

CLAIMS

1. An *in vitro* method of producing an activated T cell or an activated T cell preparation, comprising:

(a) forming an *ex vivo* reaction mixture by mixing a sample comprising at least one T cell, a sample comprising at least one cancer cell, and a bispecific or multispecific antibody, and incubating the mixture under conditions and for a period of time sufficient to activate the T cell, thereby producing at least one activated T cell; and

(b) selecting the activated T cell from (a), wherein the selection of the activated T cell is based on its increased cancer cell killing activity determined by measuring an Effective E:T ratio, wherein said Effective E:T ratio is a ratio between effector cells (E) to target cells (T), effector cells (E) being defined as activated T cells and target cells (T) being defined as cancer cells killed by said activated T cells.

2. The method of claim 1, wherein in step (a) the *ex vivo* reaction mixture is incubated under conditions and for a period of time sufficient to allow the at least one T cell to acquire a cell surface marker from the at least one cancer cell.

3. The method of claim 1 or 2, further comprising one, two or all of the following *in vitro* steps:

- (i) expanding the activated T cells from (b);
- (ii) enriching for the activated T cell from (b); or
- (iii) purifying the activated T cell from (b).

4. The method of any of claims 1-3, wherein the bispecific or multispecific antibody is selected from: a bispecific immunoglobulin (BsIgG), an immunoglobulin operatively linked to additional antigen-binding molecule, a bispecific antibody (BsAb) fragment comprising at least two scFv fragments operative linked by a linker selected from a dual-affinity retargeting (DART) antibody fragment or a Bispecific T cell Engager (BiTE), a bispecific fusion protein, or a BsAb conjugate.

5. The method of any of claims 1-4, wherein the bispecific or multispecific antibody is selected from the list consisting of BsMAb CD123/CD3, BsMAb CD19/CD3 and EpCAM/CD3.

6. The method of any of claims 3-5, wherein the selecting step (b) and/or enriching step ii) comprises using a fluorescently labeled compound that binds to i) at least one cancer antigen, or diffuses into the cancer cell membrane or ii) at least one marker of activated T cells, or both i) and ii); or using a bead coated with an antibody or fragment thereof that binds to i) at least one cancer antigen or ii) at least one marker of activated T cells, or both i) and ii).

7. The method of any of claims 1-6, wherein the activated T cell or preparation comprises at least one CD8⁺ T cell and/or at least one CD25⁺ T cell, and/or at least one CD8⁺/CD25⁺ T

cell and/or at least one CD4+/CD25+ T cell, and or at least one cytotoxic T lymphocyte (CTL) or at least one tumor infiltrating lymphocyte (TIL) and/or at least one trogocytotic T cell.

8. The method of any of claims 1-7, further comprising separating individual clones from the activated T cell preparation, wherein the separating step comprises clonal expansion of single cells by:

- (i) separating the preparation of activated T cells into single cells and
- (ii) expanding the single cells to generate at least one preparation of activated T cells.

9. The method of any of claims 1-8, wherein step (a) comprises mixing a sample comprising both the cancer cell and the T cell, and a bispecific or multispecific antibody.

10. The method of any of claims 1-9, wherein the sample comprising the at least one T cell is derived from a tissue with a microenvironment, wherein substantially no components have been removed or isolated from the sample, selected from: whole blood, peripheral blood, bone marrow, lymph node, a biopsy of a primary tumor, or a biopsy of a metastasis or spleen.

11. The method of any of claims 1-10, wherein the cancer of the sample comprising the at least one cancer cell is a hematological cancer selected from: Hodgkin's lymphoma, Non-Hodgkin's lymphoma (B cell lymphoma, diffuse large B cell lymphoma, follicular lymphoma, chronic lymphocytic leukemia, mantle cell lymphoma, marginal zone B-cell lymphoma, Burkitt lymphoma, lymphoplasmacytic lymphoma, hairy cell leukemia), acute myeloid leukemia, chronic myeloid leukemia, myelodysplastic syndrome, multiple myeloma, or acute lymphocytic leukemia.

