Combination therapy comprising a bispecific anti-VEGF-A and anti-PD-L1 antibody and a chemotherapy for cancer treatment

FIELD OF THE INVENTION

The invention relates to methods for treating cancer in a subject using a bispecific antibody that specifically binds to programmed death-ligand 1 (PD-L1) and Vascular Endothelial Growth Factor (VEGF) in combination with chemotherapy, optionally wherein the subject has a PD-L1 expression score before the treatment, as determined by a combined positive score (CPS) of ≥ 1 or other integrating scoring algorithm defining a similar PD-L1 expression score. The invention further concerns a method for determining whether a cancer in a subject is susceptible to treatment with a bispecific antibody that specifically binds to PD-L1 and VEGF, wherein the method comprises detecting in a sample a PD-L1 expression score before the treatment as determined by a combined positive score (CPS) of ≥ 1 or other integrating scoring algorithm defining a similar PD-L1 expression score, wherein the CPS of ≥ 1 indicates that the subject is susceptible to treatment with the bispecific antibody and the chemotherapy.

BACKGROUND OF THE INVENTION

The approval of immune checkpoint inhibitors (ICIs) targeting programmed cell death protein 1 (PD-1) and programmed death-ligand 1 (PD-L1) has dramatically changed the treatment landscape for cancer subjects. Immune checkpoint therapies targeting the PD-1:PD-L1 pathway have resulted in groundbreaking improvements in clinical response in multiple human cancers (Brahmer et al., N Engl J Med 2012, 366: 2455-65; Garon et al. N Engl J Med 2015, 372: 2018-28; Hamid et al, N Engl J Med 2013, 369: 134-44; Robert et al, Lancet 2014, 384: 1109-17; Robert et al, N Engl J Med 2015, 372: 2521-32; Robert et al. , N Engl J Med 2015 , 372: 320-30; Topalian et al, N Engl J Med 2012, 366: 2443-54; Topalian et al, J Clin Oncol 2014, 32: 1020-30; Wolchok et al, N Engl J Med 2013, 369: 122-33).

Numerous studies have found higher response rates and more favorable survival outcomes when subjects were treated with ICI therapy in combination with chemotherapy as compared with the conventional chemotherapy alone.

Despite these promising improvements, response to ICI treatment is not guaranteed in all cancer subjects. Predictive biomarkers, such as scoring PD-L1 expression in cancer tissue, are therefore used to evaluate the likelihood to respond to ICI treatment alone or in combination with chemotherapy, thereby defining treatment-eligible patient groups (Ulas, Ezgi B et al. "Predictive Value of Combined Positive Score and Tumour Proportion Score for Immunotherapy Response in Advanced NSCLC." JTO clinical and research reports vol. 4,9 100532. 25 May. 2023, doi:10.1016/j.jtocrr.2023.100532).

Cancer types are scored for PD-L1 expression for example by using the combined positive score (CPS), which covers the PD-L1 expression on both the tumour cells and the immune cells in the tumour microenvironment. Studies have explored the relationship between the expression of programmed death ligand 1 (PD-L1) and prognosis in cancer, and high PD-L1 expression determined by immunohistochemistry is suggested to be a predictive biomarker of response to immunotherapy in several cancers (Cortes J, Rugo HS, Cescon DW, et al. Pembrolizumab plus Chemotherapy in Advanced Triple-Negative Breast Cancer. N Engl J Med. 2022;387(3):217-226. Doi:10.1056/NEJMoa2202809, Chen, Xiao-Jiang et al. "Prognostic Significance of PD-L1 Expression in Gastric Cancer Subjects with Peritoneal Metastasis." Biomedicines vol. 11,7 2003. 15 Jul. 2023, doi:10.3390/biomedicines11072003). High expression of PD-L1 on tumour cells is at the same time, however, associated with poor prognosis in cancer subjects.

Currently used ICI therapy targeting the PD-1:PD-L1 pathway in combination with chemotherapy leads to unsatisfactory responses especially in subjects having a low PD-L1 expression on cancer cells.

For example, the approved anti-PD-L1 antibody pembrolizumab plus chemotherapy regime is currently considered the standard of care (SOC) in the first-line treatment of patients with metastatic TNBC whose tumours express PD-L1 with a CPS score of $\geq$10; and clinically significant improvements have been observed in progression-free survival (PFS) (9.7 months for pembrolizumab plus chemotherapy versus 5.6 months for chemotherapy alone) and overall survival (OS) (23.0 months for pembrolizumab plus chemotherapy versus 16.1 months for chemotherapy alone). However, these benefits have not been observed in patients with a CPS of <10. For this patient group, the SOC remains chemotherapy alone,

with a median PFS of only about6 months and a median OS of about16 months (Cortes et al. 2022).

There is therefore an unmet need for improved methods for treatment of cancer especially for cancers having a low PD-L1 expression score for which the SOC momentarily is chemotherapy alone with associated poor prognosis of PFS.

SUMMARY OF THE INVENTION

Against the aforementioned background, it is an object of the present invention to provide effective pharmacological means to treat cancer, preferably cancer having a low PD-L1 expression score in a subject. It is also an object of the present invention to provide pharmacological means to elicit a therapeutically effective immune response for the treatment of cancer, preferably cancer having a low PD-L1 expression score.

These objects are achieved by the invention set forth in the claims and embodiments explained in more detail below.

The invention provides a bispecific antibody that specifically binds to programmed death-ligand 1 (PD-L1) and Vascular Endothelial Growth Factor (VEGF) for use in a method of treating a subject with cancer, the method comprising administering to the subject:

a. the bispecific antibody; and

b. a chemotherapy, preferably a chemotherapy agent;

optionally wherein the subject has a PD-L1 expression score before the treatment as determined by a combined positive score (CPS) of ≥ 1 or other integrating scoring algorithm defining a similar PD-L1 expression score.

In other words, the present application provides in an aspect a bispecific antibody that specifically binds to programmed death-ligand 1 (PD-L1) and Vascular Endothelial Growth Factor (VEGF) for use in a method of treating a subject with cancer, the method comprising administering to the subject:

a. the bispecific antibody; and

b. a chemotherapy, preferably a chemotherapy agent.

It can be preferred that the subject has a PD-L1 expression score before the treatment as determined by a combined positive score (CPS) of ≥ 1 or other integrating scoring algorithm defining a similar PD-L1 expression score.

Furthermore, the invention concerns a method of treating cancer in a subject, the method comprising administering to the subject a bispecific antibody that specifically binds to PD-L1 and VEGF in combination with chemotherapy, optionally wherein the subject has a PD-L1 expression score before the treatment as determined by a combined positive score (CPS) of ≥ 1 or other integrating scoring algorithm defining a similar PD-L1 expression score.

In other words, the present application provides in an aspect a method of treating cancer in a subject, the method comprising administering to the subject a bispecific antibody that specifically binds to PD-L1 and VEGF in combination with chemotherapy. It can be preferred that the subject has a PD-L1 expression score before the treatment as determined by a combined positive score (CPS) of ≥ 1 or other integrating scoring algorithm defining a similar PD-L1 expression score.

The invention also concerns a method for determining whether a cancer in a subject is susceptible to treatment with a bispecific antibody that specifically binds to PD-L1 and VEGF and a chemotherapy, wherein the method comprises detecting in a sample of the subject a PD-L1 expression score before the treatment as determined by a combined positive score (CPS) of ≥ 1 or other integrating scoring algorithm defining a similar PD-L1 expression score, wherein the CPS of ≥ 1 indicates that the subject is susceptible to treatment with the bispecific antibody and the chemotherapy.

The invention also concerns a chemotherapy agent for use in a method of treating a subject with cancer, the method comprising administering to the subject:

a. a bispecific antibody that specifically binds to PD-L1 and VEGF; and
b. the chemotherapy agent;

optionally wherein the subject has a PD-L1 expression score before the treatment as determined by a combined positive score (CPS) of ≥ 1 or other integrating scoring algorithm defining a similar PD-L1 expression score.

In some aspects of the invention, there is provided use of a bispecific antibody that specifically binds to PD-L1 and VEGF and a chemotherapy agent in the manufacture of a

medicament for treating cancer in a subject, the use comprising administering to the subject:

a. the bispecific antibody; and
b. the chemotherapy agent.

In some aspects of the invention, there is provided use of a bispecific antibody that specifically binds to PD-L1 and VEGF in the manufacture of a medicament for treating cancer in a subject, the use comprising administering to the subject:

a. the bispecific antibody; and
b. a chemotherapy agent.

In some aspects of the invention, there is provided use of a bispecific antibody that specifically binds to PD-L1 and VEGF and a chemotherapy agent in the manufacture of a medicament for treating cancer in a subject, the use comprising administering to the subject:

a. the bispecific antibody; and
b. the chemotherapy agent.

In some aspects of the invention, there is provided use of a chemotherapy agent in the manufacture of a medicament for treating cancer in a subject, the use comprising administering to the subject:

a. a bispecific antibody that specifically binds to PD-L1 and VEGF; and
b. the chemotherapy agent.

Further, the invention also provides a kit of parts comprising the bispecific antibody that specifically binds to PD-L1 and VEGF and a chemotherapy agent.

DETAILED DESCRIPTION

Although certain embodiments of the present invention are described in detail below, it is to be understood that this invention is not limited to the particular embodiments, methodologies, protocols and reagents described herein as these may vary within the scope set by the claims. It is also to be understood that terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the

present invention which is defined by the appended claims. Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art.

In the following description, certain elements of the present invention will be described. These elements may be discussed with specific embodiments, however, it should be understood that they may be combined in any manner and in any number to create additional embodiments. The variously described examples, features and particular embodiments should not be construed to limit the present invention to only the explicitly described embodiments or to the explicitly described combination of features. This description should be understood to disclose and encompass embodiments which combine the explicitly described embodiments with any number of the disclosed and/or preferred elements. Furthermore, any permutations and combinations of all described elements in this application should be considered disclosed by this description unless the context indicates otherwise.

The above objects are achieved by the following embodiments in accordance with the invention:

1. A bispecific antibody that specifically binds to PD-L1 and VEGF for use in a method of treating a subject with cancer, the method comprising administering to the subject:

   a. the bispecific antibody; and

   b. a chemotherapy, preferably a chemotherapy agent;

   optionally wherein the subject has a PD-L1 expression score before the treatment as determined by a combined positive score (CPS) of ≥ 1 or other integrating scoring algorithm defining a similar PD-L1 expression score.

2. The bispecific antibody for use according to embodiment 1, wherein the other integrating scoring algorithms are selected from TAP and TPS.

3. The bispecific antibody for use according to embodiment 1 or 2, wherein the CPS has been determined in a test sample of the subject by determining the number of PD-L1 staining cells (tumour cells, lymphocytes, macrophages) and the total

number of viable tumour cells in a cancer tissue sample from the subject; and calculating the CPS for the cancer tissue sample using the formula:

$$\text{CPS} = \frac{\#\ \text{PD-L1 staining cells (tumour cells, lymphocytes, macrophages)}}{\text{total \# viable tumour cells}} * 100$$

4. The bispecific antibody for use according to embodiment 3, wherein the PD-L1 staining cells are tumour cells with partial or complete linear membrane staining that is perceived distinct from cytoplasmic staining, and lymphocytes and macrophages within the tumour nests and/or adjacent supporting stroma with membrane and/or cytoplasmic staining.

5. The bispecific antibody for use according to embodiment 3 or 4, wherein the cancer tissue sample is a tissue section of a tumour biopsy.

6. The bispecific antibody for use according to any of the preceding embodiments, wherein the PD-L1 expression is detected by immunohistochemistry (IHC) staining.

7. The bispecific antibody for use according to embodiment 5 or 6, wherein the tissue section is a formalin fixed and embedded in paraffin wax (FFPE) tissue section.

8. The bispecific antibody for use according to any of embodiments 5-7, wherein the tissue section is stained.

9. The bispecific antibody for use according to embodiment 8, wherein the stain comprises a hematoxylin and eosin (H&E) stain.

10. The bispecific antibody for use according to any of embodiments 3-9, wherein the viable tumour cells and the number of lymphocytes and macrophages are counted in the tumour nests and the adjacent supporting stroma of the tumour tissue sample.

11. The bispecific antibody for use according to any of embodiments 3-10, wherein the number of viable tumour cells in the tumour tissue sample is determined by flow cytometry.

12. The bispecific antibody for use according to any of the preceding embodiments, wherein the PD-L1 expression score has been determined using a TAP scoring algorithm in a test sample of the subject by determining the percentage of PD-L1 positive tumour cells and immune cells per tumour area in a cancer tissue sample

from the subject; and calculating the TAP for the cancer tissue sample using the formula:

$$\text{TAP} = \frac{\%\ \text{PD-L1 positive tumour cells and immune cells}}{\text{tumour area}}$$

13. The bispecific antibody for use according to embodiment 12, wherein the tumour area is determined using a hematoxylin and eosin (H&E) stain.

14. The bispecific antibody for use according to embodiment 12 or 13, wherein the PD-L1 positive tumour cells and immune cells are PD-L1 staining cells with partial or complete linear membrane staining that is perceived distinct from cytoplasmic staining, and immune cells (lymphocytes and macrophages) within the tumour nests and/or adjacent supporting stroma with membrane and/or cytoplasmic staining.

15. The bispecific antibody for use according to any of the preceding embodiments, wherein the PD-L1 expression score has been determined using a TPS scoring algorithm by determining in a test sample of the subject the number of tumour cells positive for PD-L1 and the total number of viable tumour cells in a cancer tissue sample from the subject; and calculating the TPS for the cancer tissue sample using the formula:

$$\text{TPS} = \frac{\#\ viable\ tumor\ cells\ positive\ for\ PD\text{-}L1}{\text{total \# viable tumour cells}} * 100$$

16. The bispecific antibody for use according to any of the preceding embodiments, wherein the bispecific antibody and the chemotherapy are separately administered.

17. The bispecific antibody for use according to any of the preceding embodiments, wherein a dosage of the bispecific antibody and a dosage of the chemotherapy are administered concurrently or consecutively.

18. The bispecific antibody for use according to any of the preceding embodiments, wherein the chemotherapy comprises a platinum-based chemotherapy.

19. The bispecific antibody for use according to any of the preceding embodiments, wherein the subject has a PD-L1 expression score as determined by a CPS of up to 20, preferably from 1 to 20, more preferably from 1 to <10, before the treatment or other integrating scoring algorithm defining a similar PD-L1 expression score.

20. The bispecific antibody for use according to any of the preceding embodiments, wherein the bispecific antibody dosage is in a range of from 0.1 mg/kg to 45 mg/kg body weight, preferably 1 mg/kg to 30 mg/kg body weight.

21. The bispecific antibody for use according to any of the preceding embodiments, wherein the bispecific antibody and/or the chemotherapy is administered intravenously, preferably wherein the bispecific antibody and the chemotherapy are administered intravenously.

22. The bispecific antibody for use according to any of the preceding embodiments, wherein the bispecific antibody and/or the chemotherapy is administered via an IV injection or IV infusion.

23. The bispecific antibody for use according to any of the preceding embodiments, wherein a treatment cycle is repeated at least 1, 2, 3, 4, 5, 6, 7, or 8 times.

24. The bispecific antibody for use according to any of the preceding embodiments, wherein each cycle has up to 28 days, preferably 28 or 21 days.

25. The bispecific antibody for use according to any of the preceding embodiments, wherein the subject has not been previously treated for cancer.

26. The bispecific antibody for use according to any of embodiments 1-24, wherein the subject has been previously treated for cancer, preferably wherein the subject had at least one previous chemotherapy treatment.

27. The bispecific antibody for use according to any of the preceding embodiments, wherein the bispecific antibody is administered every 6 weeks, preferably every 4 weeks, more preferably every 3 weeks or every 2 weeks.

28. The bispecific antibody for use according to any of the preceding embodiments, wherein the bispecific antibody is administered every 2 weeks at a dosage ranging from 10 mg/kg to 30 mg/kg, preferably ranging from 15 mg/kg to 25 mg/kg, more preferably at a dosage of 20 mg/kg.

29. The bispecific antibody for use according to any of the preceding embodiments, wherein the bispecific antibody is administered every 3 weeks at a dosage ranging

from 20 mg/kg to 40 mg/kg, preferably ranging from 25 mg/kg to 35 mg/kg, more preferably at a dosage of 30 mg/kg.

30. The bispecific antibody for use according to any of the preceding embodiments, wherein the chemotherapy is administered once or more within the first 20 days or the first 21 days of each cycle, wherein the chemotherapy is administered twice or more within the first 20 days or the first 21 days of each cycle, more preferably wherein the chemotherapy is administered at least thrice within the first 20 days or the first 21 days of each cycle.

31. The bispecific antibody for use according to any of the preceding embodiments, wherein the chemotherapy is administered once or more within the first 14 days or the first 15 days of each cycle, wherein the chemotherapy is administered twice or more within the first 14 days or the first 15 days of each cycle, more preferably wherein the chemotherapy is administered at least thrice within the first 14 days or the first 15 days of each cycle.

32. The bispecific antibody for use according to any of the preceding embodiments, wherein the chemotherapy is administered on the 1st, 8th, and 15th days of each cycle.

33. The bispecific antibody for use according to any of the preceding embodiments, wherein overall survival is increased in said subject compared to the chemotherapy or the bispecific antibody or an anti-PD-L1 antibody or an anti-PD-1 antibody treatment alone or compared to a standard treatment comprising the chemotherapy and the anti-PD-L1 antibody or the chemotherapy and the anti-PD-1 antibody, wherein the anti-PD-L1 antibody is preferably atezolizumab and the anti-PD-1 antibody is preferably pembrolizumab.

34. The bispecific antibody for use according to any of the preceding embodiments, wherein median progression-free survival is increased in said subject compared to the chemotherapy or the bispecific antibody or an anti-PD-L1 antibody or an anti-PD-1 antibody treatment alone or compared to a standard treatment comprising the chemotherapy and the anti-PD-L1 antibody or the chemotherapy and the anti-PD-1 antibody, wherein the anti-PD-L1 antibody is preferably atezolizumab and the anti-PD-1 antibody is preferably pembrolizumab.

35. The bispecific antibody for use according to any of the preceding embodiments, wherein the cancer comprises one or more solid tumours.

36. The bispecific antibody for use according to any of the preceding embodiments, wherein the cancer is selected from the group consisting of melanoma, lung, liver, stomach, renal cell, urothelial, cervical, colorectal, ovarian, colon, breast, esophagus, mesothelioma, and head and neck cancers, preferably wherein the cancer is selected from urothelial, breast and esophagus cancer.

37. The bispecific antibody for use according to any of the preceding embodiments, wherein the cancer is small cell lung cancer (SCLC), non-small cell lung cancer (NSCLC) or triple-negative breast cancer (TNBC), preferably advanced triple-negative breast cancer.

38. The bispecific antibody for use according to embodiment 37, wherein the NSCLC has a squamous histology.

39. The bispecific antibody for use according to embodiment 37, wherein the NSCLC has a non-squamous histology.

40. The bispecific antibody for use according to embodiment 37, wherein the NSCLC is an EGFR mutation-positive NSCLC.

41. The bispecific antibody for use according to any of the preceding embodiments, wherein the bispecific antibody comprises an anti-PD-L1 antibody or fragment thereof.

42. The bispecific antibody for use according to any of the preceding embodiments, wherein the bispecific antibody comprises an anti-VEGF antibody or fragment thereof.

43. The bispecific antibody for use according to any of the preceding embodiments, wherein the bispecific antibody comprises a Fab, Fab', F(ab')2, Fd, Fv, dAb, complementarity determining region fragment, single chain antibody, humanized antibody, chimeric antibody or diabody antibody, preferably a single domain antibody, more preferably a VHH.

44. The bispecific antibody for use according to any of the preceding embodiments, wherein the bispecific antibody comprises two anti-PD-L1 single domain antibodies, preferably two VHHs, preferably wherein each VHH is fused to the C-terminus of an anti-VEGF antibody.

45. The bispecific antibody for use according to any of the preceding embodiments, wherein the bispecific antibody comprises an anti-PD-L1 single domain antibody comprising a heavy chain variable region, and the heavy chain variable region comprises: (i) a complementarity-determining region 1 (HCDR1) whose amino acid sequence is shown in SEQ ID NO: 1 or 18, (ii) a complementarity-determining region 2 (HCDR2) whose amino acid sequence is shown in SEQ ID NO: 2 or 19, and (iii) a complementarity-determining region 3 (HCDR3) whose amino acid sequence is shown in SEQ ID NO: 3 or 38.

46. The bispecific antibody for use according to embodiment 45, wherein the amino acid sequence of the anti-PD-L1 single domain antibody is shown in SEQ ID NO: 9 or wherein the amino acid sequence of the anti-PD-L1 single domain antibody has at least 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 9.

47. The bispecific antibody for use according to any of embodiments 42-46, wherein the anti-VEGF antibody or fragment thereof comprises a constant region preferably derived from a human antibody, preferably the constant region is selected from the constant region of human IgG1, IgG2, IgG3 or IgG4.

48. The bispecific antibody for use according to any of embodiments 42-47, wherein the anti-VEGF antibody or fragment thereof comprises a IgG1 Fc region, preferably having the amino acid sequence shown in SEQ ID NO: 13 or having at least 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence shown in SEQ ID NO: 13.

49. The bispecific antibody for use according to any of the preceding embodiments, wherein the bispecific antibody specifically binds to VEGF-A.

50. The bispecific antibody for use according to any of embodiments 42-49, wherein a heavy chain variable region of the anti-VEGF antibody comprises: (i) a complementarity-determining region 1 (HCDR1) whose amino acid sequence is

shown in SEQ ID NO: 4, (ii) a complementarity-determining region 2 (HCDR2) whose amino acid sequence is shown in SEQ ID NO: 5, and (iii) a complementarity-determining region 3 (HCDR3) whose amino acid sequence is shown in SEQ ID NO: 6; and

wherein a light chain variable region of the anti-VEGF antibody comprises: (i) a complementarity-determining region 1 (LCDR1) whose amino acid sequence is shown in SEQ ID NO: 7, (ii) a complementarity-determining region 2 (LCDR2) whose amino acid sequence is shown in SEQ ID NO: 44, and (iii) a complementarity-determining region 3 (LCDR3) whose amino acid sequence is shown in SEQ ID NO: 8.

51. The bispecific antibody for use according to embodiment 50, wherein the amino acid sequence of the heavy chain variable region of the anti-VEGF antibody is shown in SEQ ID NO: 10, and the amino acid sequence of the light chain variable region of the anti-VEGF antibody is shown in SEQ ID NO: 11; or
wherein the amino acid sequence of the heavy chain variable region of the anti-VEGF antibody has at least 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 10, and the amino acid sequence of the light chain variable region of the anti-VEGF antibody has at least 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 11.

52. The bispecific antibody for use according to any of the preceding embodiments, wherein the amino acid sequence of the heavy chain of the bispecific antibody is shown in SEQ ID NO: 16, and the amino acid sequence of the light chain variable region of the bispecific antibody is shown in SEQ ID NO: 17; or
wherein the amino acid sequence of the heavy chain of the bispecific antibody has at least 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 16, and the amino acid sequence of the light chain variable region of the bispecific antibody has at least 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 17.

53. The bispecific antibody for use according to any of embodiments 42-52, wherein the anti-VEGF antibody is bevacizumab.

54. The bispecific antibody for use according to any of the preceding embodiments, wherein the bispecific antibody is encoded by one or more nucleic acid molecules.

55. The bispecific antibody for use according to any of the preceding embodiments, wherein the chemotherapy comprises a chemotherapy agent selected from lurbinectedin, topotecan, taxane such as paclitaxel, docetaxel, or nanoparticle albumin-bound paclitaxel (nab-paclitaxel), pemetrexed, 5-fluoruracil, irinotecan, etoposide, gemcitabine, anthracyclines, capecitabine, vinorelbine, eribulin, ixabepilone or combinations thereof.

56. The bispecific antibody for use according to any of embodiments 18-55, wherein the platinum-based chemotherapy comprises cisplatin, oxaliplatin or carboplatin.

57. The bispecific antibody for use according to any of the preceding embodiments, wherein the method of treatment comprises administering the bispecific antibody in combination with paclitaxel to a subject having small cell lung cancer.

58. The bispecific antibody for use according to any of embodiments 1-55, wherein the method of treatment comprises administering the bispecific antibody in combination with pemetrexed and carboplatin (preferably as first line treatment of non-squamous NSCLC) or a taxane (preferably as first line treatment of squamous NSCLC), such as paclitaxel or docetaxel (preferably as second line treatment of NSCLC), to a subject having NSCLC, preferably as second line treatment, or wherein the method of treatment comprises administering the bispecific antibody in combination with pemetrexed and carboplatin to a subject having malignant mesothelioma.

59. The bispecific antibody for use according to any of embodiments 1-55, wherein the method of treatment comprises administering the bispecific antibody in combination with nab-paclitaxel to a subject having triple-negative breast cancer.

60. The bispecific antibody for use according to any of embodiments 1-55, wherein the method of treatment comprises administering the bispecific antibody in combination with oxaliplatin, calcium folinate, and 5-fluorouracil to a subject having hepatocellular carcinoma, preferably wherein the method of treatment comprises administering the bispecific antibody every 2 weeks at a dosage ranging from 20 mg/kg to 30 mg/kg (with 20 mg/kg being particularly preferred).

61. The bispecific antibody for use according to any of embodiments 1-55, wherein the method of treatment comprises administering the bispecific antibody in combination

with irinotecan, 5-fluorouracil, calcium folinate to a subject having unresectable neuroendocrine neoplasm.

62. The bispecific antibody for use according to any of embodiments 1-55, wherein the method of treatment comprises administering the bispecific antibody in combination with etoposide and platinum to a subject having small cell lung cancer, preferably as first line treatment and/or extensive-stage small cell lung cancer, most preferably as first line treatment of extensive-stage small cell lung cancer or as second-line treatment of limited-stage small cell lung cancer.

63. The bispecific antibody for use according to embodiment 62, wherein the method of treatment comprises administering the bispecific antibody every 3 weeks at a dosage ranging from 20 mg/kg to 30 mg/kg (with 20 mg/kg or 30 mg/kg being particularly preferred) or at a dose ranging from 1400 mg to 2000 mg in combination with etoposide (preferably at a dosage of 100 mg/m^2) and with carboplatin (preferably at a dosage of AUC=5 (total dose $\leq$ 750 mg) to a subject having small cell lung cancer, preferably as first line treatment and/or extensive-stage small cell lung cancer, most preferably as first line treatment of extensive-stage small cell lung cancer or as second-line treatment of limited-stage small cell lung cancer.

64. The bispecific antibody for use according to any of embodiments 1-55, wherein the method of treatment comprises administering the bispecific antibody every 3 weeks at a dosage ranging from 20 mg/kg to 30 mg/kg in combination with paclitaxel, lurbinectedin, or topotecan to a subject having small cell lung cancer, preferably as second line treatment and/or extensive-stage small cell lung cancer, more preferably wherein the method of treatment comprises administering to a patient suffering from advanced small cell lung cancer as second line treatment the bispecific antibody every 3 weeks at a dosage of 20 mg/kg or 30 mg/kg in combination with (i) paclitaxel every 3 weeks at a dosage of 175 mg/m^2, optionally for 5 cycles, or (ii) topotecan at a dosage of 1.5 mg/m^2 administered IV or 2.3 mg/m^2 administered orally once per day on days 1 to 5 of every 3 weeks cycle.

65. The bispecific antibody for use according to any of embodiments 1-55, wherein the method of treatment comprises administering the bispecific antibody every 2 weeks

at a dosage ranging from 10 mg/kg to 20 mg/kg in combination with nab-paclitaxel, paclitaxel, or gemcitabine with carboplatin to a subject having triple-negative breast cancer.

66. The bispecific antibody for use according to any of embodiments 1-55, wherein the method of treatment comprises administered the bispecific antibody every 2 weeks at a dosage of 1000 mg to 2000 mg to a subject having triple-negative breast cancer, preferably on the 1st and 15th day of a 28-day treatment cycle, preferably wherein the bispecific antibody is administered in combination with a chemotherapy, preferably on the 1st, 8th, and 15th day of a 28-day treatment cycle.

67. A method of treating cancer in a subject comprising administering to the subject a bispecific antibody that specifically bind to PD-L1 and VEGF in combination with chemotherapy, optionally wherein the subject has a PD-L1 expression score before the treatment as determined by a combined positive score (CPS) of ≥ 1, preferably from 1 to 20, preferably from 1 to <10, or other integrating scoring algorithm defining a similar PD-L1 expression score.

68. The method according to embodiment 67, wherein the method is a method for extending progression-free survival in said subject compared to the chemotherapy or an anti-PD-L1 antibody or an anti-PD-1 antibody or the bispecific antibody treatment alone or compared to a standard treatment comprising the chemotherapy and the anti-PD-L1 antibody or the chemotherapy and the anti-PD-1 antibody, wherein the anti-PD-L1 antibody is preferably atezolizumab and the anti-PD-1 antibody is preferably pembrolizumab.

69. The method according to embodiment 67 or 68, wherein the method is a method for increased overall survival in said subject compared to the chemotherapy or the bispecific antibody or an anti-PD-L1 antibody or an anti-PD-1 antibody treatment alone or compared to a standard treatment comprising the chemotherapy and the anti-PD-L1 antibody or the chemotherapy and the anti-PD-1 antibody, wherein the anti-PD-L1 antibody is preferably atezolizumab and the anti-PD-1 antibody is preferably pembrolizumab.

70. A method for determining whether a cancer in a subject is susceptible to treatment with a bispecific antibody that specifically binds to PD-L1 and VEGF and a

chemotherapy, wherein the method comprises detecting in a sample of the subject a PD-L1 expression score before the treatment as determined by a combined positive score (CPS) of ≥ 1 or other integrating scoring algorithm defining a similar PD-L1 expression score, wherein the CPS of ≥ 1 indicates that the subject is susceptible to treatment with the bispecific antibody and the chemotherapy.

71. The method according to embodiment 70, wherein the cancer is selected from the group consisting of melanoma, lung, liver, stomach, renal cell, urothelial, cervical, colorectal, ovarian, colon, breast, esophagus, mesothelioma, and head and neck cancers, preferably wherein the cancer is selected from urothelial, breast and esophagus cancer.

72. The method according to embodiment 70 or 71, wherein the sample is a cancer tissue sample.

73. The method according to any of embodiments 70-72, wherein the bispecific antibody comprises an anti-PD-L1 antibody or fragment thereof.

74. The method according to any of embodiments 70-73, wherein the bispecific antibody comprises an anti-VEGF antibody or fragment thereof.

75. The method according to any of embodiments 70-74, wherein the bispecific antibody comprises a Fab, Fab', F(ab')2, Fd, Fv, sdAb, complementarity determining region fragment, single chain antibody, humanized antibody, chimeric antibody or diabody antibody, preferably a single domain antibody, more preferably a VHH.

76. The method according to any of embodiments 70-75, wherein the bispecific antibody comprises two anti-PD-L1 single domain antibodies, preferably two VHHs, preferably each VHH is fused to the C-terminus of the anti-VEGF antibody.

77. The method according to any of embodiments 70-76, wherein the bispecific antibody comprises an anti-PD-L1 single domain antibody comprising a heavy chain variable region, and the heavy chain variable region comprises: (i) a complementarity-determining region 1 (HCDR1) whose amino acid sequence is shown in SEQ ID NO: 1 or 18, (ii) a complementarity-determining region 2

(HCDR2) whose amino acid sequence is shown in SEQ ID NO: 2 or 19, and (iii) a complementarity-determining region 3 (HCDR3) whose amino acid sequence is shown in SEQ ID NO: 3 or 38.

78. The method according to embodiment 77, wherein the amino acid sequence of the anti-PD-L1 single domain antibody is shown in SEQ ID NO: 9 or wherein the amino acid sequence of the anti-PD-L1 single domain antibody has at least 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 9.

79. The method according to any of embodiments 74-78, wherein the anti-VEGF antibody or fragment thereof comprises a constant region preferably derived from a human antibody, preferably, the constant region is selected from the constant region of human IgGl, IgG2, IgG3 or IgG4.

80. The method according to any of embodiments 74-79, wherein the anti-VEGF antibody or fragment thereof comprises a IgG1 Fc region, preferably having the amino acid sequence shown in SEQ ID NO: 13 or having at least 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence shown in SEQ ID NO: 13.

81. The method according to any of embodiments 70-80, wherein the bispecific antibody specifically binds to VEGF-A.

82. The method according to any of embodiments 74-81, wherein a heavy chain variable region of the anti-VEGF antibody comprises: (i) a complementarity-determining region 1 (HCDR1) whose amino acid sequence is shown in SEQ ID NO: 4, (ii) a complementarity-determining region 2 (HCDR2) whose amino acid sequence is shown in SEQ ID NO: 5, and (iii) a complementarity-determining region 3 (HCDR3) whose amino acid sequence is shown in SEQ ID NO: 6; and a light chain variable region of the anti-VEGF antibody comprises: (i) a complementarity-determining region 1 (LCDR1) whose amino acid sequence is shown in SEQ ID NO: 7, (ii) a complementarity-determining region 2 (LCDR2) whose amino acid sequence is shown in SEQ ID NO: 44, and (iii) a complementarity-determining region 3 (LCDR3) whose amino acid sequence is shown in SEQ ID NO: 8.

