

antibody. In other words, the bivalent monospecific PD-L1 antibody is able to reduce T cell-mediated cell killing of cells that express PD-L1 but do not express EGFR or express only a low amount of EGFR. By combining the trispecific antibody with a bivalent monospecific antibody the trispecific antibody is more specifically targeted to the desired TA1, TA2 positive target cell.

In a second experiment, a cytotoxicity assay was used to determine the effect of a monospecific antibody on the capability of trispecific antibodies to induce T cell-mediated killing of HCT116 cells and BxPC3 cells. For the trispecific antibodies and control, an 8-fold 8-step dilution was used, starting at a concentration of 20.5 nM.

Human T cells were co-cultured with BxPC3 or HCT116 target cells in the presence of three different PD-L1 = CD3 x EGFR trispecific antibodies. A first trispecific antibody comprised a PD-L1 binding domain comprising a heavy chain variable region having SEQ ID NO: 38; a CD3 binding domain comprising a heavy chain variable region having SEQ ID NO: 8; and an EGFR binding domain comprising a heavy chain variable region having SEQ ID NO: 56. A second trispecific antibody comprised a PD-L1 binding domain comprising a heavy chain variable region having SEQ ID NO: 38; a CD3 binding domain comprising a heavy chain variable region having SEQ ID NO: 22; and an EGFR binding domain comprising a heavy chain variable region having SEQ ID NO: 56. A third trispecific antibody comprised a PD-L1 binding domain comprising a heavy chain variable region having SEQ ID NO: 42; a CD3 binding domain comprising a heavy chain variable region having SEQ ID NO: 22; and an EGFR binding domain comprising a heavy chain variable region having SEQ ID NO: 56. Apart from the PD-L1 = CD3 x TT negative control, also a TT = CD3 x EGFR control was included. The percentage cell killing is again set off against a negative control TT = CD3 x TT trispecific antibody, the TT binding arm comprising SEQ ID NO: 68, and the CD3 binding domain comprising SEQ ID NO: 8 or 22.

Figure 7 shows that the trispecific PD-L1 = CD3 x mock antibody induces T cell-mediated cell killing in the absence of a monospecific PD-L1 antibody. This T cell-mediated target cell killing is due to the antibody binding to PD-L1 only, which can be on normal, non-tumor, cells expressing PD-L1 but no or low amounts of EGFR as well as on tumor cells expressing both PD-L1 and EGFR. This T cell-mediated target cell killing is strongly reduced or diminished in the presence of a monospecific PD-L1 antibody. This is believed to be due to less PD-L1 being available to the trispecific PD-L1 = CD3 x mock antibody as it has to compete with the monospecific PD-L1 antibody.

The PD-L1 = CD3 x EGFR trispecific antibody also induces T cell-mediated cell killing in the absence of a monospecific PD-L1 antibody. This T cell-mediated cell killing is not reduced, or to a lesser extent, compared to the trispecific PDL1 = CD3 x mock antibody, in the presence of a monospecific PD-L1 antibody. Also here, less PD-L1 will be available for binding of the trispecific PD-L1 = CD3 x EGFR

Claims

1. A therapeutic composition comprising a multivalent antibody comprising a first variable domain that binds a first tumor antigen (TA1), a second variable domain that binds a second tumor antigen (TA2) and a third variable domain that binds an immune cell engaging antigen (IEA);
 - wherein the third variable domain that binds an IEA and the second variable domain that binds a TA2 are linked to an Fc region, and the first variable domain that binds a TA1 is linked to the third variable domain that binds an IEA, or
 - wherein the third variable domain that binds an IEA and the first variable domain that binds a TA1 are linked to an Fc region, and the second variable domain that binds a TA2 is linked to the third variable domain that binds an IEA; or
 - wherein the first variable domain that binds a TA1 and the second variable domain that binds a TA2 are linked to an Fc region, and the third variable domain that binds an IEA is linked to the first variable domain that binds a TA1 or linked to the second variable domain that binds a TA2; and
 - wherein the composition further comprises a second binding molecule that binds TA1 or TA2 and which competes with the multivalent antibody for binding to TA1 or TA2.
2. The therapeutic composition of claim 1, wherein the third variable domain that binds an immune cell engaging antigen (IEA) and the second variable domain that binds a second tumor antigen (TA2) are linked to an Fc region, and the first variable domain that binds a first tumor antigen (TA1) is linked to the third variable domain that binds an immune cell engaging antigen (IEA).
3. The therapeutic composition of claim 1, wherein the third variable domain that binds an immune cell engaging antigen (IEA) and the first variable domain that binds a first tumor antigen (TA1) are linked to an Fc region, and the second variable domain that binds a second tumor antigen (TA2) is linked to the third variable domain that binds an immune cell engaging antigen (IEA).
4. The therapeutic composition of any one of claims 1-3, wherein the first, second, and third variable domains comprise a common light chain variable region.
5. The therapeutic composition of any one of claims 1-4, wherein the variable domain that binds an immune cell engaging antigen binds to CD3, TCR- α chain, TCR- β chain, CD2, CD4, CD5, CD7, CD8, CD137, CD28, CD16, CD16A, CD64, OX40, CD27, CD40, ICOS, GITR, NKG2D, NKp46, NKp44, or NKp30; preferably to CD3, TCR- α chain, TCR- β chain, CD2, or CD5; more preferably to CD3.
6. The therapeutic composition of any one of claims 1-5, wherein the variable domain that binds a first tumor antigen (TA1) binds to PD-L1, PD-L2, HVEM, CD47, B7-H3, B7-H4, B7-H7, or Siglec-15; preferably PD-L1 or PD-L2; more preferably PD-L1.

