp, NKp44, NKp30,
 NKp46, NKG2D, and NKG2C.
4. The method of claim 1, wherein the CAR further comprises a costimulatory domain.
- 25 5. The method of claim 4, wherein the costimulatory domain comprises a functional
 signaling domain of a polypeptide selected from the group consisting of: CD27,
 CD28, 4-1BB, OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated
 antigen-1, CD2, CD7, LIGHT, NKG2C, B7-H3, a ligand that specifically binds with
 CD83, CDS, ICAM-1, GITR, BAFFR, HVEM, SLAMF7, NKp80, CD160, CD19,
 30 CD4, CD8 α , CD8 β , IL2R β , IL2R γ , IL7R α , ITGA4, VLA1, CD49a, ITGA4, IA4,
 CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a,
 LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, LFA-1,
 ITGB7, TNFR2, TRANCE/RANKL, DNAM1, SLAMF4, CD84, CD96, CEACAM1,
 CRTAM, Ly9, CD160, PSGL1, CD100, CD69, SLAMF6, SLAM, BLAME,

SELPLG, LTBR, LAT, GADS, SLP-76, PAG/Cbp, NKp44, NKp30, NKp46, and NKG2D.

6. The method of claim 1, wherein the CAR further comprises an extracellular spacer.
7. The method of claim 6, wherein the extracellular spacer comprises all or a portion of
5 a hinge region of human IgD or human IgG1 and optionally all or a portion of an immunoglobulin heavy chain constant region from human IgG1.
8. The method of claim 6, wherein the CAR further comprises a costimulatory domain.
9. The method of claim 1, wherein the CAR further comprises a flexible linker and an extracellular spacer.
10. The method of claim 9, wherein the flexible linker has the sequence of SEQ ID NO: 1 or SEQ ID NO: 2, present from 1 to 10 copies.
11. The method of claim 9, wherein the extracellular spacer comprises all or a portion of a hinge region of human IgD or human IgG1 and optionally all or a portion of an immunoglobulin heavy chain constant region from human IgG1.
12. The method of claim 9, wherein the CAR further comprises a costimulatory domain.
13. The method of claim 1 wherein the immune effector cell is a T-cell.
14. The method of claim 1 wherein the immune effector cell is a natural killer cell.
15. The method of claim 1 wherein the immune effector cell is a $\gamma\delta$ T-cell.
16. The method of claim 1, wherein the immune effector cell further comprises a gene
20 encoding a survival factor.
17. The method of claim 16, wherein the survival factor is selected from the group consisting of: *O*⁶-Methylguanine-DNA methyltransferase, multidrug resistance protein 1, 5' nucleotidase II, dihydrofolate reductase and thymidylate synthase.
18. The method of claim 16, wherein the immune effector cell naturally expresses a stress-
25 induced antigen receptor.
19. The method of claim 18, wherein the stress-induced antigen receptor is an NKG2D receptor.
20. The method of claim 16, wherein the immune effector cell is administered in combination with an additional therapeutic treatment.
- 30 21. The method of claim 20, wherein the additional therapeutic treatment is a treatment with an agent selected from the group consisting of: trimethotrexate, temozolomide, raltitrexed, S-(4-Nitrobenzyl)-6-thioinosine, 6-benzylguanidine, nitrosoureas, fotemustine, cytarabine, camptothecin, and a therapeutic derivative of any of the foregoing.

