# CLAIMS

1. A method for detecting the presence in a sample of clustering of at least two cell surface moieties, comprising a first cell surface moiety and a second cell surface moiety, the method comprising:

contacting a sample in which the first and second cell surface moieties have been exposed to an agent having binding specificity for at least the first and second cell surface moieties with a first binding molecule that specifically binds to the first cell surface moiety and a second binding molecule that specifically binds to the second cell surface moiety, wherein at least one of the first binding molecule and the second binding molecule comprises a molecular tag which is not detected unless the first and second cell surface moieties are in proximity of each other; and

detecting the presence or absence of the molecular tag to detect the presence in the sample of clustering of the first cell surface moiety and the second cell surface moiety.

2. A method for quantifying the presence in a sample of clustering of at least two cell surface moieties, comprising a first cell surface moiety and a second cell surface moiety, the method comprising:

contacting a sample in which the first and second cell surface moieties have been exposed to an agent having binding specificity for at least the first and second cell surface moieties with a first binding molecule that specifically binds to the first cell surface moiety and a second binding molecule that specifically binds to the second cell surface moiety, wherein at least one of the first binding molecule and the second binding molecule comprises a molecular tag which is not detected unless the first and second cell surface moieties are in proximity of each other; and

measuring the amount of the molecular tag to quantify the presence in the sample of clustering of the first cell surface moiety and the second cell surface moiety.

3. The method according to claim 1 or 2, wherein the method comprises:

- a) contacting the sample with a first binding molecule that specifically binds to the first cell surface moiety and a second binding molecule that specifically binds to the second cell surface moiety,

wherein the first binding molecule comprises a molecular tag attached thereto via a cleavable linker and the second binding molecule comprises a cleavage inducing moiety; or

b) contacting the sample with a first binding molecule that specifically binds to the first cell surface moiety and a second binding molecule that specifically binds to the second cell surface moiety,

wherein the second binding molecule comprises a molecular tag attached thereto via a cleavable linker and the first binding molecule comprises a cleavage inducing moiety;

- inducing cleavage of the molecular tag; and
- detecting the presence or absence, or measuring the amount, of released molecular tag thereby detecting or quantifying clustering of the first cell surface moiety with the second cell surface moiety in the sample.

4. The method according to claim 1 or 2, wherein the method comprises:

- a) contacting the sample with a first binding molecule that specifically binds to the first cell surface moiety, a second binding molecule that specifically binds to the second cell surface moiety, and a third binding molecule,

wherein the first binding molecule comprises a molecular tag attached thereto via a cleavable linker and the third binding molecule binds to the second binding molecule and comprises a cleavage inducing moiety; or

b) contacting the sample with a first binding molecule that specifically binds to the first cell surface moiety, a second binding molecule that specifically binds to the second cell surface moiety, and a third binding molecule,

wherein the second binding molecule comprises a molecular tag attached thereto via a cleavable linker and the third binding molecule binds to the first binding molecule and comprises a cleavage inducing moiety; or

c) contacting the sample with a first binding molecule that specifically binds to the first cell surface moiety, a second binding molecule that specifically binds to the second cell surface moiety, and a third binding molecule,

wherein the first binding molecule comprises a cleavage inducing moiety and the third binding molecule binds to the second binding molecule and comprises a molecular tag attached thereto via a cleavable linker; or

d) contacting the sample with a first binding molecule that specifically binds to the first cell surface moiety, a second binding molecule that specifically binds to the second cell surface moiety, and a third binding molecule,

wherein the second binding molecule comprises a cleavage inducing moiety and the third binding molecule binds to the first binding molecule and comprises a molecular tag attached thereto via a cleavable linker;

- inducing cleavage of the molecular tag; and
- detecting the presence or absence, or measuring the amount, of released molecular tag thereby detecting or quantifying clustering of the first cell surface moiety with the second cell surface moiety in the sample.