83. The method according to any of embodiments 74-82, wherein the amino acid sequence of the heavy chain variable region of the anti-VEGF antibody is shown in SEQ ID NO: 10, and the amino acid sequence of the light chain variable region of the anti-VEGF antibody is shown in SEQ ID NO: 11; or
wherein the amino acid sequence of the heavy chain variable region of the anti-VEGF antibody has at least 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 10, and the amino acid sequence of the light chain variable region of the anti-VEGF antibody has at least 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 11.

84. The method according to any of embodiments 70-83, wherein the amino acid sequence of the heavy chain of the bispecific antibody is shown in SEQ ID NO: 16, and the amino acid sequence of the light chain variable region of the bispecific antibody is shown in SEQ ID NO: 17; or
wherein the amino acid sequence of the heavy chain of the bispecific antibody has at least 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 16, and the amino acid sequence of the light chain variable region of the bispecific antibody has at least 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 17.

85. The method according to any of embodiments 70-84, wherein the anti-VEGF antibody is bevacizumab.

86. The method according to any of embodiments 70-85, wherein the method comprises the step of determining the CPS in a test sample of the subject by determining the number of PD-L1 staining cells (tumour cells, lymphocytes, macrophages) and the total number of viable tumour cells in a cancer tissue sample from the subject; and calculating the CPS for the cancer tissue sample using the formula:

$$\text{CPS} = \frac{\#\ \text{PD-L1 staining cells (tumour cells, lymphocytes, macrophages)}}{\text{total}\ \#\ \text{viable tumour cells}} * 100$$

87. The method according to embodiment 86, wherein PD-L1 staining cells are tumour cells with partial or complete linear membrane staining that is distinct from cytoplasmic staining, and lymphocytes and macrophages within the tumour nests and/or adjacent supporting stroma with membrane and/or cytoplasmic staining.

88. The method according to embodiment 86 or 87, wherein the cancer tissue sample is a tissue section of a tumour biopsy.

89. The method according to any of embodiments 70-88, wherein PD-L1 expression is detected by immunohistochemistry (IHC) staining.

90. The method according to embodiment 88 or 89, wherein the tissue section is a formalin fixed and embedded in paraffin wax (FFPE) tissue section.

91. The method according to any of embodiments 88-90, wherein the tissue section is stained.

92. The method according to embodiment 91, wherein the stain comprises a hematoxylin and eosin (H&E) stain.

93. The method according to any of embodiments 86-92, wherein the viable tumour cells and the number of lymphocytes and macrophages are counted in the tumour nests and the adjacent supporting stroma of the tumour tissue sample.

94. The method according to any of embodiments 86-93, wherein the number of viable tumour cells in the tumour tissue sample is determined by flow cytometry.

95. A kit of parts comprising a bispecific antibody that specifically binds to PD-L1 and VEGF and a chemotherapy agent.

96. The kit of parts according to embodiment 95, wherein the bispecific antibody and the chemotherapy agent are comprised in separate container.

97. The kit of parts according to embodiment 95 or 96, further comprising instructions for use.

98. A chemotherapy agent for use in a method of treating a subject with cancer, the method comprising administering to the subject:

a. a bispecific antibody that specifically binds to PD-L1 and VEGF; and

b. the chemotherapy agent;

optionally wherein the subject has a PD-L1 expression score before the treatment as determined by a combined positive score (CPS) of ≥ 1 or other integrating scoring algorithm defining a similar PD-L1 expression score.

99. The chemotherapy agent for use according to embodiment 98, wherein the other integrating scoring algorithms are selected from TAP and TPS.

100. The chemotherapy agent for use according to embodiment 98 or 99, wherein the CPS has been determined in a test sample of the subject by determining the number of PD-L1 staining cells (tumour cells, lymphocytes, macrophages) and the total number of viable tumour cells in a cancer tissue sample from the subject; and calculating the CPS for the cancer tissue sample using the formula:

$$\text{CPS} = \frac{\#\ \text{PD-L1 staining cells (tumour cells, lymphocytes, macrophages)}}{\text{total}\ \#\ \text{viable tumour cells}} * 100$$

101. The chemotherapy agent for use according to embodiment 100, wherein the PD-L1 staining cells are tumour cells with partial or complete linear membrane staining that is perceived distinct from cytoplasmic staining, and lymphocytes and macrophages within the tumour nests and/or adjacent supporting stroma with membrane and/or cytoplasmic staining.

102. The chemotherapy agent for use according to embodiments 100 or 101, wherein the cancer tissue sample is a tissue section of a tumour biopsy.

103. The chemotherapy agent for use according to any of embodiments 98-102, wherein the PD-L1 expression is detected by immunohistochemistry (IHC) staining.

104. The chemotherapy agent for use according to any of embodiments 102 or 103, wherein the tissue section is a formalin fixed and embedded in paraffin wax (FFPE) tissue section.

105. The chemotherapy agent for use according to any of embodiments 102-104, wherein the tissue section is stained.

106. The chemotherapy agent for use according to embodiment 105, wherein the stain comprises a hematoxylin and eosin (H&E) stain.

107. The chemotherapy agent for use according to any of embodiments 100-106, wherein the viable tumour cells and the number of lymphocytes and macrophages are counted in the tumour nests and the adjacent supporting stroma of the tumour tissue sample.

108. The chemotherapy agent for use according to any of embodiments 100-107, wherein the number of viable tumour cells in the tumour tissue sample is determined by flow cytometry.

109. The chemotherapy agent for use according to any of embodiments 98-108, wherein the PD-L1 expression score has been determined using a TAP scoring algorithm in a test sample of the subject by determining the percentage of PD-L1 positive tumour cells and immune cells per tumour area in a cancer tissue sample from the subject; and calculating the TAP for the cancer tissue sample using the formula:

$$\text{TAP} = \frac{\%\ \text{PD-L1 positive tumour cells and immune cells}}{\text{tumour area}}$$

110. The chemotherapy agent for use according to embodiment 109, wherein the tumour area is determined using a hematoxylin and eosin (H&E) stain.

111. The chemotherapy agent for use according to embodiment 109 or 110, wherein the PD-L1 positive tumour cells and immune cells are PD-L1 staining cells with partial or complete linear membrane staining that is perceived distinct from cytoplasmic staining, and immune cells (lymphocytes and macrophages) within the tumour nests and/or adjacent supporting stroma with membrane and/or cytoplasmic staining.

112. The chemotherapy agent for use according to any of embodiments 98-111, wherein the PD-L1 expression score has been determined using a TPS scoring algorithm by determining in a test sample of the subject the number of viable tumour cells positive for PD-L1 (PD-L1 staining tumour cells)tumour and the total number of viable tumour cells in a cancer tissue sample from the subject; and calculating the TPS for the cancer tissue sample using the formula:

$$\text{TPS} = \frac{\#\ viable\ tumor\ cells\ positive\ for\ PD\text{-}L1}{\text{total \# viable tumour cells}} * 100$$

113. The chemotherapy agent for use according to any of embodiments 98-112, wherein the bispecific antibody and the chemotherapy are separately administered.

114. The chemotherapy agent for use according to any of embodiments 98-113, wherein a dosage of the bispecific antibody and a dosage of the chemotherapy are administered concurrently or consecutively.

115. The chemotherapy agent for use according to any of embodiments 98-114, wherein the chemotherapy comprises a platinum-based chemotherapy.

116. The chemotherapy agent for use according to any of embodiments 98-115, wherein the subject has a PD-L1 expression score before the treatment as determined by a CPS of up to 20, preferably from 1 to 20, more preferably from 1 to <10, or other integrating scoring algorithm defining a similar PD-L1 expression score.

117. The chemotherapy agent for use according to any of embodiments 98-116, wherein the bispecific antibody dosage is in a range of from 0.1 mg/kg to 45 mg/kg body weight, preferably 1 mg/kg to 30 mg/kg body weight.

118. The chemotherapy agent for use according to any of embodiments 98-117, wherein the bispecific antibody and/or the chemotherapy is administered intravenously, preferably wherein the bispecific antibody and the chemotherapy are administered intravenously.

119. The chemotherapy agent for use according to any of embodiments 98-118, wherein the bispecific antibody and/or the chemotherapy is administered via an IV injection or IV infusion.

120. The chemotherapy agent for use according to any of embodiments 98-119, wherein a treatment cycle is repeated at least 1, 2, 3, 4, 5, 6, 7 or 8 times.

121. The chemotherapy agent for use according to any of embodiments 98-120, wherein each cycle has up to 28 days, preferably 28 or 21 days.

122. The chemotherapy agent for use according to any of embodiments 98-121, wherein the subject has not been previously treated for cancer.

123. The chemotherapy agent for use according to any of embodiments 98-121, wherein the subject has been previously treated for cancer, preferably wherein the subject had at least one previous chemotherapy treatment.

124. The chemotherapy agent for use according to any of embodiments 98-123, wherein the bispecific antibody is administered every 6 weeks, preferably every 4 weeks, more preferably every 3 weeks or every 2 weeks.

125. The chemotherapy agent for use according to any of embodiments 98-124, wherein the bispecific antibody is administered every 2 weeks at a dosage ranging from 10 mg/kg to 30 mg/kg, preferably ranging from 15 mg/kg to 25 mg/kg, more preferably at a dosage of 20 mg/kg.

126. The chemotherapy agent for use according to any of embodiments 98-125, wherein the bispecific antibody is administered every 3 weeks at a dosage ranging from 20 mg/kg to 40 mg/kg, preferably ranging from 25 mg/kg to 35 mg/kg, more preferably at a dosage of 30 mg/kg.

127. The chemotherapy agent for use according to any of embodiments 98-126, wherein the chemotherapy is administered once or more within the first 20 days or the first 21 days of each cycle, wherein the chemotherapy is administered twice or more within the first 20 days or the first 21 days of each cycle, more preferably wherein the chemotherapy is administered at least thrice within the first 20 days or the first 21 days of each cycle.

128. The chemotherapy agent for use according to any of embodiments 98-127, wherein the chemotherapy is administered once or more within the first 14 days or the first 15 days of each cycle, wherein the chemotherapy is administered twice or more within the first 14 days or the first 15 days of each cycle, more preferably wherein the chemotherapy is administered at least thrice within the first 14 days or the first 15 days of each cycle.

129. The chemotherapy agent for use according to any of embodiments 98-128, wherein the chemotherapy is administered on the 1st, 8th, and 15th days of each cycle.

130. The chemotherapy agent for use according to any of embodiments 98-129, wherein overall survival is increased in said subject compared to the chemotherapy or the bispecific antibody or an anti-PD-L1 antibody or an anti-PD-1 antibody treatment alone or compared to a standard treatment comprising the chemotherapy and the anti-PD-L1 antibody or the chemotherapy and the anti-PD-1 antibody, wherein the anti-PD-L1 antibody is preferably atezolizumab and the anti-PD-1 antibody is preferably pembrolizumab.

131. The chemotherapy agent for use according to any of embodiments 98-130, wherein median progression-free survival is increased in said subject compared to the chemotherapy or the bispecific antibody or an anti-PD-L1 antibody or an anti-PD-1 antibody treatment alone or compared to a standard treatment comprising the chemotherapy and the anti-PD-L1 antibody or the chemotherapy and the anti-PD-1 antibody, wherein the anti-PD-L1 antibody is preferably atezolizumab and the anti-PD-1 antibody is preferably pembrolizumab.

132. The chemotherapy agent for use according to any of embodiments 98-131, wherein the cancer comprises one or more solid tumours.

133. The chemotherapy agent for use according to any of embodiments 98-132, wherein the cancer is selected from the group consisting of melanoma, lung, liver, stomach, renal cell, urothelial, cervical, colorectal, ovarian, colon, breast, esophagus, mesothelioma, and head and neck cancers, preferably wherein the cancer is selected from urothelial, breast and esophagus cancer.

134. The chemotherapy agent for use according to any of embodiments 98-133, wherein the cancer is small cell lung cancer (SCLC), non-small cell lung cancer (NSCLC) or triple-negative breast cancer (TNBC), preferably advanced triple-negative breast cancer.

135. The chemotherapy agent for use according to embodiment 134, wherein the NSCLC has a squamous histology.

136. The chemotherapy agent for use according to embodiment 134, wherein the NSCLC has a non-squamous histology.

137. The chemotherapy agent for use according to embodiment 134, wherein the NSCLC is an EGFR mutation-positive NSCLC.

138. The chemotherapy agent for use according to any of embodiments 98-137, wherein the bispecific antibody comprises an anti-PD-L1 antibody or fragment thereof.

139. The chemotherapy agent for use according to any of embodiments 98-138, wherein the bispecific antibody comprises an anti-VEGF antibody or fragment thereof.

140. The chemotherapy agent for use according to any of embodiments 98-139, wherein the bispecific antibody comprises a Fab, Fab', F(ab')2, Fd, Fv, sdAb, complementarity determining region fragment, single chain antibody, humanized antibody, chimeric antibody or diabody antibody, preferably a single domain antibody, more preferably a VHH.

141. The chemotherapy agent for use according to any of embodiments 98-140, wherein the bispecific antibody comprises two anti-PD-L1 single domain antibodies, preferably two VHHs, preferably wherein each VHH is fused to the C-terminus of the anti-VEGF antibody.

142. The chemotherapy agent for use according to any of embodiments 98-141, wherein the bispecific antibody comprises an anti-PD-L1 single domain antibody comprising a heavy chain variable region, and the heavy chain variable region comprises: (i) a complementarity-determining region 1 (HCDR1) whose amino acid sequence is shown in SEQ ID NO: 1 or 18, (ii) a complementarity-determining region 2 (HCDR2) whose amino acid sequence is shown in SEQ ID NO: 2 or 19, and (iii) a complementarity-determining region 3 (HCDR3) whose amino acid sequence is shown in SEQ ID NO: 3 or 38.

143. The chemotherapy agent for use according to embodiment 142, wherein the amino acid sequence of the anti-PD-L1 single domain antibody is shown in SEQ ID NO: 9 or wherein the amino acid sequence of the anti-PD-L1 single domain antibody has at least 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 9.

144. The chemotherapy agent for use according to any of embodiments 139-143, wherein the anti-VEGF antibody or fragment thereof comprises a constant region preferably derived from a human antibody, preferably the constant region is selected from the constant region of human IgG1, IgG2, IgG3 or IgG4.

145. The chemotherapy agent for use according to any of embodiments 139-144, wherein the anti-VEGF antibody or fragment thereof comprises a IgG1 Fc region, preferably having the amino acid sequence shown in SEQ ID NO: 13 or having at least 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence shown in SEQ ID NO: 13.

146. The chemotherapy agent for use according to any of embodiments 98-145, wherein the bispecific antibody specifically binds to VEGF-A.

147. The chemotherapy agent for use according to any of embodiments 139-146, wherein a heavy chain variable region of the anti-VEGF antibody comprises: (i) a complementarity-determining region 1 (HCDR1) whose amino acid sequence is shown in SEQ ID NO: 4, (ii) a complementarity-determining region 2 (HCDR2) whose amino acid sequence is shown in SEQ ID NO: 5, and (iii) a complementarity-determining region 3 (HCDR3) whose amino acid sequence is shown in SEQ ID NO: 6; and
a light chain variable region of the anti-VEGF antibody comprises: (i) a complementarity-determining region 1 (LCDR1) whose amino acid sequence is shown in SEQ ID NO: 7, (ii) a complementarity-determining region 2 (LCDR2) whose amino acid sequence is shown in SEQ ID NO: 44, and (iii) a complementarity-determining region 3 (LCDR3) whose amino acid sequence is shown in SEQ ID NO: 8.

148. The chemotherapy agent for use according to embodiments 147, wherein the amino acid sequence of the heavy chain variable region of the anti-VEGF antibody is shown in SEQ ID NO: 10, and the amino acid sequence of the light chain variable region of the anti-VEGF antibody is shown in SEQ ID NO: 11; or
wherein the amino acid sequence of the heavy chain variable region of the anti-VEGF antibody has at least 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 10, and the amino acid sequence of the light chain variable region of the anti-VEGF antibody has at least 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 11.

149. The chemotherapy agent for use according to any of embodiments 98-148, wherein the amino acid sequence of the heavy chain of the bispecific antibody is shown in SEQ ID NO: 16, and the amino acid sequence of the light chain variable region of the bispecific antibody is shown in SEQ ID NO: 17; or
wherein the amino acid sequence of the heavy chain of the bispecific antibody has at least 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 16, and the amino acid sequence of the light chain variable region of the bispecific antibody has at least 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 17.

150. The chemotherapy agent for use according to any of embodiments 139-149, wherein the anti-VEGF antibody is bevacizumab.

151. The chemotherapy agent for use according to any of embodiments 98-150, wherein the bispecific antibody is encoded by one or more nucleic acid molecules.

152. The chemotherapy agent for use according to any of embodiments 98-151, wherein the chemotherapy agent is selected from lurbinectedin, topotecan, taxane such as paclitaxel, nanoparticle albumin-bound paclitaxel (nab-paclitaxel), or docetaxel, pemetrexed, 5-fluoruracil, irinotecan, etoposide, gemcitabine, anthracyclines, capecitabine, vinorelbine, eribulin, ixabepilone or combinations thereof.

153. The chemotherapy agent for use according to any of embodiments 115-152, wherein the platinum-based chemotherapy comprises cisplatin, oxaliplatin or carboplatin.

154. The chemotherapy agent for use according to any of embodiments 98-153, wherein the method of treatment comprises administering the bispecific antibody in combination with paclitaxel to a subject having small cell lung cancer.

155. The chemotherapy agent for use according to any of embodiments 98-154, wherein the method of treatment comprises administering the bispecific antibody in combination with pemetrexed and carboplatin (preferably as first line treatment of non-squamous NSCLC) or a taxane (preferably as first line treatment of squamous NSCLC), such paclitaxel or docetaxel (preferably as second line treatment of NSCLC), to a subject having NSCLC, preferably as second line treatment, or wherein the method of treatment comprises administering the bispecific antibody in combination with pemetrexed and carboplatin to a subject having malignant mesothelioma.

156. The chemotherapy agent for use according to any of embodiments 98-154, wherein the method of treatment comprises administering the bispecific antibody in combination with nab-paclitaxel to a subject having triple-negative breast cancer.

157. The chemotherapy agent for use according to any of embodiments 98-154, wherein the method of treatment comprises administering the bispecific antibody in combination with oxaliplatin, calcium folinate, and 5-fluorouracil to a subject having hepatocellular carcinoma, preferably wherein the method of treatment

comprises administering the bispecific antibody every 2 weeks at a dosage ranging from 20 mg/kg to 30 mg/kg (with 20 mg/kg being particularly preferred).

158. The chemotherapy agent for use according to any of embodiments 98-154, wherein the method of treatment comprises administering the bispecific antibody in combination with irinotecan, 5-fluorouracil, calcium folinate to a subject having unresectable neuroendocrine neoplasm.

159. The chemotherapy agent for use according to any of embodiments 98-154, wherein the method of treatment comprises administering the bispecific antibody in combination with etoposide and platinum to a subject having small cell lung cancer, preferably as first line treatment and/or extensive-stage small cell lung cancer, most preferably as first line treatment of extensive-stage small cell lung cancer or as second-line treatment of limited-stage small cell lung cancer.

160. The chemotherapy agent for use according to embodiment 159, wherein the method of treatment comprises administering the bispecific antibody every 3 weeks at a dosage ranging from 20 mg/kg to 30 mg/kg (with 20 mg/kg or 30 mg/kg being particularly preferred) or at a dose ranging from 1400 mg to 2000 mg in combination with etoposide (preferably at a dosage of 100 mg/m^2) with carboplatin (preferably at a dosage of AUC=5 (total dose $\leq$ 750 mg) to a subject having small cell lung cancer, preferably as first line treatment and/or extensive-stage small cell lung cancer, most preferably as first line treatment of extensive-stage small cell lung cancer or as second-line treatment of limited-stage small cell lung cancer.

161. The chemotherapy agent for use according to any of embodiments 98-154, wherein the method of treatment comprises administering the bispecific antibody every 3 weeks at a dosage ranging from 20 mg/kg to 30 mg/kg in combination with paclitaxel, lurbinectedin, or topotecan to a subject having small cell lung cancer, preferably as second line treatment and/or extensive-stage small cell lung cancer, more preferably wherein the method of treatment comprises administering to a patient suffering from advanced small cell lung cancer as second line treatment the bispecific antibody every 3 weeks at a dosage of 20 mg/kg or 30 mg/kg in combination with (i) paclitaxel every 3 weeks at a dosage of 175 mg/m^2, optionally

for 5 cycles, or (ii) topotecan at a dosage of 1.5 mg/m^2 administered IV or 2.3 mg/m^2 administered orally once per day on days 1 to 5 of every 3 weeks cycle.

162. The chemotherapy agent for use according to any of embodiments 98-154, wherein the method of treatment comprises administering the bispecific antibody every 2 weeks at a dosage ranging from 10 mg/kg to 20 mg/kg in combination with nab-paclitaxel, paclitaxel, or gemcitabine with carboplatin to a subject having triple-negative breast cancer.

163. The chemotherapy agent for use according to any of embodiments 98-154, wherein the method of treatment comprises administered the bispecific antibody every 2 weeks at a dosage of 1000 mg to 2000 mg to a subject having triple-negative breast cancer, preferably on the 1st and 15th day of a 28-day treatment cycle and preferably wherein the bispecific antibody is administered in combination with a chemotherapy, preferably on the 1st, 8th, and 15th day of a 28-day treatment cycle.

Definitions

The terms indicated for explanation of the invention and the disclosure have the following meaning, unless otherwise indicated in the description or the claims. Additional definitions are set forth throughout the detailed description.

Terms “a” and “an” and “the” and similar reference used in the context of describing the invention (especially in the context of the claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context.

The terms “about” or “approximately” as used herein denotes a range of ±10% of a reference value. For examples, “about 10” defines a range of 9 to 11. In general, those skilled in the art, familiar with the context, will appreciate the relevant degree of variance encompassed by “about” or “approximately” in that context.

The term “adjuvant” relates to a compound which prolongs, enhances or accelerates an immune response. Adjuvants comprise a heterogeneous group of compounds such as oil emulsions (e.g., Freund’s adjuvants), mineral compounds (such as alum), bacterial products (such as Bordetella pertussis toxin), or immune-stimulating complexes. Examples of adjuvants include, without limitation, LPS, GP96, CpG oligodeoxynucleotides, growth factors, and cytokines, such as monokines, lymphokines, interleukins, chemokines. The

chemokines may be IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, INFa, INF-γ, GM-CSF, LT-a. Further known adjuvants are aluminum hydroxide, Freund's adjuvant or oil such as Montanide® ISA51. Other suitable adjuvants for use in the present disclosure include lipopeptides, such as Pam3Cys, as well as lipophilic components, such as saponins, trehalose-6,6-dibehenate (TDB), monophosphoryl lipid-A (MPL), monomycoloyl glycerol (MMG), or glucopyranosyl lipid adjuvant (GLA).

"Antibodies" are glycoproteins belonging to the immunoglobulin superfamily. The term "full-length antibody" may refer to an immunoglobulin molecule that binds to a target molecule and contains four peptide chains: two heavy chains and two light chains which are connected to each other through disulfide bonds. An antibody may recognise an antigen via the fragment antigen-binding (Fab) variable region. The fragment crystallizable region (Fc region) is the tail region of an antibody that may allow antibodies to activate the immune system. The hinge region is a stretch of heavy chains linking the Fab and Fc regions. The heavy chain and light chain may each comprise a variable region and one or more constant domains. For example, in IgG antibodies, a heavy chain comprises a variable region (VH) and three constant domains (CH1, CH2, and CH3) and a light chain comprises a variable region (VL) and one constant domain (CL). Examples antibodies include a human antibody, a humanized antibody, a chimeric antibody, a multispecific antibody, a monoclonal antibody, and a polyclonal antibody.

The term "antibody fragment" may refer to a fragment of an antibody, or a genetically engineered product of one of more fragments of an antibody, which fragment is involved in binding with the target molecule. Examples of antibody fragments include an antigen-binding fragment (Fab), a Fab', a Fab'-SH, a fragment antibody (F(ab')2), a variable region (Fv), a single chain antibody (scFv), a single-domain antibody (sdAb), and a camelid antibody (VHH). The term "Antigen-binding fragment" or "Fab" refers to a region of an antibody that binds to antigens and is composed of one constant and one variable region of each of the heavy and the light chain. The term "fragment antibody" or "F(ab')2" refers to a region of an antibody that remains following digestion of the Fc region while leaving intact some of the hinge region. The term "Fab'" refers to a fragment formed by the reduction of a F(ab')2 fragment. The term "Fab'-SH" refers to a Fab' fragment with a free sulfhydryl group.

The term "carrier" refers to a component which may be natural, synthetic, organic, inorganic in which the active component is combined in order to facilitate, enhance or enable administration of the pharmaceutical composition. A carrier as used herein may be one or more compatible solid or liquid fillers, diluents or encapsulating substances, which are suitable for administration to subject. Suitable carriers include, without limitation, sterile water, Ringer, Ringer lactate, sterile sodium chloride solution, isotonic saline, polyalkylene glycols, hydrogenated naphthalenes and, in particular, biocompatible lactide polymers, lactide/glycolide copolymers or polyoxyethylene/polyoxy-propylene copolymers. In some embodiments, the pharmaceutical composition of the present disclosure includes isotonic saline.

Pharmaceutically acceptable carriers, excipients or diluents for therapeutic use are well known in the pharmaceutical art, and are described, for example, in Remington's Pharmaceutical Sciences, Mack Publishing Co. (A. R Gennaro edit. 1985). Pharmaceutical carriers, excipients or diluents can be selected with regard to the intended route of administration and standard pharmaceutical practice.

"CDR" or "CDRs" means complementarity determining region(s) in an immunoglobulin variable region. The variable regions of the heavy and light chains each contain three CDRs, designated CDR1, CDR2 and CDR3. The precise boundaries of these CDRs can be defined according to various numbering systems known in the art (see e.g. Dondelinger, M., et al., 2018. Frontiers in immunology, 9, p.2278), such as the Kabat numbering system (Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md., 1991; Kabat et al., 1992, Sequences of Proteins of Immunological Interest, DIANE Publishing: 2719), the Chothia numbering system (Chothia & Lesk (1987) J. Mol. Biol. 196:901-917; Chothia et al. (1989) Nature 342:878-883) or the IMGT numbering system (Lefranc et al., Dev.Comparat.Immunol.27:55-77, 2003; Ehrenmann F, Kaas Q, Lefranc M P. IMGT/3Dstructure-DB and IMGT/DomainGapAlign: a database and a tool for immunoglobulins or antibodies, T cell receptors, MHC, IgSF and MhcSF[J]. Nucleic acids research, 2009;38(suppl_1):D301-D307). For a given antibody, those skilled in the art will readily identify the CDRs defined by each numbering system. Also, the correspondence between different numbering systems is well known to those skilled in the art (for example, see Lefranc et al., Dev. Comparat. Immunol. 27:55-77, 2003).

The terms “chemotherapeutic agent” or “chemotherapeutical agent” or “chemotherapy agent” can be used interchangeably herein. A chemotherapeutic agent is a chemical compound useful in the treatment of cancer. Classes of chemotherapeutic agents include, but are not limited to: alkylating agents, antimetabolites, kinase inhibitors, spindle poison plant alkaloids, cytotoxic/antitumour antibiotics, topoisomerase inhibitors, photosensitizers, anti-estrogens and selective estrogen receptor modulators (SERMs), anti-progesterones, estrogen receptor down-regulators (ERDs), estrogen receptor antagonists, leutinizing hormone-releasing hormone agonists, anti-androgens, aromatase inhibitors, EGFR inhibitors, VEGF inhibitors, anti-sense oligonucleotides that that inhibit expression of genes implicated in abnormal cell proliferation or tumour growth. Chemotherapeutic agents useful in the treatment methods of the present invention include cytostatic and/or cytotoxic agents. Chemotherapeutic agents as used herein do not include antibodies.

“Chimeric antibody” refers to an antibody in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in an antibody derived from a particular species (e.g., human) or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in an antibody derived from another species (e.g., mouse) or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity.

Unless expressly specified otherwise, the term “comprising” is used in the context of the present disclosure to indicate that further members may optionally be present in addition to the members of the list introduced by “comprising”. It is, however, contemplated as specific embodiments of the present invention that each time the term “comprising” is used, this shall also encompass the possibility of no further members being present, i.e., for the purpose of this embodiment “comprising” can be understood as having the meaning of “consisting of”.

“Combination therapy” refers to the bispecific antibody disclosed herein that specifically binds to PD-L1 and VEGF in combination with the chemotherapy disclosed herein for use in a method of treating a subject with cancer, optionally wherein the subject has been determined to have a combined positive score (CPS) of ≥ 1 before the treatment. Each

component of the combination therapy, i.e., the bispecific antibody and the chemotherapy may be administered separately.

The "combined positive score" or "CPS," refers to a well-known algorithm for determining a PD-L1 expression score from a tumour sample of a subject (see for example Kulangara, Karina & Hanks, Debra & Waldroup, Stephanie & Peltz, Lindsay & Shah, Supriya & Roach, Charlotte & Juco, Jonathan & Emancipator, Kenneth & Stanforth, Dave. (2017), Development of the combined positive score (CPS) for the evaluation of PD-L1 in solid tumours with the immunohistochemistry assay PD-L1 IHC 22C3 pharmDx. Journal of Clinical Oncology). When using CPS, the PD-L1 expression score is determined by taken into account the number of PD-L1 staining cells (i.e., tumour cells, lymphocytes, macrophages) and the total number of viable tumour cells in a cancer tissue sample from the subject; and calculating the CPS for the cancer tissue sample using the formula:

$$\text{CPS} = \frac{\#\ \text{PD-L1 staining cells (tumour cells, lymphocytes, macrophages)}}{\text{total}\ \#\ \text{viable tumour cells}} * 100,$$

optionally wherein

(i) the test sample is a cancer tissue sample; and/or

(ii) the cancer tissue sample comprises at least 100 viable tumour cells as well as (tumour infiltrating) lymphocytes and macrophages; and/or

(iii) PD-L1 staining cells are determined by PD-L1 immunohistochemistry staining; and/or

(iv) viable tumour cells are determined by staining with a viability dye; and/or

(v) the number of PD-L1 staining cells is the sum of PD-L1 staining tumour cells, PD-L1 staining lymphocytes and PD-L1 staining macrophages.

It can be preferred that the CPS has been determined in a test sample of the subject by determining the number of PD-L1 staining cells (tumour cells, lymphocytes, macrophages) and the total number of viable tumour cells in a cancer tissue sample from the subject; and calculating the CPS for the cancer tissue sample using the formula:

$$\text{CPS} = \frac{\#\ \text{PD-L1 staining cells (tumour cells, lymphocytes, macrophages)}}{\text{total}\ \#\ \text{viable tumour cells}} * 100$$

wherein

(i) the test sample is a cancer tissue sample;

(ii) the cancer tissue sample comprises at least 100 viable tumour cells as well as (tumour infiltrating) lymphocytes and macrophages;

(iii) PD-L1 staining cells are determined by PD-L1 immunohistochemistry staining;

(iv) viable tumour cells are determined by staining with a viability dye; and

(v) the number of PD-L1 staining cells is the sum of PD-L1 staining tumour cells, PD-L1 staining lymphocytes and PD-L1 staining macrophages.

The CPS is approved as companion diagnostic for the treatment of cancer using pembrolizumab.

The term "diluent" relates to a diluting and/or thinning agent. Moreover, the term "diluent" includes any one or more of fluid, liquid or solid suspension and/or mixing media. Examples of suitable diluents include ethanol, glycerol, and water.

Herein, the term "DNA" relates to a nucleic acid molecule which is entirely or at least substantially composed of deoxyribonucleotide residues. In preferred embodiments, the DNA contains all or a majority of deoxyribonucleotide residues. As used herein, "deoxyribonucleotide" refers to a nucleotide which lacks a hydroxyl group at the 2'-position of a β-D-ribofuranosyl group. DNA encompasses without limitation, double stranded DNA, single stranded DNA, isolated DNA such as partially purified DNA, essentially pure DNA, synthetic DNA, recombinantly produced DNA, as well as modified DNA that differs from naturally occurring DNA by the addition, deletion, substitution and/or alteration of one or more nucleotides. Such alterations may refer to addition of non-nucleotide material to internal DNA nucleotides or to the end(s) of DNA. It is also contemplated herein that nucleotides in DNA may be non-standard nucleotides, such as chemically synthesized nucleotides or ribonucleotides. For the present disclosure, these altered DNAs are considered analogs of naturally-occurring DNA. A molecule contains "a majority of deoxyribonucleotide residues" if the content of deoxyribonucleotide residues in the molecule is more than 50% (such as at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%), based on the total number of nucleotide residues in the molecule. The total number of nucleotide residues in a molecule is the sum of all nucleotide residues (irrespective of whether the nucleotide residues are standard (i.e., naturally occurring) nucleotide residues or analogs thereof). DNA may be recombinant

DNA and may be obtained by cloning of a nucleic acid, in particular cDNA. The cDNA may be obtained by reverse transcription of RNA.

As used herein, the term "effective amount" refers to an amount of a given substance that is sufficient in quantity to produce a desired effect, including an improvement or remediation of the disease, disorder, or symptoms of the disease or condition. The combination therapy described herein is to be administered to a patient in need therefore in an effective amount.