22. The method of claim 20, wherein the additional therapeutic treatment is a treatment with an agent selected from the group consisting of: temozolomide, doxorubicin, melphalan, nitrosoureas and any combination thereof.
23. The method of claim 1, wherein the immune effector cell comprises a gene encoding a stress-induced antigen receptor.
24. The method of claim of claim 23, wherein the stress-induced antigen receptor is an NKG2D receptor.
25. The method of claim 1, wherein the immune effector cell further comprises a gene encoding a survival factor and a gene encoding a stress-induced antigen receptor.
26. The method of claim 25, wherein the survival factor is selected from the group consisting of: *O*⁶-Methylguanine-DNA methyltransferase, multidrug resistance protein 1, 5' nucleotidase II, dihydrofolate reductase and thymidylate synthase and the stress-induced antigen receptor is an NKG2D receptor.
27. The method of claim 25, wherein the immune effector cell is administered in combination with an additional therapeutic treatment.
28. The method of claim 27, wherein the additional therapeutic treatment is a treatment with an agent selected from the group consisting of: trimethotrexate, temozolomide, raltitrexed, S-(4-Nitrobenzyl)-6-thioinosine, 6-benzylguanidine, nitrosoureas, fotemustine, cytarabine, camptothecin, and a therapeutic derivative of any of the foregoing.
29. The method of claim 27, wherein the additional therapeutic treatment is a treatment with an agent selected from the group consisting of: temozolomide, doxorubicin, melphalan, nitrosoureas and any combination thereof.
30. The method of claim 1, wherein the immune effector cell comprising the CAR is a component of a composition comprising an additional immune system cell.
31. The method of claim 30, wherein the immune effector cell comprising the CAR is present at greater than or equal to 60% of the total cell population in the composition.
32. The method of claim 30, wherein the immune effector cell comprising the CAR is a $\gamma\delta$ T cell and the additional immune system cell is a natural killer cell, an $\alpha\beta$ T cell or a combination of a natural killer cell and an $\alpha\beta$ T cell.
33. The method of claim 32, wherein the $\gamma\delta$ T cell is present at greater than or equal to 60% of the total cell population in the composition.

34. The method of claim 32, wherein the $\alpha\beta$ T cells are present at less than or equal to 5% of the total cell population and the natural killer cells are present at less than or equal to 25% of the total cell population in the composition.
35. The method of claim 1, wherein the cancer is of neuroectodermal origin.
- 5 36. The method of claim 1, wherein the cancer is malignant glioma, melanoma, neuroblastoma, medulloblastoma or small cell lung carcinoma.
37. A $\gamma\delta$ T cell comprising at least one vector, the at least one vector directing the expression of a chimeric antigen receptor (CAR) and a survival factor, wherein the CAR comprises an extracellular domain comprising chlorotoxin, a functional variant of chlorotoxin, a chlorotoxin-like peptide, functional variant of the chlorotoxin-like peptide or a combination thereof, a transmembrane domain and at least one intracellular signaling domain.
- 10 38. The $\gamma\delta$ T cell of claim 37, wherein the signaling domain is selected from the group consisting of: CD3 zeta, MB1 chain, B29, FcRIII, FcRI, and combinations of the foregoing.
- 15 39. The $\gamma\delta$ T cell of claim 37, wherein the transmembrane domain is selected from the group consisting of: T-cell receptor alpha chain, T-cell receptor beta chain, T-cell receptor zeta chain, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, CD154, KIRDS2, OX40, CD2, CD27, LFA-1, ICOS, 4-1BB, GITR, CD40, BAFFR, HVEM, SLAMF7, NKp80, CD160, CD19, IL2R β , IL2R γ , IL7R α , ITGA1, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, TNFR2, DNAM1, SLAMF4, CD84, CD96, CEACAM1, CRTAM, Ly9, CD160, PSGL1, CD100, SLAMF6, SLAM, BLAME, SELPLG, LTBR, PAG/Cbp, NKp44, NKp30, NKp46, NKG2D, and NKG2C.
- 20 40. The $\gamma\delta$ T cell of claim 37, wherein the CAR further comprises a costimulatory domain.
41. The $\gamma\delta$ T cell of claim 40, wherein the costimulatory domain comprises a functional signaling domain of a polypeptide selected from the group consisting of: CD27, CD28, 4-1BB (CD137), OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, a ligand that specifically binds with CD83, CDS, ICAM-1, GITR, BAFFR, HVEM (LIGHTR), SLAMF7, NKp80 (KLRF1), CD160, CD19, CD4, CD8 α , CD8 β , IL2R β , IL2R γ ,
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- IL7R α , ITGA4, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRTAM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, NKp44, NKp30, NKp46, and NKG2D.
42. The $\gamma\delta$ T cell of claim 37, wherein the CAR further comprises an extracellular spacer.
43. The $\gamma\delta$ T cell of claim 42, wherein the extracellular spacer comprises all or a portion of a hinge region of human IgD or human IgG1 and optionally all or a portion of an immunoglobulin heavy chain constant region from human IgG1.
44. The $\gamma\delta$ T cell of claim 42, wherein the CAR further comprises a costimulatory domain.
45. The $\gamma\delta$ T cell of claim 37, wherein the CAR further comprises a flexible linker and an extracellular spacer.
46. The $\gamma\delta$ T cell of claim 45, wherein the flexible linker has the sequence of SEQ ID NO: 1 or SEQ ID NO: 2, present from 1 to 10 copies.
47. The $\gamma\delta$ T cell of claim 45, wherein the extracellular spacer comprises all or a portion of a hinge region of human IgD or human IgG1 and optionally all or a portion of an immunoglobulin heavy chain constant region from human IgG1.
48. The $\gamma\delta$ T cell of claim 45, wherein the CAR further comprises a costimulatory domain.
49. The $\gamma\delta$ T cell of claim 37, wherein the survival factor is selected from the group consisting of: *O*⁶-Methylguanine-DNA methyltransferase, multidrug resistance protein 1, 5' nucleotidase II, dihydrofolate reductase and thymidylate synthase.
50. The $\gamma\delta$ T cell of claim 37, wherein the $\gamma\delta$ T cell naturally expresses a stress-induced antigen receptor.
51. The $\gamma\delta$ T cell of claim of claim 50, wherein the stress-induced antigen receptor is an NKG2D receptor.
52. The $\gamma\delta$ T cell of claim 37, wherein the survival factor allows the $\gamma\delta$ T cell to survive in a treatment environment created by an additional therapeutic treatment.
53. The $\gamma\delta$ T cell of claim 52, wherein the additional therapeutic treatment is a treatment with an agent selected from the group consisting of: trimethotrexate, temozolomide, raltitrexed, S-(4-Nitrobenzyl)-6-thioinosine, 6-benzylguanidine, nitrosoureas, fotemustine, cytarabine, camptothecin, and any combination of the foregoing.