5. The method according to claim 1 or 2, wherein the method comprises:

- a) contacting the sample with a first binding molecule that specifically binds to the first cell surface moiety, a second binding molecule that specifically binds to the second cell surface moiety, a third binding molecule, and a fourth binding molecule,

wherein the third binding molecule binds to the first binding molecule and comprises a molecular tag attached thereto via a cleavable linker, and the fourth binding molecule binds to the second binding molecule and comprises a cleavage inducing moiety; or

b) contacting the sample with a first binding molecule that specifically binds to the first cell surface moiety, a second binding molecule that specifically binds to the second cell surface moiety, a third binding molecule, and a fourth binding molecule,

wherein the third binding molecule binds to the first binding molecule and comprises a cleavage inducing moiety; and the fourth binding molecule binds to the second binding molecule and comprises a molecular tag attached thereto via a cleavable linker,

- inducing cleavage of the molecular tag; and
- detecting the presence of absence, or measuring the amount, of released molecular tag thereby detecting or quantifying clustering of the first cell surface moiety with the second cell surface moiety in the sample.

6. The method according to any one of claims 1-5, wherein the sample is a tissue sample, a blood sample, or cultured cells, preferably from a subject or patient.

7. The method according to claim 6, wherein the sample is a fresh sample or a fixated sample.

8. The method according to any one of claims 1-7, wherein the sample is a tumor biopsy sample from a subject having cancer.

9. A method for detecting expression in a sample of at least two cell surface moieties, comprising a first cell surface moiety and a second cell surface moiety, the method comprising

contacting a tumor biopsy sample from a subject having cancer with at least one binding molecule that detects a first cell surface moiety and at least one binding molecule that detects a second cell surface moiety,

wherein at least one binding molecule that detects the first cell surface moiety and at least one binding molecule that detects the second cell surface moiety comprise a molecular tag;

and

detecting the presence or absence of the molecular tags to detect the expression of the first cell surface moiety and of the second cell surface moiety in the sample.

10. A method for quantifying expression in a sample of at least two cell surface moieties, comprising a first cell surface moiety and a second cell surface moiety, the method comprising

contacting a tumor biopsy sample from a subject having cancer with at least one binding molecule that detects a first cell surface moiety and at least one binding molecule that detects a second cell surface moiety,

wherein at least one binding molecule that detects the first cell surface moiety and at least one binding molecule that detects the second cell surface moiety comprise a molecular tag;

and

measuring the amount of the molecular tags to quantify the expression of the first cell surface moiety and of the second cell surface moiety in the sample.

11. The method according to any one of claims 1-10, wherein the first and second cell surface moieties are expressed by the same cell or same type of cell.

12. The method according to any one of claims 1-10, wherein the first and second cell surface moieties are expressed by different cells or different types of cells.

13. The method according to any one of claims 1-10, wherein the first cell surface moiety is expressed by an immune effector cell, in particular a NK cell, a T cell, a B cell, a monocyte, a macrophage, a dendritic cell or a neutrophilic granulocyte, and the second cell surface moiety is expressed by a tumor cell or an immune cell.

14. The method according to any one of claims 1-13, wherein at least one cell surface moiety is CD137 or another immune effector cell co-stimulatory moiety.

15. The method according to any one of claims 1-14, wherein at least one cell surface moiety is PD-L1 or another tumor-associated moiety or immune checkpoint moiety

16. The method according to any one of claims 1-15, wherein at least one cell surface moiety is CD137 or another immune effector cell co-stimulatory moiety, and at least one cell surface moiety is PD-L1 or another tumor-associated moiety or immune checkpoint moiety.

17. The method according to any one of claims 1-8 and 11-16, wherein the agent having binding specificity for the cell surface moieties is a multispecific antibody, such as a bispecific or trispecific antibody.

18. The method according to claim 17, wherein the multispecific antibody is a bispecific antibody that binds to CD137 or another immune effector cell co-stimulatory molecule and PD-L1 or another tumor-associated moiety or immune checkpoint moiety.

19. The method according to claim 18, wherein the binding domain of the bispecific antibody that binds to CD137 comprises a heavy chain variable region having a heavy chain CDR3 (HCDR3) with an amino acid sequence as set forth in any one of SEQ ID NO: 4, 8, 12, 16, 19, 23, 27, 30, 34, 38, 42, 45, 48, or 52, allowing for one, two, or three amino acid substitutions therein, preferably one amino acid substitution.