As used herein, the term "encode" or "encoding" refers to sequence information of a first molecule that guides production of a second molecule having a defined sequence of nucleotides (e.g., mRNA) or a defined sequence of amino acids. For example, a DNA molecule can encode an RNA molecule (e.g., by a transcription process that includes a DNA-dependent RNA polymerase enzyme). An RNA molecule can encode a polypeptide (e.g., by a translation process). Thus, a gene, a cDNA, or a single-stranded RNA (e.g., an mRNA) encodes a polypeptide if transcription and translation of mRNA corresponding to that gene produces the polypeptide in a cell or other biological system. In some embodiments, a coding region of a single-stranded RNA encoding a target polypeptide agent refers to a coding strand, the nucleotide sequence of which is identical to the mRNA sequence of such a target polypeptide agent. In some embodiments, a coding region of a single-stranded RNA encoding a target polypeptide agent refers to a non-coding strand of such a target polypeptide agent, which may be used as a template for transcription of a gene or cDNA. As is understood in the art, the phrase "nucleic acid encoding a peptide or protein" means that the polynucleotide, if present in the appropriate environment, for example within a cell and/or in a cell-free translation system, can direct the assembly of amino acids to produce the peptide or protein via a process of translation.

The term "epitope" refers to the part of an antigen that as used herein, refers to an agent that elicits an immune response; and/or (ii) an agent that binds to a T cell receptor (*e.g.,* when presented by an MHC molecule) or to an antibody. For example, epitopes are the discrete, three-dimensional sites on an antigen, which are recognized by the immune system. Epitopes usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and usually have specific three-dimensional structural characteristics, as well as specific charge characteristics. Conformational and non-

conformational epitopes are distinguished in that the binding to the former but not the latter is lost in the presence of denaturing solvents.

The term "excipient" as used herein refers to a substance which may be present in a pharmaceutical composition of the present disclosure but is not an active ingredient. Examples of excipients, include without limitation, carriers, binders, diluents, lubricants, thickeners, surface active agents, preservatives, stabilizers, emulsifiers, buffers, flavoring agents, or colorants.

As used herein, the term "gene" refers to a DNA sequence in a chromosome that codes for a protein. In some embodiments, a gene includes coding sequence (i.e., sequence that encodes a particular protein); in some embodiments, a gene includes non-coding sequence. In some particular embodiments, a gene may include both coding (e.g., exonic) and non-coding (e.g., intronic) sequences. In some embodiments, a gene may include one or more regulatory elements that, for example, may control or impact one or more aspects of gene expression (e.g., cell-type- specific expression, inducible expression, etc.).

"Human antibody" refers to an antibody that comprises human immunoglobulin protein sequences only. A human antibody may contain murine carbohydrate chains if produced in a mouse, in a mouse cell, or in a hybridoma derived from a mouse cell. Similarly, "mouse antibody" or "rat antibody" refer to an antibody that comprises only mouse or rat immunoglobulin sequences, respectively.

"Humanized antibody" refers to forms of antibodies that contain sequences from non human (e.g., murine) antibodies as well as human antibodies. Such antibodies contain minimal sequence derived from non-human immunoglobulin. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable regions, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin sequence.

The term "immune cell" means any cell of hematopoietic lineage involved in regulating an immune response against an antigen (e.g., a bacterial or viral infection or an auto-antigen). In typical embodiments, an immune cell is a leukocyte, such as a white blood cell. Immune cells include neutrophils, eosinophils, basophils, lymphocytes, and/or monocytes.

Lymphocytes include T lymphocytes and B lymphocytes. Immune cells can also be dendritic cells, natural killer (NK) cells, and/or a mast cell.

Mononuclear inflammatory cells (MIC) cells as used herein refer to lymphocytes and macrophages.

The term "monoclonal antibody" refers to an antibody obtained from a substantially homogeneous population of antibodies. The individual antibodies composing the population may be identical except for possible naturally occurring mutations, which may be present in minor amounts. Monoclonal antibodies are highly specific and target a single antigenic epitope. In contrast, polyclonal antibody preparations typically include a large number of antibodies which are specific for different epitopes.

The term “nucleoside” relates to compounds which can be thought of as nucleotides without a phosphate group. While a nucleoside is a nucleobase linked to a sugar (e.g., ribose or deoxyribose), a nucleotide is composed of a nucleoside and one or more phosphate groups. Examples of nucleosides include cytidine, uridine, pseudouridine, adenosine, and guanosine. The five standard nucleosides which usually make up naturally occurring nucleic acids are uridine, adenosine, thymidine, cytidine and guanosine. The five nucleosides are commonly abbreviated to their one letter codes U, A, T, C and G, respectively. However, thymidine is more commonly written as “dT” (“d” represents “deoxy”) as it contains a 2’-deoxyribofuranose moiety rather than the ribofuranose ring found in uridine. This is because thymidine is found in deoxyribonucleic acid (DNA) and not ribonucleic acid (RNA). Conversely, uridine is found in RNA and not DNA. The remaining three nucleosides may be found in both RNA and DNA. In RNA, they would be represented as A, C and G, whereas in DNA they would be represented as dA, dC and dG. A modified purine (A or G) or pyrimidine (C, T, or U) base moiety is, in some embodiments, modified by one or more alkyl groups, e.g., one or more $C_{1\text{-}4}$ alkyl groups, e.g., one or more methyl groups. Particular examples of modified purine or pyrimidine base moieties include N^7-alkyl-guanine, N^6-alkyl-adenine, 5-alkyl-cytosine, 5-alkyl-uracil, and N(1)-alkyl-uracil, such as N^7-$C_{1\text{-}4}$ alkyl-guanine, N^6-$C_{1\text{-}4}$ alkyl-adenine, 5-$C_{1\text{-}4}$ alkyl-cytosine, 5-$C_{1\text{-}4}$ alkyl-uracil, and N(1)-$C_{1\text{-}4}$ alkyl-uracil, preferably N^7-methyl-guanine, N^6-methyl-adenine, 5-methyl-cytosine, 5-methyl-uracil, and N(1)-methyl-uracil.

PD-L1 expression score as used herein can be determined using a PD-L1 scoring algorithm such as CPS, TPS, or TAP. As known to the skilled person, the same PD-L1 expression can lead to different numerical values depending on the PD-L1 scoring algorithm.

The term "pharmaceutical composition" relates to a composition comprising a therapeutically effective agent, preferably together with pharmaceutically acceptable carriers, diluents and/or excipients. Said pharmaceutical composition is useful for treating, preventing, or reducing the severity of a disease by administration of said pharmaceutical composition to a subject.

The term "pharmaceutically acceptable carrier" or "pharmaceutically acceptable excipient" means solvents, dispersion media, coatings, antibacterial agents and antifungal agents, isotonic agents, and absorption delaying agents, and the like, that are compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. In certain embodiments, the pharmaceutically acceptable carrier or excipient is not naturally occurring.

"Platinum-containing chemotherapy" (also known as platins) refers to the use of chemotherapeutic agent(s) used to treat cancer that are coordination complexes of platinum. Platinum-containing chemotherapeutic agents are alkylating agents that crosslink DNA, resulting in ineffective DNA mismatch repair and generally leading to apoptosis. Examples of platins include cisplatin, carboplatin, and oxaliplatin.

The term "plurality" refers to the state of being plural.

The terms "polynucleotide" and "nucleic acid" can be used interchangeably herein to refer to polymers of nucleotides. The term "polynucleotide" comprises deoxyribonucleic acid (DNA), ribonucleic acid (RNA), combinations thereof, and modified forms thereof. The term comprises genomic DNA, cDNA, mRNA, recombinantly produced and chemically synthesized molecules. In some embodiments, a polynucleotide is DNA. In some embodiments, a polynucleotide is RNA. In some embodiments, a polynucleotide is a mixture of DNA and RNA. A polynucleotide may be present as a single-stranded or double-stranded and linear or covalently circularly closed molecule. A polynucleotide can be isolated. The term "isolated polynucleotide " means, according to the present disclosure, that the polynucleotide (i) was amplified in vitro, for example via polymerase chain reaction (PCR) for DNA or in vitro transcription (using, e.g., an RNA polymerase) for

RNA, (ii) was produced recombinantly by cloning, (iii) was purified, for example, by cleavage and separation by gel electrophoresis, or (iv) was synthesized, for example, by chemical synthesis.

The terms "polypeptide," "peptide," and "protein" are used interchangeably herein to refer to polymers of amino acids.

"Kabat," as used herein, means an immunoglobulin alignment and numbering system pioneered by Elvin a. Kabat ((1991) Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md.) or Kabat et al., 1992, Sequences of Proteins of Immunological Interest, DIANE Publishing: 2719.

The term "recombinant" when used in the context of a polynucleotide means a polynucleotide having nucleotide sequences that are not naturally joined together and can be made by artificially combining two otherwise separated segments of sequence. This artificial combination is often accomplished by chemical synthesis or, more commonly, by the artificial manipulation of isolated segments of nucleic acids, for example, by genetic engineering techniques. Recombinant polynucleotides include vectors comprising an amplified or assembled polynucleotide, which can be used to transform or transfect a suitable host cell. A host cell that comprises the recombinant polynucleotide is referred to as a "recombinant host cell." The polynucleotide is then expressed in the recombinant host cell to produce a "recombinant polypeptide." A recombinant polynucleotide can also comprise a non-coding function.

A "single domain antibody" (sdAb) is an antibody composed of a single variable region (e.g., heavy chain variable region) composed of antibody fragments. Typically, a single domain antibody, domain antibody or nanobody consists of 4 framework regions and 3 complementarity determining regions, the 4 framework regions are respectively FR1-FR4, and the 3 complementarity determining regions are respectively CDR1 -CDR3. In certain embodiments, the single domain antibody of the present application may have a structure of FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4. These antibodies do not require light chain variable regions to bind antigens with high affinity and specificity. Compared with antibodies composed of heavy chain and light chain, single domain antibodies have high solubility, high stability to heat, pH, protease and other deforming agents, and only need single-chain expression to facilitate large-scale production. As used herein, the term

"framework region" or "FR" residues refers to those amino acid residues in an antibody variable region other than the CDR residues as defined above.

An antibody that "specifically binds to" a specified target protein is an antibody that exhibits preferential binding to that target as compared to other proteins, but this specificity does not require absolute binding specificity. An antibody is considered "specific" for its intended target if its binding is determinative of the presence of the target protein in a sample, e.g., without producing undesired results such as false positives. Antibodies, or binding fragments thereof, useful in the present invention will bind to the target protein with an affinity that is at least two fold greater, preferably at least ten times greater, more preferably at least 20-times greater, and most preferably at least 100-times greater than the affinity with non-target proteins. As used herein, an antibody is said to bind specifically to a polypeptide comprising a given amino acid sequence, e.g., the amino acid sequence of a mature human PD-L1 molecule, if it binds to polypeptides comprising that sequence but does not bind to proteins lacking that sequence.

As used herein, a "subject" is a human of either gender (a male or a female). The subject may be of any age. In some embodiments, the subject is female. In another embodiment, the subject is male. In some embodiments, the subject is a subject having cancer, in particular a female subject having cancer and/or a male subject having cancer.

As disclosed in more detail herein below, the term "TAP" score refers to the "Tumour Area Positivity" score. The PD-L1 expression score can be determined using a TAP scoring algorithm in a test sample of the subject by determining the percentage of PD-L1 positive tumour cells and immune cells per tumour area in a cancer tissue sample from the subject; and calculating the TAP for the cancer tissue sample using the formula:

$$\text{TAP} = \frac{\%\ \text{PD-L1 positive tumour cells and immune cells}}{\text{tumour area}},$$

optionally wherein

(i) the test sample is a cancer sample; and/or

(ii) the tumour area is the area occupied by all viable tumour cells and the tumour-associated stroma containing tumour-associated immune cells; and/or

(iii) PD-L1 positive tumour cells and immune cells are determined by PD-L1 immunohistochemistry staining (preferably by using the anti-PD-L1 antibody SP263); and/or

(iv) PD-L1 positive tumour cells and immune cells means the area covered by such PD-L1 positive tumour cells and immune cells; and/or

(v) % PD-L1 positive tumour cells and immune cells / tumour area means the area of PD-L1 positive tumour cells and immune cells relative to the tumour area expressed as %.

It can be preferred that the PD-L1 expression score has been determined using a TAP scoring algorithm in a test sample of the subject by determining the percentage of PD-L1 positive tumour cells and immune cells per tumour area in a cancer tissue sample from the subject; and calculating the TAP for the cancer tissue sample using the formula:

$$\text{TAP} = \frac{\text{\% PD-L1 positive tumour cells and immune cells}}{\text{tumour area}},$$

wherein

(i) the test sample is a cancer sample;

(ii) the tumour area is the area occupied by all viable tumour cells and the tumour-associated stroma containing tumour-associated immune cells (wherein viable tumour cells are determined by staining with a viability dye);

(iii) PD-L1 positive tumour cells and immune cells are determined by PD-L1 immunohistochemistry staining (preferably by using the anti-PD-L1 antibody SP263);

(iv) PD-L1 positive tumour cells and immune cells means the area covered by such PD-L1 positive tumour cells and immune cells; and

(v) % PD-L1 positive tumour cells and immune cells / tumour area means the area of PD-L1 positive tumour cells and immune cells relative to the tumour area expressed as %.

Alternatively, the TAP score might be referred to as total percentage of the tumour area (tumour and any desmoplastic stroma) covered with tumour cells with PD-L1 membranous staining at any intensity and tumour-associated immune cells with any pattern.

As disclosed in more detail herein below, the term "TPS" refers to the "Tumour Proportion Score". The PD-L1 expression score can be determined using a TPS scoring algorithm by determining in a test sample of the subject the number of viable tumour cells positive for PD-L1 and the total number of viable tumour cells in a cancer tissue sample from the subject; and calculating the TPS for the cancer tissue sample using the formula:

$$\text{TPS} = \frac{\#\ viable\ tumor\ cells\ positive\ for\ PD{-}L1}{\text{total \# viable tumour cells}} * 100$$

optionally wherein

(i) the test sample is a cancer tissue sample; and/or

(ii) the cancer tissue sample comprises at least 100 viable tumour cells; and/or

(iii) viable tumour cells are determined by staining with a viability dye; and/or

(iv) PD-L1 positive cells are determined by PD-L1 immunohistochemistry staining.

It can be preferred that the PD-L1 expression score has been determined using a TPS scoring algorithm by determining in a test sample of the subject the number of viable tumour cells positive for PD-L1 and the total number of viable tumour cells in a cancer tissue sample from the subject; and calculating the TPS for the cancer tissue sample using the formula:

$$\text{TPS} = \frac{\#\ viable\ tumor\ cells\ positive\ for\ PD{-}L1}{\text{total \# viable tumour cells}} * 100$$

wherein

(i) the test sample is a cancer tissue sample;

(ii) the cancer tissue sample comprises at least 100 viable tumour cells;

(iii) viable tumour cells are determined by staining with a viability dye; and

(iv) PD-L1 positive cells are determined by PD-L1 immunohistochemistry staining.

The term "treating" when used in the context of a disease or disease condition means ameliorating, improving or remedying a disease, disorder, or symptom of a disease or condition associated with the disease, or can mean completely or partially stopping, on a molecular level, the biochemical basis of the disease, etc. It describes an act that leads to the elimination, reduction, alleviation, reversal, or prevention or delay of onset or recurrence of any symptom of a disease.

The term "triple-negative breast cancer" as used herein is used in the usually sense and refers to the cancer class that tests negative for estrogen receptors and progesterone receptors expression, and HER2 overexpression or gene amplification. TNBC is human epidermal growth factor receptor 2 (HER2) negative and has <1% expression of estrogen receptors (ER) and progesterone receptors (PR) by immunostaining. It is a biologically aggressive tumour, characterized by moderate/high grade and highly proliferative cancer cells, which, together with limited treatment options leads to the poorest prognosis among the breast cancer subtypes.

"Tumour" as it applies to a subject diagnosed with, or suspected of having, a cancer refers to a malignant or potentially malignant neoplasm or tissue mass of any size and includes primary tumours and secondary neoplasms. A solid tumour is an abnormal growth or mass of tissue that usually does not contain cysts or liquid areas. Different types of solid tumours are named for the type of cells that form them. Examples of solid tumours are sarcomas, carcinomas, and lymphomas. Leukemias (cancers of the blood) generally do not form solid tumours (National Cancer Institute, Dictionary of Cancer Terms).

"Variable regions" as used herein means the segment of an antibody which contains three CDRs, designated CDR1, CDR2 and CDR3. A "variable region" of an antibody refers to the variable region of the antibody light chain or the variable region of the antibody heavy chain, either alone or in combination. The variable region of the heavy chain may be referred to as "VH." The variable region of the light chain may be referred to as "VL." Typically, the variable regions of both the heavy and light chains comprise three hypervariable regions, the CDRs, which are located within relatively conserved framework regions (FR). The CDRs are usually aligned by the framework regions, enabling binding to a specific epitope. In general, from N-terminal to C-terminal, both light and heavy chains variable regions comprise FR1, CDR1, FR2, CDR2, FR3, CDR3, and FR4.Recitation of ranges of values herein is merely intended to serve as a shorthand method of referring individually to each separate value falling within the range. Unless otherwise indicated herein, each individual value is incorporated into the specification as if it was individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., "such as"), provided herein is intended merely to better illustrate the invention and does not pose a limitation on the scope of the invention

otherwise claimed. No language in the specification should be construed as indicating any non-claimed element essential to the practice of the invention.

All patents, patent applications, and other publications cited in this application are incorporated by reference in the entirety for all purposes.

The combination therapy of the invention

The present invention provides a bispecific antibody that specifically binds to PD-L1 and VEGF for use in a method of treating a subject with cancer, the method comprising administering to the subject:

a. the bispecific antibody; and

b. a chemotherapy, preferably a chemotherapy agent

optionally wherein the subject has a PD-L1 expression score before the treatment as determined by the combined positive score (CPS) of ≥ 1, preferably 1 to 20, more preferably 1 to <10 or other integrating scoring algorithm defining a similar PD-L1 expression score. It can be preferred that the subject has the afore-mentioned PD-L1 expression score before the treatment.

For example, the present invention provides a bispecific antibody that specifically binds to PD-L1 and VEGF for use in a method of treating a subject with cancer, the method comprising administering to the subject:

a. the bispecific antibody; and

b. a chemotherapy, preferably a chemotherapy agent

optionally wherein the subject has a PD-L1 expression score before the treatment as determined by the combined positive score (CPS) of ≥ 1, preferably 1 to 20, more preferably 1 to <10.

As further example, the present invention provides a bispecific antibody that specifically binds to PD-L1 and VEGF for use in a method of treating a subject with cancer, the method comprising administering to the subject:

a. the bispecific antibody; and

b. a chemotherapy, preferably a chemotherapy agent

optionally wherein the subject has a PD-L1 expression score before the treatment as determined by an integrating scoring algorithm defining a PD-L1 expression score similar or identical to the CPS of ≥ 1, preferably 1 to 20, more preferably 1 to <10.

In some embodiments, a PD-L1 expression score determined by an integrating scoring algorithm and defining a PD-L1 expression score similar to the CPS means an ≥ 80%, preferably ≥85%, more preferably ≥90%, most preferably ≥95% average positive agreement (APA) and ≥ 80%, preferably ≥85%, more preferably ≥90%, most preferably ≥95% average negative agreement (ANA), and ≥ 80%, preferably ≥85%, more preferably ≥90%, most preferably ≥95% overall percent agreement (OPA) between and within readers with 95% confidence intervals (Cis) when comparing the scoring algorithm with the CPS. For example, the PD-L1 expression score determined by an integrating scoring algorithm and defining a PD-L1 expression score similar to the CPS can mean an ≥85% average positive agreement (APA) and ≥ 85% average negative agreement (ANA), and ≥ 85% overall percent agreement (OPA) between and within readers with ≥95% confidence intervals (Cis) when comparing the scoring algorithm with the CPS. In some embodiments, the PD-L1 expression score determined by an integrating scoring algorithm and defining a PD-L1 expression score similar to the CPS can mean an ≥90% average positive agreement (APA) and ≥ 90% average negative agreement (ANA), and ≥ 90% overall percent agreement (OPA) between and within readers with ≥95% confidence intervals (Cis) when comparing the scoring algorithm with the CPS. In some embodiments, the PD-L1 expression score determined by an integrating scoring algorithm and defining a PD-L1 expression score similar to the CPS can mean an ≥95% average positive agreement (APA) and ≥ 95% average negative agreement (ANA), and ≥ 95% overall percent agreement (OPA) between and within readers with ≥95% confidence intervals (Cis) when comparing the scoring algorithm with the CPS.

In some embodiments, the other integrating scoring algorithms are selected from TAP and TPS. In some preferred embodiments, the other integrating scoring algorithm is TAP.

The integrating scoring algorithm defining a similar PD-L1 expression score preferably is a tumour cell integrating scoring algorithm such TPS or a tumour cell and immune cell integrating scoring algorithm such as CPS or TAP.

Vascular endothelial growth factor (VEGF), also known as vascular permeability factor (VPF) or vasculotropin, is a highly specific homodimer that promotes the growth of vascular endothelial cells. Body protein. VEGF family proteins include VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, VEGF-F and placental growth factor (PIGF), among which VEGF-A is involved in the early formation of blood vessels play an important role. In 1983, Senger et al. first isolated liver cancer cells from guinea pigs, which can increase the permeability of venules and venules, promote the division and proliferation of vascular endothelial cells, and induce the formation of blood vessels. Meanwhile, VEGF is involved in the pathogenesis and progression of many angiogenesis-dependent diseases, including cancer, certain inflammatory diseases, and diabetic retinopathy. Therefore, VEGF is an important target in antitumour drug research.

The main receptors of VEGF proteins are VEGFR1, VEGFR2, VEGFR3, NRP1, NRP2 and NRP3. However, the binding of VEGF family protein members to VEGF receptors is selective, and VEGFA can bind to VEGFR1 and VEGFR2, activate endogenous kinase activation, and promote new blood vessels. Blocking the binding of VEGF to the receptor can be applied to the treatment of various cancers, such as breast cancer, colon cancer, lung cancer, colorectal, ovarian cancer, endometrial cancer, mesothelioma, cervical cancer, kidney cancer (Rakesh R. Ramjiawan, Arjan W. Griffioen, and Dan G. Duda, Angiogenesis. 2017 20(2):185–204.).

Apart of its angiogenesis-modulating capabilities VEGF, in particular VEGF-A, is associated with a range of immunosuppressive effects at successive steps in the cancer-immunity cycle, such as diminished antigen presentation, T cell priming, T cell trafficking, and T cell tumour infiltration. Hence, the bispecific antibody binding to VEGF can release immunosuppression. For example, an anti-VEGF antibody treatment alone results in increased gene expression associated with Th1 chemokines involved with T-cell trafficking, tumour MHC-I protein expression and infiltration of tumour-specific T-cell clones demonstrating that an anti-VEGF antibody is capable of inducing anti-tumour immune responses.

By blocking the VEGF-A/VEGFR-2 interaction the bispecific antibody may reverse VEGF-A mediated inhibition of monocyte-to-dendritic cell maturation thereby increasing numbers of dendritic cells (DCs), and reverse VEGF-A mediated inhibition of dendritic cell

maturation thereby increasing tumour-antigen presentation. The bispecific antibody may further reverse or reduce VEGF-A mediated enhanced T cell exhaustion, as VEGF-A binding to VEGFR-2 on the surface of CD8+ T cells has been shown in preclinical studies to result in upregulated expression of the immune-checkpoint molecules (and exhaustion markers) PD-1, Cytotoxic T Lymphocyte antigen 4 (CTLA-4), and T cell immunoglobulin mucin receptor 3 (TIM3) as well as that of lymphocyte activation gene 3 protein (LAG3). The bispecific antibody may further reverse or reduce VEGF-A mediated proliferation of Treg cells, and reverse VEGF-A mediated downregulation of adhesion molecules (e.g. ICAM-1, or CD34) to allow for more efficient immune cell infiltration of the tumour. VEGF thus has a major role in the generation of an immunosuppressive tumour microenvironment.

In addition, blocking the VEGF-A/VEGFR-2 interaction can induce physical changes in the tumour vasculature such as vascular normalization, a process whereby hypoxia is transiently alleviated and the tumour vasculature reverts back to resemble that of a nonmalignant tissue (Jain, R. K. Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy. Science 307, 58–62 (2005)).

Programmed death-ligand 1 (PD-L1), also known as CD274, is a member of the B7 family and is a ligand of PD-1. PD-L1 is a type I transmembrane protein with a total of 290 amino acids, including an IgV-like domain, an IgC-like domain, a transmembrane hydrophobic domain and an intracellular domain consisting of 30 amino acids. Unlike other B7 family molecules, PD-L1 negatively regulates immune responses. Studies have found that PD-L1 is mainly expressed in activated T cells, B cells, macrophages and dendritic cells, etc. In addition to lymphocytes, PD-L1 is also expressed in other tissues such as thymus, heart, placenta, etc. endothelial cells, as well as various non-lymphoid lineages such as melanoma, lung cancer, liver cancer, gastric cancer, renal cell cancer, urothelial cancer, cervical cancer, colorectal cancer, ovarian cancer, colon cancer, breast cancer, esophageal cancer, head and neck cancer, etc. (Akintunde Akinleye&Zoaib Rasool, Journal of Hematology&Oncology volume 12, Article number: 92 (2019)). PD-L1 regulates autoreactive T- and B-cells, and immune tolerance, and plays a role in peripheral tissue T- and B-cell responses. Interfering with the PD-1:PD-L1 interaction reinvigorates exhausted T cells, as PD-1 is a major regulator of T-cell exhaustion. Hence blocking the PD-1:PD-L1 pathway restores T-cell function and improves tumour eradication.

High expression of PD-L1 on tumour cells is associated with poor prognosis in cancer subjects. For a better prognosis in cancer subjects, it is therefore highly desired to provide an effective treatment for low PD-L1 expressing cancer.

Surprisingly, the combination therapy disclosed herein comprising the bispecific antibody targeting specifically PD-L1 and VEGF combined with chemotherapy shows particularly encouraging anti-tumour activity even when the subject has a low PD-L1 expression score. This can distinguish it from treatments of the prior art for the same cancer in which only patient groups with higher PD-L1 expression scores could be effectively targeted by immunotherapy. The combination of the PD-L1 and VEGF bispecific antibody of the invention with the chemotherapy is highly effective in cancer treatment, even in subjects having a low PD-L1 expression score as, e.g., determined by the PD-L1 scoring algorithm CPS of ≥ 1. In addition, the combination of the bispecific antibody and chemotherapy shows a good safety profile for cancer subjects.

The release in immunosuppression by blocking VEGF and PD-L1 and the consequential influx of immune cells is contemplated to lead to an increased expression in PD-L1 on tumour cells, further enriching for the bispecific antibody in the tumour microenvironment. Hence, the bispecific antibody is contemplated to transform PD-L1 low-expressing tumours into more inflamed, immune cell infiltrated “hot tumours”.

The combination therapy comprising the bispecific antibody and chemotherapy can be used in a method of treating cancer in a subject as disclosed herein. Features described herein in more detail in connection with the “bispecific antibody for use in a method of treating” embodiments equally apply to the corresponding method of treatment embodiments.

The bispecific antibody and chemotherapy disclosed herein can provide for substantial improvement in a subject’s overall survival (OS), progression-free survival (PFS), objective response rate (ORR), duration of response, and/or disease control rate. As regards PFS, the bispecific antibody and chemotherapy disclosed herein can provide for a PFS of at least about 9 months, preferably of at least about 13 months in patients suffering from TNBC (see also example 3 herein).

Objective Response Rate (ORR) refers to the number (%) of subjects with at least one visit response of Complete Response (CR) or Partial Response (PR) per RECIST 1.1. Duration of Response (DoR) refers to the time from the date of first documented response until the

first date of documented progression or death in the absence of disease progression (i.e., date of PFS event or censoring – date of first response + 1). Disease Control Rate (DCR) refers to the rate of best objective response of CR, PR, or stable disease (SD) according to RECIST 1.1.

The PD-L1 expression score as described herein is determined by histologically analyzing a tumour sample and applying a scoring algorithm, the scoring algorithm according to the invention is the combined positive score (CPS) or any other scoring algorithm defining a similar PD-L1 expression score as the CPS. CPS is a well-established scoring algorithm which is approved for the treatment of cancer using pembrolizumab in multiple indications. In some embodiments, the treatment method comprises the step of applying the scoring algorithm to determine the PD-L1 expression score and thereby whether the subject shall be treated with the combination therapy of the invention. In other embodiments, the PD-L1 expression score (e.g., CPS or similar score type) is already known and provided before the start of the method of the invention.

The PD-L1 expression score can be determined in form of the CPS in a test sample of the subject by determining the number of PD-L1 staining cells (tumour cells, lymphocytes, macrophages) and the total number of viable tumour cells in a cancer tissue sample from the subject; and calculating the CPS for the cancer tissue sample using the formula:

$$\text{CPS} = \frac{\#\ \text{PD-L1 staining cells (tumour cells, lymphocytes, macrophages)}}{\text{total}\ \#\ \text{viable tumour cells}} * 100$$

wherein preferably the PD-L1 staining cells are tumour cells with partial or complete linear membrane staining that is perceived distinct from cytoplasmic staining, and lymphocytes and macrophages within the tumour nests and/or adjacent supporting stroma with membrane and/or cytoplasmic staining. The lymphocytes and macrophages are preferably directly associated with the response against the cancer. For example, the PD-L1 staining cells are tumour cells with convincing partial or complete linear membrane staining (at any intensity) that is perceived distinct from cytoplasmic staining, and lymphocytes and macrophages within the tumour nests and/or adjacent supporting stroma with membrane and/or cytoplasmic staining (at any intensity). The PD-L1 staining cells are preferably viable PD-L1 staining cells.

Categorization using CPS scoring algorithm is for example described in Kulangara, Karina & Hanks, Debra & Waldroup, Stephanie & Peltz, Lindsay & Shah, Supriya & Roach, Charlotte & Juco, Jonathan & Emancipator, Kenneth & Stanforth, Dave. (2017), Development of the combined positive score (CPS) for the evaluation of PD-L1 in solid tumours with the immunohistochemistry assay PD-L1 IHC 22C3 pharmDx. Journal of Clinical Oncology, which disclosure is incorporated herein in its entirety.

In some preferred embodiments, the number of viable tumour cells in the tumour tissue sample are determining by flow cytometry. For example, a tissue sample analyzed by flow cytometry can be contacted with a viability dye prior to analysis, e.g., propidium iodide. Any convenient viability stain may be employed, with many examples known in the art.

In some embodiments, the tumour proportion score (TPS) (an integrating scoring algorithm) is used instead of the CPS. The TPS can be obtained by determining in a test sample of the subject the number of viable tumour cells positive for PD-L1 (PD-L1 staining tumour cells) and the total number of viable tumour cells in a cancer tissue sample from the subject; and calculating the TPS for the cancer tissue sample using the formula:

$$\text{TPS} = \frac{\#\ viable\ tumor\ cells\ positive\ for\ PD{-}L1}{\text{total \# viable tumour cells}} * 100$$

Categorization using TPS scoring algorithm is described in Roach C, Zhang N, Corigliano E, et al. Development of a Companion Diagnostic PD-L1 Immunohistochemistry Assay for Pembrolizumab Therapy in Non-Small-cell Lung Cancer. Appl Immunohistochem Mol Morphol. 2016;24(6):392-397 (doi:10.1097/PAI.0000000000000408), which disclosure is incorporated herein in its entirety.

For example, the number of total tumour cells and tumour cells positive for PD-L1 (i.e., PD-L1 staining tumour cells) can be assessed using the PD-L1 IHC 22C3 pharmDx assay (Agilent Technologies, Carpinteria, CA, USA) according to the manufacturer's Instructions for Use. In some exemplary embodiments, the number of tumour cells can be measured in formalin-fixed tumour samples obtained by core-needle or excisional biopsy of a tumour lesion or from tissue resected at or after the time the cancer was diagnosed. In some such embodiments, for determination of PD-L1 expression, positivity is defined as complete circumferential or partial cell membrane staining of viable tumour cells with 1+ to 3+ intensity. Nonspecific staining can be recorded on a 0 to 3 intensity scale, in 0.25 grade

increments. Tumour-associated immune cells are preferably excluded from PD-L1 scoring. Cytoplasmic staining, if present, is preferably excluded from the scoring. Scoring can be recorded as percentage of PD-L1-positive tumour cells over total tumour cells in the denominator (TPS).

In some embodiments, the TAP scoring algorithm is used as the integrating scoring algorithm instead of CPS. The TAP score can be obtained by determining in a test sample of the subject the number of PD-L1 positive tumour and immune cells (PD-L1 staining tumour and immune cells) and the tumour area in a cancer tissue sample from the subject; and calculating the TAP for the cancer tissue sample using the formula:

$$\text{TAP} = \frac{\%\ \text{PD-L1 positive tumour cells and immune cells}}{\text{tumour area}}$$

The TAP score can be determined as described in Liu, Chunyan et al. "Tumour Area Positivity (TAP) score of programmed death-ligand 1 (PD-L1): a novel visual estimation method for combined tumour cell and immune cell scoring." Diagnostic pathology vol. 18,1 48. 19 Apr. 2023, doi:10.1186/s13000-023-01318-8, which disclosure is incorporated by reference herein in its entirety.

In some embodiments, the tumour area is determined using the hematoxylin and eosin (H&E) stain. In some embodiments, the PD-L1 positive tumour cells and immune cells are PD-L1 staining cells with partial or complete linear membrane staining that is distinct from cytoplasmic staining, and immune cells (lymphocytes and macrophages) within the tumour nests and/or adjacent supporting stroma with membrane and/or cytoplasmic staining.

