

CLAIMS

1. A method for stimulating a universal immune receptor-mediated immune response to a tumor in a mammal, wherein the tumor co-expresses at least two different antigens,
5 the method comprising
 - (a) administering to the mammal a cell genetically modified to express an immune receptor comprising a T cell receptor intracellular signaling domain, a transmembrane domain, and an extracellular domain comprising an adaptor molecule,
 - 10 (b) administering to the mammal a first agent linked to a reciprocal adaptor molecule, wherein the first agent specifically binds a first antigen expressed by the tumor, and
 - (c) administering to the mammal a second agent linked to a reciprocal adaptor molecule, wherein the second agent specifically binds a second antigen
15 expressed by the tumor and wherein the first antigen and the second antigen are different antigens.
2. A method of treating a cancer in a mammal in need thereof, wherein the cancer co-expresses at least two different antigens, the method comprising
 - 20 (a) administering to the mammal an effective amount of a cell genetically modified to express an immune receptor comprising a T cell receptor intracellular signaling domain, a transmembrane domain, and an extracellular domain comprising an adaptor molecule,
 - (b) administering to the mammal an effective amount of a first agent linked to a reciprocal adaptor molecule, wherein the first agent specifically binds a first antigen expressed by the cancer, and
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 - (c) administering to the mammal an effective amount of a second agent linked to a reciprocal adaptor molecule, wherein the second agent specifically binds a second antigen expressed by the cancer and wherein the first antigen and the second antigen are different antigens.
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3. The method of claim 1 or 2, wherein the extracellular domain comprises a SpyCatcher or SpyTag.

4. The method of any one of claims 1-3, wherein the first and/or second agent is linked to a SpyTag or SpyCatcher.
5. The method of any one of claims 1-4, wherein the first and/or second agent is an antibody, an antibody fragment, a scFv, or a DARPin.
6. The method of any one of claims 1-5, wherein the first and/or second agent is an antibody and is human IgG.
- 10 7. The method of any one of claims 1-6, wherein the reciprocal adaptor molecule, SpyTag, or SpyCatcher is linked to the first and/or second agent via light activated site-specific conjugation (LASIC).
- 15 8. The method of any one of claims 1-7, wherein the cell is a T cell, a Natural Killer (NK) cell, a cytotoxic T lymphocyte (CTL), a macrophage, a stem cell, or a regulatory T cell.
9. The method of any one of claims 1-8, wherein the cell is an autologous cell.
- 20 10. The method of any one of claims 1-9, wherein the immune receptor further comprises an intracellular domain of a costimulatory molecule.
11. The method of claim 10, wherein the costimulatory molecule is 4-1BB, CD28, CD27, CD2, CD3, OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated
25 antigen-1 (LFA-1), CD7, LIGHT, NKG2C, B7-H3, or a ligand that specifically binds with CD83.
12. The method of any one of claims 1-11, wherein the cell is contacted with the first and/or second agent prior to administration to the mammal to generate a pre-armed
30 cell and the pre-armed cell is subsequently administered to the mammal.
13. The method of any one of claims 1-11, wherein the cell is administered to the mammal prior to administering the first and/or second agent to the mammal.

