

CLAIMS

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1. A multispecific antigen-binding fragment comprising at least two Fab fragments with different CH1 and CL domains, wherein each Fab fragment recognizes a different epitope of interest, and said Fab fragments are tandemly arranged in any order, the C-terminal end of the CH1 domain of a first Fab fragment being linked to the N-terminal end of the VH domain of the following Fab fragment through a polypeptide linker, characterized in that the polypeptide linker sequence comprises or consists of amino acid sequence EPKX₁CDKX₂HX₃X₄PPX₅PAPELLGGPX₆X₇PPX₈PX₉PX₁₀GG (SEQ ID NO: 1), wherein X₁, X₂, X₃, X₄, X₅, X₆, X₇, X₈, X₉, X₁₀, identical or different, are any amino acid; with the proviso that the linker sequence does not comprise nor consist of sequences

10 of the following Fab fragment through a polypeptide linker,

characterized in that the polypeptide linker sequence comprises or consists of amino acid sequence EPKX₁CDKX₂HX₃X₄PPX₅PAPELLGGPX₆X₇PPX₈PX₉PX₁₀GG (SEQ ID NO: 1), wherein X₁, X₂, X₃, X₄, X₅, X₆, X₇, X₈, X₉, X₁₀, identical or different, are any amino acid; with the proviso that the linker sequence does not comprise nor consist of sequences

15 EPKSCDKTHTCPPCPAPELLGGPSTPPTSPSGG (SEQ ID NO: 5) or EPKSCDKTHTSPSPAPPELLGGPSTPPTSPSGG (SEQ ID NO: 6).

2. A multispecific antibody having two identical antigen-binding arms, each consisting of a multispecific antigen-binding fragment of claim 1.

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3. The multispecific antibody of claim 2, which has an immunoglobulin-like structure, comprising:

- two identical antigen-binding arms each consisting of a multispecific antigen-binding fragment as defined in claim 1;
- 25 - the dimerized CH2 and CH3 domains of an immunoglobulin;
- the hinge region of an IgA, IgG, or IgD, linking the C-terminal ends of CH1 domains of the antigen-binding arms to the N-terminal ends of the CH2 domains.

4. A multispecific antibody, preferably a bispecific antibody, comprising two heavy chains and four light chains,

30 wherein each heavy chain comprises

- a. a Fc region of an immunoglobulin comprising Hinge-CH2-CH3 domains,
- b. which Fc region is linked to Fab heavy chain CH1-VH of antibody 1 (Ab1) by said Hinge domain,
- 35 c. which in turn is linked to Fab heavy chain CH1-VH of antibody 2 (Ab2), by a polypeptide linker sequence, wherein the polypeptide linker sequence links the N-terminus of said Fab heavy chain VH domain of Ab1 with the C-terminus of said CH1 domain of Ab2,

and the four light chains comprise light chains of Ab1 and light chains of Ab2 associated with their cognate heavy chain domains;

wherein the polypeptide linker sequence comprises or consists of amino acid sequence EPKX₁CDKX₂HX₃X₄PPX₅PAPELLGGPX₆X₇PPX₈PX₉PX₁₀GG (SEQ ID NO: 1),

- 5 wherein X₁, X₂, X₃, X₄, X₅, X₆, X₇, X₈, X₉, X₁₀, identical or different, are any amino acid; with the proviso that the linker sequence does not comprise nor consist of sequences EPKSCDKTHTCPPCPAPELLGGPSTPPTSPSPSGG (SEQ ID NO: 5) or EPKSCDKTHTSPSPAPPELLGGPSTPPTSPSPSGG (SEQ ID NO: 6).

- 10 5. The multispecific antigen-binding fragment, or multispecific antibody of any of claims 1 to 4, wherein X₁, X₂ and X₃, identical or different, are Threonine (T) or Serine (S).

6. The multispecific antigen-binding fragment, or multispecific antibody of any of claims 1 to 4, wherein X₁, X₂ and X₃, identical or different, are any amino acid other than Threonine
15 (T) or Serine (S), preferably wherein X₁, X₂ and X₃, identical or different, are selected from the group consisting of Ala (A), Gly (G), Val (V), Asn (N), Asp (D) and Ile (I).

7. The multispecific antigen-binding fragment, or multispecific antibody of any of claims 1 to 6, wherein X₄ and X₅, identical or different, are any amino acid selected from the group
20 consisting of Serine (S), Cysteine (C), Alanine (A), and Glycine (G).

8. The multispecific antigen-binding fragment, or multispecific antibody of any of claims 1 to 7, wherein X₆, X₇, X₈, X₉, X₁₀, identical or different, are any amino acid other than Threonine (T) or Serine (S).