For example, to determine the TAP score, a hematoxylin and eosin stained slide can be first examined to identify the tumour area (area occupied by all viable tumour cells (TC) and the tumour-associated stroma containing tumour-associated immune cells (IC)). The immune cells measured in the nominator are tumour-associated stroma containing tumour-associated immune cells. If tumour nests are separated by non-neoplastic tissue, they can be included as part of the tumour area as long as the tumour nests are bordered on both sides of a 10x field; the intervening non-neoplastic tissue can also be included in the tumour area (commonly abbreviated as 10x field rule). Necrosis, crush, and cautery artifacts are preferably excluded from tumour area. Tumour-associated IC are preferably intra- and peri-tumoural, including those present within the tumour proper, between tumour nests, and

within any tumour-associated reactive stroma. In lymph nodes with focal or discrete tumour metastases, only IC immediately adjacent to the leading edge of the metastatic tumour nest are preferably defined as tumour-associated IC.

The TAP can be determined on the IHC slide by visually aggregating/estimating the area covered by PD-L1 positive TC (PD-L1 staining TC) and tumour-associated IC relative to the total tumour area. Both circumferential and partial/lateral membrane staining of TC at any intensity can be regarded as positive PD-L1 staining, while cytoplasmic staining of TC can be disregarded; membranous, cytoplasmic, and punctate staining of tumour-associated IC at any intensity can be regarded as PD-L1 positive staining.

Intra-luminal macrophage staining is preferably not included in the TAP score unless the macrophages completely fill the luminal space and are in direct contact with the TC. Staining of multi-nucleated giant cells, granulomas, and IC located within blood vessels and lymphatics are preferably not included in the TAP. Off-target staining (e.g., fibroblasts, endothelial cells, neuroendocrine cells, smooth muscle, and nerves) is preferably not included in the TAP.

In some embodiments, the cancer tissue sample is a tissue section of a tumour biopsy, preferably a solid tumour biopsy. Biopsies of interest can include tumour and/or non-neoplastic biopsies of skin (melanomas, carcinomas, etc.), soft tissue, bone, breast, colon, liver, kidney, adrenal, gastrointestinal, pancreatic, gall bladder, salivary gland, cervical, ovary, uterus, testis, prostate, lung, thymus, thyroid, parathyroid, pituitary (adenomas, etc.), brain, spinal cord, ocular, nerve, and skeletal muscle, etc. In some embodiments, the subject from which the biopsy is obtained has a malignancy is selected from the list consisting of gastric cancer, esophageal carcinomas, head and neck cancer (e.g., head and neck squamous cell carcinoma, or HNSCC), renal cell carcinoma, urothelial/bladder carcinoma, colorectal cancer, ovarian carcinoma, myeloma, melanoma, lung cancer, classical Hodgkin lymphoma, and breast cancer (e.g., triple-negative breast cancer, hormone receptor positive (ER and/or PR) and Her2 positive breast cancer), small cell lung cancer including extensive stage small cell lung cancer and limited stage small cell lung cancer, salivary gland carcinoma, vulvar carcinoma, thyroid carcinoma, anal canal carcinoma, biliary carcinoma, mesothelioma, cervical carcinoma, and neuroendocrine carcinoma. In some embodiments, the subject from which the biopsy is obtained has a malignancy selected from melanoma,

liver, stomach, renal cell, colorectal, ovarian, colon, breast, esophagus, mesothelioma, and head and neck cancer. In some preferred embodiments, the biopsy of interest can include a tumour and optionally non-neoplastic biopsy of any of a melanoma, liver, stomach, renal cell, colorectal, ovarian, colon, breast, esophagus, mesothelioma, and head and neck solid tumour. In some preferred embodiments, the biopsy is obtained from a subject having urothelial, breast, or esophageal cancer, most preferably breast cancer such as TNBC.

Detecting PD-L1 positive cells in a tumour sample is meanwhile clinical routine and required as companion diagnostic for several approved cancer treatments. It can be done in any convenient manner. In certain embodiments, the CPS is calculated from a stained tumour tissue biopsy section (e.g., on a slide) or serial tumour tissue biopsy sections by immunohistochemistry (IHC) staining, in-situ hybridization (ISH; e.g., fluorescence-in-situ-hybridization, or FISH), histological stain, and a combination thereof. In certain embodiments, a tumour tissue biopsy section is analyzed by IHC. In certain embodiments, the percentage of viable PD-L1 positive and negative tumour cells and PD-L1 positive mononuclear inflammatory cells (MIC), i.e., lymphocytes and macrophages, is determined within the tumour nests and the adjacent supporting stroma. In such embodiments, cells are positive for PD- L1 staining if they display partial or complete membrane staining relative to all viable tumour cells present in the sample. In some preferred embodiments, PD-L1 is detected by immunohistochemistry (IHC) staining. In some preferred embodiments, the number of viable tumour cells can be determining by flow cytometry. For example, a tissue sample analyzed by flow cytometry can be contacted with a viability dye prior to analysis, e.g., propidium iodide or any convenient viability stain known in the art.

Interpretation of stained slides can preferably be performed using a light microscope with an objective of 20x magnification. All viable tumour cells on the entire slide are preferably evaluated and included in the CPS or TPS PD-L1 scoring assessment optionally together with tumour-associated PD-L1 positive lymphocytes and macrophages for the CPS. A minimum of 100 viable tumour cells are preferably present in the PD-L1 stained slide to be considered adequate for evaluation. For slides comprising less than 100 viable tumour cells, tissue from a deeper level of the block or potentially another block, could present sufficient tumour cells for PD-L1 evaluation.

In some embodiments, the tumour tissue section is formalin fixed and embedded in paraffin wax (FFPE). In alternative embodiments, the tissue section has been fixed in a different way, including tissue sections that have been fixed in, e.g., acrolein, glyoxal, osmium tetroxide, carbodiimide, mercuric chloride, zinc salts, picric acid, potassium di chromate, ethanol, methanol, acetone, and/or acetic acid.

In other embodiments, the PD-L1 scoring algorithm (such as CPS, TPS, or TAP) is calculated from a tumour tissue sample that is not a fixed section on a slide. For example, in certain embodiments, the CPS is calculated using flow cytometric analysis of a cell suspension from the tumour tissue sample. In these embodiments, the tumour tissue cell suspension can be stained with a detectable PD-L1 binding agent (e.g., a fluorescently labeled antibody) and analyzed on a flow cytometer for counting the number of tumour cells and MIC cells (i.e., lymphocytes and macrophages) expressing PD-L1. Tumour cells and MIC cells in the sample can be distinguished using any convenient flow cytometric parameter, e.g., forward scatter (FS), side scatter (SS), or by the expression of one or more additional markers using corresponding detectable binding agents for the one or more additional markers, e.g., markers specific or MIC or tumour cells. In other embodiments, the cells in the tumour tissue sample can be analyzed on a cell-by-cell basis for mRNA expression of PD-L1 and any other desired target, e.g., using single-cell nucleic acid sequencing methods for gene expression profiling (e.g., next generation sequencing methods).

In some embodiments, the tissue section is stained. In some embodiments, the stain comprises a hematoxylin and eosin (H&E) stain. Hematoxylin, a basic dye, stains nuclei blue due to an affinity to nucleic acids in the cell nucleus; eosin, an acidic dye, stains the cytoplasm pink. A hematoxylin and eosin (H&E) stained section can be used for the evaluation of an acceptable tumour tissue sample or of a tumour area.

There are many other staining techniques known to those of skill in the art that can be used to selectively stain cells and cellular components that find use in the present disclosure, and as such no limitation in this regard is intended. The staining of a target (e.g., PD-L1) in cells from a tumour tissue biopsy is generally done by contacting the cells with one or more detectable target-specific binding agents under suitable conditions to allow for binding of the target-specific binding agent to its desired target (while minimizing nontarget binding).

The term "target-specific binding agent" means any agent that specifically binds to a target or analyte of interest, e.g., a target of interest that is present in a tissue section as described herein (e.g., a polypeptide or polynucleotide). In some embodiments, the target-specific binding agent is an antibody (or target-binding fragments thereof), e.g., as used in IHC and flow cytometry.

Staining may be performed with primary and secondary antibodies or without using secondary antibodies (e.g., where the primary antibody is detectably labeled). Non-limiting examples of anti-PD-L1 antibodies include, but are not limited to, clone 22C3 (Merck & Co.), clone 28-8 (Bristol-Myers Squibb), clones SP263 (Roche), SP142 (Spring Biosciences), and clone E1L3N (Cell Signaling Technology). A preferred anti-PD-L1 antibody is clone SP263 (Roche), another preferred anti-PD-L1 antibody is clone E1L3N (Cell Signaling Technology) .

Clone 22C3 is a well-known, commercially available anti-PD-L1 antibody described e.g., in US 9,709,568 B2, which disclosure is incorporated by reference in its entirety.

Clone 22C3 comprises a heavy and light chain variable region, wherein the heavy chain variable region comprises: (i) a complementarity-determining region 1 (HCDR1) whose amino acid sequence is shown in SEQ ID NO: 23, (ii) a complementarity-determining region 2 (HCDR2) whose amino acid sequence is shown in SEQ ID NO: 24, and (iii) a complementarity-determining region 3 (HCDR3) whose amino acid sequence is shown in SEQ ID NO: 25; and the light chain variable region comprises: (i) a complementarity-determining region 1 (LCDR1) whose amino acid sequence is shown in SEQ ID NO: 20, (ii) a complementarity-determining region 2 (LCDR2) whose amino acid sequence is shown in SEQ ID NO: 21, and (iii) a complementarity-determining region 3 (LCDR3) whose amino acid sequence is shown in SEQ ID NO: 22. The heavy and light chain variable regions are set forth in SEQ ID NO: 27 and 26.

Clone 28-8 is a well-known commercially available anti-PD-L1 antibody described e.g., in US 9,212,224 B2, which disclosure is incorporated by reference in its entirety.

Clone 28-8 comprises a heavy and light chain variable region (i.e., VH and VL) set forth in SEQ ID NO: 28 and 29.

SP263 is a well-known commercially available anti-PD-L1 antibody described e.g., in US 2010/0343556 A1

Clone SP263 comprises a heavy and light chain variable region, wherein the heavy chain variable region comprises: (i) a complementarity-determining region 1 (HCDR1) whose amino acid sequence is shown in SEQ ID NO: 33, (ii) a complementarity-determining region 2 (HCDR2) whose amino acid sequence is shown in SEQ ID NO: 34, and (iii) a complementarity-determining region 3 (HCDR3) whose amino acid sequence is shown in SEQ ID NO: 35; and the light chain variable region comprises: (i) a complementarity-determining region 1 (LCDR1) whose amino acid sequence is shown in SEQ ID NO: 30, (ii) a complementarity-determining region 2 (LCDR2) whose amino acid sequence is shown in SEQ ID NO: 31, and (iii) a complementarity-determining region 3 (LCDR3) whose amino acid sequence is shown in SEQ ID NO: 32. The heavy and light chain variable region are set forth in SEQ ID NO: 36 and 37.

In certain other embodiments, a target specific binding agent is a nucleic acid or nucleic acid binding agent, e.g., as employed in in situ hybridization (ISH) reactions. For example, the target binding reagent can be a DNA, RNA, DNA/RNA hybrid molecule, peptide nucleic acid (PNA), and the like. No limitation in the metes and bounds of a target-specific binding agent that finds use in the subject disclosure is intended.

The target-specific binding agent (or any secondary reagent used to detect the target-specific binding agent) may be attached to any suitable detectable label (or chromogen) or enzyme capable of producing a detectable label. Thus, in certain embodiments, the first or second label is produced by an enzymatic reaction, e.g., by the activity of horseradish peroxidase, alkaline phosphatase, and the like. Any convenient enzymatic label/chromogen deposition system can be employed (e.g., as used in standard IHC methods), and as such, no limitation in this regard is intended. In some embodiments, the detectable label is a fluorescent tag.

In some embodiments, the staining reagents used may include a target-specific antibody (e.g., a PD-L1 specific antibody). Where an additional target is to be detected, the staining reagents used may include one or more additional antibodies that each bind to a different antigen. For example, a set of antibodies may include a first antibody that binds to a first antigen (e.g., PD-L1), a second antibody that binds to a second antigen, optionally a third

antibody that binds to a third antigen and, optionally a fourth antibody that binds to a fourth antigen and/or further antibodies that bind to further antigens. In some embodiments, the antibody/antibodies used are primary antibodies that are detected by use of a secondary antibody (or other reagent). The staining steps thus may be done by incubating the cells of the tissue sample, e.g., a tissue section or cell suspension, with the primary antibody/antibodies and then, after the primary antibody has bound to the desired target in/on the cells, incubating the cells with the labeled secondary antibody/ antibodies (e.g., as is done in standard IHC protocols). In some embodiments, each of the primary antibodies for each different target is from a different species (e.g., goat, rabbit, mouse, camel, chicken, donkey, etc.) and the corresponding secondary antibodies specific for each different primary antibody are distinguishably labeled from each other.

In some preferred embodiments, the viable tumour cells and the number of lymphocytes and macrophages are counted in the tumour nests and the adjacent supporting stroma of the tumour tissue sample.

Although the result of a CPS calculation can exceed 100, the maximum score is preferably defined as 100 and a minimum of 100 viable tumour cells are preferably required to calculate the score. See Park et al. Cancer Res. Treat., 2020; 52(3):661-670 and Yamashita et al., Gastric Cancer, 2020, 23: 95-104.

When an integrating scoring algorithm different than the CPS is used in the context of the methods disclosed herein, an *in vitro* diagnostic device (IVD) bridging study can be performed to determine whether that scoring algorithm defines a similar PD-L1 expression score as the established CPS. How to conduct such bridging assays is well known in the field of companion diagnostics and described, e.g., in detail in the FDA authorized "Principles for Codevelopment of an In Vitro Companion Diagnostic Device with a Therapeutic Product" issued on July 15, 2016, which disclosure is incorporated by reference in its entirety.

As disclosed therein, a test for determining PD-L1 expression score other than the CPS companion diagnostic (e.g., determining PD-L1 expression score using the IHC assay PD-L1 IHC 22C3 pharmDx in accordance with the manufacturer's guide) can be used, if it can be demonstrated that the other IVD companion diagnostic has performance characteristics that are very similar to those of the CPS companion diagnostic. This is generally

demonstrated through a bridging study between the two tests, using the original clinical trial samples and a pre-specified statistical analysis plan, to show that results with the candidate IVD companion diagnostic are very similar to those of the CPS companion diagnostic. A bridging study can evaluate efficacy of the therapeutic product in subjects whose marker status is determined by the candidate IVD companion diagnostic by assessing both concordance and discordance between the two tests using the same specimens from subjects who were tested for trial eligibility. The analysis needs to consider any potential impact of missing samples not available for the concordance study. The ability of the candidate IVD companion diagnostic to predict the efficacy of the therapeutic product can be supported indirectly by high analytical concordance with the CPS companion diagnostic on a large number of representative samples, including samples from subjects excluded from the trial because they were marker-negative by the CPS companion diagnostic. The assessment of the clinical validity of the candidate IVD companion diagnostic can rely on extrapolating the clinical performance characteristics of the CPS companion diagnostic to the clinical performance characteristics of the candidate IVD companion diagnostic. The ideal bridging study is one in which all samples tested with the trial test are retested with the candidate IVD companion diagnostic and valid test results are obtained and used to assess comparative performance. A bridging study with specimens from an all-comers trial also allows an analysis of efficacy using the results of the candidate IVD companion diagnostic. Note, however, that care should preferably be taken in understanding the analytical performance of the IVD prior to the bridging study because adjustments to the IVD should not be made from results obtained with the clinical trial samples. Whether a clinical trial enrolls subjects irrespective of the test result or enrolls only the subset of subjects identified by the test result, both the test-negative and test-positive clinical trial samples should preferably be included in bridging studies to avoid bias due to prescreening.

It is, however, recognized (e.g., by the FDA) that there are many reasons why all the samples tested with the CPS companion diagnostic may not be available for retesting, including that samples are missing, not accessible, or insufficient in quantity to retest, and it may not be possible to retest all samples. If only a subset of samples is retested, it should preferably be ensured that the characteristics of the subset adequately reflect the characteristics that affect test performance (e.g., tumour size, histology, melanin content,

necrotic tissue, resected tissue versus core needle biopsy) and that the characteristics of the subjects that may affect therapeutic product efficacy (e.g., patient demographics, stage of disease, stratification factors) are proportionally preserved in the retest sample set when compared to the samples in the original set. In addressing baseline imbalance between the retested and non-retested analysis sets, FDA recommends that any covariates are identified that can affect the test result and then check for baseline imbalance between the retested and non-retested analysis sets using the set of covariates identified. A re-analysis of the primary outcome data should preferably be made according to the final test results with the retest sample set in order to assure that any reclassification that occurs does not alter conclusions about the safety and efficacy of the therapeutic product in the selected population. When all samples are not retested, a second re-analysis can be conducted in which missing data for the final test are imputed. The nature of the re-analysis will be product-specific and may be discussed with the appropriate IVD review center. Finally, additional analytical validation can potentially be requested to support satisfactory concordance across methods where discordance may arise, e.g., precision, limit of detection, and accuracy. In the event there is discordance in a marker-positive-only trial, it is possible that the candidate IVD companion diagnostic will more accurately predict responders, a difference that would represent an advantage for optimal use of the therapeutic product.

For example, when using a PD-L1 expression score obtained from the TAP scoring algorithm, it can be demonstrated that the TAP has performance characteristics that are very similar to those of the CPS companion diagnostic using the described bridging study. The same holds true for a PD-L1 expression score determined using different anti-PD L1 antibodies, such as SP263 or 22C3 or E1L3N. For example, it can be demonstrated that a PD-L1 expression score obtained from using 22C3 and the CPS scoring algorithm has characteristics that are very similar to those of a PD-L1 expression score obtain from using 28-8 and the CPS scoring algorithm using the described bridging study.

In some embodiments, the bispecific antibody and the chemotherapy are separately administered. In some embodiments, a dosage of the bispecific antibody and a dosage of the chemotherapy are separately administered. In some embodiments, a dosage of the bispecific antibody and a dosage of the chemotherapy are administered using a single composition.

In some embodiments, a dosage of the bispecific antibody and a dosage of the chemotherapy are administered concurrently or consecutively.

The bispecific antibody and the chemotherapy provided herein can be administered via any suitable enteral route or parenteral route of administration. The term "enteral route" of administration refers to the administration via any part of the gastrointestinal tract. Examples of enteral routes include oral, mucosal, buccal, and rectal route, or intragastric route. "Parenteral route" of administration refers to a route of administration other than enteral route. Examples of parenteral routes of administration include intravenous, intramuscular, intradermal, intraperitoneal, intratumour, intravesical, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, transtracheal, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrastemal, subcutaneous, or topical administration. The bispecific antibody and the chemotherapy of the disclosure can be administered using any suitable method, such as by oral ingestion, nasogastric tube, gastrostomy tube, injection, infusion, implantable infusion pump, and osmotic pump. The suitable route and method of administration may vary depending on a number of factors such as the specific therapeutic agent being used, the rate of absorption desired, specific formulation or dosage form used, type or severity of the disorder being treated, the specific site of action, and conditions of the subject, and can be readily selected by a person skilled in the art. The terms "treatment regimen," "dosing protocol," and "dosing regimen" are used interchangeably to refer to the dosage and timing of administration of each dosage bispecific antibody and the chemotherapy in a combination therapy of the disclosure.

In some preferred embodiments, the bispecific antibody and/or the chemotherapy is administered intravenously, preferably wherein the bispecific antibody and the chemotherapy are administered intravenously. In some embodiments, the bispecific antibody and/or the chemotherapy is administered via an IV injection or IV infusion. Preferably, the bispecific antibody and the chemotherapy are administered via an IV injection or IV infusion. For example, the bispecific antibody and the chemotherapy can be administered concurrently or consecutively via an IV infusion.

In one embodiment, the bispecific antibody is administered in a step-up dosing regimen to a subject or patient, e.g., with a first and one or more subsequent dose regimens. For example, in the first dose regimen the dosage of the bispecific antibody is lower than the

dosage of the bispecific antibody in a second dose regimen. If the bispecific antibody is administered in more than two dose regimens, the dosage of the bispecific antibody in each subsequent dosage regimen is higher compared to the dosage of the bispecific antibody in the dose regimen that precedes each subsequent dose regimen. In some embodiments of the afore mentioned examples, the dosage of the chemotherapeutic agent may be the same in the first and one or more subsequent dose regimens. In some embodiments of the afore mentioned examples, in the first dose regimen the dosage of the chemotherapeutic agent may be lower than the dosage of the chemotherapeutic agent in a second dose regimen. If the chemotherapeutic agent is administered in more than two dose regimens in some embodiment of the afore mentioned examples, the dosage of the chemotherapeutic agent in each subsequent dosage regimen may be higher compared to the dosage of the chemotherapeutic agent in the dose regimen that precedes each subsequent dose regimen.

In some embodiments, the subject has been determined to have a PD-L1 expression score before the treatment as determined by the combined positive score (CPS) of ≤ 30, preferably ≤ 20, more preferably ≤ 10, and most preferably < 10 or even < 1 or other integrating scoring algorithm defining a similar PD-L1 expression score such as TPS or TAP, preferably TAP. For example, the subject has been preferably determined to have a PD-L1 expression score before the treatment as determined by a CPS of up to 20, preferably from 1 to 20 or > 0 to 20, more preferably from 1 to <10, such as 1 to 9, or other integrating scoring algorithm defining a similar PD-L1 expression score. In some preferred embodiments, the subject has been determined to have a PD-L1 expression score as determined by a CPS from 1 to 9, 1 to 8, 1 to 7, 1 to 6, 1 to 5, 1 to 4, or 1 to 3 or other integrating scoring algorithm defining a similar PD-L1 expression score. For example, the subject has a PD-L1 expression score as determined by a CPS of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20, preferably a CPS of 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10, more preferably a CPS of 1, 2, 3, 4, 5, 6, 7, 8, or 9 or other integrating scoring algorithm defining a similar PD-L1 expression score such as TPS or TAP. In some particularly preferred embodiments, the subject has been determined to have a PD-L1 expression score as determined by CPS of < 10, < 9, < 8, < 7, < 6, < 5, < 4, or < 3 before the treatment or other integrating scoring algorithm defining a similar PD-L1 expression score such as TPS or TAP. In some particularly preferred embodiments, the subject has been determined to have a PD-L1 expression score as determined by CPS of > 0 before the treatment or other

integrating scoring algorithm defining a similar PD-L1 expression score such as TPS or TAP.

In some embodiments, the subject is part of a patient group to be treated with the combination therapy disclosed herein. For example, this patient group comprises one or more subjects having a PD-L1 expression score as determined by a CPS of ≤ 30, preferably ≤ 20, more preferably ≤ 10, and most preferably < 10 or even < 1, before the treatment or other integrating scoring algorithm defining a similar PD-L1 expression score such as TPS or TAP. This patient group may further comprise one or more subjects having a PD-L1 expression score as determined by a CPS of > 30, preferably >20, more preferably > 10, and most preferably ≥ 10 or other integrating scoring algorithm defining a similar PD-L1 expression score such as TPS or TAP.

In some embodiments, the bispecific antibody is administered in a dosage ranging from 0.1 mg/kg to 45 mg/kg body weight, preferably 1 mg/kg to 30 mg/kg body weight per treatment.

In some embodiments, the treatment comprises repeated treatments, wherein the treatment cycle is repeated at least 1, 2, 3, 4, 5, 6, 7, or 8 times, wherein each cycle preferably has up to 30, preferably 28 days or 21 days. However, the treatment can be continued until disease progression or the initiation of a new anti-tumour treatment.

In some embodiments, the subject has not been previously treated for cancer, i.e., is treatment naive. In some embodiments, the subject has been previously treated for cancer, in particular the subject has had at least one previous chemotherapy treatment.

In some embodiment, the subject has not been previously treated with a PD-1/PD-L1 inhibitor, such as pembrolizumab or atezolizumab.

In some embodiment, the subject had one or more been previous treatments with a PD-1/PD-L1 inhibitor, such as pembrolizumab or atezolizumab.

In some embodiments, the bispecific antibody is administered every 6 weeks, preferably every 4 weeks, more preferably every 3 weeks, and most preferably every 2 weeks.

In some embodiments, the bispecific antibody is administered every 2 weeks at a dosage ranging from 10 mg/kg to 30 mg/kg, preferably ranging from 15 mg/kg to 25 mg/kg, more preferably being 20 mg/kg.

In some embodiments, the bispecific antibody is administered every 3 weeks at a dosage ranging from 20 mg/kg to 40 mg/kg, preferably ranging from 25 mg/kg to 35 mg/kg, more preferably being 30 mg/kg.

In some embodiments, the chemotherapy is administered once or more within the first 20 days or the first 21 days of each cycle, wherein the chemotherapy is administered twice or more within the first 20 days or the first 21 days of each cycle, more preferably wherein the chemotherapy is administered at least thrice within the first 20 days or the first 21 days of each cycle.

In some embodiments, the chemotherapy is administered once or more within the first 14 days or the first 15 days of each cycle, wherein the chemotherapy is administered twice or more within the first 14 days or the first 15 days of each cycle, more preferably wherein the chemotherapy is administered at least thrice within the first 14 days or the first 15 days of each cycle. For example, the chemotherapy can be administered on the 1st, 8th, and 15th day of each cycle, wherein each cycle has 21 days.

In some embodiments, the treatment method disclosed herein results in increased overall survival in said subject compared to the chemotherapy or the bispecific antibody or an anti-PD-L1 antibody or an anti-PD-1 antibody treatment alone or compared to a standard treatment comprising the chemotherapy and an anti-PD-L1 antibody or chemotherapy and an anti-PD-1 antibody, wherein the anti-PD-L1 antibody is preferably atezolizumab and the anti-PD-1 antibody is preferably pembrolizumab. In some embodiments, the treatment method disclosed herein results in increased median progression-free survival said subject compared to the chemotherapy or the bispecific antibody or anti-PD-L1 antibody or anti-PD-1 antibody treatment alone or compared to a standard treatment comprising the chemotherapy and the anti-PD-L1 antibody or the chemotherapy and the anti-PD-1 antibody, wherein the anti-PD-L1 antibody is preferably atezolizumab and the anti-PD-1 antibody is preferably pembrolizumab.

In some embodiments, the cancer is a solid tumour.

In some embodiments, the cancer is selected from the group consisting of melanoma, lung, liver, stomach, renal cell, urothelial, cervical, colorectal, ovarian, colon, breast, esophagus, mesothelioma, and head and neck cancers, preferably the cancer is selected from urothelial, breast and esophagus cancer. For example, the cancer can be selected from non-small cell lung cancer (NSCLC), small cell lung cancer (SCLC) including extensive stage small cell lung cancer and limited stage small cell lung cancer, urothelial carcinoma, esophageal cancer, head and neck squamous cell carcinoma (HNSCC), triple-negative breast cancer (TNBC), or cervical cancer. The cancer can preferably be TBNC, gastric adenocarcinoma, gastroesophageal junction (GEJ) adenocarcinoma, esophageal squamous-cell carcinoma, cervical cancer, urothelial carcinoma, or HNSCC. Most preferably, the cancer is TBNC. The TNBC may be a TNBC of the subtype (1) luminal androgen receptor (LAR), (2) immunomodulatory (IM), (3) basal-like immune-suppressed (BLIS), or (4) mesenchymal-like (MES) according to the Fudan University Shanghai Cancer Center (FUSCC) classification (see Jiang YZ, Ma D, Suo C, et al. Genomic and transcriptomic landscape of triple-negative breast cancers: subtypes and treatment strategies. Cancer Cell. 2019; 35(3):428-440.e5), preferably TNBC of the subtype IM or MES.

In some preferred embodiments, the cancer is small cell lung cancer (SCLC), non-small cell lung cancer (NSCLC) or triple-negative breast cancer (TNBC), preferably the cancer is triple-negative breast cancer, for example advanced or metastatic triple-negative breast cancer.

In some embodiments, the NSCLC has a squamous histology. In some embodiments, the NSCLC has a non-squamous histology. In some embodiments, the NCLC is an EGFR mutation-positive NSCLC.

In some preferred embodiments, the bispecific antibody comprises an anti-PD-L1 antibody or fragment thereof. In some preferred embodiments, the bispecific antibody comprises an anti-VEGF antibody or fragment thereof, preferably an anti-VEGF-A antibody or fragment thereof.

In some embodiments, the bispecific antibody comprises a Fab, F'b', F(ab')2, Fd, Fv, sdAb, complementarity determining region fragment, single chain antibody, humanized antibody, chimeric antibody or diabody antibody, preferably a single domain antibody, more preferably a VHH.

In some embodiments, the bispecific antibody comprises two anti-PD-L1 single domain antibodies, preferably two VHHs, preferably fused to the N- or C-terminus of the anti-VEGF antibody heavy or light chain. In some embodiments, the bispecific antibody comprises two anti-PD-L1 single domain antibodies, preferably two VHHs, preferably fused to the C-terminus of the anti-VEGF antibody heavy chain.

In some embodiments, the anti-PD-L1 antibody or fragment thereof is a monovalent or bivalent antibody or fragment thereof. In some embodiments, the anti-VEGF antibody or fragment thereof is a monovalent or bivalent antibody or fragment thereof,

In some preferred embodiments, the bispecific antibody comprises an anti-PD-L1 single domain antibody comprising a heavy chain variable region, and the heavy chain variable region comprises: (i) a complementarity-determining region 1 (HCDR1) whose amino acid sequence is shown in SEQ ID NO: 1, (ii) a complementarity-determining region 2 (HCDR2) whose amino acid sequence is shown in SEQ ID NO: 2, and (iii) a complementarity-determining region 3 (HCDR3) whose amino acid sequence is shown in SEQ ID NO: 3, or a variant thereof having up to three, e.g. one or two, amino acid substitutions, additions or deletions, wherein the CDRs are defined according to the IMGT numbering system (see Ehrenmann F, Kaas Q, Lefranc M P. IMGT/3Dstructure-DB and IMGT/DomainGapAlign: a database and a tool for immunoglobulins or antibodies, T cell receptors, MHC, IgSF and MhcSF[J]. Nucleic acids research, 2009;38(suppl_1):D301-D307).

In an alternative example, the bispecific antibody comprises an anti-PD-L1 single domain antibody comprising a heavy chain variable region, and the heavy chain variable region comprises: (i) a complementarity-determining region 1 (HCDR1) whose amino acid sequence is shown in SEQ ID NO: 18, (ii) a complementarity-determining region 2 (HCDR2) whose amino acid sequence is shown in SEQ ID NO: 19, and (iii) a complementarity-determining region 3 (HCDR3) whose amino acid sequence is shown in SEQ ID NO: 38, or a variant thereof having up to three, e.g. one or two, amino acid substitutions, additions or deletions, wherein the CDRs are defined according to the Kabat numbering system.

In some embodiments, the CDR variant has up to two amino acid substitutions, additions or deletions. In some embodiments, the CDR variant has up to one amino acid substitution,

addition or deletion. In some embodiments, the CDR variant has up to three amino acid substitutions. In some embodiments, the CDR variant has up to two amino acid substitutions. In some embodiments, the CDR variant has up to one amino acid substitution.

In some embodiments, the amino acid sequence of the anti-PD-L1 single domain antibody is shown in SEQ ID NO: 9. In some embodiments, the amino acid sequence of the anti-PD-L1 single domain antibody has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 9 and/or one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) amino acid additions, deletions and/or substitutions in a framework region thereof. For example, the bispecific antibody can comprise one anti-VEGF antibody and two anti-PD-L1 single domain antibodies, preferably fused to the C-terminus of the anti-VEGF antibody, wherein each of these anti-PD-L1 single domain antibodies comprises or consists of the amino acid sequence shown in SEQ ID NO: 9 or wherein the amino acid sequence of the anti-PD-L1 single domain antibody has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 9 In some embodiments, the amino acid sequence of the anti-PD-L1 single domain antibody has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 9 and/or one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) amino acid additions, deletions and/or substitutions in a framework region thereof.

In some embodiments, the anti-PD-L1 antibody or fragment thereof comprises or consists of the amino acid sequence of SEQ ID NO: 9, or an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto and/or one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) amino acid additions, deletions and/or substitutions in a framework region thereof, and comprises a heavy chain variable region comprising: a HCDR1 comprising or consisting of the amino acid sequence of SEQ ID NO: 1; a HCDR2 comprising or consisting of the amino acid sequence of SEQ ID NO: 2; and a HCDR3 comprising or consisting of the amino acid sequence of SEQ ID NO: 3.

In some embodiments, the anti-PD-L1 antibody or fragment thereof comprises or consists of the amino acid sequence of SEQ ID NO: 9, or an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto and/or one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) amino acid additions, deletions and/or substitutions in a framework region thereof, and comprises a heavy chain variable region comprising: a HCDR1 comprising or consisting of the amino acid sequence of SEQ ID NO: 18; a HCDR2

comprising or consisting of the amino acid sequence of SEQ ID NO: 19; and a HCDR3 comprising or consisting of the amino acid sequence of SEQ ID NO: 38.

In some embodiments, the anti-PD-L1 antibody or fragment thereof comprises or consists of the amino acid sequence of SEQ ID NO: 9. In some embodiments, the anti-PD-L1 antibody or fragment thereof consists of the amino acid sequence of SEQ ID NO: 9.

In some preferred embodiments, the bispecific antibody specifically binds to VEGF-A.