14. A method of generating a level of lytic activity against a tumor, the method comprising
- 5 (a) contacting an amount of cells with an amount of an agent linked to a reciprocal adaptor molecule, wherein the cells are genetically modified to express an immune receptor comprising a T cell receptor intracellular signaling domain, a transmembrane domain, and an extracellular domain wherein the extracellular domain comprises an adaptor molecule, wherein the agent specifically binds an antigen expressed by the tumor, and wherein the amount of the agent and/or the amount of cells is selected to
- 10 generate the level of lytic activity against the tumor.
15. A method of treating a cancer in a mammal in need thereof, the method comprising
- 15 (a) administering to the mammal an amount of cells genetically modified to express an immune receptor comprising a T cell receptor intracellular signaling domain, a transmembrane domain, and an extracellular domain comprising an adaptor molecule, and
- 20 (b) administering to the mammal an amount of an agent linked to a reciprocal adaptor molecule, wherein the agent specifically binds an antigen expressed by the cancer, wherein the amount of the cells and/or the amount of the agent is selected to provide a level of lytic activity against the cancer.
16. The method of any one of claims 14-15, wherein increasing the amount of agent relative to the amount of the cells increases the level of lytic activity and decreasing the amount of agent relative to the amount of the cells decreases the level of lytic
- 25 activity.
17. The method of any one of claims 14 -16, wherein the extracellular domain comprises a SpyCatcher or SpyTag.
- 30 18. The method of any one of claims 14-17, wherein the agent is linked to a SpyTag or SpyCatcher.
19. The method of any one of claims 14-18, wherein the agent is an antibody, an antibody fragment, a scFv, or a DARPin.

20. The method of any one of claims 14-19, wherein the agent is an antibody and is human IgG.
- 5 21. The method of any one of claims 14-20, wherein the reciprocal adaptor molecule, SpyTag or SpyCatcher is linked to the agent via light activated site-specific conjugation (LASIC).
- 10 22. The method of any one of claims 14-21, wherein the cell is a T cell, a Natural Killer (NK) cell, a cytotoxic T lymphocyte (CTL), a macrophage, a stem cell, or a regulatory T cell.
23. The method of any one of claims 14-22, wherein the cell is an autologous cell.
- 15 24. The method of any one of claims 14-23, wherein the immune receptor further comprises an intracellular domain of a costimulatory molecule.
25. The method of any one of claims 14-24, wherein the costimulatory molecule is 4-1BB, CD28, CD27, CD2, CD3, OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD7, LIGHT, NKG2C, B7-H3, or a ligand that specifically binds with CD83.
- 20 26. The method of any one of claims 15-25, wherein the cell is contacted with the agent prior to administration to the mammal to generate a pre-armed cell and the pre-armed cell is subsequently administered to the mammal.
- 25 27. The method of any one of claims 15-26, wherein the cell is administered to the mammal prior to administering the agent to the mammal.
- 30 28. A method of stimulating a universal immune receptor-mediated immune response to a tumor in a mammal in need thereof, the method comprising
- (a) administering to the mammal a cell genetically modified to express an immune receptor comprising a T cell receptor intracellular signaling domain, a

- transmembrane domain, and an extracellular domain comprising an adaptor molecule, and
- (b) subsequently administering to the mammal an agent linked to a reciprocal adaptor molecule, wherein the agent specifically binds an antigen expressed by the tumor.
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29. A method of treating a cancer in a mammal in need thereof, the method comprising
- (a) administering to the mammal an effective amount of a T cell genetically modified to express an immune receptor comprising a T cell receptor intracellular signaling domain, a transmembrane domain, and an extracellular domain comprising an adaptor molecule, and
- 10
- (b) subsequently administering to the mammal an effective amount of an agent linked to a reciprocal adaptor molecule, wherein the agent specifically binds an antigen expressed by the cancer.
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30. The method of claim 28 or 29, wherein the extracellular domain comprises a SpyCatcher or SpyTag.
31. The method of any one of claims 28-30, wherein the agent is linked to a SpyTag or SpyCatcher.
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32. The method of any one of claims 28-31, wherein the agent is an antibody, an antibody fragment, a scFv, or a DARPin.
33. The method of any one of claims 28-32, wherein the agent is an antibody and is human IgG.
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34. The method of any one of claims 28-33, wherein the reciprocal adaptor molecule, SpyTag or SpyCatcher is linked to the agent via light activated site-specific conjugation (LASIC).
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35. The method of any one of claims 28-34, wherein the cell is a T cell, a Natural Killer (NK) cell, a cytotoxic T lymphocyte (CTL), a macrophage, a stem cell, or a regulatory T cell.