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9. The multispecific antigen-binding fragment, or multispecific antibody of any of claims 1 to 8, wherein X₄ is Serine (S) or Cysteine (C).

10. The multispecific antigen-binding fragment, or multispecific antibody of any of claims
30 1 to 9, wherein X₅ is Alanine (A) or Cysteine (C).

11. The multispecific antigen-binding fragment, or multispecific antibody of any of claims 1 to 9, wherein X₆, X₇, X₈, X₉, X₁₀, identical or different, are selected from the group consisting of Ala (A), Gly (G), Val (V), Asn (N), Asp (D) and Ile (I).

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12. The multispecific antigen-binding fragment, or multispecific antibody of claim 11, wherein X₆, X₇, X₈, X₉, X₁₀, identical or different, are selected from the group consisting of Ala (A) and Gly (G).

13. The multispecific antigen-binding fragment, or multispecific antibody of claim 12, wherein X_6 and X_7 are identical and are preferably selected from the group consisting of Ala (A) and Gly (G).
- 5 14. The multispecific antigen-binding fragment, or multispecific antibody of claim 12, wherein the polypeptide linker sequence comprises or consists of a sequence selected from the group consisting of
EPKSCDKTHTSPAPAPELLGGPAAPPAPAPAGG (SEQ ID NO: 3);
10 EPKSCDKTHTSPAPAPELLGGPGGPPGPGPGGG (SEQ ID NO: 2);
and
EPKSCDKTHTSPAPAPELLGGPAAPPGPAPGGG (SEQ ID NO: 4).
- 15 15. The multispecific antigen-binding fragment, or multispecific antibody of any of claims 1 to 14, which recognizes both EGFR and HER2/neu.
16. The multispecific antibody of claim 4, wherein Ab1 and Ab2, being different, independently are selected from the group consisting of cetuximab or a mutated derivative thereof, on the one hand, and trastuzumab, or a mutated derivative thereof, on the other
20 hand.
17. The multispecific antigen-binding fragment, or multispecific antibody of any of claims 1 to 14, which recognizes both CD38 and PD-L1.
- 25 18. A polypeptide which comprises, preferably consists of, a heavy chain of the antigen-binding fragment or a heavy chain of the multispecific antibody as defined in any of claims 1 to 17.
19. A polynucleotide comprising a sequence encoding the polypeptide of claim 18.
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20. A host cell transfected with an expression vector comprising the polynucleotide of claim 19.
21. The host cell of claim 20, further transformed with at least two polynucleotides
35 encoding two different light chains: a first light chain pairing specifically with a first VH/CH1 region of said heavy chain; a second light chain pairing specifically with a second VH/CH1 region of said heavy chain.

22. The host cell of claim 21, which is additionally transformed with a third polynucleotide encoding a third light chain which is different from the first and second light chains, and which pairs specifically with a third VH/CH1 region of said heavy chain.
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23. A method for preparing an antigen-binding fragment or a multispecific antibody as defined in any of claims 1 to 17, wherein said method comprising culturing a host cell of any of claims 20 to 22 and recovering said antigen-binding fragment or antibody from said culture.
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24. The multispecific antigen-binding fragment or a multispecific antibody as defined in any of claims 1 to 17, for use as a medicament.
25. The multispecific antigen-binding fragment or a multispecific antibody as defined in any of claims 15 to 17, for use in treating a cancer.
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26. A polypeptide which comprises or consists of amino acid sequence EPKX₁CDKX₂HX₃X₄PPX₅PAPELLGGPX₆X₇PPX₈PX₉PX₁₀GG (SEQ ID NO: 1), wherein X₁, X₂, X₃, X₄, X₅, X₆, X₇, X₈, X₉, X₁₀, identical or different, are any amino acid as defined in any of claims 1 to 10; with the proviso that the polypeptide does not comprise nor consist of sequences
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- EPKSCDKTHTCPPCPAPELLGGPSTPPTPSPSGG (SEQ ID NO: 5) or
EPKSCDKTHTSPPSPAPELLGGPSTPPTPSPSGG (SEQ ID NO: 6).
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27. The polypeptide of claim 26, which sequence comprises or consists of a sequence selected from the group consisting of
- EPKSCDKTHTSPPAPPELLGGPAAPPAPAPAGG (SEQ ID NO: 3);
EPKSCDKTHTSPPAPPELLGGPGGPPGPGPGGG (SEQ ID NO: 2);
and
- 30
- EPKSCDKTHTSPPAPPELLGGPAAPPGPAPGGG (SEQ ID NO: 4).