12. The method of any of claims 1-11, wherein the cancer is a solid cancer selected from: ovarian cancer, rectal cancer, stomach cancer, testicular cancer, cancer of the anal region, uterine cancer, colon cancer, renal-cell carcinoma, liver cancer, non-small cell carcinoma of the lung, cancer of the small intestine, cancer of the esophagus, melanoma, Kaposi's sarcoma, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, cutaneous or intraocular malignant melanoma, brain stem glioma, pituitary adenoma, epidermoid cancer, carcinoma of the cervix squamous cell cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the vagina, sarcoma of soft tissue, cancer of the urethra, carcinoma of the vulva, cancer of the penis, cancer of the bladder, cancer of the kidney or ureter, carcinoma of the renal pelvis, spinal axis tumor, neoplasm of the central nervous system (CNS), primary CNS lymphoma, tumor angiogenesis, metastatic lesions of said cancers, or combinations thereof.

13. The method of any of claims 1-12, wherein the subject providing sample comprising the at least one T cell and/or sample comprising the at least one cancer cell:

- (i) has not received a prior treatment for the cancer;
- (ii) has received at least one previous treatment for the cancer; or
- 5 (iii) has minimal residual disease (MRD).

14. The method of any of claims 1-13, further comprising evaluating the cancer-killing activity of the activated T cell or activated T cell preparation.

15. The method of claim 14, wherein evaluating comprises:

- 10 (a) contacting an activated T cell or preparation thereof obtainable according to the method of claims 1-14 with a sample comprising at least one cancer cell, wherein the cancer cell is from the same subject as the activated T cell or preparation thereof, for a period of time sufficient to allow the activated T cell to kill the at least one cancer cell;
- 15 (b) determining the level of cancer cells after step (a), and optionally determining the level of activated T cells after step (a); and optionally,
- (c) determining the ratio of either cancer cell to T cell, or T cell to cancer cell, from step (b).

16. The method of claim 15, wherein a decrease in the level or amount of cancer cells, relative to a reference level, is indicative of increased cancer cell killing, or wherein a reduced change or no substantial change in the level or amount of cancer cells relative to a reference level, is
20 indicative of decreased cancer cell killing.

17. The method of claim 15 or 16, wherein an Effective E:T ratio of 1:10 or lower indicates that the activated T cell or preparation thereof is an effective killer of cancer cells, and wherein a ratio of 1:1, 1:3, or 1:5 is indicative of a poor T cell killing activity.

25 18. The method of any of claims 1-17, wherein Effective E:T ratio is calculated from mathematical fitting of the experimental values of a dose response curve of multiple doses of the bispecific or multispecific antibody.

19. The method of any of claims 1-18, wherein in step (a) immunomodulatory agents are added to the mixture, wherein said immunomodulatory agents are selected from the group consisting
30 of immune checkpoint inhibitors, agonists of T cells and immunomodulatory drugs.

20. The method of claim 19, wherein the immune checkpoint inhibitors inhibit immune checkpoints selected from the group consisting of CTLA4, PD-1, PD-L1, PD-L2, TIM3, LAG3,

CD160, 2B4, CD80, CD86, B7-H3 (CD276), B7-H4 (VTCN1), HVEM (TNFRSF14 or CD270), BTLA, KIR, MHC class I, MHC class II, GAL9, VISTA, BTLA, TIGIT, LAIR1 and A2aR.

