

CLAIMS

1. A bi-specific antigen binding molecule comprising:

(i) a receptor tyrosine kinase-like orphan receptor 1 (ROR1) specific antigen binding molecule comprising an amino acid sequence represented by the formula (I):

5 FW1-CDR1-FW2-HV2-FW3a-HV4-FW3b-CDR3-FW4 (I)

in which

FW1 is a framework region

CDR1 is a CDR sequence

FW2 is a framework region

10 HV2 is a hypervariable sequence

FW3a is a framework region

HV4 is a hypervariable sequence

FW3b is a framework region

CDR3 is a CDR sequence

15 FW4 is a framework region;

and

(ii) an epidermal growth factor receptor (EGFR) specific antigen binding molecule;
or a functional variant thereof.

2. A bi-specific antigen binding molecule according to Claim 1, in which

20 (i) the ROR1-specific antigen binding molecule does not bind to receptor tyrosine kinase-like orphan receptor 2 (ROR2),

(ii) the ROR1-specific antigen binding molecule binds to both human ROR1 and murine ROR1 (mROR1),

25 (iii) the ROR1-specific antigen binding molecule binds to deglycosylated ROR1,
and/or

(iv) the ROR1-specific antigen binding molecule does not bind to a linear peptide sequence selected from:

YMESLHMQGEIENQI (SEQ ID NO: 34)

CQPWNSQYPHTHTFTALRFP (SEQ ID NO: 35)

5 RSTIYGSRLRIRNLDTTDTGYFQ (SEQ ID NO: 36)

QCVATNGKEVVSSSTGVLFVKFGPPPTASPGYSDEYE (SEQ ID NO: 37)

3. A bi-specific antigen binding molecule according to Claim 1 or 2 comprising a ROR1-specific antigen binding molecule in which

FW1 is a framework region of from 20 to 28 amino acids

10 CDR1 is a CDR sequence selected from DTSYGLYS (SEQ ID NO: 1), GAKYGLAA (SEQ ID NO: 2), GAKYGLFA (SEQ ID NO: 3), GANYGLAA (SEQ ID NO: 4), or GANYGLAS (SEQ ID NO: 5)

FW2 is a framework region of from 6 to 14 amino acids

15 HV2 is a hypervariable sequence selected from TTDWERMSIG (SEQ ID NO: 6), SSNQERISIS (SEQ ID NO: 7), or SSNKEQISIS (SEQ ID NO: 8)

FW3a is a framework region of from 6 to 10 amino acids

HV4 is a hypervariable sequence selected from NKRAK (SEQ ID NO: 9), NKRTM (SEQ ID NO: 10), NKGAK (SEQ ID NO: 11), or NKGTK (SEQ ID NO: 12)

FW3b is a framework region of from 17 to 24 amino acids

20 CDR3 is a CDR sequence selected from QSGMAISTGSGHGYNWY (SEQ ID NO: 13), QSGMAIDIGSGHGYNWY (SEQ ID NO: 14), YPWAMWGQWY (SEQ ID NO: 15), VFMPQHWHPAAHWY (SEQ ID NO: 16), REARHPWLRQWY (SEQ ID NO: 17), or YPWGAGAPWLVQWY (SEQ ID NO: 18)

FW4 is a framework region of from 7 to 14 amino acids

25 or a functional variant thereof with at least 45% sequence identity thereto, optionally in which FW1 is selected from: ASVNQTPRTATKETGESLTINCVLT (SEQ ID NO: 19), AKVDQTPRTATKETGESLTINCVLT (SEQ ID NO: 20), TRVDQTPRTATKETGESLTINCVVT (SEQ ID NO: 21), TRVDQTPRTATKETGESLTINCVLT (SEQ ID NO: 22),

ASVNQTPRTATKETGESLTINCVVT (SEQ ID NO: 23), or
 TRVDQSPSSLSASVGDRVITITCVLT (SEQ ID NO: 24), FW2 is selected from:
 TSWFRKNPG (SEQ ID NO: 25), or TYWYRKNPG (SEQ ID NO: 26); FW3a is
 selected from: GRYVESV (SEQ ID NO: 27), or GRYSESV (SEQ ID NO: 28), FW3b is
 selected from: SFSRLRIKDLTVADSATYYCKA (SEQ ID NO: 29),
 SFTLTISSLQPEDSATYYCRA (SEQ ID NO: 30), or SFTLTISSLQPEDFATYYCKA
 (SEQ ID NO: 31), and FW4 is selected from: DGAGTVLTVN (SEQ ID NO: 32), or
 DGAGTKVEIK (SEQ ID NO: 33); or functional variants thereof with a sequence
 identity of at least 45%.

