

CLAIMS

1. A multispecific antibody comprising:

- a) one or two binding domains, which specifically bind to the extracellular domain of ROR1 (ROR1-BDs); and
- b) one binding domain, which specifically binds to CD3 (CD3-BD);

wherein

- the multispecific antibody does not comprises an immunoglobulin Fc region;
- said ROR1-BDs comprise independently from each other a set of CDR sequences

selected from the set consisting of

the HCDR1 sequence of SEQ ID NO: 1,

the HCDR2 sequence of SEQ ID NO: 2,

the HCDR3 sequence of SEQ ID NO: 3,

the LCDR1 sequence of SEQ ID NO: 4,

the LCDR2 sequence of SEQ ID NO: 5, and

the LCDR3 sequence of SEQ ID NO: 6;

and/or the set consisting of

the HCDR1 sequence of SEQ ID NO: 13 or 14,

the HCDR2 sequence of SEQ ID NO: 15,

the HCDR3 sequence of SEQ ID NO: 16,

the LCDR1 sequence of SEQ ID NO: 17,

the LCDR2 sequence of SEQ ID NO: 18, and

the LCDR3 sequence of SEQ ID NO: 19;

- said CD3-BD comprises the set of CDR sequences consisting of

the HCDR1 sequence of SEQ ID NO: 30,

the HCDR2 sequence of SEQ ID NO: 31,

the HCDR3 sequence of SEQ ID NO: 32,

the LCDR1 sequence of SEQ ID NO: 33,

the LCDR2 sequence of SEQ ID NO: 34, and

the LCDR3 sequence of SEQ ID NO: 35.

2. The multispecific antibody of claim 1, where in case said multispecific antibody comprises two ROR1-BDs, said two ROR1-BDs comprise the same sets of CDRs, *i. e.* both ROR1-BDs either comprise CDRs of SEQ ID NOs: 1 to 6 or CDRs of SEQ ID NOs: 13/14 to 19.

3. The multispecific antibody of claim 1 or 2, wherein said antibody further comprises one binding domain, which specifically binds to human serum albumin (hSA-BD).

4. The multispecific antibody of any one of the preceding claims, wherein the multispecific antibody has one or more of the following features 1) to 5):

1) said multispecific antibody does not comprise CH1 and/or CL regions;

2) said one or two ROR1-BDs, said CDR-BD and said hSA-BD, if present, comprise VH1a, VH1b, VH3 or VH4 domain framework sequences FR1 to FR4; particularly VH3 or VH4 domain framework sequences FR1 to FR4; particularly VH3 domain framework sequences FR1 to FR4; or

said one or two ROR1-BDs, said CDR-BD and said hSA-BD, if present, comprise a VH domain comprising VH framework regions FR1, FR2, FR3 and FR4, which are selected from a VH framework subtype, particularly from the VH framework subtypes VH1a, VH1b, VH3 and VH4, particularly from the VH framework subtypes VH3 and VH4, particularly are of the VH3 subtype; wherein said VH framework regions FR1, FR2, FR3 and FR4 have the following substitutions (AHO numbering): an arginine (R) at amino acid position 12; a threonine (T) at amino acid position 103 and a glutamine (Q) at amino acid position 144;

3) said one or two ROR1-BDs, said CDR-BD and said hSA-BD, if present, comprise a VL domain comprising VL framework regions FR1, FR2 and FR3, which are selected from Vk subtypes, particularly from the Vk1 and Vk3 subtypes, particularly are of the Vk1 subtype, and a VL framework FR4, which is a Vλ FR4, particularly is a Vλ FR4 comprising an amino acid sequence having at least 70, 80, 90 percent identity to any of SEQ ID NO: 76 to SEQ ID NO: 83, more particularly a Vλ FR4 selected from any of SEQ ID NO: 76 to SEQ ID NO: 83, particularly a Vλ FR4 according to SEQ ID NO: 76 or 83;

4) said multispecific antibody is humanized, in particular said multispecific antibody is humanized and comprises rabbit derived CDRs;

5) said multispecific antibody is trispecific, in particular trispecific and trivalent or trispecific and tetravalent.

5. The multispecific antibody of any one of the preceding claims, wherein the format of said multispecific antibody is selected from an scDb-scFv, an scMATCH3, a MATCH3 and a MATCH4.

6. The multispecific antibody of any one of the preceding claims, wherein said one or two ROR1-BDs comprise

a) a VH sequence being at least 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99 percent identical to any one of the amino acid sequences selected from SEQ ID NOs: 7 and 10; and

b) a VL sequence being at least 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99 percent identical to any one of the amino acid sequences selected from SEQ ID NOs: 9 and 12;

or

a) a VH sequence being at least 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99 percent identical to any one of the amino acid sequences selected from SEQ ID NOs: 20, 23, 26 and 28; and

b) a VL sequence being at least 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99 percent identical to any one of the amino acid sequences selected from SEQ ID NOs: 22, 25, 27 and 29;

or

a) a VH sequence being at least 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99 percent identical to any one of the amino acid sequences selected from SEQ ID NOs: 8 and 11; and

b) a VL sequence being at least 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99 percent identical to any one of the amino acid sequences selected from SEQ ID NOs: 9 and 12;

or

a) a VH sequence being at least 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99 percent identical to any one of the amino acid sequences selected from SEQ ID NOs: 21 and 24; and

b) a VL sequence being at least 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99 percent identical to any one of the amino acid sequences selected from SEQ ID NOs: 22 and 25.