20. The method according to claim 18 or 19, wherein the binding domain of the bispecific antibody that binds to CD137 comprises a heavy chain CDR1 (HCDR1) with an amino acid sequence as set forth in any one of SEQ ID NO: 2, 6, 10, 14, 18, 21, 25, 32, 36, 40, 44, or 50, allowing for one, two, or three amino acid substitutions therein, preferably one amino acid substitution; and/or a heavy chain CDR2 (HCDR2) with an amino acid sequence as set forth in any one of SEQ ID NO: 3, 7, 11, 15, 22, 26, 29, 33, 37, 41, 47, or 51, allowing for one, two, or three amino acid substitutions therein, preferably one amino acid substitution.

21. The method according to any one of claims 18-20, wherein the binding domain of the bispecific antibody that binds to CD137 comprises a heavy chain variable region having any one of SEQ ID NO: 1, 5, 9, 13, 17, 20, 24, 28, 31, 35, 39, 43, 46, or 49, or having at least 80%, 85%, 90%, 95%, 99% sequence identity thereto, in particular having at least 80%, 85%, 90%, 95%, 99% sequence identity to the framework regions thereof.

22. The method according to any one of claims 18-21, wherein the binding domain of the bispecific antibody that binds to PD-L1 comprises a heavy chain variable region having a heavy chain CDR3 (HCDR3) with an amino acid sequence as set forth in any one of SEQ ID NO: 56, 58, 61, 72, 76, 80, 84, 88, 91, 95, 99, 102, or 106, allowing for one, two, or three amino acid substitutions therein, preferably one amino acid substitution.

23. The method according to any one of claims 18-22, wherein the binding domain of the bispecific antibody that binds to PD-L1 comprises a heavy chain CDR1 (HCDR1) with an amino acid sequence as set forth in any one of SEQ ID NO: 54, 60, 65, 68, 70, 74, 78, 82, 86, 90, or 93, allowing for one, two, or three amino acid substitutions therein, preferably one amino acid substitution; and/or a heavy chain CDR2 (HCDR2) with an amino acid sequence as set forth in any one of SEQ ID NO: 55, 3, 63, 66, 71, 75, 79, 83, 87, 94, 98, 101, 105, or 108, allowing for one, two, or three amino acid substitutions therein, preferably one amino acid substitution.

24. The method according to any one of claims 18-23, wherein the binding domain of the bispecific antibody that binds to PD-L1 comprises a heavy chain variable region having any one

of SEQ ID NO: 53, 57, 59, 62, 64, 67, 69, 73, 77, 81, 85, 89, 92, 96, 97, 100, 103, 104, or 107, or having at least 80%, 85%, 90%, 95%, 99% sequence identity thereto, in particular having at least 80%, 85%, 90%, 95%, 99% sequence identity to the framework regions thereof.

25. A method for predicting the responsiveness of a subject, in particular a cancer patient, to an agent or agents binding a first cell surface moiety and a second cell surface moiety, in particular a moiety expressed on an immune effector cell and a moiety expressed on a tumor cell or immune cell, the method comprising:

- detecting the expression levels of a first cell surface moiety and a second cell surface member in a biological sample from a subject;
- determining whether the expression levels of the first cell surface moiety and the second cell surface moiety in the subject's sample is above or below a threshold level; and
- predicting that the subject is likely to respond to an agent or agents binding the first cell surface moiety and the second cell surface moiety if the expression levels of the first cell surface moiety and the second cell surface moiety in the subject's sample is equal to or above the threshold level.

26. The method according to claim 25, wherein the expression levels of a first cell surface moiety and a second cell surface member are measured using the method according to any one of claims 9-16.

27. A method for treating a subject in need thereof, in particular a subject having cancer, the method comprising:

-predicting responsiveness of a subject to an agent or agents binding a first cell surface moiety and a second cell face moiety using the method according to claim 25 or 26; and

-administering an agent or agents binding the first cell surface moiety and the second cell surface to a subject that is likely to respond.

28. The method according to any one of claims 25-27, wherein at least one cell surface moiety is CD137 or another immune effector cell co-stimulatory moiety.