In some embodiments, the anti-VEGF antibody or fragment thereof comprises a constant region preferably derived from a human antibody, preferably, the constant region is selected from the constant region of human IgG1, IgG2, IgG3 or IgG4. In some embodiments, the anti-VEGF-A antibody or fragment thereof comprises a constant region preferably derived from a human antibody, preferably, the constant region is selected from the constant region of human IgG1, IgG2, IgG3 or IgG4.

In some embodiments, the anti-VEGF antibody or fragment thereof comprises an Fc region comprising one or more mutations which reduce or abolish immune effect functions. Suitable mutations are known in the art and may include one or more amino acid substitutions (see e.g. Wilkinson, I., et al., 2021. FcPLoS One, 16(12), p.e0260954; and Liu, R., et al., 2020. Antibodies, 9(4), p.64). In some embodiments, the anti-VEGF antibody or fragment thereof comprises an IgG1 Fc region comprising a L234A (with the numbering according to the EU-index) and/or a L235A mutation (with the numbering according to the EU-index). In some embodiments, the anti-VEGF antibody or fragment thereof comprises an IgG1 Fc region comprising L234A and L235A mutations (with the numbering according to the EU-index). As noted, amino acids are numbered according to the Eu numbering (Eu-index) as set forth in Kabat (Kabat, E.A.; National Institutes of Health (U.S.) Office of the Director. *Sequences of Proteins of Immunological Interest*, 5th ed.; DIANE Publishing: Collingdale, PA, USA, 1991).

In some embodiments, the anti-VEGF antibody or fragment thereof comprises a IgG1 Fc region, preferably having the amino acid sequence shown in SEQ ID NO: 13. In some embodiments, the anti-VEGF-A antibody or fragment thereof comprises a IgG1 Fc region, preferably having the amino acid sequence shown in SEQ ID NO: 13 or an amino acid

sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence shown in SEQ ID NO: 13.

The one or more anti-PD-L1 single domain antibodies may be fused to the anti-VEGF antibody by any suitable method. In some embodiments, each anti-PD-L1 single domain antibody is terminally-fused to the anti-VEGF antibody. In some embodiments, each anti-PD-L1 single domain antibody is fused to: (i) the C-terminus of a heavy chain; (ii) the N-terminus of a heavy chain; (iii) the C-terminus of a light chain; or (iv) the N-terminus of a light chain. In preferred embodiments, each anti-PD-L1 single domain antibodies is fused to the C-terminus of an anti-VEGF antibody, preferably the C-terminus of a heavy chain. The bispecific antibody preferably comprises an anti-VEGF antibody and two anti-PD-L1 VHHs, wherein each heavy chain of the anti-VEGF antibody is fused to an anti-PD-L1 VHH.

The VHH can be fused to the anti-VEGF antibody by a linker, for example a glycine and serine-rich linker (GS-linker) or any suitable linker known in the art. An example linker is provided by SEQ ID NO: 14 or an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence shown in SEQ ID NO: 14.

In some embodiments, the anti-VEGF antibody or fragment thereof comprises a CL, preferably having the amino acid sequence shown in SEQ ID NO: 15 or an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence shown in SEQ ID NO:15. In some embodiments, the anti-VEGF antibody or fragment thereof comprises a CH1, preferably having the amino acid sequence shown in SEQ ID NO: 12 or an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence shown in SEQ ID NO: 12.

In some embodiments, the anti-VEGF antibody comprises a heavy and light chain variable region, wherein the heavy chain variable region comprises: (i) a complementarity-determining region 1 (HCDR1) whose amino acid sequence is shown in SEQ ID NO: 4, (ii) a complementarity-determining region 2 (HCDR2) whose amino acid sequence is shown in SEQ ID NO: 5, and (iii) a complementarity-determining region 3 (HCDR3) whose amino acid sequence is shown in SEQ ID NO: 6, or a variant thereof having up to three e.g. one or two, amino acid substitutions, additions or deletions; and

wherein the light chain variable region comprises: (i) a complementarity-determining region 1 (LCDR1) whose amino acid sequence is shown in SEQ ID NO: 7, (ii) a complementarity-determining region 2 (LCDR2) whose amino acid sequence is shown in SEQ ID NO: 44, and (iii) a complementarity-determining region 3 (LCDR3) whose amino acid sequence is shown in SEQ ID NO: 8, or a variant thereof having up to three, e.g. one or two, amino acid substitutions, additions or deletions. These CDRs are defined according to the IMGT numbering system (see Ehrenmann F, Kaas Q, Lefranc M P. IMGT/3Dstructure-DB and IMGT/DomainGapAlign: a database and a tool for immunoglobulins or antibodies, T cell receptors, MHC, IgSF and MhcSF[J]. Nucleic acids research, 2009;38(suppl_1):D301-D307).

In some embodiments, the anti-VEGF antibody comprises a heavy and light chain variable region, wherein the heavy chain variable region comprises: (i) a complementarity-determining region 1 (HCDR1) whose amino acid sequence is shown in SEQ ID NO: 39, (ii) a complementarity-determining region 2 (HCDR2) whose amino acid sequence is shown in SEQ ID NO: 40, and (iii) a complementarity-determining region 3 (HCDR3) whose amino acid sequence is shown in SEQ ID NO: 41, or a variant thereof having up to three e.g. one or two, amino acid substitutions, additions or deletions; and
wherein the light chain variable region comprises: (i) a complementarity-determining region 1 (LCDR1) whose amino acid sequence is shown in SEQ ID NO: 42, (ii) a complementarity-determining region 2 (LCDR2) whose amino acid sequence is shown in SEQ ID NO: 43, and (iii) a complementarity-determining region 3 (LCDR3) whose amino acid sequence is shown in SEQ ID NO: 8, or a variant thereof having up to three, e.g. one or two, amino acid substitutions, additions or deletions. These CDRs are defined according to the Kabat numbering system.

In some embodiments, the CDR variant has up to two amino acid substitutions, additions or deletions. In some embodiments, the CDR variant has up to one amino acid substitution, addition or deletion. In some embodiments, the CDR variant has up to three amino acid substitutions. In some embodiments, the CDR variant has up to two amino acid substitutions. In some embodiments, the CDR variant has up to one amino acid substitution.
In some embodiments, the amino acid sequence of the heavy chain variable region of the anti-VEGF antibody (or anti-VEGF-A antibody) is shown in SEQ ID NO: 10, and the amino acid sequence of the light chain variable region of the anti-VEGF antibody is shown

in SEQ ID NO: 11; or

wherein the amino acid sequence of the heavy chain variable region of the anti-VEGF antibody has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 10, and the amino acid sequence of the light chain variable region of the anti-VEGF antibody has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 11.

In some embodiments, the amino acid sequence of the heavy chain of the bispecific antibody is shown in SEQ ID NO: 16, and the amino acid sequence of the light chain variable region of the bispecific antibody is shown in SEQ ID NO: 17; or

wherein the amino acid sequence of the heavy chain of the bispecific antibody has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 16, and the amino acid sequence of the light chain variable region of the bispecific antibody has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 17.

In some embodiments, the anti-VEGF-A antibody is bevacizumab.

In some embodiments, the bispecific antibody is encoded by one or more nucleic acid molecules.

The bispecific antibody can preferably comprise:

(a) two anti-PD-L1 single domain antibodies each comprising a heavy chain variable region, and the heavy chain variable region comprises: (i) a complementarity-determining region 1 (HCDR1) whose amino acid sequence is shown in SEQ ID NO: 1, (ii) a complementarity-determining region 2 (HCDR2) whose amino acid sequence is shown in SEQ ID NO: 2, and (iii) a complementarity-determining region 3 (HCDR3) whose amino acid sequence is shown in SEQ ID NO: 3; and

(b) an anti-VEGF-A antibody comprising a heavy chain variable region comprising: (i) a complementarity-determining region 1 (HCDR1) whose amino acid sequence is shown in SEQ ID NO: 4, (ii) a complementarity-determining region 2 (HCDR2) whose amino acid sequence is shown in SEQ ID NO: 5, and (iii) a complementarity-determining region 3 (HCDR3) whose amino acid sequence is shown in SEQ ID NO: 6; and

a light chain variable region comprising: (i) a complementarity-determining region 1 (LCDR1) whose amino acid sequence is shown in SEQ ID NO: 7, (ii) a complementarity-determining region 2 (LCDR2) whose amino acid sequence is shown in SEQ ID NO: 44, and (iii) a complementarity-determining region 3 (LCDR3) whose amino acid sequence is shown in SEQ ID NO: 8.

In particularly preferred embodiments, the bispecific antibody or bispecific antibody variants are described in WO 2022/042719, which disclosure is hereby incorporated in its entirety.

The bispecific antibody can be linked or produced by various methods, see, for example, the method of Songsivilai et al. (Clin. Exp. Immunol., 79:315-321 (1990)), and the method of Kostelny et al. (J. Immunol., 148:1547-1553 (1992) which disclosures are also hereby incorporated in their entirety.

In some embodiments, the bispecific antibody is conjugated with a chemotherapeutical agent to obtain an immunoconjugate. In some embodiments, the immunoconjugate contains:

(a) a bispecific antibody as disclosed herein; and

(b) a conjugation moiety selected from the group consisting of detectable labels, drugs, toxins, cytokines, radionuclides, or enzymes, gold nanoparticles/nanorods, nanomagnetic particles, viral coat proteins or VLPs, or their combination.

In some embodiments, the radionuclide includes:

(a) a diagnostic isotope selected from the group consisting of Tc-99m, Ga-68, F-18, I-123, I-125, I-131, In-111, Ga-67, Cu-64, Zr-89, C-11, Lu-177, Re-188, or a combination thereof; and/or

(b) a therapeutic isotope selected from the group consisting of Lu-177, Y-90, Ac-225, As-211, Bi-212, Bi-213, Cs-137, Cr-51, Co-60, Dy-165, Er-169, Fm-255, Au-198, Ho-166, I-125, I-131, Ir-192, Fe-59, Pb-212, Mo-99, Pd- 103, P-32, K-42, Re-186, Re-188, Sm-153, Ra223, Ru-106, Na24, Sr89, Tb-149, Th-227, Xe-133Yb-169, Yb-177, or a combination thereof.

In some embodiments, the coupling moiety is a drug or a toxin. In another preferred embodiment, the drug is a cytotoxic drug. In some embodiments, the cytotoxic drugs are selected from the group consisting of anti-tubulin drugs, DNA minor groove binding reagents, DNA replication inhibitors, alkylating reagents, antibiotics, folic acid antagonists, antimetabolites, chemotherapy A sensitizer, a topoisomerase inhibitor, a vinca alkaloid, or a combination thereof. Examples of particularly useful cytotoxic drugs include, for example, DNA minor groove binding agents, DNA alkylating agents, and tubulin inhibitors. Typical cytotoxic drugs include, for example, auristatins, camptothecins, duocarmycins, etoposides, maytansines and maytansinoids (eg DM1 and DM4), taxanes (taxanes), benzodiazepines, or benzodiazepine containing drugs (eg, pyrrolo[1,4]benzodiazepines (PBDs), indoline benzodiazepines indolinobenzodiazepines and oxazolidinobenzodiazepines), vinca alkaloids, or combinations thereof.

In some embodiments, the toxin is selected from the following group: Auristatins (e.g., auristatin E, auristatin F, MMAE, and MMAF), chlortetracycline, maytansoid, gamatoxin, gamatoxin A-chain, combretastatin, docarmicin, Lastatin, doxorubicin, daunorubicin, paclitaxel, cisplatin, cc1065, ethidium bromide, mitomycin, etoposide, tenoposide, vincristine, vinblastine, autumn Narcissin, Dihydroxyanthraxdione, Actinomycin, Diphtheria Toxin, Pseudomonas Exotoxin (PE) A, PE40, Acacia toxin, Acacia A chain, Capsule root toxin A chain, α - Sarcinus, gelonin, mitogellin, retstrictocin, phenomycin, enomycin, curicin, crotontoxin, calicheamicin, Sapaonaria officinalis inhibitors, glucocorticoids, or a combination thereof.

Chemotherapy

The methods of treating disclosed herein comprise a chemotherapy. In some embodiments, the chemotherapy comprises one or more platinum-based chemotherapeutic agents. In some embodiments, the one or more platinum-based chemotherapeutic agents is carboplatin, cisplatin, oxaliplatin, or combinations thereof. In some embodiments, the chemotherapy comprises one or more antimetabolite-based chemotherapy agents. In some embodiments, the one or more antimetabolite-based chemotherapeutic agent is gemcitabine. In some embodiments, chemotherapy comprises one or more platinum-based chemotherapeutic agents in combination with one or more antimetabolite-based chemotherapy agents. In some embodiments, the chemotherapy comprises cisplatin and gemcitabine.

In some embodiments, the bispecific antibody is administered concurrently with chemotherapy. In some embodiments, the bispecific antibody and chemotherapy are administered within up to 15 days of each other. In some embodiments, the bispecific antibody and chemotherapy are administered within about two days of each other. In some embodiments, the bispecific antibody and chemotherapy are administered within about one day of each other. In some embodiments, the bispecific antibody and chemotherapy are administered concurrently (for example by simultaneous (same day) administration). In some embodiments, the bispecific antibody is administered on day 1 of the chemotherapy treatment cycle.

In some embodiments, the observed toxicities of the treatment with the bispecific antibody and chemotherapy combination are similar to those commonly seen with either chemotherapy or immunotherapy alone.

In some embodiments, the chemotherapy comprises a chemotherapy agent selected from lurbinectedin, topotecan, taxane, such as paclitaxel, nanoparticle albumin-bound paclitaxel (nab-paclitaxel) or docetaxel, pemetrexed, 5-fluoruracil, irinotecan, etoposide, gemcitabine, anthracyclines, capecitabine, vinorelbine, eribulin, ixabepilone or combinations thereof. In some embodiments, the chemotherapy comprises a chemotherapy agent selected from topotecan, taxane, such as paclitaxel, nanoparticle albumin-bound paclitaxel (nab-paclitaxel) or docetaxel, pemetrexed, 5-fluoruracil, irinotecan, etoposide, gemcitabine, anthracyclines, capecitabine, vinorelbine, eribulin, ixabepilone or combinations thereof.

In some embodiments, the platinum-based chemotherapy comprises cisplatin, oxaliplatin or carboplatin.

In some preferred embodiments, the method of treatment comprises administering the bispecific antibody in combination with paclitaxel, lurbinectedin, or topotecan. In some preferred embodiments, the method of treatment comprises administering the bispecific antibody in combination with paclitaxel or topotecan. In some such embodiments, the cancer is small cell lung cancer and, preferably, the method of treatment can be a second line cancer treatment. In a particularly preferred embodiment, the method of treatment comprising administering to a patient suffering from advanced small cell lung cancer as second line treatment the bispecific antibody every 3 weeks at a dosage of 20 mg/kg or 30 mg/kg in combination with paclitaxel every 3 weeks at a dosage of 175 mg/m^2. Further, in

this particularly preferred embodiment, the combination may be administered for five cycles followed by the administration of the bispecific antibody only as maintenance therapy. In another particularly preferred embodiment, the method of treatment comprising administering to a patient suffering from advanced small cell lung cancer as second line treatment the bispecific antibody every 3 weeks at a dosage of 20 mg/kg or 30 mg/kg in combination with topotecan at a dosage of 1.5 mg/m^2 administered IV or 2.3 mg/m^2 administered orally once per day on days 1 to 5 of every 3 weeks cycle. In the afore-mentioned two particularly preferred embodiments, the patient may have progressed during or after first-line platinum-based therapy (with or without checkpoint inhibitor therapy).

In some preferred embodiments, the method of treatment comprises administering the bispecific antibody in combination with pemetrexed and carboplatin. In some such embodiments, the cancer is a malignant mesothelioma and, preferably, the method of treatment can be a first line cancer treatment.

In some preferred embodiments, the method of treatment comprises administering the bispecific antibody in combination with pemetrexed and carboplatin. In some preferred embodiments, the method of treatment comprises administering the bispecific antibody in combination with paclitaxel and carboplatin. In some such embodiments, the cancer is NSCLC, preferably the method of treatment can be a first line treatment. In one example, the cancer is non-squamous NSCLC. In another example, the cancer is squamous NSCLC. In some preferred embodiments, pemetrexed and carboplatin can be administered at a dosage of 500 mg/m^2 and AUC 5, respectively, every 3 weeks, e.g. for 4 cycles. In some such embodiments, the cancer is non-squamous NSCLC. In some preferred embodiments, paclitaxel and carboplatin can be administered at a dosage of 200 mg/m^2 and AUC 6, respectively, every 3 weeks, e.g. for 4 cycles. In some such embodiments, the cancer is squamous NSCLC.

In some preferred embodiments, the method of treatment comprises administering the bispecific antibody in combination with pemetrexed and carboplatin or in combination with a taxane, such as paclitaxel or docetaxel, preferably docetaxel. In some such embodiments, the cancer is NSCLC, preferably the method of treatment can be a second line treatment. For example, the cancer is an EGFR-mutant advanced non-squamous NSCLC and, preferably, the method of treatment can be a cancer treatment following a failed EGFR-TKI

treatment. Thus, it can be preferred that the method of treatment comprises administering the bispecific antibody in combination with pemetrexed and carboplatin to a patient suffering from NSCLC as second-line treatment, in particular to a patient suffering from NSCLC who progressed after EGFR-TKI treatment. In the afore-mentioned embodiment, it is particularly preferred that the bispecific antibody is administered at a dosage of 30 mg/kg plus AUC 5 mg/mL/min carboplatin and 500 mg/m^2 pemetrexed every three weeks, e.g. for 4 cycles. Such a treatment may be followed by a maintenance treatment using 30 mg/kg of the bispecific antibody in combination with 500 mg/m^2 pemetrexed. In another embodiment, the method of treatment comprises administering the bispecific antibody in combination with a taxane, such as paclitaxel or docetaxel, preferably docetaxel, to a patient suffering from NSCLC, e.g. squamous NSCLC, as second-line treatment, e.g., to a patient suffering from second-line stage IV or recurrent NSLC who progressed at least one prior line of an immune-oncology (IO) comprising treatment such as an immune checkpoint inhibitor comprising treatment or chemoimmunotherapy. In the afore-mentioned embodiment, it is particularly preferred that the bispecific antibody is administered at a dosage range of 1400 mg to 2000 mg (1400 mg or 2000 mg preferred) plus 75 mg/m^2 docetaxel every three weeks. For example, the bispecific antibody may be administered at a dosage of 2000 mg bispecific antibody plus 75 mg/m^2 docetaxel every three weeks. In another example, the bispecific antibody may be administered in a step-up dosing regimen with a first and a second dose regimen, preferably wherein the first dose regimen is 1400 mg bispecific antibody plus 60 mg/m^2 docetaxel every three weeks or 1400 mg bispecific antibody plus 75 mg/m^2 docetaxel every three weeks and the second dose regimen is 2000 mg bispecific antibody plus 75 mg/m^2 docetaxel every three weeks.

In some preferred embodiments, the method of treatment comprises administering the bispecific antibody in combination with anthracyclines, capecitabine, vinorelbine, eribulin, ixabepilone, nab-paclitaxel, paclitaxel, or gemcitabine and carboplatin, preferably nab-paclitaxel, paclitaxel, gemcitabine and carboplatin or eribulin. In some such embodiments, the cancer is TNBC (e.g., advanced or metastatic TNBC) and, preferably, the method of treatment can be a first line cancer treatment or second line cancer treatment. For example, nab-paclitaxel can be administered at a dosage of 100 mg/m^2 on the 1st, 8th, and 15th day of a 28-days treatment cycle (preferably in combination with the bispecific antibody at a dosage of 15 mg/kg or 20 mg/kg on the 1st and 15th day of a 28-days treatment cycle). For

example, paclitaxel can be administered at a dosage of 90 mg/m^2 on the 1st, 8th, and 15th day of a 28-days treatment cycle (preferably in combination with the bispecific antibody at a dosage of 1400 mg on the 1st and 15th day of a 28-days treatment cycle). Gemcitabine and carboplatin can be administered at a dosage of 1000 mg/m^2 and AUC 2 respectively, on the 1st and 8th day of a 21-days treatment cycle (preferably in combination with the bispecific antibody at a dosage of 2000 mg on the 1st day of a 21-days treatment cycle). Eribulin can be administered at a dosage of 1.4 mg/m^2 on the 1st and 8th day of a 21-days treatment cycle (preferably in combination with the bispecific antibody at a dosage of 2000 mg on the first day of a 21-days treatment cycle). In a preferred example, Nab-paclitaxel can be administered at a dosage of 100 mg/m^2 on the 1st, 8th, and 15th day of a 28-days treatment cycle (preferably in combination with the bispecific antibody at a dosage of 1400 mg on the 1st and 15th day of a 28-days treatment cycle). In a further preferred example, paclitaxel can be administered at a dosage of 90 mg/m^2 on the 1st, 8th, and 15th day of a 28-days treatment cycle (preferably in combination with the bispecific antibody at a dosage of 1400 mg on the 1st and 15th day of a 28-days treatment cycle). In a further preferred example, gemcitabine can be administered at a dosage of 1000 mg/m^2 and carboplatin can be administered at AUC2 on the 1st and 8th, and 15th day of a 21-days treatment cycle (preferably in combination with the bispecific antibody at a dosage of 2000 mg every 3 weeks). In the aforementioned example, the patient may have a PD-L1 expression score before the treatment as determined by a combined positive score (CPS) of < 10 or other integrating scoring algorithm defining a similar PD-L1 expression score. In the aforementioned example, the treatment may be a first line cancer treatment. In some preferred embodiments, the method of treatment comprises administering the bispecific antibody in combination with FOLFIRNOX, preferably comprising folinic acid, oxaliplatin, Irinotecan Hydrochloride, and 5-fluorouracil. In some such embodiments, the cancer is hepatocellular carcinoma and, preferably, the method of treatment can be a first line cancer treatment. In some other embodiments, the cancer is colorectal cancer.

In some preferred embodiments, the method of treatment comprises administering the bispecific antibody, e.g. at a dosage of 20 mg/kg every two weeks, in combination with oxaliplatin, calcium folinate, and 5-fluorouracil (which may also be referred to as FOLFOX4). In some such embodiments, the cancer is hepatocellular carcinoma and, preferably, the method of treatment can be a first line cancer treatment.

In some preferred embodiments, the method of treatment comprises administering the bispecific antibody in combination with irinotecan, 5-fluorouracil, calcium folinate. In some such embodiments, the cancer is unresectable neuroendocrine neoplasm and, preferably, the method of treatment can be a second line cancer treatment.

In some preferred embodiments, the method of treatment comprises administering the bispecific antibody in combination with etoposide and platinum. In some such embodiments, the cancer is small cell lung cancer, e.g., extensive-stage small cell lung cancer or limited-stage small cell lung cancer, and, preferably, the method of treatment can be a first line cancer treatment.

In some preferred embodiments, the method of treatment comprises administering the bispecific antibody every 3 weeks at a dosage ranging from 20 mg/kg to 30 mg/kg in combination with etoposide with carboplatin to a subject having small cell lung cancer, e.g., extensive-stage small cell lung cancer or limited-stage small cell lung cancer. Preferably this method of treatment is a first line treatment and for extensive-stage small cell lung cancer, wherein the bispecific antibody is administered every 3 weeks at a dosage of 20 mg/kg or 30 mg/kg together with the etoposide (preferably at a dosage of 100 mg/m^2) with carboplatin (preferably at a dosage of AUC=5 (total dose $\leq$ 750 mg).

In some preferred embodiments, the method of treatment comprises administering the bispecific antibody every 3 weeks at a dosage ranging from 20 mg/kg to 30 mg/kg (preferably at 20 mg/kg or 30 mg/kg) in combination with paclitaxel, lurbinectedin, or topotecan to a subject having small cell lung cancer, e.g., extensive-stage small cell lung cancer. Preferably this method of treatment is a second line treatment, in particular a treatment of SCLC patients who progressed during or after first-line platinum-based therapy (with or without checkpoint inhibitor therapy). Preferably this method of treatment comprises paclitaxel, in particular paclitaxel every 3 weeks at a dosage of 175 mg/m^2. Preferably this method of treatment comprises topotecan, in particular topotecan at a dosage of 1.5 mg/m^2 administered IV or 2.3 mg/m^2 administered orally once per day on days 1 to 5 of every three weeks cycle.

In some preferred embodiments, the method of treatment comprises administering the bispecific antibody every 3 weeks at a dosage ranging from 1400 mg to 2000 mg in combination with etoposide with carboplatin to a subject having small cell lung cancer,

e.g., extensive-stage small cell lung cancer. Preferably this method of treatment is a first line treatment and for extensive-stage small cell lung cancer, wherein the bispecific antibody is administered every 3 weeks at a dosage of 1400 mg or 2000 mg together with the etoposide (preferably at a dosage of 100 mg/m^2) on days 1-3 and carboplatin (preferably at a dosage of AUC=5 (total dose $\leq$ 750 mg) on day 1 of a 21 day cycle, preferably for 4 cycles followed by a 1400 mg or 2000 mg dose every 3 weeks, respectively, of the bispecific antibody. In some preferred embodiments, the method of treatment comprises administering the bispecific antibody every 2 weeks at a dosage ranging from 10 mg/kg to 20 mg/kg, e.g. 15 mg/kg or 20 mg/kg, preferably on the 1st and 15th day of a 28 day cycle, in combination with nab-paclitaxel, paclitaxel, or gemcitabine with carboplatin to a subject having triple-negative breast cancer.

In some preferred embodiments, the method of treatment comprises administering the bispecific antibody at a flat dosage, e.g. at a dosage of 500 mg to 3000 mg, 750 mg to 2500 mg, 1000 mg to 2250 mg or 1500 mg to 2000 mg to a subject. In some embodiments, the bispecific antibody is administered every week or every 2, 3 or 4 weeks, preferably every 2 weeks. Simulations using integrated PopPK (population pharmacokinetic) analysis surprisingly show that flat dosing and weight-based dosing approaches provide similar results. A fixed dosing approach is desirable to facilitate drug preparation without the need to calculate the dosage, per participant, thus lowering the potential risk of dosing errors as compared with body weight-based dosing. In addition, fixed dosing also offers several advantages over weight-based dosing including better convenience and compliance, reduction of preparation time, and a reduced amount of drug waste.

In some preferred embodiments, the method of treatment comprises administering the bispecific antibody every 2 weeks at a dosage of 500 mg to 3000 mg, 750 mg to 2500 mg, 1000 mg to 2250 mg or 1500 mg to 2000 mg to a subject, preferably of 1000 mg or 1400 mg to a subject having triple-negative breast cancer, preferably on the 1st and 15th day of a 28-day treatment cycle. It can be preferred to administer the bispecific antibody at a dosage of 1400 mg (preferably on the 1st and 15th day of a 28-days treatment cycle) or to administer the bispecific antibody at a dosage of 2000 mg (preferably on the 1st day of a 21-days treatment cycle) to a subject having triple-negative breast cancer.

In some embodiments the bispecific antibody is administered in combination with a chemotherapy.

In some embodiments, the chemotherapy is selected from the group consisting of nab-paclitaxel, paclitaxel, or gemcitabine with carboplatin. In a preferred embodiment, the chemotherapy is nab-paclitaxel. In some embodiments, the chemotherapy is administered on the 1st, 8th and 15th day of a 28-day treatment cycle. In some embodiments, the subject has not received prior systemic treatment, preferably not in the advanced setting. In some preferred embodiments, the method of treatment comprises administering the bispecific antibody every 2 weeks at a dosage of 500 mg to 3000 mg, 750 mg to 2500 mg, 1000 mg to 2250 mg or 1500 mg to 2000 mg to a subject, preferably of 1000 mg or 1400 mg to a subject having small cell lung cancer, preferably on the 1st day of a 21-day treatment cycle. In some embodiments, the bispecific antibody is administered in combination with a chemotherapy, preferably selected from paclitaxel, topotecan or lurbinectedin. In some embodiments, the bispecific antibody is administered in combination with a chemotherapy, preferably selected from paclitaxel or topotecan. In some embodiments, the chemotherapy is paclitaxel. In some embodiments, the paclitaxel is administered on the 1st day of a 21-day treatment cycle. In another embodiment, the chemotherapy is topotecan. In some embodiments, the topotecan is administered on the 1st and 5th day of a 21-day treatment cycle.

The invention further provides a chemotherapy agent for use in a method of treating a subject with cancer, the method comprising administering to the subject:

a. a bispecific antibody that specifically binds to PD-L1 and VEGF; and

b. the chemotherapy agent;

optionally wherein the subject has a PD-L1 expression score before the treatment as determined by the combined positive score (CPS) of ≥ 1 or other integrating scoring algorithm defining a similar PD-L1 expression score.

The embodiments disclosed herein for the method of treatment comprising the bispecific antibody in combination with the chemotherapy can be used in a method of treatment of the chemotherapy agent in combination with the bispecific antibody as disclosed herein. Features described herein in more detail for the “bispecific antibody for use in a method of

treating" embodiments equally apply to the chemotherapy agent for use in a method of treating disclosed herein.

Method of treatment

The invention further provides a method of treating cancer in a subject, the method comprising administering to the subject a bispecific antibody that specifically binds to PD-L1 and VEGF, in combination with chemotherapy, optionally wherein the subject has a PD-L1 expression score before the treatment as determined by the combined positive score (CPS) of ≥ 1 or other integrating scoring algorithm defining a similar PD-L1 expression score.

The embodiments disclosed herein for the method of treatment comprising the bispecific antibody in combination with the chemotherapy can be used in a method of treating cancer in a subject as disclosed herein. Features described herein in more detail for the "bispecific antibody for use in a method of treating" embodiments equally apply to the method of treatment disclosed herein.

In some embodiments, the method is a method for extending progression-free survival in said subject compared to the chemotherapy or the bispecific antibody or an anti PD-L1 antibody or an anti-PD-1 antibody treatment alone or compared to a standard treatment comprising the chemotherapy and the anti-PD-L1 antibody or the chemotherapy and the anti-PD-1 antibody, wherein the anti-PD-L1 antibody is preferably atezolizumab and the anti-PD-L1 antibody is preferably pembrolizumab. In some embodiments, the method is a method for increased overall survival in said subject compared to the chemotherapy or the bispecific antibody or an anti PD-L1 antibody or an anti-PD-1 antibody treatment alone or compared to a standard treatment comprising the chemotherapy and the anti-PD-L1 antibody or the chemotherapy and the anti-PD-1 antibody, wherein the anti-PD-L1 antibody is preferably atezolizumab and the anti-PD-L1 antibody is preferably pembrolizumab.

In some preferred embodiments, the invention provides a bispecific antibody that specifically binds to PD-L1 and VEGF for use in a method of treating a subject with triple-negative breast cancer, the method comprising administering to the subject:

a. the bispecific antibody; and

b. a chemotherapy, preferably nab-paclitaxel;

wherein the subject has a PD-L1 expression score before the treatment similar or identical to a CPS of ≥1 to <10 as determined by the TPS scoring algorithm.

In some preferred embodiments, the invention provides a bispecific antibody that specifically binds to PD-L1 and VEGF for use in a method of treating a subject with triple-negative breast cancer, the method comprising administering to the subject:

a. the bispecific antibody; and

b. a chemotherapy, preferably nab-paclitaxel;

wherein the subject has a PD-L1 expression score before the treatment similar or identical to a CPS of ≥1 to <10 as determined by the TAP scoring algorithm.

In some particularly preferred embodiments, the invention provides a bispecific antibody that specifically binds to PD-L1 and VEGF for use in a method of treating a subject with triple-negative breast cancer, the method comprising administering to the subject:

a. the bispecific antibody; and

b. a chemotherapy, preferably nab-paclitaxel;

wherein the subject has a combined positive score (CPS) of ≥1 to <10 before the treatment. The CPS is preferably determined with Dako's PD-L1 IHC 22C3 pharmDx kit (SK006) according to the manufacturer's TNBC Instructions for Use.

In some preferred embodiments, the invention provides a bispecific antibody that specifically binds to PD-L1 and VEGF for use in a method of treating a subject with triple-negative breast cancer, the method comprising administering to the subject:

a. the bispecific antibody; and

b. a chemotherapy, preferably a chemotherapy agent;

wherein the subject has a PD-L1 expression score before the treatment as determined by a combined positive score (CPS) of ≥1 to <10 or another integrating scoring algorithm defining a similar PD-L1 expression score, wherein the other integrating scoring algorithm is selected from tumour area positivity (TAP) score and tumour proportion score (TPS), preferably wherein the other integrating scoring algorithm is TAP score.

In some preferred embodiments, the invention provides a bispecific antibody that specifically binds to PD-L1 and VEGF for use in a method of treating a subject with triple-negative breast cancer, the method comprising administering to the subject:

a. the bispecific antibody; and

b. a chemotherapy, preferably a chemotherapy agent;

wherein the subject has a PD-L1 expression before the treatment that provides a score of ≥1 to <10 when determined by the combined positive score (CPS) or a score that is indicative of such a PD-L1 expression when determined by another integrating scoring algorithm, preferably when determined by the tumour area positivity (TAP) score or the tumour proportion score (TPS), more preferably when determined by the TAP score.

In some preferred embodiments, the invention provides a bispecific antibody that specifically binds to PD-L1 and VEGF for use in a method of treating a subject with cancer, the method comprising administering to the subject:

a. the bispecific antibody; and

b. a chemotherapy, preferably a chemotherapy agent;

optionally wherein the subject has a PD-L1 expression before the treatment that provides a score of ≥1 when determined by the combined positive score (CPS) or a score that is indicative of such a PD-L1 expression when determined by another integrating scoring algorithm, preferably when determined by the tumour area positivity (TAP) score or the tumour proportion score (TPS), more preferably when determined by the TAP score.