4. A bi-specific antigen binding molecule according to Claim 1, 2 or 3, in which the
 ROR1-specific antigen binding molecule comprises an amino acid sequence selected from:

ASVNQTPRTATKETGESLTINCVLTDTSYGLYSTSWFRKNPGTTDWERMISIGGR
 YVESVNKRAKSFSRLRIKDLTVADSATYYCKAQSGMAISTGSGHGYNWYDGAG
 TVLTVN (SEQ ID NO: 39);

AKVDQTPRTATKETGESLTINCVLTDTSYGLYSTSWFRKNPGTTDWERMISIGGR
 YVESVNKRAKSFSRLRIKDLTVADSATYYCKAQSGMAIDIGSGHGYNWYDGAGT
 VLTVN (SEQ ID NO: 40);

TRVDQTPRTATKETGESLTINCVVTGAKYGLAATYWYRKNPGSSNQERISISGR
 YVESVNKRTMSFSRLRIKDLTVADSATYYCKAYPWAMWGQWYDGAGTVLTVN
 (SEQ ID NO: 41);

TRVDQTPRTATKETGESLTINCVVTGAKYGLFATYWYRKNPGSSNQERISISGR
 YVESVNKRTMSFSRLRIKDLTVADSATYYCKAVFMPQHWHPAAHWYDGAGTVL
 TVN (SEQ ID NO: 42);

TRVDQTPRTATKETGESLTINCVLTDTSYGLYSTSWFRKNPGTTDWERMISIGGR
 YVESVNKRAKSFSRLRIKDLTVADSATYYCKAREARHPWLRQWYDGAGTVLTV
 N (SEQ ID NO: 43);

ASVNQTPRTATKETGESLTINCVVTGANYGLAATYWYRKNPGSSNQERISISGR
 YVESVNKRTMSFSRLRIKDLTVADSATYYCKAYPWGAGAPWLQWYDGAGTV
 LTVN (SEQ ID NO: 44);,

TRVDQSPSSLSASVGDRVITITCVLTGANYGLASTYWYRKNPGSSNKEQISISGRY
 SESVNKGTKSFTLTISSLQPEDSATYYCRAYPWGAGAPWLQWYDGAGTKVEI
 K (SEQ ID NO: 45);

TRVDQSPSSLSASVGDRVITITCVLTGANYGLASTYWYRKNPGSSNQERISISGRY
 SESVNKRRTMSFTLTISLQPEDSATYYCRAYPWGAGAPWLVQWYDGAGTKVEIK
 K (SEQ ID NO: 46);

5 TRVDQSPSSLSASVGDRVITITCVLTDTSYGLYSTSWFRKNPGTTDWERM SIGGR
 YVESVNKGAKSFTLTISLQPEDFATYYCKAREARHPWLRQWYDGAGTKVEIK
 (SEQ ID NO: 47);

TRVDQSPSSLSASVGDRVITITCVLTDTSYGLYSTYWYRKNPGSSNKEQISISGRY
 SESVNKGTKSFTLTISLQPEDSATYYCRAREARHPWLRQWYDGAGTKVEIK
 (SEQ ID NO: 48);

10 TRVDQSPSSLSASVGDRVITITCVLTDTSYGLYSTYWYRKNPGTTDWERM SIGGR
 YSESVNKGAKSFTLTISLQPEDSATYYCRAREARHPWLRQWYDGAGTKVEIK
 (SEQ ID NO: 49); or a functional variant thereof with a sequence identity of at least
 45%.

5. A bi-specific antigen binding molecule according to any preceding claim, in which the
 15 ROR1-specific antigen binding molecule is humanized, or in which the ROR1-specific
 antigen binding molecule is de-immunized.

6. A bi-specific antigen binding molecule according to any preceding claim, in which the
 bi-specific antigen binding molecule is conjugated to a detectable label, dye, toxin, drug, pro-
 drug, radionuclide or biologically active molecule.

20 7. A bi-specific antigen binding molecule according to any preceding claim, in which the
 ROR1-specific antigen binding molecule selectively interacts with ROR1 protein with an
 affinity constant of approximately 0.01 to 50 nM, preferably 0.1 to 30 nM, even more
 preferably 0.1 to 10 nM.

8. A bi-specific antigen binding molecule according to any preceding claim, in which the
 25 bi-specific antigen binding molecule is capable of

- (i) mediating killing of ROR1-expressing tumour cells,
- (ii) inhibiting cancer cell proliferation,
- (iii) being endocytosed upon binding to ROR1,
- (iv) down regulating ROR1 or EGFR upon binding, or

(v) down regulating ROR1 or EGFR signalling.