54. The $\gamma\delta$ T cell of claim 52, wherein the additional therapeutic treatment is a treatment with an agent selected from the group consisting of: temozolomide, doxorubicin, melphalan, nitrosoureas and any combination thereof.
55. The $\gamma\delta$ T cell of claim 37, wherein the at least one vector further directs the expression of a gene encoding a stress-induced antigen receptor.
56. The $\gamma\delta$ T cell of claim 56, wherein the stress-induced antigen receptor is an NKG2D receptor.
57. The $\gamma\delta$ T cell of claim 37, wherein a single vector directs the expression of the CAR and the survival factor.
58. The $\gamma\delta$ T cell of claim 55, wherein a single vector directs the expression of the CAR, the survival factor and the stress-induced antigen receptor.
59. The $\gamma\delta$ T cell of claim 58, wherein the survival factor is selected from the group consisting of: *O*⁶-Methylguanine-DNA methyltransferase, multidrug resistance protein 1, 5' nucleotidase II, dihydrofolate reductase and thymidylate synthase and the stress-induced antigen receptor is an NKG2D receptor.
60. The $\gamma\delta$ T cell of claim 37, wherein the $\gamma\delta$ T cell is isolated.
61. A pharmaceutical composition comprising a $\gamma\delta$ T cell of any one of claims 37 to 60 and a pharmaceutically acceptable carrier.
62. The pharmaceutical composition of claim 61 further comprising an additional immune system cell.
63. The pharmaceutical composition of claim 62, wherein the $\gamma\delta$ T cell is present at greater than or equal to 60% of the total cell population of the composition.
64. The pharmaceutical composition of claim 62, wherein the additional immune system cell is a natural killer cell, an $\alpha\beta$ T cell or a combination of a natural killer cell and an $\alpha\beta$ T cell.
65. The pharmaceutical composition of claim 64, wherein the $\gamma\delta$ T cell is present at greater than or equal to 60% of the total cell population of the composition.
66. The pharmaceutical composition of claim 64, wherein the $\alpha\beta$ T cell is present at less than or equal to 5% of the total cell population of the composition and the natural killer cell is present at less than or equal to 25% of the total cell population of the composition.