36. The method of any one of claims 28-35, wherein the cell is an autologous cell.
37. The method of any one of claims 28-36, wherein the immune receptor further
5 comprises an intracellular domain of a costimulatory molecule.
38. The method of any one of claims 28-37, wherein the costimulatory molecule is 4-
1BB, CD28, CD27, CD2, CD3, OX40, CD30, CD40, PD-1, ICOS, lymphocyte
10 function-associated antigen-1 (LFA-1), CD7, LIGHT, NKG2C, B7-H3, or a ligand
that specifically binds with CD83.
39. A method of quantifying turnover of a universal immune receptor on a cell surface,
the method comprising
(a) contacting a cell genetically modified to express an immune receptor
15 comprising a T cell receptor intracellular signaling domain, a transmembrane
domain, and an extracellular domain comprising an adapter molecule with an
agent linked to a reciprocal adapter molecule, thereby generating an armed
receptor; and
(b) determining an amount of the armed receptor relative to a reference amount.
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40. The method of claim 39, wherein the reference amount is an amount of the armed
receptor at a prior time.
41. The method of claim 40, wherein the amount of the armed receptor is determined by
25 labeling the agent and detecting the labeled agent.
42. The method of claim 41, wherein the agent is labeled by linking or contacting the
agent with a labeling molecule comprising a myc-tag, FLAG-tag, His-tag, HA-tag, a
fluorescent protein (e.g., a green fluorescent protein (GFP)), a fluorophore (e.g.,
30 tetramethylrhodamine (TRITC)), fluorescein isothiocyanate (FITC), dinitrophenol,
peridin chlorophyll protein complex, phycoerythrin (PE), histidine, biotin,
streptavidin, avidin, horse radish peroxidase, palmitoylation, nitrosylation, alkaline
phosphatase, glucose oxidase, Glutathione S –transferase (GST), maltose binding
protein, a radioisotope, a heavy metal, a supramagnetic nanoparticle, and any types of

compounds used for radioisotope labeling including, 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA), diethylene triamine pentaacetic acid (DTPA), 1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA), or allophycocyanin (APC).

- 5 43. The method of any one of claims 39-42, further comprising the step of (c) contacting the cell and the agent with a tumor cell.
44. The method of any one of claims 39-43, wherein the extracellular domain comprises a SpyCatcher.
- 10 45. The method of any one of claims 39-44, wherein the agent is linked to a SpyTag.
46. The method of any one of claims 39-45, wherein the agent is an antibody, an antibody fragment, a scFv, or a DARPin.
- 15 47. The method of any one of claims 39-46, wherein the agent is an antibody and is human IgG.
48. The method of any one of claims 39-47, wherein the cell is a T cell, a Natural Killer (NK) cell, a cytotoxic T lymphocyte (CTL), a macrophage, a stem cell, or a regulatory T cell.
- 20 49. The method of any one of claims 39-48, wherein the immune receptor further comprises an intracellular domain of a costimulatory molecule.
- 25 50. The method of any one of claims 39-49, wherein the costimulatory molecule is 4-1BB, CD28, CD27, CD2, CD3, OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD7, LIGHT, NKG2C, B7-H3, or a ligand that specifically binds with CD83.
- 30 51. A method of stimulating a universal immune receptor-mediated immune response to a tumor in a mammal in need thereof, the method comprising
- (a) administering to the mammal an effective amount of a cell genetically modified to express an immune receptor comprising a T cell receptor