21. A composition comprising activated T cells, wherein said activated T cells have an Effective E:T ratio of 1:10 or lower and wherein said Effective E:T ratio is a ratio between effector cells (E) to target cells (T), effector cells (E) being defined as the number of activated T cells (E) with a bispecific or multispecific antibody and target cells (T) being defined as the number of cancer cells killed by said activated T cells.
22. The composition of claim 21, wherein said composition comprises trogocytotic activated T cells.
23. The composition of activated T cells according to claim 21 or 22, wherein the activated T cells comprise at least 100 copies of the cancer cell surface marker and also comprise a detectable amount of bispecific or multispecific antibody.
24. The composition of activated T cells of any of claims 21-23, wherein the activated T cells are cytotoxic T lymphocytes or helper T cells selected from CD8+ T cells or CD4+ T cells.
25. The composition of activated T cells of any of claims 22-24, comprising trogocytotic T cells at a concentration of at least 30% of the total number of cells in the composition or preparation.
26. The composition of activated T cells of any of claims 21-25, comprising immunomodulatory agents selected from the group consisting of immune checkpoint inhibitors, agonists of T cells and immunomodulatory drugs.
27. The composition of activated T cells of claim 26, wherein immune checkpoint inhibitors inhibit immune checkpoints selected from the group consisting of CTLA4, PD-1, PD-L1, PD-L2, TIM3, LAG3, CD160, 2B4, CD80, CD86, B7-H3 (CD276), B7-H4 (VTCN1), HVEM (TNFRSF14 or CD270), BTLA, KIR, MHC class I, MHC class II, GAL9, VISTA, BTLA, TIGIT, LAIR1 and A2aR.
28. Pharmaceutical composition comprising the composition of any of claims 21-27 and a pharmaceutically acceptable carrier.
29. Use of the pharmaceutical composition according to claim 28 for the preparation of a medicament for the treatment of cancer in a subject, wherein the treatment of cancer consists of an Adoptive Cancer Therapy, wherein the subject is the same subject as that of the sample comprising the at least one T cell and/or wherein the subject is the same subject as that of the sample comprising the at least one cancer cell and/or wherein the subject is different from the

subject as that of the sample comprising the at least one T cell and of the sample comprising the at least one cancer cell.

30. Use of the pharmaceutical composition according to claim 29, wherein the cancer is a hematological cancer selected from chronic lymphocytic leukemia, acute lymphocytic leukemia, Non-Hodgkin's Lymphoma, acute myeloid leukemia, myelodysplastic syndrome or multiple myeloma.

31. *An in vitro* method of evaluating the responsiveness of a subject to a therapy comprising:

(a) forming an *ex vivo* reaction mixture by mixing a sample comprising at least one T cell, a sample comprising at least one cancer cell, a bispecific or multispecific antibody, and the therapy, and incubating the mixture under conditions and for a period of time sufficient to activate the T cell, thereby producing at least one activated T cell;

(b) selecting the activated T cell from (a), wherein the selection of the activated T cell is based on increased cancer cell killing activity determined by measuring an Effective E:T ratio, wherein said Effective E:T ratio is a ratio between effector cells (E) to target cells (T), effector cells (E) being defined as activated T cells and target cells (T) being defined as cancer cells killed by said activated T cells and wherein an increase in cancer cell killing activity of the reaction mixture in the presence of the therapy, relative to a reference value in absence of the therapy is indicative of a higher responsiveness to the therapy.

32. The method of claim 31, wherein in step (a) the *ex vivo* reaction mixture is incubated under conditions and for a period of time sufficient to allow the at least one T cell to acquire a cell surface marker from the at least one cancer cell.

33. The method of claim 31 or 32, further comprising one, two or all of the following *in vitro* steps:

- (i) expanding the activated T cells from (b);
- (ii) enriching for the activated T cell from (b); or
- (iii) purifying the activated T cell from (b).

34. The method of any of claims 31-33, wherein step (b) additionally comprises determining the level of cancer cells after step (a), and optionally determining the level of activated T cells after step (a).

35. The method of any of claims 31-34, wherein the bispecific or multispecific antibody is selected from: a bispecific immunoglobulin (BsIgG), an immunoglobulin operatively linked to

additional antigen-binding molecule, a bispecific antibody (BsAb) fragment, a bispecific fusion protein, or a BsAb conjugate.