29. The method according to any one of claims 25-28, wherein at least one cell surface moiety is PD-L1 or another tumor-associated moiety or immune checkpoint moiety

30. The method according to any one of claims 25-29, wherein at least one cell surface moiety is CD137 and at least one cell surface moiety is PD-L1.

31. The method according to any one of claims 25-30, wherein the agent binding the cell surface moieties is a multispecific antibody, such as a bispecific or trispecific antibody, in particular a bispecific antibody that specifically binds to CD137 or another immune effector cell co-stimulatory moiety and PD-L1 or another tumor-associated moiety or immune checkpoint moiety, as defined in any one of claims 17-24.

32. A method for determining the effectiveness of an agent, the agent comprising at least a binding domain that specifically binds to a first cell surface moiety and a binding domain that specifically binds to a second cell surface moiety, the method comprising detecting clustering of a first cell surface moiety with a second cell surface moiety in a biological sample of a subject under treatment with the agent by using the method according to any one of claims 1-24.

33. A method for determining the effectiveness of an agent, the agent comprising at least a binding domain that specifically binds to a first cell surface moiety and a binding domain that specifically binds to a second cell surface moiety, the method comprising quantifying clustering of a first cell surface moiety with a second cell surface moiety in a biological sample of a subject under treatment with the agent by using the method according to any one of claims 1-24.

34. A method for confirming the mode of action of an agent, the agent comprising at least a binding domain that specifically binds to a first cell surface moiety and a binding domain that specifically binds to a second cell surface moiety, the method comprising detecting clustering of a first cell surface moiety with a second cell surface moiety in a biological sample of a subject under treatment with the agent by using the method according to any one of claims 1-24.

35. A method for confirming the mode of action of an agent, the agent comprising at least a binding domain that specifically binds to a first cell surface moiety and a binding domain that specifically binds to a second cell surface moiety, the method comprising quantifying clustering of a first cell surface moiety with a second cell surface moiety in a biological sample of a subject under treatment with the agent by using the method according to any one of claims 1-24.

36. The method according to claim 34 or 35, wherein the mode of action is the simultaneous binding of the agent to the first and second cell surface moiety.

37. The method according to claim 34 or 35, wherein the mode of action is clustering of two or more cell surface moieties.

38. The method according to 37, wherein the clustering is of two or more cell surface moieties expressed on an immune effector cell, in particular the clustering of two or more CD137 proteins.

39. A method for treating a subject in need thereof, in particular a subject having cancer, the method comprising:

-treating a subject with an agent binding a first cell surface moiety and a second cell surface moiety;

-analyzing the effectiveness of the agent or the mode of action of the agent using the method according to any one of claims 32-38.

40. The method according to claim 39, further comprising continuing or adapting the treatment based on the outcome of the analysis or confirmation.

41. A method for screening one or more test agents for the ability to induce clustering of at least a first cell surface moiety with a second cell surface moiety, the method comprising:

- contacting one or more test cell cultures with a test agent,

wherein the test cell culture comprises a cell expressing a first cell surface moiety, and a cell expressing a second cell surface moiety;

- detecting or quantifying the level of clustering of the first and second cell surface moieties using a method according to any one of claims 1-24; and
- comparing the level of clustering with the level of clustering detected for the clustering in a control cell culture not contacted with the test agent or contacted with a reference agent,

wherein the control cell culture comprises the first cell surface moiety, and the second cell surface moiety.

42. The method according to claim 41, further comprising selecting a test agent that induces an equal or higher level of clustering than the level of clustering in the control cell culture.

43. The method according to claim 41 or 42, wherein at least one cell surface moiety is CD137 or another immune effector co-stimulatory moiety.

44. The method according to any one of claims 41-43, wherein at least one cell surface moiety is PD-L1 or another tumor-associated moiety or immune checkpoint molecule.

45. The method according to any one of claims 41-44,wherein at least one cell surface moiety is CD137 and at least one cell surface moiety is PD-L1.

46. The method according to any one of claims 41-45, wherein the reference agent is a multispecific antibody that specifically binds to CD137 or another immune effector co-stimulatory moiety and PD-L1 or another tumor-associated moiety or immune checkpoint moiety, as defined in any one of claims 17-24.