- intracellular signaling domain, an intracellular domain of 4-1BB, a transmembrane domain, and an extracellular domain comprising an adaptor molecule, and
- 5 (b) administering to the mammal an effective amount of an agent linked to a reciprocal adaptor molecule, wherein the agent specifically binds an antigen expressed by the tumor, wherein the tumor is pre-determined to express the antigen at an increased level relative to a reference level.
- 10 52. A method of treating a cancer in a mammal in need thereof, the method comprising
- (a) administering to the mammal an amount of a cell genetically modified to express an immune receptor comprising a T cell receptor intracellular signaling domain, an intracellular domain of 4-1BB, a transmembrane domain, and an extracellular domain comprising an adaptor molecule, and
- 15 (b) administering to the mammal an amount of an agent linked to a reciprocal adaptor molecule, wherein the agent specifically binds an antigen expressed by the cancer, wherein the cancer is pre-determined to express the antigen at an increased level relative to a reference level.
- 20
53. The method of claim 51 or 52, wherein the reference level is a level of expression of the antigen on healthy tissue.
54. The method of any one of claims 51-53, wherein the extracellular domain comprises a SpyCatcher or SpyTag.
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55. The method of any one of claims 51-54, wherein the agent is linked to a SpyTag or SpyCatcher.
- 30 56. The method of any one of claims 51-55, wherein the agent is an antibody, an antibody fragment, a scFv, or a DARPin.
57. The method of any one of claims 51-56, wherein the agent is an antibody and is human IgG.

58. The method of any one of claims 51-57, wherein the SpyTag or SpyCatcher is linked to the agent via light activated site-specific conjugation (LASIC).
- 5 59. The method of any one of claims 51-58, wherein the cell is a T cell, a Natural Killer (NK) cell, a cytotoxic T lymphocyte (CTL), a macrophage, a stem cell, or a regulatory T cell.
60. The method of any one of claims 51-59, wherein the cell is an autologous cell.
- 10 61. The method of any one of claims 51-60, wherein the cell is contacted with agent prior to administration to the mammal to generate a pre-armed cell and the pre-armed cell is subsequently administered to the mammal.
- 15 62. The method of any one of claims 51-61, wherein the cell is administered to the mammal prior to administering the agent to the mammal.
63. A genetically modified cell, a first agent, and a second agent, for use in stimulating a universal immune receptor-mediated immune response to a tumor in a mammal in
20 need thereof, wherein the tumor co-expresses at least two different antigens,
wherein the cell is genetically modified to express an immune receptor comprising a T cell receptor intracellular signaling domain, a transmembrane domain, and an extracellular domain comprising an adaptor molecule,
wherein the first agent is linked to a reciprocal adaptor molecule, and wherein
25 the first agent specifically binds a first antigen expressed by the tumor;
wherein the second agent is linked to a reciprocal adaptor molecule, and
wherein the second agent specifically binds a second antigen expressed by the tumor;
wherein the first antigen and the second antigen are different antigens, and
30 wherein the immune receptor, first agent, and second agent are administered to the mammal.
64. A genetically modified cell, a first agent, and a second agent, for use in treating cancer in a mammal in need thereof, wherein the cancer co-expresses at least two different antigens,

wherein the cell is genetically modified to express an immune receptor comprising a T cell receptor intracellular signaling domain, a transmembrane domain, and an extracellular domain comprising an adaptor molecule,

5 wherein the first agent is linked to a reciprocal adaptor molecule, and wherein the first agent specifically binds a first antigen expressed by the cancer;

wherein the second agent is linked to a reciprocal adaptor molecule, and wherein the second agent specifically binds a second antigen expressed by the cancer; and wherein the first antigen and the second antigen are different antigens.

10 65. A genetically modified cell and an agent for use in generating a level of lytic activity against a tumor, wherein the cell is genetically modified to express an immune receptor comprising a T cell receptor intracellular signaling domain, a transmembrane domain, and an extracellular domain comprising an adaptor molecule, wherein the agent is linked to a reciprocal adaptor molecule, and wherein the agent specifically
15 binds a an antigen expressed by the tumor; wherein an amount of the cell is contacted with an amount of the agent and wherein the amount of the agent relative to the amount of cells is selected to generate the level of lytic activity against the tumor.