9. A recombinant fusion protein comprising a bi-specific antigen binding molecule according to any preceding claim.

10. A recombinant fusion protein according to Claim 9, in which the bi-specific antigen binding molecule is fused to one or more biologically active proteins, optionally in which

(i) the specific antigen binding molecule is fused to one or more biologically active proteins via one or more linker domains, and/or

(ii) at least one biologically active protein is an immunoglobulin, an immunoglobulin Fc region, an immunoglobulin Fab region, a single chain Fv (scFv), a diabody, a triabody, a tetrabody, a bispecific t-cell engager (BiTE), an intein, a VNAR domain, a single domain antibody (sdAb), a VH domain, or a scaffold protein.

11. A chimeric antigen receptor (CAR), comprising at least one bi-specific antigen binding molecule as defined in any of Claims 1 to 5, fused or conjugated to at least one transmembrane region and at least one intracellular domain.

12. A cell comprising a chimeric antigen receptor according to Claim 11, which cell is preferably an engineered T-cell.

13. A nucleic acid sequence comprising a polynucleotide sequence that encodes a bi-specific antigen binding molecule, recombinant fusion protein or chimeric antigen receptor according to any of Claims 1 to 11, a vector comprising the nucleic acid sequence and optionally further comprising one or more regulatory sequences, or a host cell comprising the vector.

14. A method for preparing a bi-specific antigen binding molecule, recombinant fusion protein or chimeric antigen receptor, comprising cultivating or maintaining a host cell comprising a polynucleotide according to Claim 13 under conditions such that the host cell produces the binding molecule, optionally further comprising isolating the binding molecule.

15. A pharmaceutical composition comprising a specific antigen binding molecule, recombinant fusion protein or chimeric antigen receptor according to any of Claims 1 to 11.

16. A bi-specific antigen binding molecule, recombinant fusion protein or chimeric antigen receptor according to any of Claims 1 to 11, for use in therapy.

17. A bi-specific antigen binding molecule, recombinant fusion protein or chimeric antigen receptor according to any of Claims 1 to 11, for use in the treatment of cancer, optionally in which the cancer is a ROR1-positive cancer type or the cancer is selected from the group comprising blood cancers such as lymphomas and leukemias, chronic lymphocytic leukaemia (CLL), mantle cell lymphoma (MCL), B-cell acute lymphoblastic leukaemia (B-ALL), marginal zone lymphoma (MZL), non-Hodgkin lymphomas (NHL), acute myeloid leukemia (AML) and solid tumours including neuroblastoma, renal cancer, lung cancer, colon cancer, ovarian cancer, pancreatic cancer, breast cancer, skin cancer, uterine cancer, prostate cancer, thyroid cancer, Head and Neck cancer, bladder cancer, stomach cancer or liver cancer.
18. A method of assaying for the presence of a target analyte in a sample, comprising the addition of a detectably labelled bi-specific antigen binding molecule according to any of Claims 1 to 8 or a recombinant fusion protein according to Claim 9 or 10 to the sample and detecting the binding of the molecule to the target analyte.
19. A method of imaging a site of disease in a subject, comprising administration of a detectably labelled bi-specific antigen binding molecule according to any of Claims 1 to 8 or a detectably labelled recombinant fusion protein according to Claim 9 or 10 to a subject.
20. A method of diagnosis of a disease or medical condition in a subject comprising administration of a detectably labelled bi-specific antigen binding molecule according to any of Claims 1 to 8 or a recombinant fusion protein according to Claim 9 or 10 to the subject and detecting the molecule.
21. An antibody, antibody fragment or antigen-binding molecule that competes for binding to ROR1 with a ROR1-specific antigen binding molecule according to any of Claims 1 to 8; or a ROR1 and EGFR bi-specific antibody, ROR1 and EGFR bi-specific antibody fragment or ROR1 and EGFR bi-specific antigen-binding molecule that competes for binding to ROR1 and/or EGFR with a bi-specific antigen binding molecule according to any of Claims 1 to 8.
22. A kit for diagnosing a subject suffering from cancer, or a pre-disposition thereto, or for providing a prognosis of the subject's condition, the kit comprising detection means for detecting the concentration of antigen present in a sample from a test subject, in which the detection means comprises a bi-specific antigen binding molecule as defined in any of Claims 1 to 8, a recombinant fusion protein as defined in Claim 9 or 10, a CAR as defined in Claim 11, or a nucleic acid as defined in Claim 13, each being optionally derivatized, in which

presence of antigen in the sample suggests that the subject suffers from cancer, optionally in which

- (i) the antigen comprises ROR1 protein, more preferably an extracellular domain thereof,
- (ii) the kit is used to identify the presence or absence of ROR1-positive cells in the sample, or determine the concentration thereof in the sample,
- (iii) the kit comprises a positive control and/or a negative control against which the assay is compared, or
- (iv) the kit further comprises a label which may be detected.