47. A kit of parts comprising at least two binding molecules that specifically bind to a first and second cell surface moiety, optionally wherein one of the binding molecules comprises a molecular tag attached thereto via a cleavable linker and the other binding molecule comprises a cleavage inducing moiety, and instructions to contact a patient sample with the at least two binding molecules, optionally to induce cleavage of the molecular tag; and to detect the presence

or absence of a signal induced by contacting the patient sample with the at least two binding molecules.

## Claims

1. A method for detecting the presence in a sample of clustering of at least two cell surface moieties, comprising a first cell surface moiety and a second cell surface moiety, the method comprising:

contacting a sample in which the first and second cell surface moieties have been exposed to an agent having binding specificity for at least the first and second cell surface moieties with a first binding molecule that specifically binds to the first cell surface moiety and a second binding molecule that specifically binds to the second cell surface moiety, wherein at least one of the first binding molecule and the second binding molecule comprises a molecular tag which is not detected unless the first and second cell surface moieties are in proximity of each other; and

detecting the presence or absence of the molecular tag to detect the presence in the sample of clustering of the first cell surface moiety and the second cell surface moiety.

2. A method for quantifying the presence in a sample of clustering of at least two cell surface moieties, comprising a first cell surface moiety and a second cell surface moiety, the method comprising:

contacting a sample in which the first and second cell surface moieties have been exposed to an agent having binding specificity for at least the first and second cell surface moieties with a first binding molecule that specifically binds to the first cell surface moiety and a second binding molecule that specifically binds to the second cell surface moiety, wherein at least one of the first binding molecule and the second binding molecule comprises a molecular tag which is not detected unless the first and second cell surface moieties are in proximity of each other; and

measuring the amount of the molecular tag to quantify the presence in the sample of clustering of the first cell surface moiety and the second cell surface moiety.

3. The method according to claim 1 or 2, wherein the method comprises:

- a) contacting the sample with a first binding molecule that specifically binds to the first cell surface moiety and a second binding molecule that specifically binds to the second cell surface moiety,

wherein the first binding molecule comprises a molecular tag attached thereto via a cleavable linker and the second binding molecule comprises a cleavage inducing moiety; or

b) contacting the sample with a first binding molecule that specifically binds to the first cell surface moiety and a second binding molecule that specifically binds to the second cell surface moiety,

wherein the second binding molecule comprises a molecular tag attached thereto via a cleavable linker and the first binding molecule comprises a cleavage inducing moiety;

- inducing cleavage of the molecular tag; and

- detecting the presence or absence, or measuring the amount, of released molecular tag thereby detecting or quantifying clustering of the first cell surface moiety with the second cell surface moiety in the sample.

4. The method according to claim 1 or 2, wherein the method comprises:

- a) contacting the sample with a first binding molecule that specifically binds to the first cell surface moiety, a second binding molecule that specifically binds to the second cell surface moiety, and a third binding molecule,

wherein the first binding molecule comprises a molecular tag attached thereto via a cleavable linker and the third binding molecule binds to the second binding molecule and comprises a cleavage inducing moiety; or

b) contacting the sample with a first binding molecule that specifically binds to the first cell surface moiety, a second binding molecule that specifically binds to the second cell surface moiety, and a third binding molecule,

wherein the second binding molecule comprises a molecular tag attached thereto via a cleavable linker and the third binding molecule binds to the first binding molecule and comprises a cleavage inducing moiety; or

c) contacting the sample with a first binding molecule that specifically binds to the first cell surface moiety, a second binding molecule that specifically binds to the second cell surface moiety, and a third binding molecule,

wherein the first binding molecule comprises a cleavage inducing moiety and the third binding molecule binds to the second binding molecule and comprises a molecular tag attached thereto via a cleavable linker; or

d) contacting the sample with a first binding molecule that specifically binds to the first cell surface moiety, a second binding molecule that specifically binds to the second cell surface moiety, and a third binding molecule,

wherein the second binding molecule comprises a cleavage inducing moiety and the third binding molecule binds to the first binding molecule and comprises a molecular tag attached thereto via a cleavable linker;

- inducing cleavage of the molecular tag; and

- detecting the presence or absence, or measuring the amount, of released molecular tag thereby detecting or quantifying clustering of the first cell surface moiety with the second cell surface moiety in the sample.