7. The multispecific antibody of any one of the preceding claims, wherein said one or two ROR1-BDs comprise

a) a VH sequence of SEQ ID NO: 7 and a VL sequence of SEQ ID NO: 9; or

b) a VH sequence of SEQ ID NO: 10 and a VL sequence of SEQ ID NO: 12; or

c) a VH sequence of SEQ ID NO: 20 and a VL sequence of SEQ ID NO: 22; or

- d) a VH sequence of SEQ ID NO: 23 and a VL sequence of SEQ ID NO: 25; or
 - e) a VH sequence of SEQ ID NO: 8 and a VL sequence of SEQ ID NO: 9; or
 - f) a VH sequence of SEQ ID NO: 11 and a VL sequence of SEQ ID NO: 12; or
 - g) a VH sequence of SEQ ID NO: 21 and a VL sequence of SEQ ID NO: 22; or
 - 5 h) a VH sequence of SEQ ID NO: 24 and a VL sequence of SEQ ID NO: 25; or
 - i) a VH sequence of SEQ ID NO: 26 and a VL sequence of SEQ ID NO: 27; or
 - j) a VH sequence of SEQ ID NO: 28 and a VL sequence of SEQ ID NO: 29;
- particularly wherein said one or two ROR1-BDs comprise
- a) a VH sequence of SEQ ID NO: 7 and a VL sequence of SEQ ID NO: 9; or
 - 10 b) a VH sequence of SEQ ID NO: 10 and a VL sequence of SEQ ID NO: 12; or
 - c) a VH sequence of SEQ ID NO: 23 and a VL sequence of SEQ ID NO: 25; or
 - d) a VH sequence of SEQ ID NO: 8 and a VL sequence of SEQ ID NO: 9; or
 - e) a VH sequence of SEQ ID NO: 11 and a VL sequence of SEQ ID NO: 12; or
 - f) a VH sequence of SEQ ID NO: 24 and a VL sequence of SEQ ID NO: 25.
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8. The multispecific antibody of any one of the preceding claims, wherein said CD3-BD comprises
- a) a VH sequence being at least 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99 percent identical to any one of the amino acid sequences selected from SEQ ID NO: 36 or 37; and
 - 20 b) a VL sequence being at least 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99 percent identical to any one of the amino acid sequences selected from SEQ ID NO: 38;
- in particular
- a) a VH domain comprising the amino acid sequence of SEQ ID NO: 36 or 37, and
 - b) a VL domain comprising the amino acid sequence of SEQ ID NO: 38.
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9. The multispecific antibody of claim 4, wherein said hSA-BD comprises
- (i) a VH domain comprising the amino acid sequence of SEQ ID NO: 47 and a VL domain comprising the amino acid sequence of SEQ ID NO: 48; or
 - (ii) a VH domain comprising the amino acid sequence of SEQ ID NO: 45 and a VL domain comprising the amino acid sequence of SEQ ID NO: 46; or
 - 30 (iii) a VH domain comprising the amino acid sequence of SEQ ID NO: 55 and a VL domain comprising the amino acid sequence of SEQ ID NO: 56; or
 - (iv) a VH domain comprising the amino acid sequence of SEQ ID NO: 57 and a VL domain comprising the amino acid sequence of SEQ ID NO: 58; or

- (v) a VH domain comprising the amino acid sequence of SEQ ID NO: 65 and a VL domain comprising the amino acid sequence of SEQ ID NO: 67; or
- (vi) a VH domain comprising the amino acid sequence of SEQ ID NO: 66 and a VL domain comprising the amino acid sequence of SEQ ID NO: 67; or
- (vii) a VH domain comprising the amino acid sequence of SEQ ID NO: 68 and a VL domain comprising the amino acid sequence of SEQ ID NO: 70;
- (viii) a VH domain comprising the amino acid sequence of SEQ ID NO: 69 and a VL domain comprising the amino acid sequence of SEQ ID NO: 70; or

in particular

- (v) a VH domain comprising the amino acid sequence of SEQ ID NO: 65 and a VL domain comprising the amino acid sequence of SEQ ID NO: 67; or
- (vi) a VH domain comprising the amino acid sequence of SEQ ID NO: 66 and a VL domain comprising the amino acid sequence of SEQ ID NO: 67; or
- (vii) a VH domain comprising the amino acid sequence of SEQ ID NO: 68 and a VL domain comprising the amino acid sequence of SEQ ID NO: 70
- (viii) a VH domain comprising the amino acid sequence of SEQ ID NO: 69 and a VL domain comprising the amino acid sequence of SEQ ID NO: 70.

10. A ROR1-BD as defined in any one of claims 1, 4, 6 and 7.

11. A nucleic acid or two nucleic acids encoding the multispecific antibody of any one of claims 1 to 9 or the ROR1-BD of claim 10.

12. A vector or two vectors comprising the nucleic acid or the two nucleic acids of claim 11.

13. A host cell or host cells comprising the vector or the two vectors of claim 12.

14. A pharmaceutical composition comprising the multispecific antibody of any one of claims 1 to 9 and a pharmaceutically acceptable carrier.

15. Use of The the multispecific antibody of any one of claims 1 to 9 or the pharmaceutical composition of claim 14 for use in the treatment of in the manufacture of a medicament for treating a ROR1-expressing cancer, in particular a ROR1-expressing cancer selected from chronic lymphocytic leukemia / small lymphocytic lymphoma (CLL/SLL), acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), mantle cell lymphoma

(MCL), hairy cell leukemia, follicular lymphoma (FL), marginal zone lymphoma (MZL), Non-Hodgkin lymphoma (NHL), diffuse large B cell lymphoma (DLBCL), Richter's syndrome (RS), lung cancer, pancreatic cancer, prostate cancer, colon cancer, bladder cancer, breast cancer, ovarian cancer, glioblastoma, testicular cancer, uterine cancer, adrenal cancer, melanoma, neuroblastoma, sarcoma and renal cancer.

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