36. The method of any of claims 31-35, wherein the therapy is an immunomodulatory agent or a combination of multiple immunomodulatory agents selected from: an inhibitor of an immune
5 checkpoint inhibitor, or an agonist of T cells, or an inhibitor of MDSCs and/or Treg cells, or lenalidomide, or a vaccine, or a cytokine, or an autologous T cell, or a modulator of a component associated with amino acid catabolism, signalling of tumor-derived extracellular ATP, adenosine signalling, adenosine production, chemokine and chemokine receptor, recognition of foreign organisms, or kinase signalling activity, or an inhibitor of IDO, COX2,
10 ARG1, ARG2, iNOS, or phosphodiesterase; or a TLR agonist, or a chemokine antagonist.

37. The method of claim 36, wherein said immunomodulatory agents are immune checkpoint inhibitors.

38. The method of claim 37, wherein the immune checkpoint inhibitors inhibit immune checkpoints selected from the group consisting of CTLA4, PD-1, PD-L1, PD-L2, TIM3, LAG3,
15 CD160, 2B4, CD80, CD86, B7-H3 (CD276), B7-H4 (VTCN1), HVEM (TNFRSF14 or CD270), BTLA, KIR, MHC class I, MHC class II, GAL9, VISTA, BTLA, TIGIT, LAIR1 and A2aR.

39. Use of an activated T cell obtainable by the method of claims 1-20 or the composition of activated T cells of claims 21-27 or the pharmaceutical composition comprising the same of claim 28 in the manufacture of a medicament for the treatment of cancer.

20 40. Use according to claim 39, wherein said treatment of cancer comprises:

- (a) forming an *ex vivo* reaction mixture by mixing a sample comprising at least one T cell, and a bispecific or multispecific antibody, and incubating the mixture under conditions and for a period of time sufficient to activate the T cell, thereby producing at least one activated T cell;
- 25 (b) selecting the activated T cell from (a), wherein the selection of the activated T cell is based on increased cancer cell killing activity determined by measuring an Effective E:T ratio; and
- (c) administering an effective amount of the activated T cells to a subject.

41. Use according to claim 40, wherein in step (a) the *ex vivo* reaction mixture is incubated
30 under conditions and for a period of time sufficient to allow the at least one T cell to acquire a cell surface marker from the at least one cancer cell.

42. Use according to claim 40 or 41, further comprising one, two or all of the following *in vitro* steps:

- (i) expanding the activated T cells from (b);
- (ii) enriching for the activated T cell from (b); or
- 5 (iii) purifying the activated T cell from (b).

43. Use according to any of claims 40-42, further comprising administering a second therapeutic agent or procedure.

44. Use according to claim 43, wherein the second therapeutic agent or procedure is chosen from at least one of: chemotherapy, a targeted anti-cancer therapy, an oncolytic drug, a cytotoxic agent, an immune-based therapy, a cytokine, a surgical procedure, a radiation procedure, an agonist of T cells, an inhibitor of an immune checkpoint inhibitor, an immunomodulatory agent or a combination of multiple immunomodulatory agents, a vaccine, or a cellular immunotherapy.

45. Use according to claim 44, wherein said immunomodulatory agents are immune checkpoint inhibitors.

46. Use according to claim 45, wherein the immune checkpoint inhibitors inhibit immune checkpoints selected from the group consisting of CTLA4, PD-1, PD-L1, PD-L2, TIM3, LAG3, CD160, 2B4, CD80, CD86, B7-H3 (CD276), B7-H4 (VTCN1), HVEM (TNFRSF14 or CD270), BTLA, KIR, MHC class I, MHC class II, GAL9, VISTA, BTLA, TIGIT, LAIR1 and A2aR.