20 66. A genetically modified cell and an agent for use in treating cancer in a mammal in need thereof, wherein the cell is genetically modified to express an immune receptor comprising a T cell receptor intracellular signaling domain, a transmembrane domain, and an extracellular domain comprising an adaptor molecule, wherein the agent is linked to a reciprocal adaptor molecule, and wherein the agent specifically binds an antigen expressed by the cancer; wherein an amount of the cell and an amount of the
25 agent is administered to the mammal, wherein the amount of the cell and/or the amount of the agent is selected to provide a level of lytic activity against the cancer.

30 67. A genetically modified cell and an agent for use in stimulating a universal immune receptor-mediated immune response to a tumor in a mammal in need thereof, wherein the cell is genetically modified to express an immune receptor comprising a T cell receptor intracellular signaling domain, a transmembrane domain, and an extracellular domain comprising an adaptor molecule, wherein the agent is linked to a reciprocal adaptor molecule, and wherein the agent specifically binds a an antigen expressed by

the tumor; wherein an amount of the cell is administered to the mammal and an amount of the agent is subsequently administered to the mammal.

68. A genetically modified cell and an agent for use in treating cancer in a mammal in
5 need thereof, wherein the cell is genetically modified to express an immune receptor
comprising a T cell receptor intracellular signaling domain, a transmembrane domain,
and an extracellular domain comprising an adaptor molecule, wherein the agent is
linked to a reciprocal adaptor molecule, and wherein the agent specifically binds an
10 antigen expressed by the cancer; wherein an amount of the cell is administered to the
mammal and an amount of the agent is subsequently administered to the mammal.
69. A genetically modified cell and an agent for use in stimulating a universal immune
receptor-mediated immune response to a tumor in a mammal in need thereof,
15 wherein the cell is genetically modified to express an immune receptor
comprising a T cell receptor intracellular signaling domain, an intracellular domain of
4-1BB, a transmembrane domain, and an extracellular domain comprising an adaptor
molecule,
wherein the agent is linked to a reciprocal adaptor molecule,
wherein the agent specifically binds a an antigen expressed by the tumor;
20 wherein an effective amount of the cell is administered to the mammal and an
effective amount of the agent is administered to the mammal, and
wherein the tumor is pre-determined to express the antigen at an increased
level relative to a reference level.
70. A genetically modified cell and an agent for use in treating a cancer in a mammal in
25 need thereof, wherein the cell is genetically modified to express an immune receptor
comprising a T cell receptor intracellular signaling domain, an intracellular domain of
4-1BB, a transmembrane domain, and an extracellular domain comprising an adaptor
molecule, wherein the agent is linked to a reciprocal adaptor molecule, and wherein
30 the agent specifically binds a an antigen expressed by the tumor; wherein an effective
amount of the cell is administered to the mammal and an effective amount of the
agent is administered to the mammal, and wherein the tumor is pre-determined to
express the antigen at an increased level relative to a reference level.