In some aspects of the invention, there is provided use of a bispecific antibody that specifically binds to PD-L1 and VEGF and a chemotherapy agent in the manufacture of a medicament for treating cancer in a subject, the use comprising administering to the subject:

a. the bispecific antibody; and

b. the chemotherapy agent.

In some aspects of the invention, there is provided use of a bispecific antibody that specifically binds to PD-L1 and VEGF in the manufacture of a medicament for treating cancer in a subject, the use comprising administering to the subject:

a. the bispecific antibody; and
b. a chemotherapy agent.

In some aspects of the invention, there is provided use of a bispecific antibody that specifically binds to PD-L1 and VEGF and a chemotherapy agent in the manufacture of a medicament for treating cancer in a subject, the use comprising administering to the subject:

a. the bispecific antibody; and
b. the chemotherapy agent.

In some aspects of the invention, there is provided use of a chemotherapy agent in the manufacture of a medicament for treating cancer in a subject, the use comprising administering to the subject:

a. a bispecific antibody that specifically binds to PD-L1 and VEGF; and
b. the chemotherapy agent.

Other methods

The invention further provides a method for determining whether a cancer in a subject is susceptible to treatment with a bispecific antibody that specifically binds to PD-L1 and VEGF and a chemotherapy, wherein the method comprises detecting in a sample of the subject a PD-L1 expression score before the treatment as determined by the combined positive score (CPS) of ≥ 1 or other integrating scoring algorithm defining a similar PD-L1 expression score, wherein the CPS of ≥ 1 indicates that the subject is susceptible to treatment with the bispecific antibody and the chemotherapy.

The embodiments for determining PD-L1 expression score in cancer tissue disclosed herein for the method of treatment comprising the bispecific antibody in combination with the chemotherapy can be used in the method for determining whether a cancer in a subject is susceptible to treatment with a bispecific antibody that specifically binds to PD-L1 and VEGF and a chemotherapy. Features described herein in more detail for the “bispecific

antibody for use in a method of treating" embodiments equally apply to the determining method disclosed herein.

In some preferred embodiments, the method for determining whether a cancer in a subject is susceptible to treatment with a bispecific antibody that specifically binds to PD-L1 and VEGF and a chemotherapy is conducted using the IHC assay PD-L1 IHC 22C3 pharmDx in accordance with the manufacturer's guide.

Kits-of-part of the invention

The invention further provides kit-of-parts comprising the bispecific antibody disclosed herein that specifically binds to PD-L1 and VEGF and the chemotherapy agent disclosed herein.

The embodiments disclosed herein for the method of treatment comprising the bispecific antibody in combination with the chemotherapy can be used in the kit-of-parts disclosed herein. Features described herein in more detail for the "bispecific antibody for use in a method of treating" embodiments equally apply to the kit-of-parts disclosed herein.

In some embodiments, the bispecific antibody and the chemotherapy agent are comprised in separate container.

In some embodiments, the kit-of parts further comprise instructions for use.

Further aspects of the combination therapy

In an aspect, the present application is directed to a bispecific antibody that specifically binds to PD-L1 and VEGF (preferably PM8002 comprising the heavy and light chain amino acid sequences as set forth in SEQ ID NOs: 16 and 17) for use in a method of treating a subject with small cell lung cancer, the method comprising administering to the subject the bispecific antibody in combination with paclitaxel, wherein the antibody is administered every three weeks at a dosage of about 20 mg/kg or about 30 mg/kg and the paclitaxel is administered every three weeks at a dosage of about 175 mg/m^2. Preferably, the patient progressed during or after first-line platinum-based therapy (with or without Immuno-oncology (IO), e.g., checkpoint inhibitor therapy), i.e. the patient failed first-line platinum-based chemotherapy. Accordingly, on a more general level, it is preferred that the method of the present paragraph is a second-line treatment for a patient suffering from

small cell lung cancer. It is also preferred that the combination is administered intravenously. The treatment may take place for 5 cycles (3 weeks/cycle), followed by a maintenance therapy by the antibody at a dosage of about 20 mg/kg or about 30 mg/kg every three weeks.

In another aspect, the present application is directed to a bispecific antibody that specifically binds to PD-L1 and VEGF (preferably PM8002 comprising the heavy and light chain amino acid sequences as set forth in SEQ ID NOs: 16 and 17) for use in a method of treating a subject with small cell lung cancer, the method comprising administering to the subject the bispecific antibody in combination with topotecan, e.g., wherein the antibody is administered every three weeks at a dosage of about 20 mg/kg or about 30 mg/kg and the topotecan is administered every three weeks at days 1 to 5 once daily at a dosage of about 1.5 mg/m^2 IV or about 2.3 mg/m^2 orally. Preferably, the patient progressed during or after first-line platinum-based therapy (with or without checkpoint inhibitor therapy), i.e. the patient failed first-line platinum-based chemotherapy. Accordingly, on a more general level, it is preferred that the method of the present paragraph is a second-line treatment for a patient suffering from small cell lung cancer. It is also preferred that the antibody is administered intravenously.

In another aspect, the present application is directed to a bispecific antibody that specifically binds to PD-L1 and VEGF (preferably PM8002 comprising the heavy and light chain amino acid sequences as set forth in SEQ ID NOs: 16 and 17) for use in a method of treating a subject with extensive-stage small cell lung cancer, the method comprising administering to the subject the bispecific antibody in combination with systemic chemotherapy (in particular with platinum-etoposide), wherein the antibody is administered every three weeks at a dosage of about 20 mg/kg or about 30 mg/kg in combination with systemic chemotherapy (in particular with platinum [preferably carboplatin at a dosage of about AUC=5 (total dose $\leq$ about 750 mg) at day 1 of each cycle] and etoposide [preferably at a dosage of about 100 mg/m^2 on days 1 to 3 of each cycle]). Preferably, the patient has not received systemic treatment for extensive-stage small cell lung cancer prior to the administration, i.e. the treatment is a first-line treatment. Accordingly, on a more general level, it is preferred that the method of the present paragraph is a first-line treatment for a patient suffering from extensive-stage small cell lung cancer. It is also preferred that the antibody is administered intravenously. The treatment may take place for 4 cycles (3

weeks/cycle), followed by a maintenance therapy by the antibody at a dosage of about 20 mg/kg or about 30 mg/kg every three weeks.

In another aspect, the present application is directed to a bispecific antibody that specifically binds to PD-L1 and VEGF (preferably PM8002 comprising the heavy and light chain amino acid sequences as set forth in SEQ ID NOs: 16 and 17) for use in a method of treating a subject with limited-stage small cell lung cancer (LS-SCLC), the method comprising administering to the subject the bispecific antibody in combination with systemic chemotherapy (in particular with platinum-etoposide), wherein the antibody is administered every three weeks at a dosage of about 20 mg/kg or about 30 mg/kg in combination with systemic chemotherapy (in particular with platinum [preferably carboplatin at a dosage of about AUC=5 (total dose ≤ about 750 mg) at day 1 of each cycle] and etoposide [preferably at a dosage of about 100 mg/m^2 on days 1 to 3 of each cycle]). Preferably, the patient had TFI≤6 months since the last chemotherapy, chemoradiotherapy or radiotherapy. It is also preferred that the antibody is administered intravenously. The treatment may take place for 4 cycles (3 weeks/cycle), followed by a maintenance therapy by the antibody at a dosage of about 20 mg/kg or about 30 mg/kg every three weeks.

In another aspect, the present application is directed to a bispecific antibody that specifically binds to PD-L1 and VEGF (preferably PM8002 comprising the heavy and light chain amino acid sequences as set forth in SEQ ID NOs: 16 and 17) for use in a method of treating a subject with extensive-stage small cell lung cancer, the method comprising administering to the subject the bispecific antibody in combination with systemic chemotherapy (in particular with platinum-etoposide), e.g., wherein the antibody is administered every three weeks at a dosage of about 1400 mg or about 2000 mg in combination with systemic chemotherapy (in particular with platinum [preferably carboplatin at a dosage of about AUC=5 (total dose ≤ about 750 mg) at day 1 of each cycle] and etoposide [preferably at a dosage of about 100 mg/m^2 on days 1 to 3 of each cycle]). Preferably, the patient has not received systemic treatment for extensive-stage small cell lung cancer prior to the administration, i.e. the treatment is a first-line treatment. Accordingly, on a more general level, it is preferred that the method of the present paragraph is a first-line treatment for a patient suffering from extensive-stage small cell lung cancer. It is also preferred that the antibody is administered intravenously. The

treatment may take place for 4 cycles (3 weeks/cycle), followed by a maintenance therapy by the antibody at a dosage of about 1400 mg or about 2000 mg every three weeks.

In another aspect, the present application is directed to a bispecific antibody that specifically binds to PD-L1 and VEGF (preferably PM8002 comprising the heavy and light chain amino acid sequences as set forth in SEQ ID NOs: 16 and 17) for use in a method of treating a subject with triple negative breast cancer (TNBC), the method comprising administering to the subject the bispecific antibody in combination with systemic chemotherapy, wherein the systemic chemotherapy is nab-paclitaxel, which can preferably be administered at a dosage of about 100 mg/m^2 on the 1st, 8th, and 15th day of a 28-days treatment cycle (preferably in combination with the bispecific antibody at a dosage of about 15 mg/kg or about 20 mg/kg on the 1st and 15th day of a 28-days treatment cycle); or wherein the systemic chemotherapy is paclitaxel, which can preferably be administered at a dosage of about 90 mg/m^2 on the 1st, 8th, and 15th day of a 28-days treatment cycle (preferably in combination with the bispecific antibody at a dosage of about 1400 mg every two weeks of a 28-days treatment cycle); or wherein the systemic chemotherapy is gemcitabine and carboplatin which can preferably be administered at a dosage of about 1000 mg/m^2 and AUC 2 respectively, on the 1st and 8th day of a 21-days treatment cycle (preferably in combination with the bispecific antibody at a dosage of about 2000 mg on the 1st day of a 28-days treatment cycle); or wherein the systemic chemotherapy is eribulin, which can preferably be administered at a dosage of about 1.4 mg/m^2 on the 1st and 8th day of a 21-days treatment cycle (preferably in combination with the bispecific antibody at a dosage of about 2000 mg on the first day of a 21-days treatment cycle). It is preferred that the antibody is administered intravenously.

In another aspect, the present application is directed to a bispecific antibody that specifically binds to PD-L1 and VEGF (preferably PM8002 comprising the heavy and light chain amino acid sequences as set forth in SEQ ID NOs: 16 and 17) for use in a method of treating a subject with non-small cell lung cancer, the method comprising administering to the subject the bispecific antibody in combination with chemotherapy (in particular carboplatin and pemetrexed), wherein the antibody is preferably administered every three weeks at a dosage of about 30 mg/kg together with the chemotherapy (in particular carboplatin and pemetrexed at a dosage of about AUC 5 mg/mL/min carboplatin and about 500 mg/m^2 pemetrexed). Preferably, the patient progressed after first-line EGFR-TKI

therapy, i.e. the patient failed first-line EGFR-TKI therapy. Accordingly, on a more general level, it is preferred that the method of the present paragraph is a second-line treatment for a patient suffering from non-small cell lung cancer. Furthermore, it can be preferred that the patient suffers from a NSCLC with PD-L1 expressed, i.e. with a PD-L1 expression score before the treatment as determined by a CPS of ≥ 1 or other integrating scoring algorithm defining a similar PD-L1 expression score.

In another aspect, the present application is directed to a bispecific antibody that specifically binds to PD-L1 and VEGF (preferably PM8002 comprising the heavy and light chain amino acid sequences as set forth in SEQ ID NOs: 16 and 17) for use in a method of treating a subject with non-small cell lung cancer (NSCLC), e.g., to a patient suffering from NSCLC as second-line treatment, e.g., to a patient suffering from second-line stage IV or recurrent NSCLC or, e.g., to a patient who progressed at least one prior line of an immune-oncology (IO)-comprising treatment (such as an immune checkpoint inhibitor-comprising treatment), wherein the method comprises administering to the subject the bispecific antibody (preferably at a dosage range of about 1400 mg to about 2000 mg [with about 1400 mg or about 2000 mg being more preferred]) and docetaxel (preferably at about 75 mg/m^{2}every three weeks). For example, the bispecific antibody may be administered at a dosage of about 2000 mg bispecific antibody plus about 75 mg/m^2 docetaxel every three weeks. In another example, the bispecific antibody may be administered in a step-up dosing regimen with a first and a second dose regimen, preferably wherein the first dose regimen is about 1400 mg bispecific antibody plus about 60 mg/m^2 docetaxel every three weeks or about 1400 mg bispecific antibody plus about 75 mg/m^2 docetaxel every three weeks or about 2000 mg bispecific antibody plus about 60 mg/m^2 docetaxel every three weeks and the second dose regimen is about 2000 mg bispecific antibody plus about 75 mg/m^2 docetaxel every three weeks. In another example, the bispecific antibody may be administered in a step-down dosing regimen with a first and a second dose regimen, preferably wherein the first dose regimen is about 2000 mg bispecific antibody plus about 75 mg/m^2 docetaxel every three weeks and the second dose regimen is about 1400 mg bispecific antibody plus about 75 mg/m^2 docetaxel every three weeks or about 2000 mg bispecific antibody plus about 60 mg/m^2 docetaxel every three weeks.

In another aspect, the present application is directed to a bispecific antibody that specifically binds to PD-L1 and VEGF (preferably PM8002 comprising the heavy and light

chain amino acid sequences as set forth in SEQ ID NOs: 16 and 17) for use in a method of treating a subject with non-small cell lung cancer (NSCLC), e.g., to a treatment-naïve patient suffering from Stage IIIB/IIIC or IV NSCLC, wherein the method comprises administering to the subject the bispecific antibody (preferably at a dosage range of about 1400 mg to about 2000 mg [with about 1400 mg or about 2000 mg being more preferred]) and (i) platinum (preferably carboplatin at a dosage of about AUC=5) in combination with pemetrexed (preferably at a dosage of about 500 mg/m^2) or (ii) platinum (preferably carboplatin at a dosage of about AUC=5) in combination with paclitaxel (preferably at a dosage of about 200 mg/m^2), wherein the combination is preferably administered every three weeks. Combination (i) may in particular be administered to a patient suffering from non-squamous NSCLC, whereas combination (ii) may in particular be administered to a patient suffering from squamous NSCLC, wherein the maintenance therapy may be at a dosage of about 1400 mg or about 2000 mg bispecific antibody plus pemetrexed (preferably at a dosage of about 500 mg/m^2) for patient (i) and a dosage of about 1400 mg or about 200 mg bispecific antibody for patient (ii).

In another aspect, the present application is directed to a bispecific antibody that specifically binds to PD-L1 and VEGF (preferably PM8002 comprising the heavy and light chain amino acid sequences as set forth in SEQ ID NOs: 16 and 17) for use in a method of treating a subject with hepatocellular cancer, the method comprising administering to the subject the bispecific antibody in combination with chemotherapy (in particular FOLFOX4, i.e. oxaliplatin, leucovorin and 5-fluorouracil), wherein the antibody is administered every two weeks at a dosage of about 20 mg/kg together with the chemotherapy. Preferably, the patient has not received systemic treatment for hepatocellular cancer prior to the administration, i.e. the treatment is a first-line treatment. Accordingly, on a more general level, it is preferred that the method of the present paragraph is a first-line treatment for a patient suffering from hepatocellular cancer.

In another aspect, the present application is directed to a bispecific antibody that specifically binds to PD-L1 and VEGF (preferably PM8002 comprising the heavy and light chain amino acid sequences as set forth in SEQ ID NOs: 16 and 17) for use in a method of treating a subject with hepatocellular cancer, the method comprising administering to the subject the bispecific antibody (preferably as the sole active agent), wherein the antibody is administered every two weeks at a dosage of about 20 mg/kg. Preferably, the patient has

not received systemic treatment for hepatocellular cancer prior to the administration, i.e. the treatment is a first-line treatment. Accordingly, on a more general level, it is preferred that the method of the present paragraph is a first-line treatment for a patient suffering from hepatocellular cancer.

Preferred combinations

In the light of the above-stated further aspects of the combination, it is evident that the following combinations (preferably in the respective dosages as it can be derived from the above-stated further aspects of the combination and preferably administered as separate dosage forms) are particularly preferred:

The bispecific antibody that specifically binds to PD-L1 and VEGF (most preferably PM8002 comprising the heavy and light chain amino acid sequence set forth in SEQ ID NO: 16 and 17) in combination with (i) etoposide and carboplatin; (ii) paclitaxel; (iii) topotecan; (iv) Nab-paclitaxel; (v) paclitaxel; (vi) gemcitabine; (vii) eribulin; (viii) carboplatin and pemetrexed; (ix) carboplatin and paclitaxel; or (x) docetaxel.

A further aspect relating to the bispecific antibody

While the bispecific antibody has been disclosed above as specifically binding to PD-L1 and VEGF, the bispecific antibody may comprise in a general aspect an anti-VEGF antibody (e.g., an anti-VEGF-A antibody) or fragment thereof, and an antagonist of the PD1/PD-L1 interaction and/or a PD-1 or PD-L1 blocking antibody. In some embodiments relating to this general aspect, the bispecific antibody comprises an anti-VEGF antibody (e.g., an anti-VEGF-A antibody) or fragment thereof, and a PD-1 or PD-L1 blocking antibody. In some preferred embodiments relating to this general aspect, the bispecific antibody comprises an anti-VEGF antibody (e.g., an anti-VEGF-A antibody) or fragment thereof, and an anti-PD-1 or anti-PD-L1 antibody or fragment thereof. For example, the anti-PD-1 antibody can be selected from any anti-PD-1 antibody known in the art such as pembrolizumab or nivolumab. An exemplary anti-PD-L1 antibody includes atezolizumab. For example, the anti-VEGF antibody can be selected from any VEGF antibody known in the art such as bevacizumab, ranibizumab or an VEGF binding region derived from the anti-VEGF fusion protein Aflibercept (see, e.g., US 7070959). An exemplary anti-VEGF antibody includes bevacizumab.

The embodiments as set out herein for the bispecific antibody specifically binding to PD-L1 and VEGF as disclosed above are generally also applicable for the antibody of the present general aspect, unless the context clearly indicates otherwise.

DESCRIPTION OF THE DRAWING

Fig. 1. (A)-(C) depicts boxplots of $AUC_{tau,ss}$ by 20 mg/kg Q2W (A) versus 1200 mg Q2W (B) or 1500 mg Q2W (C) dosing regimens. N is the number of participants; the x-axis represents baseline body weight, the horizontal line at the center of the box is the median of $AUC_{tau,ss}$, the box represents the inter-quartile distance, and the whiskers represent $\leq$1.5 times the inter-quartile range (75th - 25th quartile). The number at the top of the figure represents the number of participants included in each category. Abbreviations: $AUC_{tau,ss}$ = area under the curve during the dosing interval at steady-state; N = number; Q2W = every two weeks.

SEQUENCE LISTING

This application contains a Sequence Listing which has been submitted electronically and is hereby incorporated by reference in its entirety. Said Sequence Listing file is named B19165WO.xml and 39.3 Bytes in size.

SEQ ID NO: 1-3 are exemplary CDR1-3 amino acid sequences of an anti-PD-L1 antibody using the IMGT numbering system.

SEQ ID NO: 4-6 are exemplary HCDR1-3 amino acid sequences of an anti-VEGF antibody using the IMGT numbering system.

SEQ ID NO: 7 and 8 are exemplary LCDR1 and 3 amino acid sequences of an anti-VEGF antibody using the IMGT numbering system.

SEQ ID NO: 9 is an exemplary amino acid sequence of an anti-PD-L1 VHH.

SEQ ID NO: 10 is an exemplary VH amino acid sequence of an anti-VEGF antibody.

SEQ ID NO: 11 is an exemplary VL amino acid sequence of an anti-VEGF antibody.

SEQ ID NO: 12 is an exemplary CH1 amino acid sequence of an anti-VEGF antibody.

SEQ ID NO: 13 is an exemplary IgG1 Fc region amino acid sequence of an anti-VEGF antibody.

SEQ ID NO: 14 is an exemplary linker amino acid sequence of the bispecific antibody as disclosed herein.

SEQ ID NO: 15 is an exemplary CL amino acid sequence of an anti-VEGF antibody.

SEQ ID NO: 16 is an exemplary heavy chain amino acid sequence of the bispecific antibody as disclosed herein comprising an exemplary anti-PD-L1 VHH amino acid sequence.

SEQ ID NO: 17 is an exemplary light chain amino acid sequence of the bispecific antibody as disclosed herein.

SEQ ID NO: 18, 19 and 38 are exemplary CDR1-3 amino acid sequences of an anti-PD-L1 antibody using the Kabat numbering system.

SEQ ID NO: 20-25 are exemplary LCDR1-3 and HCDR1-3 amino acid sequences of the 22C3 antibody.

SEQ ID NO: 26 is an exemplary VL amino acid sequence of the 22C3 antibody.

SEQ ID NO: 27 is an exemplary VH amino acid sequence of the 22C3 antibody.

SEQ ID NO: 28 is an exemplary VH amino acid sequence of the 28-8 antibody.

SEQ ID NO: 29 is an exemplary VL amino acid sequence of the 28-8 antibody.

SEQ ID NO: 30-35 are exemplary LCDR1-3 and HCDR1-3 amino acid sequences of the SP263 antibody.

SEQ ID NO: 36 is an exemplary VH amino acid sequence of the SP263 antibody.

SEQ ID NO: 37 is an exemplary VL amino acid sequence of the SP263 antibody.

SEQ ID NO: 39-41 are exemplary HCDR1-3 amino acid sequences of an anti-VEGF-A antibody using the Kabat numbering system.

SEQ ID NO: 42 and 43 are exemplary LCDR1 and 2 amino acid sequences of an anti-VEGF-A antibody using the Kabat numbering system.

An exemplary anti-VEGF-A LCDR2 is the amino acid sequence FTS referred to herein as SEQ ID NO: 44 using the IGMT numbering system.

The sequences of SEQ ID NO: 1-44 are shown in the table below.

Table 1: Exemplary sequences disclosed herein

SEQ ID NO	Amino acid sequence	Description
1	GFTFSSYW	Anti-PD-L1 CDR1/IMGT
2	INSDSSST	Anti-PD-L1 CDR2/IMGT
3	AKDPGGYA	Anti-PD-L1 CDR3/IMGT
4	GYTFTNYG	Anti-VEGF-A HCDR1/IMGT
5	INTYTGEPT	Anti-VEGF-A HCDR2/IMGT
6	AKYPHYYGSSHWYFDV	Anti-VEGF-A HCDR3/IMGT
7	QDISNY	Anti-VEGF-A LCDR1/IMGT
8	QQYSTVPWT	Anti-VEGF-A LCDR3/IMGT/Kabat
9	EVQLQESGGGLVQPGGSLRLSCAASGFTFSSYWMYWLRQAPGKGLEWVSSINSDSSSTYYRDSVKGRFTISRDNAKNTLYLQMNSLKSEDTAVYYCAKDPGGYAKGQGTQVTVSS	Anti-PD-L1 VHH
10	EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTYAADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTLVTVSS	Anti-VEGF VH
11	DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIK	Anti-VEGF VL
12	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC	CH1
13	DKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG	IgG1 (LALA) Fc
14	GGGGSGGGGSG	Linker

15	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKA DYEKHKVYACEVTHQGLSSPVTKSFNRGEC	CL
16	EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWV RQAPGKGLEWVGWINTYTGEPTYAADFKRRFTFSLDTS KSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDV WGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCL VKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKT HTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK AKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDI AVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGGSGG GGSGEVQLQESGGGLVQPGGSLRLSCAASGFTFSSYWM YWLRQAPGKGLEWVSSINSDSSSTYYRDSVKGRFTISRD NAKNTLYLQMNSLKSEDTAVYYCAKDPGGYAKGQGTQ VTVSS	Heavy chain
17	DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQK PGKAPKVLIYFTSSLHSGVPSRFSGSGSGTDFTLTISSLQP EDFATYYCQQYSTVPWTFGQGTKVEIKRTVAAPSVFIFP PSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSG NSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEV THQGLSSPVTKSFNRGEC	Light chain
18	SYWMY	Anti-PD-L1 CDR1/Kabat
19	SINSDSSSTYYRDSVKG	Anti-PD-L1 CDR2/Kabat
20	KSSQSLLHTSTRKNYLA	22C3 LCDR1
21	WASTRES	22C3 LCDR2
22	KQSYDVVT	22C3 LCDR3
23	SYWIH	22C3 HCDR1
24	YINPSSGYHEYNQKFID	22C3 HCDR2
25	SGWLIHGDYYFDF	22C3 HCDR3
26	DIVMSQSPSSLAVSAGEKVTMTCKSSQSLLHTSTRKNYL AWYQQKPGQSPKLLIYWASTRESGVPDRFTGSGSGTDFT LTISSVQAEDLAVYYCKQSYDVVTFGAGTKLELK	22C3 VL
27	QVHLQQSGAELAKPGASVKMSCKASGYTFTSYWIHWIK QRPGQGLEWIGYINPSSGYHEYNQKFIDKATLTADRSSS TAYMHLTSLTSEDSAVYYCARSGWLIHGDYYFDFWGQ GTTLTVSS	22C3 VH
28	METGLRWLLLVAVLKGVQCLSVEESGGRLVTPGTPLTL TCTASGFTITNYHMFWVRQAPGKGLEWIGVITSSGIGSSS TTYYATWAKGRFTISKTSTTVNLRITSPTTEDTATYFCAR DYFTNTYYALDIWGPGTLVTVSS	28-8 VH

29	MDTRAPTQLLGLLLLWLPGARCALVMTQTPSSTSTAVG GTVTIKCQASQSISVYLAWYQQKPGQPPKLLIYSASTLAS GVPSRFKGSRSGTEYTLTISGVQREDAATYYCLGSAGS	28-8 VL
30	QASQSIYNNNWLS	SP263 LCDR1
31	LASTLAS	SP263 LCDR2
32	IGGESSNNDGIA	SP263 LCDR3
33	NHAIS	SP263 HCDR1
34	TINSDTHTYYATWP	SP263 HCDR2
35	RIFSSSNI	SP263 HCDR3
36	QSLEESGGRLVTPGTPLTLTCTASGFSLSNHAISWVRQAP GKGLEWIGTINSDTHTYYATWPKGRFTISKTSSTTVDLK MTSPTTEDTATYFCARRIFSSSNIWGPGTLVTVSS	SP263 VH
37	AIVMTQTSSPVSAVVGGTVAINCQASQSIYNNNWLSWF QQKPGQPPKLLIYLASTLASGVPSRFKGSGSGTQFTLTIS DVVCDDAATYYCIGGGESSNNDGIAFGGGTEVVVK	SP263 VL
38	DPGGYA	Anti-PD-L1 CDR3/Kabat
39	NYGMN	Anti-VEGF-A HCDR1/Kabat
40	WINTYTGEPTYAADFKR	Anti-VEGF-A HCDR2/ Kabat
41	YPHYYGSSHWYFDV	Anti-VEGF-A HCDR3/ Kabat
42	SASQDISNYLN	Anti-VEGF-A LCDR1/ Kabat
43	FTSSLHS	Anti-VEGF-A LCDR2/ Kabat
44	FTS	Anti-VEGF-A LCDR2/IMGT

EXAMPLES

Example 1: Determining the CPS of a cancer tissue sample using Dako's PD-L1 IHC 22C3 pharmDx kit in combination with anti-PD-L1 antibody clone 22C3.

This example is to provide guidelines for evaluating PD-L1 expression on formalin-fixed, paraffin-embedded (FFPE) tumour tissue section with Dako's PD-L1 IHC 22C3 pharmDx kit (SK006) according to the manufacturer's TNBC Instructions for Use. This immunohistochemical (IHC) assay has been performed using the Dako Auto-stainer Link 48 automated staining system.

The embodiments below are described with respect to the use of the PD-L1 IHC 22C3 pharmDx kit, which is a qualitative immunohistochemical assay using Monoclonal Mouse

Anti-PD-L1, Clone 22C3. This kit is intended for use in the detection of PD-L1 protein in formalin- fixed, paraffm-30 embedded (FFPE) tumour tissue using EnVision FLEX visualization system on Autostainer Link 48. Here, PD-L1 protein expression was used to determine a Combined Positive Score (CPS).

PD-L1 IHC 22C3 pharmDx contains optimized reagents to perform an IHC staining procedure using a linker and a chromogen enhancement reagent. Deparaffinization, rehydration, and target retrieval was performed using a 3-in-1 procedure on PT Link.

Following peroxidase block, specimens were incubated with the monoclonal mouse primary antibody to PD-L1 or the Negative Control Reagent. Specimens were then incubated with a Mouse LINKER, followed by incubation with a ready-to-use Visualization Reagent consisting of secondary antibody molecules and horseradish peroxidase molecules coupled to a dextran polymer backbone. The enzymatic conversion of the subsequently added chromogen resulted in precipitation of a visible reaction product at the site of the antigen. The color of the chromogenic reaction was modified by a chromogen enhancement reagent. The specimen was counterstained and coverslipped and results were interpreted using a light microscope.

Clinical Interpretation Guidelines for PD-L1 IHC 22C3 pharmDx in Tumour Tissue Specimen Criteria

A hematoxylin and eosin (H&E) stain of the tissue specimen were evaluated first to assess tissue histology and preservation quality. PD-L1 IHC 22C3 pharmDx and the H&E staining were performed on serial sections from the same paraffin block of the specimen (sample). Tissue specimens were intact, well preserved, and confirmed tumour indication.

The specimen contained a minimum of 100 viable tumour cells to determine the percentage of positive cells. For specimens with less than 100 viable tumour cells, tissue from a deeper level of the block, or potentially another block, presented sufficient number of viable tumour cells for PD-L1 IHC 22C3 pharmDx testing.

PD-L1 IHC 22C3 pharmDx Control Cell Line Slide

The PD-L1 IHC 22C3 pharmDx Control Cell Line Slide were examined to determine that reagents are functioning properly. Each slide contained sections of cell pellets with positive and negative PD-L1 expression. The percentage of positive cells, staining intensity, and

non-specific staining were assessed in both cell pellets. If any staining of the Control Cell Line Slide was not satisfactory, all results with the subject specimens were considered invalid. The Control Cell Line Slide has not been used as an aid in interpretation of subject results.

The overall staining intensity was evaluated using the following guide:

0	Negative
1+	Weak intensity
2+	Moderate intensity
3+	Strong intensity

Positive Control Cell Pellet:

The following stainings were acceptable for the PD-L1 positive cell pellet:

- Cell membrane staining of ≥70% of cells
- ≥2+ average staining intensity
- Non-specific staining < 1+ intensity

Negative Control Cell Pellet:

For the PD-L1 negative cell pellet, the following stainings were acceptable:

- No specific staining
- Non-specific staining < 1+ intensity. Note that staining of a few cells in the MCF-7 cell pellet may occasionally be observed. The following acceptance criteria are applicable: the presence of ≤10 total cells with distinct plasma membrane staining, or cytoplasmic staining with ≥1+ intensity within the boundaries of the MCF-7 cell pellet are acceptable

Positive and Negative User Control Tissue (TNBC):

The TNBC Positive Control Tissue Slides were examined to verify that the fixation method and epitope retrieval process are effective. The Positive Control Tissue Slides were stained

with both PD-L1 primary antibody and Negative Control Reagent. The ideal positive control tissue provided a complete dynamic representation of weak-to-moderate staining of tumour cells and tumour-associated mononuclear inflammatory cells (MICs). Known positive tissue controls were utilized for monitoring the correct performance of processed tissues and test reagents, not as an aid in formulating a specific diagnosis of subject samples. If stainings of positive in-house control tissue were not satisfactory, all results with the subject specimen were considered invalid.

- Requirements for slide stained with PD-L1: Presence of brown plasma membrane staining were observed. Non-specific staining ≤1+
- Requirements for slide stained with Negative Control Reagent: No membrane staining. Non-specific staining ≤1+

Optional Control Tissue:

In addition to the Control Cell Line Slide and in-house control tissues, FFPE tonsil were used as an optional control specimen. Tonsil stained with PD-L1 exhibited strong membrane staining in portions of the crypt epithelium and weak-to-moderate membrane staining of the follicular macrophages in the germinal centers.

PD-L1 expression of the endothelium, fibroblasts, and the surface epithelium was absent.

Negative Control Reagent (NCR):

The slides stained with the NCR were examined to identify non-specific background staining that may interfere with PD-L1 staining interpretation, making the specimen non-evaluable. Satisfactory performance was indicated by 0 specific staining and ≤1+ non-specific staining.

The subject specimens stained were examined with the NCR to determine if there is any non-specific staining that may interfere with interpreting the PD-L1 stained slide. Non-specific staining was ≤1+.

NCR-stained slides indicated non-specific background staining and allowed for better interpretation of subject specimens stained with the primary antibody.

Scoring Guidelines

PD-L1 expression score in TNBC was determined by using combined positive score (CPS), which is the number of PD-L1 staining cells (tumour cells, lymphocytes, macrophages) divided by the total number of viable tumour cells, multiplied by 100. Although the result of the calculation can exceed 100, the maximum score was defined as CPS 100.