47. An *in vitro* method of evaluating a subject's ability to generate activated T cells, comprising:

- (a) forming an *ex vivo* reaction mixture by mixing a sample comprising at least one T cell, a sample comprising at least one cancer cell, and a bispecific or multispecific antibody, and incubating the mixture under conditions and for a period of time sufficient to activate the T cell, thereby producing at least one activated T cell;
- 25 (b) selecting the activated T cell from (a), wherein the selection of the activated T cell is based on increased cancer cell killing activity determined by measuring an Effective E:T ratio, wherein said Effective E:T ratio is a ratio between effector cells (E) to target cells (T), effector cells (E) being defined as activated T cells (E) and target cells (T) being defined as cancer cells (T) killed by said activated T cells; and
- 30 (c) quantifying the level of the activated T cells with a high Effective E:T ratio in the *ex vivo* reaction mixture, wherein an elevated level of activated T cells with a high Effective E:T ratio in the *ex vivo* reaction mixture compared to a reference level of activated T cells, generated in an *ex vivo* reaction mixture in the absence of the T cell, the cancer cell or the bispecific or multispecific antibody, indicates that the subject is capable of

generating activated T cells with a high Effective E:T ratio and is identified as being responsive to the method of claims 39-46, and a low level of activated T cells with a high Effective E:T ratio in the *ex vivo* reaction mixture indicates that the subject is less capable of generating activated T cells and said subject is identified as being non-responsive to the method of claims 39-46.

48. The method of claim 47, wherein in step (a) the *ex vivo* reaction mixture is incubated under conditions and for a period of time sufficient to allow the at least one T cell to acquire a cell surface marker from the at least one cancer cell.

49. The method of claim 47 or 48, further comprising one, two or all of the following *in vitro* steps:

- (i) expanding the activated T cells from (b);
- (ii) enriching for the activated T cell from (b); or
- (iii) purifying the activated T cell from (b).

50. The method of any of claims 47-49, wherein the cancer cell is a cell selected from a hematological cancer, a solid cancer or a metastatic cancer, or a circulating tumor cells or a combination thereof.

51. The method of any of claims 47-50, wherein the T cell is a cell selected from: a peripheral blood sample, a bone marrow sample, a lymph node sample, a tumor sample comprising a CTL and/or a TIL, or a combination thereof.

52. The method of claim 51, wherein the T cell expresses CD8 and/or CD25 and/or the T cell expresses CD4 and/or CD25.

53. The method of any of claims 47-52, wherein the presence of a tumor antigen on the activated T cell is detected.

54. The method of any of claims 47-53, wherein an elevated level of activated T cells in the *ex vivo* reaction mixture indicates that the subject is capable of generating activated T cells.

55. The method of any of claims 47-54, wherein the activated T cells comprises trogocytotic activated T cells.

56. The method of any of claims 47-55, wherein an elevated level of cancer cell killing in the presence of the *ex vivo* reaction mixture is indicative of the activity of the activated T cells in the reaction mixture.

57. An *in vitro* method of identifying the effectiveness of an activated T cell or preparation thereof in killing a cancer cell *in vivo*, comprising:

- (a) providing a population of activated T cells specific for the cancer cell; and
- (b) determining the parameter Effective E:T Ratio.

5 58. An *in vitro* method of evaluating the potency of an activated T cell or preparation thereof, comprising:

- (a) contacting at least one activated T cell or the preparation thereof obtainable according to the method of claims 1-20 with a sample comprising at least one cancer cell, wherein the cancer cell is from the same subject as the activated T cell or preparation thereof,
10 under conditions and for a period of time sufficient to allow the activated T cell to kill the cancer cells;
 - (b) determining the level of cancer cells after step (a), and optionally determining the level of activated T cells after step (a); and
 - (c) determining the Effective E:T ratio of either cancer cell to T cell, or T cell to cancer cell,
15 from step (b);
- wherein a decrease in the level or amount of cancer cells is indicative of increased cancer cell killing, and wherein no substantial change in the level or amount of cancer cells is indicative of decreased cancer cell killing.

59. The method of claim 58, wherein the contacting step further comprises the addition of a
20 bispecific or multispecific antibody at increasing dosages to generate a dose response curve.

60. The method of claim 58 or 59, wherein an Effective E:T ratio of 1:10 or lower is indicative of potent cancer cell killing activity.

61. The method of any of claims 58-60, wherein if the T cells have potent cancer cell killing activity, the subject is identified as being a strong responder to the bispecific or multispecific
25 antibody.