5. The method according to claim 1 or 2, wherein the method comprises:

- a) contacting the sample with a first binding molecule that specifically binds to the first cell surface moiety, a second binding molecule that specifically binds to the second cell surface moiety, a third binding molecule, and a fourth binding molecule,

wherein the third binding molecule binds to the first binding molecule and comprises a molecular tag attached thereto via a cleavable linker, and the fourth binding molecule binds to the second binding molecule and comprises a cleavage inducing moiety; or

b) contacting the sample with a first binding molecule that specifically binds to the first cell surface moiety, a second binding molecule that specifically binds to the second cell surface moiety, a third binding molecule, and a fourth binding molecule,

wherein the third binding molecule binds to the first binding molecule and comprises a cleavage inducing moiety; and the fourth binding molecule binds to the second binding molecule and comprises a molecular tag attached thereto via a cleavable linker,

- inducing cleavage of the molecular tag; and

- detecting the presence of absence, or measuring the amount, of released molecular tag thereby detecting or quantifying clustering of the first cell surface moiety with the second cell surface moiety in the sample.

6. The method according to any one of claims 1-5, wherein the sample is a tissue sample, a blood sample, or cultured cells, preferably from a subject or patient, in particular wherein the sample is a fresh sample or a fixated sample.

7. The method according to any one of claims 1-6, wherein the sample is a tumor biopsy sample from a subject having cancer.

8. A method for detecting expression in a sample of at least two cell surface moieties, comprising a first cell surface moiety and a second cell surface moiety, the method comprising

contacting a tumor biopsy sample from a subject having cancer with at least one binding molecule that detects a first cell surface moiety and at least one binding molecule that detects a second cell surface moiety,

wherein at least one binding molecule that detects the first cell surface moiety and at least one binding molecule that detects the second cell surface moiety comprise a molecular tag;

and

detecting the presence or absence of the molecular tags to detect the expression of the first cell surface moiety and of the second cell surface moiety in the sample.

9. A method for quantifying expression in a sample of at least two cell surface moieties, comprising a first cell surface moiety and a second cell surface moiety, the method comprising

contacting a tumor biopsy sample from a subject having cancer with at least one binding molecule that detects a first cell surface moiety and at least one binding molecule that detects a second cell surface moiety,

wherein at least one binding molecule that detects the first cell surface moiety and at least one binding molecule that detects the second cell surface moiety comprise a molecular tag;

and

measuring the amount of the molecular tags to quantify the expression of the first cell surface moiety and of the second cell surface moiety in the sample.

10. The method according to any one of claims 1-9, wherein the first and second cell surface moieties are expressed by the same cell or same type of cell, or wherein the first and second cell surface moieties are expressed by different cells or different types of cells.

11. The method according to any one of claims 1-10, wherein the first cell surface moiety is expressed by an immune effector cell, in particular a NK cell, a T cell, a B cell, a monocyte, a macrophage, a dendritic cell or a neutrophilic granulocyte, and the second cell surface moiety is expressed by a tumor cell or an immune cell.

12. The method according to any one of claims 1-11, wherein at least one cell surface moiety is CD137 or another immune effector cell co-stimulatory moiety, and/or wherein at least one cell surface moiety is PD-L1 or another tumor-associated moiety or immune checkpoint moiety.

13. The method according to any one of claims 1-7 and 10-12, wherein the agent having binding specificity for the cell surface moieties is a multispecific antibody, such as a bispecific or trispecific antibody, in particular wherein the multispecific antibody is a bispecific antibody that binds to CD137 or another immune effector cell co-stimulatory molecule and PD-L1 or another tumor-associated moiety or immune checkpoint moiety.