71. Use of a genetically modified cell, a first agent, and a second agent, in stimulating a universal immune receptor-mediated immune response to a tumor in a mammal in need thereof, wherein the tumor co-expresses at least two different antigens,
wherein the cell is genetically modified to express an immune receptor comprising a T cell receptor intracellular signaling domain, a transmembrane domain, and an extracellular domain comprising an adaptor molecule,
wherein the first agent is linked to a reciprocal adaptor molecule, and wherein the first agent specifically binds a first antigen expressed by the tumor;
wherein the second agent is linked to a reciprocal adaptor molecule, and wherein the second agent specifically binds a second antigen expressed by the tumor;
wherein the first antigen and the second antigen are different antigens, and wherein the immune receptor, first agent, and second agent are administered to the mammal.
72. Use of a genetically modified cell, a first agent, and a second agent, in the treatment of cancer in a mammal in need thereof, wherein the cancer co-expresses at least two different antigens,
wherein the cell is genetically modified to express an immune receptor comprising a T cell receptor intracellular signaling domain, a transmembrane domain, and an extracellular domain comprising an adaptor molecule,
wherein the first agent is linked to a reciprocal adaptor molecule, and wherein the first agent specifically binds a first antigen expressed by the cancer;
wherein the second agent is linked to a reciprocal adaptor molecule, and wherein the second agent specifically binds a second antigen expressed by the cancer;
and wherein the first antigen and the second antigen are different antigens.
73. Use of a genetically modified cell and an agent in generating a level of lytic activity against a tumor, wherein the cell is genetically modified to express an immune receptor comprising a T cell receptor intracellular signaling domain, a transmembrane domain, and an extracellular domain comprising an adaptor molecule, wherein the agent is linked to a reciprocal adaptor molecule, and wherein the agent specifically binds a an antigen expressed by the tumor; wherein an amount of the cell is contacted with an amount of the agent and wherein the amount of the agent relative to the amount of cells is selected to generate the level of lytic activity against the tumor.

74. Use of a genetically modified cell and an agent in the treatment of cancer in a mammal in need thereof, wherein the cell is genetically modified to express an immune receptor comprising a T cell receptor intracellular signaling domain, a transmembrane domain, and an extracellular domain comprising an adaptor molecule, wherein the agent is linked to a reciprocal adaptor molecule, and wherein the agent specifically binds an antigen expressed by the cancer; wherein an amount of the cell and an amount of the agent is administered to the mammal, wherein the amount of the cell and/or the amount of the agent is selected to provide a level of lytic activity against the cancer.
75. Use of a genetically modified cell and an agent in stimulating universal immune receptor-mediated immune response to a tumor in a mammal in need thereof, wherein the cell is genetically modified to express an immune receptor comprising a T cell receptor intracellular signaling domain, a transmembrane domain, and an extracellular domain comprising an adaptor molecule, wherein the agent is linked to a reciprocal adaptor molecule, and wherein the agent specifically binds a an antigen expressed by the tumor; wherein an amount of the cell is administered to the mammal and an amount of the agent is subsequently administered to the mammal.
76. Use of a genetically modified cell and an in the treatment of cancer in a mammal in need thereof, wherein the cell is genetically modified to express an immune receptor comprising a T cell receptor intracellular signaling domain, a transmembrane domain, and an extracellular domain comprising an adaptor molecule, wherein the agent is linked to a reciprocal adaptor molecule, and wherein the agent specifically binds an antigen expressed by the cancer; wherein an amount of the cell is administered to the mammal and an amount of the agent is subsequently administered to the mammal.
77. Use of a genetically modified cell and an agent in stimulating a universal immune receptor-mediated immune response to a tumor in a mammal in need thereof, wherein the cell is genetically modified to express an immune receptor comprising a T cell receptor intracellular signaling domain, an intracellular domain of 4-1BB, a transmembrane domain, and an extracellular domain comprising an adaptor molecule, wherein the agent is linked to a reciprocal adaptor molecule, and wherein the agent

specifically binds a an antigen expressed by the tumor; wherein an effective amount of the cell is administered to the mammal and an effective amount of the agent is administered to the mammal, and wherein the tumor is pre-determined to express the antigen at an increased level relative to a reference level.

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78. Use of a genetically modified cell and an agent in the treatment of a cancer in a mammal in need thereof, wherein the cell is genetically modified to express an immune receptor comprising a T cell receptor intracellular signaling domain, an intracellular domain of 4-1BB, a transmembrane domain, and an extracellular domain comprising an adaptor molecule, wherein the agent is linked to a reciprocal adaptor molecule, and wherein the agent specifically binds a an antigen expressed by the tumor; wherein an effective amount of the cell is administered to the mammal and an effective amount of the agent is administered to the mammal, and wherein the tumor is pre-determined to express the antigen at an increased level relative to a reference level.

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