Any perceptible and convincing partial or complete linear membrane staining (≥1+) of viable tumour cells that was perceived as distinct from cytoplasmic staining was considered PD-L1 staining and was included in the scoring. Any membrane and/or cytoplasmic staining (≥1+) of lymphocytes and macrophages (mononuclear inflammatory cells, MICs) within tumour nests and/or adjacent supporting stroma was considered PD-L1 staining and included in the CPS numerator. Only MICs directly associated with the response against the tumour were scored. Additional CPS inclusion/exclusion criteria is summarized in tables 1 and 2.

- At lower magnifications, all well-preserved tumour areas were examined. Overall areas of PD-L1 staining and non-staining tumour cells were evaluated, keeping in mind that partial membrane staining or 1+ membrane staining could be difficult to see at low magnifications. There were at least 100 viable tumour cells in the sample
    - A minimum of 100 viable tumour cells must be present in the PD-L1 stained slide (biopsy and resection) for the specimen to be considered adequate for evaluation
- For specimens with less than 100 viable tumour cells, tissue from a deeper level of the block or potentially another block has a sufficient number of tumour cells for evaluation of PD-L1 expression
- At higher magnification (20×), PD-L1 expression was evaluated and CPS calculated:
    - The total number of viable tumour cells was determined, both PD-L1 staining and non-staining (CPS denominator)
    - The number of PD-L1 staining cells was determined (tumour cells, lymphocytes, macrophages) (CPS numerator; see Tables 1 and 2 for additional CPS inclusion/exclusion criteria)
    - CPS was calculated

– Evaluation of membrane staining was performed at no higher than 20× magnification.

Table 2: CPS Numerator Inclusion/Exclusion Criteria for TNBC

Tissue Elements	**Included in the Numerator**	**Excluded from the Numerator**
Tumour cells	Convincing partial or complete linear membrane staining (at any intensity) of viable invasive tumour cells	- Non-staining tumour cells - Tumour cells with only cytoplasmic staining - Carcinoma in situ (ductal carcinoma in situ (DCIS) and lobular carcinoma in situ (LCIS))
Immune cells	Membrane and/or cytoplasmic* staining (at any intensity) of mononuclear inflammatory cells (MICs) within tumour nests and adjacent supporting stroma†: - Lymphocytes (including lymphocyte aggregates) - Macrophages‡ Only MICs directly associated with theresponse to the tumour are scored	- Non-staining MICs - MICs associated with DCIS and LCIS - MICs associated with benign structures - MICs (including lymphoid aggregates) not directly associated with the response to the tumour - Neutrophils, eosinophils, and plasma cells
Other cells	Not included	- Benign epithelial cells - Stromal cells (including fibroblasts)

		- Necrotic cells and/or cellular debris

* In MICs, membrane and cytoplasmic staining can be indistinguishable due to high nuclear to cytoplasmic ratio. Therefore, membrane and/or cytoplasmic staining of MICs was included in the score.

† Adjacent MICs were defined as being within the same 20× field as the tumour. However, MICs that were NOT directly associated with the response against the tumour were excluded.

‡ Macrophages and histiocytes have been considered the same cells.

Table 3: CPS Denominator Inclusion/Exclusion Criteria for TNBC

Tissue Elements	**Included in the denumerator**	**Excluded from the denumerator**
Tumour cells	All viable invasive tumour cells	- Non-viable tumour cells - Carcinoma in situ (DCIS and LCIS)
Immune cells	Not included	All immune cells
Other cells	Not included	- Benign cells - Stromal cells (including fibroblasts) - Necrotic cells and/or cellular debris

Example 2: Determining the CPS of a cancer tissue sample using PD-L1 IVD Kit (MEDx Inc.) in combination with anti-PD-L1 antibody clone E1L3N.

The PD-L1 expression score was determined using the PD-L1 IVD Kit (MEDx Inc.) and clone E1L3N according to the Manufacturer's Instructions for Use. CPS definition and

scoring process has been conducted according to a similar method as the PD-L1 IHC 22C3 pharmDx's Instructions for Use described in example 1.

Example 3: Efficacy of a First-Line therapy of a bispecific anti VEGF-A anti PD-L1 antibody (PM8002) in Combination with Chemotherapy for Subjects with Triple-Negative Breast Cancers (TNBC)

This is a Phase Ib/II study of a bispecific anti-VEGF-A anti-PD-L1 antibody (i.e., PM8002 comprising the heavy and light chain amino acid sequence set forth in SEQ ID NO: 16 and 17) in combination with nab-paclitaxel in subjects with locally advanced or metastatic triple-negative breast cancer without previous systematic treatment. This study determines the efficacy and safety of first-line treatment with PM8002 in combination with nab-paclitaxel in subjects with previously unresectable locally advanced or metastatic TNBC.

Approximately 42 subjects (intention-to-treat population (ITT)) with previously unresectable locally advanced or metastatic TNBC were treated. TNBC is confirmed by histology or cytology, i.e., ER, PR, HER-2 are all negative. Negative ER and PR are defined as: IHCER < 1%, IHCPR < 1%. HER-2 negative is defined as: IHCHER-2 (-) or (1+), HER-2 (2+) must be tested by FISH and the result is negative.

Subjects who had not received systemic treatment for advanced TNBC in the past were allowed to use taxane anti-tumour therapy in the previous neoadjuvant and/or adjuvant treatment stage, but had to meet the end time of taxane neoadjuvant and/or adjuvant treatment recurrence/metastasis interval $\geq$ 12 months.

Study arm: PM8002 at 20 mg/kg (Q2W) and nab-paclitaxel at 100 mg/m^2 on the 1st, 8th, and 15th days of each cycle until unacceptable toxicity or disease progression were observed. Each cycle contains 28 days.

The primary endpoint of the study was the Objective Response Rate (ORR) assessed by investigators per RECIST v1.1, the incidence and severity of Treatment-Related Adverse Events (TRAEs) graded according to NCI-CTCAE v5.0. Objective response rate (ORR) is the proportion of subjects with complete response (CR) or partial response (PR), based on RECIST v1.1.

The secondary endpoint of the study was the Progression-free survival (PFS) and the Disease Control rate (DCR) based on Investigator assessments per RECIST v1.1.

The study population included subjects aged 18 to 75 (including boundary value). Each subject met all of the inclusion criteria and none of the exclusion criteria for this study in order to be randomized to a study intervention.

Inclusion Criteria:

1) Ability to understand and willing to provide written informed consent and to comply with scheduled visits and study procedures.
2) Female or male, ages 18 to 75 years.
3) Histologically or cytologically confirmed unresectable locally advanced or metastatic breast cancer with negative ER (estrogen receptor, ER <1% tumour cells), PR (progesterone receptor, PR <1% tumour cells) and HER-2(IHC 0/IHC 1+/IHC 2+/FISH not-amplified). Testing results for all three markers conducted within 36 months prior to the initiation of the study by a local facility accredited by clinical research center are acceptable. If deemed necessary by the investigator during screening, subjects may provide additional biopsy to confirm the latest pathological.
4) Subjects who have not received prior systemic treatment for advanced TNBC are eligible for the study. Subjects who have received taxane-based chemotherapy or pembrolizumab during the neoadjuvant and/or adjuvant treatment phase are eligible if the occurrence of relapse or metastasis was more than 12 months after the end of such treatment(s).
5) Subjects should provide a fresh tumour biopsy during the screening period (bone biopsies, fine-needle aspiration biopsies, and samples from pleural or peritoneal fluid are not acceptable, subjects with only one target lesion are not eligible to provide biopsy). Sufficient qualified tumour tissue specimens should be obtained for biomarker analysis including PD-L1 expression levels. If a subject is unable to provide a fresh biopsy, a recent tumour sample (up to a maximum of 24 months prior to the start of the study) processed through formalin-fixed paraffin embedding (FFPE) or unstained slides (3-5 μm) is acceptable for the corresponding biomarker analysis. If a subject is unable to provide specimens that meet the aforementioned requirements, she/he may still participate in the screening process with the consent of the Sponsor.
6) Adequate organ function, as defined below:

a. Hematology (RBC or platelet transfusions, granulocyte colony-stimulating factors treatment, or other medical supports are not allowed within 14 days prior to the study treatment initiation):
   i. Neutrophil count (ANC) ≥1.5 × 109/L.
   ii. Platelet count (PLT) ≥100 × 109/L.
   iii. Hemoglobin (Hb) ≥90 g/L.
b. Liver function:
   i. Total bilirubin (TBIL) ≤1.5 × upper limit of normal (ULN), objects with liver metastasis ≤2 × ULN, subjects with a previous diagnosis of Gilbert's syndrome ≤3 × ULN.
   ii. Aspartate aminotransferase (AST) or alanine aminotransferase (ALT) ≤2.5 × ULN, objects with liver metastasis ≤5 × ULN.
c. Renal function:
   i. Serum creatinine ≤1.5 × ULN or Creatinine Clearance (CrCl) ≥50 ml/min {Cockcroft-Gault formula: [(140 - age) × weight (kg) × (0.85, for women only)]/[72 × creatinine (mg/dL)] (conversion of creatinine unit: 1 mg/dL = 88.4 μmol/L)}.
   ii. Qualitative urine protein ≤1+; If qualitative urine protein ≥ 2+, 24 h urine protein quantitative test is required, if the result is < 1g, it is acceptable.
d. Coagulation function: International normalized ratio (INR) ≤1.5, activated partial thromboplastin time (APTT) ≤1.5 × ULN, objects with liver metastasis INR and APTT ≤2 × ULN.

7) Performance status as assessed by the Eastern Cooperative Oncology Group (ECOG): 0-1.
8) Life expectancy ≥12 weeks.
9) According to RECIST 1.1, the subject has at least 1 measurable lesion as the targeted lesion (a measurable lesion at the previously irradiated radiation field or other local treatment area should not be selected as targeted lesion, the only bone metastasis or the only central nervous system metastasis should not be considered as a measurable lesion).
10) Female subjects of childbearing potential have a negative blood pregnancy test result within 7 days prior to the study treatment and are willing to follow medically

approved highly effective contraceptive measures (such as intrauterine device and condom) from signing the informed consent form to until 6 months after the last dosage of treatment.

11) Male subjects are willing to follow medically approved highly effective contraceptive measures from signing the informed consent form to until 6 months after the last dosage, and do not donate sperm during this period.

Exclusion Criteria:

1) History of severe allergic disease, severe drug allergy (including allergy to any investigational products) or known allergy to any component of the investigational product, or its excipients.
2) Adverse events resulting from prior anti-tumour therapies should be assessed and graded according to the CTCAE 5.0 criteria, subjects whose AEs have not returned to Grade 1 or below (unless the investigator determines that the current AEs pose no safety risk to the patients, such as hair loss or stable hypothyroidism under hormone replacement therapy) are not eligible for the study.
3) Those who have previously received any antibody- or inhibitor-based therapy targeting PD-1/PD-L1 or VEGF.
4) Those with a history of pulmonary fibrosis, or currently diagnosed with severe lung diseases such as interstitial pneumonia, pneumoconiosis, chemical pneumonitis, or any other condition resulting in significant impairment in lung function. Exception: asymptomatic interstitial changes caused by previous radiation therapy, chemotherapy, or other factors such as smoking are acceptable.
5) Those who have received any of the following therapies or drugs prior to study initiation:
    a. Have received immunotherapy, nitrosoureas, or mitomycin C within 6 weeks prior to the initiation of the study treatment, or have received oral fluoropyrimidine-based chemotherapy or small molecule targeted therapy within 2 weeks prior to the start of the study treatment (or within 5 half-lives of the drugs, whichever is longer), or have received palliative radiotherapy within 7 days prior to the initiation of the study treatment, or have received any other chemotherapy, curative/palliative radiotherapy, endocrine therapy, biologic therapy (including tumour vaccines, cytokines, or growth factors

for tumour control) or any experimental anti-tumour drugs within 28 days prior to the initiation of the study treatment.

b. Have undergone major organ surgery (excluding needle biopsies) within 28 days prior to the study treatment or need to undergo elective surgery during the trial.

c. Have clinically significant unhealed wounds, ulcers, or fractures.

d. Have received systemic corticosteroids (at a dosage greater than 10 mg/day of prednisone or an equivalent dosage of other corticosteroids) within 14 days prior to the initiation of the study treatment. Have received other systemic immunostimulatory agents or immunosuppressive therapies (such as IFN-α, IL-2, or methotrexate) within 4 weeks prior to the initiation of the study treatment or are within 5 half-lives of the treatment drug (whichever is longer).

Exception: excluding local, intranasal, intraocular, intra-articular or inhaled corticosteroids, short-term use (≤7 days) of corticosteroids for prophylaxis (e.g., prevention of contrast agent allergy) or treatment of non-autoimmune conditions (e.g., delayed hypersensitivity reactions caused by exposure to allergens).

e. Have been vaccinated with live attenuated vaccine(s) within 28 days prior to the study treatment.

f. Have used systemic broad-spectrum antibiotics for ≥7 days within 14 days prior to the study treatment.

6) Those who have meningeal metastases, uncontrolled or symptomatic central nervous system (CNS) metastases.

7) Those who have active infections requiring intravenous antibiotic therapy at the time of study.

8) Those who had or have active autoimmune disease or a history of autoimmune diseases with anticipated relapse (such as systemic lupus erythematosus, rheumatoid arthritis, vasculitis, etc.), except for those with clinically stable autoimmune thyroid disease or type 1 diabetes.

9) Those who have had other active malignant tumours within 5 years prior to the study treatment, except for those that can be treated locally and have been cured (such as basal cell or squamous cell carcinoma of the skin, superficial or non-

invasive bladder cancer, carcinoma in situ of the cervix, and papillary carcinoma of thyroid).

10) Those with any of the following conditions within 6 months prior to the study treatment:
    a. Acute coronary syndrome, coronary artery bypass grafting, congestive heart failure (CHF), aortic dissection, stroke, or other grade 3 and above cardiovascular and cerebrovascular events.
    b. New York Heart Association (NYHA) functional classification ≥II heart failure (as defined by the NYHA), or left ventricular ejection fraction (LVEF) < 50%.
    c. Those who have ventricular arrhythmias requiring clinical intervention, second- to third-degree atrioventricular block, or congenital long QT syndrome.
    d. Mean QT interval corrected by Fridericia's method (QTcF) > 450 ms for males and > 470 ms for females.
    e. Use of cardiac pacemaker.
    f. Cardiac troponin (cTn) I or N > 2 × ULN.
11) Those who have uncontrollable pleural, pericardial, or abdominal effusions.
12) Those with fever of unknown origin > 38.5 °Cprior to the study treatment (subjects with fever caused by tumours can be enrolled as judged by the investigator).
13) Presence any of the following conditions prior to study treatment:
    a. Poorly controlled diabetes (fasting blood glucose ≥13.3 mmol/L).
    b. Poorly controlled hypertension (systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg).
    c. Those with a history of hypertensive crisis or hypertensive encephalopathy.
    d. Those with a history of abdominal fistula, tracheoesophageal fistula, gastrointestinal perforation, or intra-abdominal abscess within the last 6 months prior to the start of the study treatment.
    e. Those who have undergone core needle biopsy or other minor surgical procedures within the last 7 days prior to the start of the study treatment (excluding placement of vascular infusion devices).
14) Those with uncontrolled tumour-related pain requiring analgesic treatment should have a stable analgesic regimen at screening. For asymptomatic metastatic lesion, if

its growth may cause dysfunction or intractable pain (e.g., current epidural metastasis unrelated to spinal cord compression), local treatment should be considered before screening, if appropriate.

15) Those who present with a history of major bleeding diathesis or other significant risk of bleeding such as:
   a. History of intracranial hemorrhage or intraspinal hemorrhage.
   b. With tumour lesions invading large blood vessels and are at significant risk of bleeding.
   c. Have had thrombosis or embolism (except intramuscular venous thrombosis that is asymptomatic and does not require treatment) within 6 months prior to the study treatment.
   d. Have had clinically significant hemoptysis or tumour hemorrhage of any cause within 1 month prior to the study treatment.
   e. Have had anticoagulant therapy of therapeutic purposes (except low molecular weight heparin for prophylaxis) within 14 days prior to the study treatment.
   f. Have used antiplatelet drugs including but not limited to aspirin (≥100 mg/day), clopidogrel (> 75 mg/day), dipyridamole, ticlopidine or cilostazol, within 10 days prior to the study treatment, or those requiring long-term antiplatelet therapy.
16) Those who have received allogeneic hematopoietic stem cell transplantation or organ transplantation.
17) Those who are known to have a history of alcoholic abuse, psychotropic drug abuse, or illicit drug addiction.
18) Those with a documented history of neurologic or mental disorders, such as epilepsy, dementia, schizophrenia and so on.
19) Presence of human immunodeficiency virus (HIV) infection or known acquired immunodeficiency syndrome (AIDS).
20) Those with positive result in non-specific treponemal antibody tests for syphilis (such as TRUST, PRP) or positive results in specific treponemal antibody tests (such as TPPA) are not eligible for the study, except those who have a positive result in specific treponemal antibody tests but have consistently negative results in non-specific treponemal antibody tests for one year or longer.

21) Those with active tuberculosis or a history of tuberculosis infection that was not successfully controlled.

22) Those with active hepatitis B (positive for HBsAg and HBV-DNA ≥1000 IU/ml) are not eligible for the study, unless their viral load is controlled (HBV-DNA < 1000 IU/ml) with antiviral medication. Those with active hepatitis C (HCV-RNA > lower limit of detection as determined by the research center) are also not eligible for the study.

23) Subject's underlying condition may increase the risk of research treatment or complicate the interpretation of toxicities and adverse events, as judged by the investigator.

24) Those who are expected to require other anti-tumour drug therapy during the trial.

25) Pregnant or lactating women.

26) Other situations in which investigators consider the patient unsuitable for participation in this study.

Before treatment, the CPS of each patient has been determined as described in example 2 using the anti-PD-L1 antibody clone E1L3N and the PD-L1 IVD Kit (MEDx Inc.).

Subgroups of patients having a CPS of <1, ≥1, and ≥10 have been formed. The study results are summarized in table 4. As shown in this table, the combination therapy led to an increased median progression-free survival in the CPS subgroup ≥1. Median progression-free survival (95% CI) was 9.2 months with the addition of the bispecific antibody targeting specifically PD-L1 and VEGF-A combined with chemotherapy in subjects having a CPS of ≥1, identical to the median progression-free survival of subjects having a higher PS of ≥10. Thus, contrary to the SOC treatment comprising pembrolizumab and chemotherapy, the combination treatment disclosed herein has a beneficial effect in PD-L1 low expressing cancers (CPS subgroup ≥1) similar to PD-L1 high expressing cancers (CPS subgroup ≥10). The ORR in the ITT population was 78.6%, including 1 complete response and 32 partial responses, the confirmed ORR was 73.8% with the disease control rate of 95.2%. The ORR in the CPS<1 population was 76.9%, including 10 partial responses, the confirmed ORR was 76.9% with the disease control rate of 100%. The ORR in the CPS≥10 population was 100%, including 9 partial responses, the confirmed ORR was 100% with the disease control rate of 100%. The ORR in the CPS≥1 population was 80.0%, the confirmed ORR was 72.0%. The ORR in the CPS<1 population was 76.9%, the confirmed ORR was 76.2%.

with the disease control rate of 100%. Patients were continued to be treated after obtaining the results discussed above and shown below in Tables 4 and 5, and, as of 5 July 2024, the median PFS for the ITT population was 13.5 months (compared to 9.2 months as shown in Table 4), the median time to response was 1.9 months, and the median duration of response was 11.7 months.

Table 4: Efficacy Outcomes measured by median PFS, 95% confidence interval (CI), in Subgroups according to CPS Status at Baseline

CPS	**n (%)**	**ORR**	**mPFS, month (95%CI)**
<1	13 (31.0)	76.9 %	7.4 (95%CI: 5.7,--)
≥1	25 (59.5)	80.0 %	9.2 (95%CI: 8.5,--)
≥10	9 (21.4)	100 %	9.2 (95%CI: 5.5,--)
UK*	4 (9.5)	75 %	-- (95%CI: 81,--)
ITT	42	78.6 %	9.2 (95%CI: 8.5,--)

*Unknown CPS due to lack of tissue sections

In the CPS≥1 subgroup, a further subgroup was formed, namely the subgroup of 1≤CPS<10, wherein the results are shown in **Table 4a**.

Table 4a: Efficacy Outcomes measured by median PFS, 95% confidence interval (CI), in a further subgroup according to CPS Status at Baseline

CPS	**n (%)**	**ORR**	**mPFS, month (95%CI)**
1≤CPS<10	16 (38.1)	68.8 %	14.0 (95%CI: 7.2,--)

The ORR in the ITT population 1≤CPS<10 was 68.8%, including 1 complete response and 10 partial responses, the confirmed ORR was 56.3% with the disease control rate of 93.8%.

The combination therapy disclosed herein shows an improved median progression-free survival compared to the standard treatment involving chemotherapy alone or chemotherapy in combination with the anti-PD-L1 antibody pembrolizumab as shown in table 5.

Table 5: Comparison of the current treatment of care for triple-negative breast cancer with the combination therapy disclosed herein

CPS		**Chemotherapy***	**Chemotherapy and pembrolizumab***	**Chemotherapy and the anti-VEGF-A and anti-PD-L1 antibody**
CPS ≥1	Response Rate	38.9%	44.9 %	about 75 %
	Median PFS	5.6	7.6	9.2
	6 Month PFS	46.6%	56.4%	> 85 %
CPS ≥10	Response Rate	40.8%	52.7%	> 75%
	Median PFS	5.6	9.7	9.2
	6 Month PFS	46.9%	65%	> 85%

* data derived from Cortes J, Rugo HS, Cescon DW, et al. Pembrolizumab plus Chemotherapy in Advanced Triple-Negative Breast Cancer. N Engl J Med. 2022;387(3):217-226. doi:10.1056/NEJMoa2202809.

Surprisingly, the combination therapy of the bispecific antibody targeting specifically PD-L1 and VEGF-A combined with chemotherapy showed encouraging anti-tumour activity regardless of PD-L1 status and good safety profile as a first-line therapy for TNBC cancer having a low CPS of ≥1.

The combination therapy therefore meets the high unmet need for a breakthrough therapy designation for subject afflicted with cancer and having a low CPS of ≥1.

The patients of the present study were also classified according to the Fudan University Shanghai Cancer Center (FUSCC) classification (see Jiang YZ, Ma D, Suo C, et al. Genomic and transcriptomic landscape of triple-negative breast cancers: subtypes and treatment strategies. Cancer Cell. 2019; 35(3):428-440.e5), which classifies TNBCs into four transcriptome-based subtypes with distinct molecular features: (1) luminal androgen receptor (LAR), (2) immunomodulatory (IM), (3) basal-like immune-suppressed (BLIS), and (4) mesenchymal-like (MES). 31 of the 42 patients could be classified according to these four transcriptome-based subtypes, and the results were as shown in **Table 6**.

Table 6: Efficacy in the FUDAN-subtypes

	BLIS	**IM**	**LAR**	**MES**
Population	12	4	13	2
ORR, % (95% CI)	66.7 (34.9, 90.1)	100.0 (39.8, 100.0)	76.9 (46.2, 95.0)	100.0 (15.8, 100.0)
cORR, % (95% CI)	58.3 (27.7, 84.8)	100.0 (39.8, 100.0)	69.2 (38.6, 90.9)	100.0 (15.8, 100.0)
DCR, % (95% CI)	100.0 (73.5, 100.0)	100.0 (39.8, 100.0)	92.3 (64.0, 99.8)	100.0 (15.8, 100.0)

ORR, objective response rate; cORR, confirmed ORR; disease control rate, DCR; basal-like immune-suppressed, BLIS; immunomodulatory, IM; luminal androgen receptor (LAR); mesenchymal-like, MES.

It is noteworthy that both in the IM and MES subtypes, the ORR was 100%.

All patients experienced treatment-related adverse events (TRAEs), 54.8% were Grade 3 or 4 and no Grade 5 TRAEs was observed. The most common TRAEs included neutropenia, leukocytopenia, anemia, proteinuria, alopecia and epistaxis. 35.7% patients experienced immune-related adverse events (irAEs) with Grade 3 or 4 of 9.5%, which included hyperthyroidism, hypothyroidism and rash, etc. The most common AEs related to VEGF were hypertension and proteinuria which most were Grade 1-2.

In view of the above results, PM8002 combined with nab-paclitaxel showed encouraging antitumor activity and good safety as first-line therapy for locally advanced and metastatic TNBC.

Example 4: Safety and Efficacy of PM8002 (anti-PD-L1 x VEGF-A bispecific) Combined with Paclitaxel as a Second-Line Therapy for Small Cell Lung Cancer

The present example is a Phase II study of a bispecific anti-VEGF-A anti-PD-L1 antibody (i.e., PM8002 comprising the heavy and light chain amino acid sequence set forth in SEQ ID NO: 16 and 17) in combination with paclitaxel as a second-line therapy for patients suffering from small cell lung cancer (SCLC) to assess the safety and efficacy of this treatment.

Patients with advanced SCLC who failed first-line platinum-based chemotherapy (i.e., who progressed during or after first-line platinum therapy) with or without checkpoint inhibitor therapy were enrolled in the present study. There is an unmet medical need for such patients.

Primary endpoints included the objective response rate (ORR) and safety, with progression free survival (PFS) and overall survival (OS) as the secondary endpoints. The endpoints were assessed by investigators according to commonly and routinely used standardized procedures.

All patients received PM8002 at 30 mg/kg (Q3W) and paclitaxel at 175 mg/m^2 (Q3W) for 5 cycles (PM8002 and paclitaxel were administered IV), followed by PM8002 maintenance at 30 mg/kg (Q3W) until unacceptable toxicity or disease progression.

Results: As of April 12, 2024, 60 patients have been enrolled. Among 26 immunotherapy-naïve patients, 22 patients completed at least one tumor assessment. 5 patients were still on treatment. Median PFS was 5.9 months and median OS was 14.4 months, which were still maturing. The ORR was 72.7% (16/22) with a disease control rate (DCR) of 81.8% (18/22), and the median duration of response (DOR) was 11.5 months. Among 34 immunotherapy-treated patients, 29 patients completed at least one tumor assessment. 12 patients were still on treatment. The median PFS was 6.2 months which was still maturing. The ORR was 44.8% (13/29) with a DCR of 89.7% (26/29). Any-grade treatment-related adverse events

(TEAEs) occurred in 95% (57/60) of patients. Grade ≥ 3 TEAEs related to the combination regimen occurred in 70% (42/60) of patients, with the most common grade ≥ 3 TRAEs observed being neutropenia (56.7%, 34/60) and leukopenia (31.7%, 19/60). Three patients discontinued PM8002 and/or paclitaxel administration due to TRAEs. Any-grade immune-related adverse events (irAEs) occurred in 30% (18/60) of patients, and grade ≥ 3 irAEs occurred in 3.3% (2/60) of patients.

Summarizing the above, PM8002, in combination with paclitaxel, showed encouraging antitumor activity and an acceptable safety profile for patients with advanced SCLC who failed first-line platinum-based chemotherapy with or without immunotherapy.

Example 5: Safety and Efficacy of PM8002 (anti-PD-L1 x VEGF-A bispecific) Combined with Systemic Chemotherapy as a First-Line Therapy for Extensive-Stage Small Cell Lung Cancer

The present example is an open label, single arm, multicenter Phase II study of a bispecific anti-VEGF-A anti-PD-L1 antibody (i.e., PM8002 comprising the heavy and light chain amino acid sequence set forth in SEQ ID NO: 16 and 17) in combination with systemic chemotherapy (in particular platinum-etoposide) as a first-line therapy for patients suffering from extensive-stage small cell lung cancer (ES-SCLC) to assess the safety and efficacy of this treatment.

Patients with ES-SCLC have a poor prognosis and there is an unmet medical need for such patients. The patients of the present study suffered from ES-SCLC (histologically or cytologically confirmed) and had not received systemic treatment for ES-SCLC. Further, patients were eligible for the present study if they were equal to or older than 18 years with an Eastern Cooperative Oncology Group Performance Status (ECOG PS) from 0 to 1.

Primary endpoints included the objective response rate (ORR) and safety, with progression free survival (PFS) and overall survival (OS) as the secondary endpoints. The endpoints were assessed by investigators according to RECIST v1.1 and commonly and routinely used standardized procedures as regards the TRAEs.

All patients received PM8002 at 30 mg/kg (Q3W) and platinum-etoposide (with carboplatin at AUC=5 (total dose ≤ 750 mg) on day 1 of each cycle and etoposide at 100 mg/m^2 on days 1 to 3 of each cycle) every three weeks for 4 cycles, followed by PM8002

maintenance (at 30 mg/kg) every three weeks until disease progression or unacceptable toxicity.

Results: As of April 12, 2024, 50 patients were enrolled in this study and 48 patients completed at least one tumor evaluation. The median age was 60 years, with a range from 46 to 75 years. A partial response (PR) was observed in 42 patients with a best overall objective response rate (ORR) of 87.50%, Confirmed ORR of 81.25%. The median best percentage tumor volume shrinkage from baseline was 58.48%. The disease control rate (DCR) was 100% (48/48), and the median progression free survival (PFS) was 6.93 months (95% CI: 4.27, 8.48). 26 patients were still on treatment. The censored rate of PFS was 47.92%. Median overall survival (OS) had not yet been reached. Any-Grade treatment-related adverse events (TRAEs) occurred in all patients. Grade ≥ 3 TRAEs occurred in 42 patients (84%). The most commonly observed AE at any Grade was neutrophil count decrease (90%, 45/50), white blood cell count decrease (76%, 38/50), and platelet count decrease (60%, 30/50). Immune-related adverse events (irAEs) occurred in 34% of patients (17/50) with 4% (2/50) Grade ≥ 3. Two patients (4%) discontinued treatment due to TRAEs.

Summarizing the above, PM8002 plus platinum-based chemotherapy as a first-line treatment for ES-SCLC showed some efficacy and acceptable tolerability.

Example 6: Safety and Efficacy of PM8002 (anti-PD-L1 x VEGF-A bispecific) Combined with Chemotherapy in patients with EGFR-mutated Non-Small Cell Lung Cancer (NSCLC)

The present example is a Phase II study of a bispecific anti-VEGF-A anti-PD-L1 antibody (i.e., PM8002 comprising the heavy and light chain amino acid sequence set forth in SEQ ID NO: 16 and 17) in combination with chemotherapy (in particular carboplatin and pemetrexed) as a therapy for patients suffering from EGFR-mutated NSCLC who progressed after EGFR-tyrosine kinase inhibitors (TKIs) treatment to assess the safety and efficacy of this treatment. Furthermore, the correlation between tumor PD-L1 expression and clinical response was investigated.

PD-L1 expression was determined by immunohistochemistry (IHC; using TPS) from patients (biopsy at baseline) after progression from EGFR-TKI therapy as disclosed above in Example 2. Patients were divided into 3 groups based on these TPS results (<1% defined as negative, 1 to 49% defined as low expression, and ≥50% defined as high expression,

using clone E1L3N as the detection antibody). The primary endpoint of this study was the objective response rate (ORR) assessed by investigators according to per RECIST v1.1.

Patients received 30 mg/kg PM8002 plus AUC 5 mg/mL/min carboplatin and 500mg/m^2 pemetrexed every three weeks (Q3W) for 4 cycles, followed by 30 mg/kg PM8002 and 500mg/m^2 pemetrexed as maintenance therapy.

Results: As of 24 July 2024, 64 patients were tested for PD-L1 TPS: 28 (43.8%) were negative, 23 (35.9%) had low expression, and 13 (20.3%) had high expression. All patients were evaluable for safety and efficacy. Overall confirmed ORR was 57.8% (37/64) and DCR was 95.3% (61/64). In the PD-L1 negative group, the confirmed ORR was 39.3% and DCR was 92.9%. In the PD-L1 low expression group, the confirmed ORR was 60.9% and DCR was 100%. In the PD-L1 high expression group, the confirmed ORR was 92.3% and DCR was 92.3%. Any-grade TRAEs occurred in 98.4% (63/64) and grade $\geq$ 3 TRAEs occurred in 60.9% (39/64) patients. Any-grade immune-related adverse events (irAEs) occurred in 40.6% (26/64) and grade $\geq$ 3 irAEs occurred in 6.3% (4/64) patients. 5 patients discontinued PM8002 administration due to TRAEs with 1 TRAE-related death (pneumonia).

Summarizing the above, PM8002 in combination with chemotherapy showed encouraging antitumor activity and acceptable tolerability in EGFR-mutated NSCLC patients that progressed after prior EGFR-TKI therapy. The anti-tumor activity of PM8002 therapy is positively correlated with tumor PD-L1 expression level.

Example 7: Safety and Efficacy of PM8002 (anti-PD-L1 x VEGF-A bispecific) as a Single Agent or Combined with Chemotherapy for the treatment of Hepatocellular Carcinoma (HCC)

The present example is a Phase I/IIa study of a bispecific anti-VEGF-A anti-PD-L1 antibody (i.e., PM8002 comprising the heavy and light chain amino acid sequence set forth in SEQ ID NO: 16 and 17) as a single agent for treating HCC. Furthermore, the present example is a Phase II study of a bispecific anti-VEGF-A anti-PD-L1 antibody (i.e., PM8002 comprising the heavy and light chain amino acid sequence set forth in SEQ ID NO: 16 and 17) in combination with chemotherapy (in particular FOLFOX4 [oxaliplatin, leucovorin (which may also be referred to as calcium folinate) and 5-fluorouracil]) for

treating HCC. Both studies assessed the safety and efficacy of the respective treatments as first-line treatments for HCC.