14. The method according to claim 13, wherein the binding domain of the bispecific antibody that binds to CD137 comprises a heavy chain variable region having a heavy chain CDR1 (HCDR1) with an amino acid sequence as set forth in any one of SEQ ID NO: 2, 6, 10, 14, 18, 21, 25, 32, 36, 40, 44, or 50; and/or a heavy chain CDR2 (HCDR2) with an amino acid sequence as set forth in any one of SEQ ID NO: 3, 7, 11, 15, 22, 26, 29, 33, 37, 41, 47, or 51; and/or a heavy chain CDR3 (HCDR3) with an amino acid sequence as set forth in any one of SEQ ID NO: 4, 8, 12, 16, 19, 23, 27, 30, 34, 38, 42, 45, 48, or 52, allowing for one, two, or three amino acid substitutions therein, preferably one amino acid substitution in each of said HCDR1, HCDR2 and/or HCDR3.

15. The method according to claim 13 or 14, wherein the binding domain of the bispecific antibody that binds to CD137 comprises a heavy chain variable region having any one of SEQ ID NO: 1, 5, 9, 13, 17, 20, 24, 28, 31, 35, 39, 43, 46, or 49, or having at least 80%, 85%, 90%, 95%, 99% sequence identity thereto, in particular having at least 80%, 85%, 90%, 95%, 99% sequence identity to the framework regions thereof.

16. The method according to any one of claims 13-15, wherein the binding domain of the bispecific antibody that binds to PD-L1 comprises a heavy chain variable region having a heavy chain CDR1 (HCDR1) with an amino acid sequence as set forth in any one of SEQ ID NO: 54, 60, 65, 68, 70, 74, 78, 82, 86, 90, or 93, and/or a heavy chain CDR2 (HCDR2) with an amino acid sequence as set forth in any one of SEQ ID NO: 55, 3, 63, 66, 71, 75, 79, 83, 87, 94, 98, 101, 105, or 108, and/or a heavy chain CDR3 (HCDR3) with an amino acid sequence as set forth in any one of SEQ ID NO: 56, 58, 61, 72, 76, 80, 84, 88, 91, 95, 99, 102, or 106, allowing for one, two, or three amino acid substitutions therein, preferably one amino acid substitution in each of said HCDR1, HCDR2 and/or HCDR3.

17. The method according to any one of claims 13-16, wherein the binding domain of the bispecific antibody that binds to PD-L1 comprises a heavy chain variable region having any one of SEQ ID NO: 53, 57, 59, 62, 64, 67, 69, 73, 77, 81, 85, 89, 92, 96, 97, 100, 103, 104, or 107, or having at least 80%, 85%, 90%, 95%, 99% sequence identity thereto, in particular having at least 80%, 85%, 90%, 95%, 99% sequence identity to the framework regions thereof.

18. A method for predicting the responsiveness of a subject, in particular a cancer patient, to an agent or agents binding a first cell surface moiety and a second cell surface moiety, in particular a moiety expressed on an immune effector cell and a moiety expressed on a tumor cell or immune cell, the method comprising:

- detecting the expression levels of a first cell surface moiety and a second cell surface member in a biological sample from a subject;
- determining whether the expression levels of the first cell surface moiety and the second cell surface moiety in the subject's sample is above or below a threshold level; and

- predicting that the subject is likely to respond to an agent or agents binding the first cell surface moiety and the second cell surface moiety if the expression levels of the first cell surface moiety and the second cell surface moiety in the subject's sample is equal to or above the threshold level.

19. The method according to claim 18, wherein the expression levels of a first cell surface moiety and a second cell surface member are measured using the method according to any one of claims 8-12.

20. Use of an agent or agents for the manufacture of a medicament for a method of treatment in a subject, in particular a method of treatment of cancer in a subject, the method comprising:

-predicting responsiveness of a subject to an agent or agents binding a first cell surface moiety and a second cell surface moiety using the method according to claim 18 or 19; and

-administering the agent or agents binding the first cell surface moiety and the second cell surface moiety to a subject that is likely to respond.

21. The method according to claims 18 or 19 or the use according to claim 20, wherein at least one cell surface moiety is CD137 or another immune effector cell co-stimulatory moiety and/or wherein at least one cell surface moiety is PD-L1 or another tumor-associated moiety or immune checkpoint moiety.