7. The therapeutic composition of any one of claims 1-6, wherein the variable domain that binds a second tumor antigen (TA2) binds to CLEC12A or EGFR, preferably EGFR.
8. The therapeutic composition of any one of claims 1-7, wherein the first tumor antigen (TA1) is expressed on non-tumor cells.
9. The therapeutic composition of any one of claims 1-8, wherein the second binding molecule binds to TA1.
10. The therapeutic composition of any one of claims 1-9, wherein the second binding molecule is a bivalent monospecific antibody.
11. The therapeutic composition of any one of claims 1-10, wherein the second binding molecule has a binding affinity to TA1 or TA2 that is comparable to, equal to, or lower than the binding affinity to TA1 or TA2 of the first variable domain or second variable domain of the multivalent antibody.
12. The therapeutic composition of any one of claims 1-11, wherein the second binding molecule has reduced effector function.
13. A kit of parts comprising a multivalent antibody and a second binding molecule as defined in any one of claims 1-12.
14. A kit of parts comprising a therapeutic composition of any one of claims 1-12 and instructions for administering the composition to a subject in need thereof.
15. The kit of parts of claim 13, wherein the kit comprises instructions for administering the multivalent antibody and second binding molecule simultaneously or consecutively to a subject in need thereof.
16. The kit of parts of claim 13, wherein the kit comprises instructions for administering the second binding molecule prior to administering the multivalent antibody.
17. A pharmaceutical composition comprising a multivalent antibody and a second binding molecule as defined in any one of claims 1-12, and a pharmaceutically acceptable carrier, diluent, or excipient.
18. A composition comprising a multivalent antibody and a second binding molecule as defined in any one of claims 1-12, a therapeutic composition of any one of claims 1-12, a kit of parts of claim 13, or a pharmaceutical composition of claim 17, for use as a medicament.

19. A combination of a multivalent antibody and a second binding molecule as defined in any one of claims 1-12 or a kit of parts of any one of claims 13, 15 or 16 for use in a combination therapy.
20. Use of a composition comprising a multivalent antibody and a second binding molecule as defined in any one of claims 1-12, a therapeutic composition of any one of claims 1-12, a kit of parts of claim 13, or a pharmaceutical composition of claim 17, in the manufacture of a medicament for the treatment of cancer that expresses TA1 and TA2.
21. Use of a multivalent antibody as defined in any one of claims 1-12 in the manufacture of a first medicament and use of a second binding molecule as defined in any one of claims 1-12 in the manufacture of a second medicament, wherein the first and second medicament are to be administered in a combination therapy to treat a cancer that expresses TA1 and TA2.
22. The combination or kit of parts for use of claim 19 or the use of claim 21, wherein the multivalent antibody and second binding molecule are formulated for simultaneous administration as a single composition or formulated for consecutive administration as two separate compositions.
23. The combination or kit of parts for use of claim 19 or the use of claim 21, wherein the multivalent antibody is formulated for administration prior to the second binding molecule or wherein the second binding molecule is formulated for administration prior to the multivalent antibody.
24. A vector comprising a nucleic acid encoding the heavy chain variable region of the first, second, and third variable domain of a multivalent antibody as defined in any one of claims 1-12, wherein the vector further comprises a different nucleic acid encoding the heavy chain variable region of a second binding molecule as defined in any one of claims 1-12.
25. A host cell comprising the vector of claim 24.
26. A host cell comprising a nucleic acid encoding the heavy chain variable region of the first, second, and third variable domain of a multivalent antibody as defined in any one of claims 1-12, wherein the host cell further comprises a different nucleic acid encoding the heavy chain variable region of a second binding molecule as defined in any one of claims 1-12.
27. The host cell of claim 26, wherein the host cell further comprises a nucleic acid encoding the light chain variable region of the first, second, and third variable domain of the multivalent antibody, and the light chain variable region of the second binding molecule, as defined in any one of claims 1-12.

28. Use of a multivalent antibody as defined in any one of claims 1-12 in the manufacture of a medicament for the treatment of a cancer, wherein the medicament is to be administered with a second binding molecule as defined in any one of claims 1-12.
29. Use of a second binding molecule as defined in any one of claims 1-12 in the manufacture of a medicament for the treatment of a cancer, wherein the medicament is to be administered with a multivalent antibody as defined in any one of claims 1-12.
30. The use of claim 28 or 29, wherein the multivalent antibody and the second binding molecule are administered simultaneously or consecutively to a subject in need thereof.