Patients suffering from HCC who had not been treated previously with systemic therapy were included in the two studies. Tumor responses were assessed every 6 weeks for the monotherapy study and every 8 weeks for the combination study. The primary endpoint for both studies was the objective response rate (ORR) based on RECIST v1.1, and a further endpoint was safety assessed by commonly and routinely used standardized procedures as regards the TRAEs.

In the single-agent study, patients received 20 mg/kg PM8002 every two weeks (Q2W), whereas patients in the combination study received 20 mg/kg PM8002 every two weeks (Q2W) in combination with FOLFOX4(day 1: oxaliplatin [85 mg/m^2, 2-h infusion] plus leucovorin [200 mg/m^2, 2-h infusion], followed by 5-fluorouracil [400 mg/m^2, intravenous bolus at hour 2; then 600 mg/m^2 for a22-h infusion]; day 2: leucovorin [200 mg/m^2, 2-h infusion], followed by 5-fluorouracil [400 mg/m^2, intravenous bolus at hour 2; then 600 mg/m^2 for a 22-h infusion]..

Results: As of April 15, 2024, a total of 46 patients participated in the two studies. 11 patients received at least one dose of PM8002 monotherapy, while 35 patients received at least one dose of the combination therapy. Two patients did not undergo tumor assessment. The ORR for the monotherapy was 27.3%, and 39.4% for the combination therapy. The disease control rates were 100% and 90.9%, respectively. The median progression-free survival was 15.1 months for the monotherapy and 5.8 months for the combination therapy. The median overall survival was 15.4 months for the combination therapy study and not reached for the monotherapy study. Regarding safety, 90.9% of the patients in the monotherapy study and 97.1% of the patients in the combination study experienced treatment-related adverse events (TRAE), with Grade $\geq$ 3 adverse events reported in 36.4% and 51.4% of cases for each study, respectively. The most common TRAEs in the monotherapy study were hypertension (45.5%), proteinuria (45.5%), and decreased platelet count (36.4%), while in the combination study, the most common TRAEs were decreased platelet count (71.4%), decreased neutrophil count (68.6%), decreased white blood cell count (68.6%), and anemia (40.0%).

Summarizing the above, PM8002 as monotherapy and in combination with chemotherapy has shown promising anti-tumor activity and manageable safety for the first line treatment of HCC.

CLAIMS

1. A bispecific antibody that specifically binds to PD-L1 and VEGF for use in a method of treating a subject with cancer, the method comprising administering to the subject:

   a. the bispecific antibody; and

   b. a chemotherapy, preferably a chemotherapy agent;

   optionally wherein the subject has a PD-L1 expression before the treatment that provides a score of ≥1 when determined by the combined positive score (CPS) or a score that is indicative of such a PD-L1 expression when determined by another integrating scoring algorithm.

2. The bispecific antibody for use according to claim 1, wherein the subject has a PD-L1 expression before the treatment that provides a score of ≥1 when determined by the combined positive score (CPS) or a score that is indicative of such a PD-L1 expression when determined by the tumour area positivity (TAP) score or the tumour proportion score (TPS).

3. The bispecific antibody for use according to claim 1 or 2, wherein the subject has a PD-L1 expression before the treatment that provides a score of ≥1 when determined by the combined positive score (CPS) or a score that is indicative of such a PD-L1 expression when determined by the tumour area positivity (TAP) score.

4. The bispecific antibody for use according to any of claims 1 to 3, wherein the CPS has been determined in a test sample of the subject by determining the number of PD-L1 staining cells (tumour cells, lymphocytes, macrophages) and the total number of viable tumour cells in a cancer tissue sample from the subject; and calculating the CPS for the cancer tissue sample using the formula:

$$\text{CPS} = \frac{\#\ \text{PD-L1 staining cells (tumour cells, lymphocytes, macrophages)}}{\text{total}\ \#\ \text{viable tumour cells}} * 100$$

   optionally wherein

   (i) the test sample is a cancer tissue sample; and/or

(ii) the cancer tissue sample comprises at least 100 viable tumour cells as well as (tumour infiltrating) lymphocytes and macrophages; and/or

(iii) PD-L1 staining cells are determined by PD-L1 immunohistochemistry staining; and/or

(iv) viable tumour cells are determined by staining with a viability dye; and/or

(v) the number of PD-L1 staining cells is the sum of PD-L1 staining tumour cells, PD-L1 staining lymphocytes and PD-L1 staining macrophages.

5. The bispecific antibody for use according to claim 4, wherein the CPS has been determined in a test sample of the subject by determining the number of PD-L1 staining cells (tumour cells, lymphocytes, macrophages) and the total number of viable tumour cells in a cancer tissue sample from the subject; and calculating the CPS for the cancer tissue sample using the formula:

$$\text{CPS} = \frac{\#\ \text{PD-L1 staining cells (tumour cells, lymphocytes, macrophages)}}{\text{total \# viable tumour cells}} * 100$$

wherein

(i) the test sample is a cancer tissue sample;

(ii) the cancer tissue sample comprises at least 100 viable tumour cells as well as (tumour infiltrating) lymphocytes and macrophages;

(iii) PD-L1 staining cells are determined by PD-L1 immunohistochemistry staining;

(iv) viable tumour cells are determined by staining with a viability dye; and

(v) the number of PD-L1 staining cells is the sum of PD-L1 staining tumour cells, PD-L1 staining lymphocytes and PD-L1 staining macrophages.

6. The bispecific antibody for use according to any of the preceding claims, wherein the PD-L1 expression score has been determined using a TAP scoring algorithm in a test sample of the subject by determining the percentage of PD-L1 positive tumour cells and immune cells per tumour area in a cancer tissue sample from the subject; and calculating the TAP for the cancer tissue sample using the formula:

$$\text{TAP} = \frac{\%\ \text{PD-L1 positive tumour cells and immune cells}}{\text{tumour area}}$$

optionally wherein

(i) the test sample is a cancer sample; and/or

(ii) the tumour area is the area occupied by all viable tumour cells and the tumour-associated stroma containing tumour-associated immune cells; and/or

(iii) PD-L1 positive tumour cells and immune cells are determined by PD-L1 immunohistochemistry staining (preferably by using the anti-PD-L1 antibody SP263); and/or

(iv) PD-L1 positive tumour cells and immune cells means the area covered by such PD-L1 positive tumour cells and immune cells; and/or

(v) % PD-L1 positive tumour cells and immune cells / tumour area means the area of PD-L1 positive tumour cells and immune cells relative to the tumour area expressed as %.

7. The bispecific antibody for use according to claim 6, wherein the PD-L1 expression score has been determined using a TAP scoring algorithm in a test sample of the subject by determining the percentage of PD-L1 positive tumour cells and immune cells per tumour area in a cancer tissue sample from the subject; and calculating the TAP for the cancer tissue sample using the formula:

$$\text{TAP} = \frac{\%\ \text{PD-L1 positive tumour cells and immune cells}}{\text{tumour area}}$$

wherein

(i) the test sample is a cancer sample;

(ii) the tumour area is the area occupied by all viable tumour cells and the tumour-associated stroma containing tumour-associated immune cells;

(iii) PD-L1 positive tumour cells and immune cells are determined by PD-L1 immunohistochemistry staining (preferably by using the anti-PD-L1 antibody SP263);

(iv) PD-L1 positive tumour cells and immune cells means the area covered by such PD-L1 positive tumour cells and immune cells; and

(v) % PD-L1 positive tumour cells and immune cells / tumour area means the area of PD-L1 positive tumour cells and immune cells relative to the tumour area expressed as %.

8. The bispecific antibody for use according to any of the preceding claims, wherein the PD-L1 expression score has been determined using a TPS scoring algorithm by determining in a test sample of the subject the number of viable tumour cells positive for PD-L1 and the total number of viable tumour cells in a cancer tissue sample from the subject; and calculating the TPS for the cancer tissue sample using the formula:

$$\text{TPS} = \frac{\#\ viable\ tumor\ cells\ positive\ for\ PD{-}L1}{\text{total \# viable tumour cells}} * 100$$

optionally wherein

(i) the test sample is a cancer tissue sample; and/or

(ii) the cancer tissue sample comprises at least 100 viable tumour cells; and/or

(iii) viable tumour cells are determined by staining with a viability dye; and/or

(iv) PD-L1 positive cells are determined by PD-L1 immunohistochemistry staining.

9. The bispecific antibody for use according to claim 8, wherein the PD-L1 expression score has been determined using a TPS scoring algorithm by determining in a test sample of the subject the number of viable tumour cells positive for PD-L1 and the total number of viable tumour cells in a cancer tissue sample from the subject; and calculating the TPS for the cancer tissue sample using the formula:

$$\text{TPS} = \frac{\#\ viable\ tumor\ cells\ positive\ for\ PD{-}L1}{\text{total \# viable tumour cells}} * 100$$

wherein

(i) the test sample is a cancer tissue sample;

(ii) the cancer tissue sample comprises at least 100 viable tumour cells;

(iii) viable tumour cells are determined by staining with a viability dye; and

(iv) PD-L1 positive cells are determined by PD-L1 immunohistochemistry staining.

10. The bispecific antibody for use according to any of claims 3 to 9, wherein the cancer tissue sample is a tissue section of a tumour biopsy.

11. The bispecific antibody for use according to claim 10, wherein the tissue section is a formalin fixed and embedded in paraffin wax (FFPE) tissue section.

12. The bispecific antibody for use according to claim 10 or 11, wherein the tissue section is stained.

13. The bispecific antibody for use according to claim 12, wherein the stain comprises a hematoxylin and eosin (H&E) stain.

14. The bispecific antibody for use according to any of claims 3 to 13, wherein the viability dye is propidium iodide.

15. The bispecific antibody for use according to any of claims 3 to 14, wherein the number of viable tumour cells in the tumour tissue sample is determined by flow cytometry.

16. The bispecific antibody for use according to any of the preceding claims, wherein the bispecific antibody and the chemotherapy are separately administered.

17. The bispecific antibody for use according to any of the preceding claims, wherein a dosage of the bispecific antibody and a dosage of the chemotherapy are administered concurrently or consecutively.

18. The bispecific antibody for use according to any of the preceding claims, wherein the chemotherapy comprises a platinum-based chemotherapy.

19. The bispecific antibody for use according to any of the preceding claims, wherein the subject has a PD-L1 expression before the treatment that provides a score of < 20, preferably from 1 to 20, more preferably from 1 to <10 when determined by the CPS or a score that is indicative of such a PD-L1 expression when determined by another integrating scoring algorithm, preferably when determined by the tumour area positivity (TAP) score or the tumour proportion score (TPS), more preferably when determined by the TAP score.

20. The bispecific antibody for use according to any of the preceding claims, wherein the bispecific antibody dosage is in the range of from 0.1 mg/kg to 45 mg/kg body weight, preferably 1 mg/kg to 30 mg/kg body weight.

21. The bispecific antibody for use according to any of the preceding claims, wherein the bispecific antibody and/or the chemotherapy is administered intravenously,

preferably wherein the bispecific antibody and the chemotherapy are administered intravenously.

22. The bispecific antibody for use according to any of the preceding claims, wherein the bispecific antibody and/or the chemotherapy is administered via an IV injection or IV infusion.

23. The bispecific antibody for use according to any of the preceding claims, wherein a treatment cycle is repeated at least 1, 2, 3, 4, 5, 6, 7, or 8 times.

24. The bispecific antibody for use according to any of the preceding claims, wherein each cycle has up to 28 days, preferably 28 or 21 days.

25. The bispecific antibody for use according to any of the preceding claims, wherein the subject has not been previously treated for cancer.

26. The bispecific antibody for use according to any of claims 1-24, wherein the subject has been previously treated for cancer, preferably wherein the subject had at least one previous chemotherapy treatment.

27. The bispecific antibody for use according to any of the preceding claims, wherein the bispecific antibody is administered every 6 weeks, preferably every 4 weeks, more preferably every 3 weeks or every 2 weeks.

28. The bispecific antibody for use according to any of the preceding claims, wherein the bispecific antibody is administered every 2 weeks at a dosage ranging from 10 mg/kg to 30 mg/kg, preferably ranging from 15 mg/kg to 25 mg/kg, more preferably at a dosage of 20 mg/kg.

29. The bispecific antibody for use according to any of the preceding claims, wherein the bispecific antibody is administered every 3 weeks at a dosage ranging from 20 mg/kg to 40 mg/kg, preferably ranging from 25 mg/kg to 35 mg/kg, more preferably at a dosage of 30 mg/kg.

30. The bispecific antibody for use according to any of the preceding claims, wherein the chemotherapy is administered once or more within the first 20 days or the first 21 days of each cycle, wherein the chemotherapy is administered twice or more within the first 20 days or the first 21 days of each cycle, more preferably wherein

the chemotherapy is administered at least thrice within the first 20 days or the first 21 days of each cycle.

31. The bispecific antibody for use according to any of the preceding claims, wherein the chemotherapy is administered once or more within the first 14 days or the first 15 days of each cycle, wherein the chemotherapy is administered twice or more within the first 14 days or the first 15 days of each cycle, more preferably wherein the chemotherapy is administered at least thrice within the first 14 days or the first 15 days of each cycle.

32. The bispecific antibody for use according to any of the preceding claims, wherein the chemotherapy is administered on the 1st, 8th, and 15th day of each cycle.

33. The bispecific antibody for use according to any of the preceding claims, wherein overall survival is increased in said subject compared to the chemotherapy or the bispecific antibody or an anti-PD-L1 antibody or an anti-PD-1 antibody treatment alone or compared to a standard treatment comprising the chemotherapy and the anti-PD-L1 antibody or the chemotherapy and the anti-PD-1 antibody, wherein the anti-PD-L1 antibody is preferably atezolizumab and the anti-PD-1 antibody is preferably pembrolizumab.

34. The bispecific antibody for use according to any of the preceding claims, wherein median progression-free survival is increased in said subject compared to the chemotherapy or the bispecific antibody or an anti-PD-L1 antibody or an anti-PD-1 antibody treatment alone or compared to a standard treatment comprising the chemotherapy and the anti-PD-L1 antibody or the chemotherapy and the anti-PD-1 antibody, wherein the anti-PD-L1 antibody is preferably atezolizumab and the anti-PD-1 antibody is preferably pembrolizumab.

35. The bispecific antibody for use according to any of the preceding claims, wherein the cancer comprises one or more solid tumours.

36. The bispecific antibody for use according to any of the preceding claims, wherein the cancer is selected from the group consisting of melanoma, lung, liver, stomach, renal cell, urothelial, cervical, colorectal, ovarian, colon, breast, esophagus,

mesothelioma, and head and neck cancers, preferably wherein the cancer is selected from urothelial, breast and esophagus cancer.

37. The bispecific antibody for use according to any of the preceding claims, wherein the cancer is small cell lung cancer (SCLC), non-small cell lung cancer (NSCLC) or triple-negative breast cancer (TNBC), preferably advanced triple-negative breast cancer.

38. The bispecific antibody for use according to claim 37, wherein the NSCLC has a squamous histology.

39. The bispecific antibody for use according to claim 37, wherein the NSCLC has a non-squamous histology.

40. The bispecific antibody for use according to claim 37, wherein the NSCLC is an EGFR mutation-positive NSCLC.

41. The bispecific antibody for use according to any of the preceding claims, wherein the bispecific antibody comprises an anti-PD-L1 antibody or fragment thereof.

42. The bispecific antibody for use according to any of the preceding claims, wherein the bispecific antibody comprises an anti-VEGF antibody or fragment thereof.

43. The bispecific antibody for use according to any of the preceding claims, wherein the bispecific antibody comprises a Fab, Fab', F(ab')2, Fd, Fv, dAb, complementarity determining region fragment, single chain antibody, humanized antibody, chimeric antibody or diabody antibody, preferably a single domain antibody, more preferably a VHH.

44. The bispecific antibody for use according to any of the preceding claims, wherein the bispecific antibody comprises two anti-PD-L1 single domain antibodies, preferably two VHHs, preferably wherein each VHH is fused to the C-terminus of an anti-VEGF antibody.

45. The bispecific antibody for use according to any of the preceding claims, wherein the bispecific antibody comprises an anti-PD-L1 single domain antibody comprising a heavy chain variable region, and the heavy chain variable region comprises: (i) a complementarity-determining region 1 (HCDR1) whose amino acid sequence is

shown in SEQ ID NO: 1 or 18, (ii) a complementarity-determining region 2 (HCDR2) whose amino acid sequence is shown in SEQ ID NO: 2 or 19, and (iii) a complementarity-determining region 3 (HCDR3) whose amino acid sequence is shown in SEQ ID NO: 3 or 38.

46. The bispecific antibody for use according to claim 45, wherein the amino acid sequence of the anti-PD-L1 single domain antibody is shown in SEQ ID NO: 9 or wherein the amino acid sequence of the anti-PD-L1 single domain antibody has at least 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 9.

47. The bispecific antibody for use according to any of claims 42-46, wherein the anti-VEGF antibody or fragment thereof comprises a constant region preferably derived from a human antibody, preferably the constant region is selected from the constant region of human IgGl, IgG2, IgG3 or IgG4.

48. The bispecific antibody for use according to any of claims 42-47, wherein the anti-VEGF antibody or fragment thereof comprises a IgG1 Fc region, preferably having the amino acid sequence shown in SEQ ID NO: 13 or having at least 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence shown in SEQ ID NO: 13.

49. The bispecific antibody for use according to any of the preceding claims, wherein the bispecific antibody specifically binds to VEGF-A.

50. The bispecific antibody for use according to any of claims 42-49, wherein a heavy chain variable region of the anti-VEGF antibody comprises: (i) a complementarity-determining region 1 (HCDR1) whose amino acid sequence is shown in SEQ ID NO: 4, (ii) a complementarity-determining region 2 (HCDR2) whose amino acid sequence is shown in SEQ ID NO: 5, and (iii) a complementarity-determining region 3 (HCDR3) whose amino acid sequence is shown in SEQ ID NO: 6; and a light chain variable region of the anti-VEGF antibody comprises: (i) a complementarity-determining region 1 (LCDR1) whose amino acid sequence is shown in SEQ ID NO: 7, (ii) a complementarity-determining region 2 (LCDR2) whose amino acid sequence is shown in SEQ ID NO: 44, and (iii) a complementarity-determining region 3 (LCDR3) whose amino acid sequence is shown in SEQ ID NO: 8.

51. The bispecific antibody for use according to claim 50, wherein the amino acid sequence of the heavy chain variable region of the anti-VEGF antibody is shown in SEQ ID NO: 10, and the amino acid sequence of the light chain variable region of the anti-VEGF antibody is shown in SEQ ID NO: 11; or
wherein the amino acid sequence of the heavy chain variable region of the anti-VEGF antibody has at least 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 10, and the amino acid sequence of the light chain variable region of the anti-VEGF antibody has at least 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 11.

52. The bispecific antibody for use according to any of the preceding claims, wherein the amino acid sequence of the heavy chain of the bispecific antibody is shown in SEQ ID NO: 16, and the amino acid sequence of the light chain variable region of the bispecific antibody is shown in SEQ ID NO: 17; or
wherein the amino acid sequence of the heavy chain of the bispecific antibody has at least 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 16, and the amino acid sequence of the light chain variable region of the bispecific antibody has at least 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 17.

53. The bispecific antibody for use according to any of claims 42-52, wherein the anti-VEGF antibody is bevacizumab.

54. The bispecific antibody for use according to any of the preceding claims, wherein the bispecific antibody is encoded by one or more nucleic acid molecules.

55. The bispecific antibody for use according to any of the preceding claims, wherein the chemotherapy comprises a chemotherapy agent selected from lurbinectedin, topotecan, taxane, such as paclitaxel, nanoparticle albumin-bound paclitaxel (nab-paclitaxel) or docetaxel, pemetrexed, 5-fluoruracil, irinotecan, etoposide, gemcitabine, anthracyclines, capecitabine, vinorelbine, eribulin, ixabepilone, or combinations thereof.

56. The bispecific antibody for use according to any of claims 18-55, wherein the platinum-based chemotherapy comprises cisplatin, oxaliplatin or carboplatin.

57. The bispecific antibody for use according to any of the preceding claims, wherein the method of treatment comprising administering the bispecific antibody in combination with paclitaxel to a subject having small cell lung cancer.

58. The bispecific antibody for use according to any of claims 1-55, wherein the method of treatment comprising administering the bispecific antibody in combination with pemetrexed and carboplatin or a taxane such as paclitaxel or docetaxel to a subject having NSCLC, preferably as second line treatment, or wherein the method of treatment comprises administering the bispecific antibody in combination with pemetrexed and carboplatin to a subject having malignant mesothelioma.

59. The bispecific antibody for use according to any of claims 1-55, wherein the method of treatment comprising administering the bispecific antibody in combination with nab-paclitaxel to a subject having triple-negative breast cancer.

60. The bispecific antibody for use according to any of claims 1-55, wherein the method of treatment comprising administering the bispecific antibody in combination with oxaliplatin, calcium folinate, and 5-fluorouracil to a subject having hepatocellular carcinoma.

61. The bispecific antibody for use according to any of claims 1-55, wherein the method of treatment comprising administering the bispecific antibody in combination with irinotecan, 5-fluorouracil, calcium folinate to a subject having unresectable neuroendocrine neoplasm.

62. The bispecific antibody for use according to any of claims 1-55, wherein the method of treatment comprising administering the bispecific antibody in combination with etoposide and platinum to a subject having small cell lung cancer, preferably as first line treatment and/or extensive-stage small cell lung cancer or limited-stage small cell lung cancer.

63. The bispecific antibody for use according to claim 62, wherein the method of treatment comprises administering the bispecific antibody every 3 weeks at a dosage ranging from 20 mg/kg to 30 mg/kg or a dosage ranging from 1400 mg to 2000 mg in combination with etoposide with carboplatin to a subject having small

cell lung cancer, preferably as first line treatment and/or extensive-stage small cell lung cancer.

64. The bispecific antibody for use according to any of claims 1-55, wherein the method of treatment comprises administering the bispecific antibody every 3 weeks at a dosage ranging from 20 mg/kg to 30 mg/kg in combination with paclitaxel, lurbinectedin, or topotecan to a subject having small cell lung cancer, preferably as second line treatment and/or extensive-stage small cell lung cancer.

65. The bispecific antibody for use according to any of claims 1-55, wherein the method of treatment comprises administering the bispecific antibody every 2 weeks at a dosage ranging from 10 mg/kg to 20 mg/kg in combination with nab-paclitaxel, paclitaxel, or gemcitabine with carboplatin to a subject having triple-negative breast cancer.

66. The bispecific antibody for use according to any of claims 1-55, wherein the method of treatment comprises administered the bispecific antibody every 2 weeks at a dosage of 1000 mg to 2000 mg to a subject having triple-negative breast cancer, preferably on the 1st and 15th day of a 28-day treatment cycle, preferably wherein the bispecific antibody is administered in combination with a chemotherapy, preferably on the 1st, 8th and 15th day of a 28-day treatment cycle.

67. A method of treating cancer, preferably triple-negative breast cancer (TNBC), in a subject, the method comprising administering to the subject a bispecific antibody that specifically bind to PD-L1 and VEGF in combination with chemotherapy, optionally wherein the subject has a PD-L1 expression before the treatment that provides a score of ≥ 1, preferably from 1 to 20, preferably from 1 to <10, when determined by the combined positive score (CPS) or a score that is indicative of such a PD-L1 expression when determined by another integrating scoring algorithm, preferably when determined by the tumour area positivity (TAP) score or the tumour proportion score (TPS), more preferably when determined by the TAP score.

68. The method according to claim 67, wherein the method is a method for extending progression-free survival in said subject compared to the chemotherapy or an anti-PD-L1 antibody or the bispecific antibody or the anti-PD-1 antibody treatment

alone or compared to a standard treatment comprising the chemotherapy and the anti-PD-L1 antibody or the chemotherapy and the anti-PD-1 antibody, wherein the anti-PD-L1 antibody is preferably atezolizumab and the anti-PD-1 antibody is preferably pembrolizumab.

69. The method according to claim 67 or 68, wherein the method is a method for increased overall survival in said subject compared to the chemotherapy or the bispecific antibody or an anti-PD-L1 antibody or an anti-PD-1 antibody treatment alone or compared to a standard treatment comprising the chemotherapy and the anti-PD-L1 antibody or the chemotherapy and the anti-PD-1 antibody, wherein the anti-PD-L1 antibody is preferably atezolizumab and the anti-PD-1 antibody is preferably pembrolizumab.

70. A method for determining whether a cancer in a subject is susceptible to treatment with a bispecific antibody that specifically binds to PD-L1 and VEGF and a chemotherapy, wherein the method comprises detecting in a sample of the subject a PD-L1 expression before the treatment by determining the combined positive scope (CPS) or another score, preferably the tumour area positivity (TAP) score or the tumour proportion score (TPS), wherein a CPS of ≥ 1 indicates a PD-L1 expression that renders the subject susceptible to treatment with the bispecific antibody and the chemotherapy, or a score that is indicative of such a PD-L1 expression when determined by another integrating scoring algorithm, preferably when determined by the TAP score or the TPS, more preferably when determined by the TAP score.

71. The method according to claim 70, wherein the cancer is selected from the group consisting of melanoma, lung, liver, stomach, renal cell, urothelial, cervical, colorectal, ovarian, colon, breast, esophagus, mesothelioma, and head and neck cancers, preferably wherein the cancer is selected from urothelial, breast and esophagus cancer.

72. The method according to claim 70 or 71, wherein the sample is a cancer tissue sample.

73. The method according to any of claims 70-71, wherein the bispecific antibody comprises an anti-PD-L1 antibody or fragment thereof.

74. The method according to any of claims 70-73, wherein the bispecific antibody comprises an anti-VEGF antibody or fragment thereof.

75. The method according to any of claims 70-74, wherein the bispecific antibody comprises a Fab, Fab', F(ab')2, Fd, Fv, sdAb, complementarity determining region fragment, single chain antibody, humanized antibody, chimeric antibody or diabody antibody, preferably a single domain antibody, more preferably a VHH.

76. The method according to any of claims 70-75, wherein the bispecific antibody comprises two anti-PD-L1 single domain antibodies, preferably two VHHs, preferably each VHH is fused to the C-terminus of the anti-VEGF antibody.

77. The method according to any of claims 70-76, wherein the bispecific antibody comprises an anti-PD-L1 single domain antibody comprising a heavy chain variable region, and the heavy chain variable region comprises: (i) a complementarity-determining region 1 (HCDR1) whose amino acid sequence is shown in SEQ ID NO: 1 or 18, (ii) a complementarity-determining region 2 (HCDR2) whose amino acid sequence is shown in SEQ ID NO: 2 or 19, and (iii) a complementarity-determining region 3 (HCDR3) whose amino acid sequence is shown in SEQ ID NO: 3 or 38.

78. The method according to claim 77, wherein the amino acid sequence of the anti-PD-L1 single domain antibody is shown in SEQ ID NO: 9 or wherein the amino acid sequence of the anti-PD-L1 single domain antibody has at least 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 9.

79. The method according to any of claims 74-78, wherein the anti-VEGF antibody or fragment thereof comprises a constant region preferably derived from a human antibody, preferably, the constant region is selected from the constant region of human IgG1, IgG2, IgG3 or IgG4.

80. The method according to any of claims 74-79, wherein the anti-VEGF antibody or fragment thereof comprises a IgG1 Fc region, preferably having the amino acid sequence shown in SEQ ID NO: 13 or having at least 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence shown in SEQ ID NO: 13.

81. The method according to any of claims 70-80, wherein the bispecific antibody specifically binds to VEGF-A.

82. The method according to any of claims 74-81, wherein a heavy chain variable region of the anti-VEGF antibody comprises: (i) a complementarity-determining region 1 (HCDR1) whose amino acid sequence is shown in SEQ ID NO: 4, (ii) a complementarity-determining region 2 (HCDR2) whose amino acid sequence is shown in SEQ ID NO: 5, and (iii) a complementarity-determining region 3 (HCDR3) whose amino acid sequence is shown in SEQ ID NO: 6; and
a light chain variable region of the anti-VEGF antibody comprises: (i) a complementarity-determining region 1 (LCDR1) whose amino acid sequence is shown in SEQ ID NO: 7, (ii) a complementarity-determining region 2 (LCDR2) whose amino acid sequence is shown in SEQ ID NO: 44, and (iii) a complementarity-determining region 3 (LCDR3) whose amino acid sequence is shown in SEQ ID NO: 8.

83. The method according to any of claims 74-82, wherein the amino acid sequence of the heavy chain variable region of the anti-VEGF antibody is shown in SEQ ID NO: 10, and the amino acid sequence of the light chain variable region of the anti-VEGF antibody is shown in SEQ ID NO: 11; or
wherein the amino acid sequence of the heavy chain variable region of the anti-VEGF antibody has at least 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 10, and the amino acid sequence of the light chain variable region of the anti-VEGF antibody has at least 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 11.

84. The method according to any of claims 70-83, wherein the amino acid sequence of the heavy chain of the bispecific antibody is shown in SEQ ID NO: 16, and the amino acid sequence of the light chain variable region of the bispecific antibody is shown in SEQ ID NO: 17; or
wherein the amino acid sequence of the heavy chain of the bispecific antibody has at least 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 16, and the amino acid sequence of the light chain variable region of the bispecific antibody has at least 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 17.

85. The method according to any of claims 70-84, wherein the anti-VEGF antibody is bevacizumab.

86. The method according to any of claims 70-85, wherein the method comprises the step of determining the CPS in a test sample of the subject by determining the number of PD-L1 staining cells (tumour cells, lymphocytes, macrophages) and the total number of viable tumour cells in a cancer tissue sample from the subject; and calculating the CPS for the cancer tissue sample using the formula:

$$\text{CPS} = \frac{\#\ \text{PD-L1 staining cells (tumour cells, lymphocytes, macrophages)}}{\text{total}\ \#\ \text{viable tumour cells}} * 100$$

optionally wherein

(i) the test sample is a cancer tissue sample; and/or

(ii) the cancer tissue sample comprises at least 100 viable tumour cells as well as (tumour infiltrating) lymphocytes and macrophages; and/or

(iii) PD-L1 staining cells are determined by PD-L1 immunohistochemistry staining; and/or

(iv) viable tumour cells are determined by staining with a viability dye; and/or

(v) the number of PD-L1 staining cells is the sum of PD-L1 staining tumour cells, PD-L1 staining lymphocytes and PD-L1 staining macrophages.

87. The method according to any one of claims 70-85, wherein the method comprises the step of determining the TAP score in a test sample of the subject by determining the percentage of PD-L1 positive tumour cells and immune cells per tumour area in a cancer tissue sample from the subject; and calculating the TAP for the cancer tissue sample using the formula:

$$\text{TAP} = \frac{\%\ \text{PD-L1 positive tumour cells and immune cells}}{\text{tumour area}}$$

optionally wherein

(i) the test sample is a cancer sample; and/or

(ii) the tumour area is the area occupied by all viable tumour cells and the tumour-associated stroma containing tumour-associated immune cells; and/or

(iii) PD-L1 positive tumour cells and immune cells are determined by PD-L1 immunohistochemistry staining (preferably by using the anti-PD-L1 antibody SP263); and/or

(iv) PD-L1 positive tumour cells and immune cells means the area covered by such PD-L1 positive tumour cells and immune cells; and/or

(v) % PD-L1 positive tumour cells and immune cells / tumour area means the area of PD-L1 positive tumour cells and immune cells relative to the tumour area expressed as %.

88. The method according to any one of claims 70-85, wherein the method comprises the step of determining the TPS in a test sample of the subject by determining the number of viable tumour cells positive for PD-L1 and the total number of viable tumour cells in a cancer tissue sample from the subject; and calculating the TPS for the cancer tissue sample using the formula:

$$\text{TPS} = \frac{\#\ viable\ tumor\ cells\ positive\ for\ PD{-}L1}{\text{total \# viable tumour cells}} * 100$$

optionally wherein

(i) the test sample is a cancer tissue sample; and/or

(ii) the cancer tissue sample comprises at least 100 viable tumour cells; and/or

(iii) viable tumour cells are determined by staining with a viability dye; and/or

(iv) PD-L1 positive cells are determined by PD-L1 immunohistochemistry staining.

89. The method according to any of claims 86 to 88, wherein the cancer tissue sample is a tissue section of a tumour biopsy.

90. The method according to claim 89, wherein the tissue section is a formalin fixed and embedded in paraffin wax (FFPE) tissue section.

91. The method according to claim 89 or 90, wherein the tissue section is stained.

92. The method according to claim 91, wherein the stain comprises a hematoxylin and eosin (H&E) stain.

93. The method according to any of claims 86 to 92, wherein the viability dye is propidium iodide.

94. The method according to any of claims 86 to 93, wherein the number of viable tumour cells in the tumour tissue sample is determined by flow cytometry.

95. A kit of parts comprising a bispecific antibody that specifically binds to PD-L1 and VEGF and a chemotherapy agent.

96. The kit of parts according to claim 95, wherein the bispecific antibody and the chemotherapy agent are comprised in separate container.

97. The kit of parts according to claim 95 or 96, further comprising instructions for use.

98. A chemotherapy agent for use in a method of treating a subject with cancer, the method comprising administering to the subject:

a. a bispecific antibody that specifically binds to PD-L1 and VEGF; and

b. the chemotherapy agent;

optionally wherein the subject has a PD-L1 expression before the treatment that provides a score of ≥ 1 when determined by the combined positive score (CPS) or a score that is indicative of such a PD-L1 expression when determined by another integrating scoring algorithm, preferably when determined by the tumour area positivity (TAP) score or the tumour proportion score (TPS), more preferably when determined by the TAP score.