22. The method or use according to any one of claims 18-21, wherein the agent binding the cell surface moieties is a multispecific antibody, such as a bispecific or trispecific antibody, in particular a bispecific antibody that specifically binds to CD137 or another immune effector cell co-stimulatory moiety and PD-L1 or another tumor-associated moiety or immune checkpoint moiety, as defined in any one of claims 13-17.

23. A method for determining the effectiveness of an agent, the agent comprising at least a binding domain that specifically binds to a first cell surface moiety and a binding domain that specifically binds to a second cell surface moiety, the method comprising detecting clustering of

a first cell surface moiety with a second cell surface moiety in a biological sample of a subject under treatment with the agent by using the method according to any one of claims 1-17.

24. A method for determining the effectiveness of an agent, the agent comprising at least a binding domain that specifically binds to a first cell surface moiety and a binding domain that specifically binds to a second cell surface moiety, the method comprising quantifying clustering of a first cell surface moiety with a second cell surface moiety in a biological sample of a subject under treatment with the agent by using the method according to any one of claims 1-17.

25. A method for confirming the mode of action of an agent, the agent comprising at least a binding domain that specifically binds to a first cell surface moiety and a binding domain that specifically binds to a second cell surface moiety, the method comprising detecting clustering of a first cell surface moiety with a second cell surface moiety in a biological sample of a subject under treatment with the agent by using the method according to any one of claims 1-17.

26. A method for confirming the mode of action of an agent, the agent comprising at least a binding domain that specifically binds to a first cell surface moiety and a binding domain that specifically binds to a second cell surface moiety, the method comprising quantifying clustering of a first cell surface moiety with a second cell surface moiety in a biological sample of a subject under treatment with the agent by using the method according to any one of claims 1-17.

27. The method according to claim 25 or 26, wherein the mode of action is the simultaneous binding of the agent to the first and second cell surface moiety.

28. The method according to claim 25 or 26, wherein the mode of action is clustering of two or more cell surface moieties, in particular wherein the clustering is of two or more cell surface moieties expressed on an immune effector cell, in particular the clustering of two or more CD137 proteins.

29. Use of an agent in the manufacture of a medicament, for a method of treatment of a subject, in particular a method of treatment of cancer in a subject, the method comprising:

-treating a subject with an agent binding a first cell surface moiety and a second cell surface moiety;

-analyzing the effectiveness of the agent or the mode of action of the agent using the method according to any one of claims 23-28;

and optionally further comprising continuing or adapting the treatment based on the outcome of the analysis or confirmation.

30. A method for screening one or more test agents for the ability to induce clustering of at least a first cell surface moiety with a second cell surface moiety, the method comprising:

- contacting one or more test cell cultures with a test agent,

wherein the test cell culture comprises a cell expressing a first cell surface moiety, and a cell expressing a second cell surface moiety;

- detecting or quantifying the level of clustering of the first and second cell surface moieties using a method according to any one of claims 1-17; and
- comparing the level of clustering with the level of clustering detected for the clustering in a control cell culture not contacted with the test agent or contacted with a reference agent,

wherein the control cell culture comprises the first cell surface moiety, and the second cell surface moiety.

31. The method according to claim 30, further comprising selecting a test agent that induces an equal or higher level of clustering than the level of clustering in the control cell culture.

32. The method according to claim 30 or 31, wherein at least one cell surface moiety is CD137 or another immune effector co-stimulatory moiety and/or wherein at least one cell surface moiety is PD-L1 or another tumor-associated moiety or immune checkpoint molecule.

33. The method according to any one of claims 30-32, wherein the reference agent is a multispecific antibody that specifically binds to CD137 or another immune effector co-stimulatory moiety and PD-L1 or another tumor-associated moiety or immune checkpoint moiety, as defined in any one of claims 13-17.

34. A kit of parts comprising at least two binding molecules that specifically bind to a first and second cell surface moiety, optionally wherein one of the binding molecules comprises a molecular tag attached thereto via a cleavable linker and the other binding molecule comprises a cleavage inducing moiety, and instructions to contact a patient sample with the at least two binding molecules, optionally to induce cleavage of the molecular tag; and to detect the presence or absence of a signal induced by contacting the patient sample with the at least two binding molecules.