23. A bi-specific antigen binding molecule comprising an amino acid sequence represented by the formula (II):



in which

FW1 is a framework region

CDR1 is a CDR sequence

FW2 is a framework region

HV2 is a hypervariable sequence

FW3a is a framework region

HV4 is a hypervariable sequence

FW3b is a framework region

CDR3 is a CDR sequence

FW4 is a framework region

in which Xa, Xb, Ya and Yb are either absent or an EGFR-specific binding molecule,

in which at least one of Xa, Xb, Ya and Yb is an EGFR-specific binding molecule,

X and Y are optional amino acid sequences,

in which the bi-specific antigen binding molecule is conjugated to a second moiety.

24. A bi-specific antigen binding molecule according to Claim 23, in which X or Y are individually either absent or selected from the group comprising an immunoglobulin, an immunoglobulin Fc region, an immunoglobulin Fab region, a single chain Fv (scFv), a diabody, a triabody, a tetrabody, a bispecific t-cell engager (BiTE), an intein, a VNAR domain, a single domain antibody (sdAb), a VH domain, or a scaffold protein.

25. A bi-specific antigen binding molecule according to Claim 23 or 24, in which

(i) the conjugation is via a cysteine residue in the amino acid sequence of the specific antigen binding molecule,

(ii) the conjugation is via a thiol, aminoxy or hydrazinyl moiety incorporated at the N-terminus or C-terminus of the amino acid sequence of the specific antigen binding molecule, and/or

(iii) the second moiety is selected from the group comprising an immunoglobulin, an immunoglobulin Fc region, an immunoglobulin Fab region, a single chain Fv (scFv), a diabody, a triabody, a tetrabody, a bispecific t-cell engager (BiTE), an intein, a VNAR domain, a single domain antibody (sdAb), a VH domain, a scaffold protein, detectable label, dye, toxin, drug, pro-drug, radionuclide or biologically active molecule, or at least one toxin selected from the group comprising:

- maytansinoids,
- auristatins,
- anthracyclins, preferably PNU-derived anthracyclins
- amanitin derivatives, preferably α -amanitin derivatives
- calicheamicins,
- tubulysins
- duocarmycins
- radioisotopes - such as an alpha-emitting radionuclide, such as ^{227}Th and ^{225}Ac label
- liposomes comprising a toxic payload,
- protein toxins

- taxanes
- pyrrolbenzodiazepines and/or
- indolinobenzodiazepine pseudodimers and/or
- spliceosome inhibitors
- 5 • CDK11 inhibitors
- Pyridinobenzodiazepines

26. A bi-specific antigen binding molecule according to Claim 23, 24 or 25, in which the specific antigen binding molecule is a receptor tyrosine kinase-like orphan receptor 1 (ROR1) specific antigen binding molecule, optionally in which

- 10 (i) the ROR1-specific antigen binding molecule does not bind to receptor tyrosine kinase-like orphan receptor 2 (ROR2),
- (ii) the ROR1-specific antigen binding molecule binds to both human ROR1 and murine ROR1 (mROR1),
- (iii) the ROR1-specific antigen binding molecule binds to deglycosylated ROR1,
- 15 (iv) the ROR1-specific antigen binding molecule does not bind to a linear peptide sequence selected from:

YMESLHMQGEIENQI (SEQ ID NO: 34)

CQPWNSQYPHTHTFTALRFP (SEQ ID NO: 35)

RSTIYGSRLRIRNLDTTDTGYFQ (SEQ ID NO: 36)

20 QCVATNGKEVVSSTGVLFVKFGPPPTASPGYSDEYE (SEQ ID NO: 37)

27. A bi-specific antigen binding molecule according to Claim 26, in which:

FW1 is a framework region of from 20 to 28 amino acids

CDR1 is a CDR sequence selected from DTSYGLYS (SEQ ID NO: 1), GAKYGLAA (SEQ ID NO: 2), GAKYGLFA (SEQ ID NO: 3), GANYGLAA (SEQ ID NO: 4), or

25 GANYGLAS (SEQ ID NO: 5)

FW2 is a framework region of from 6 to 14 amino acids

HV2 is a hypervariable sequence selected from TTDWERMSIG (SEQ ID NO: 6), SSNQERISIS (SEQ ID NO: 7), or SSNKEQISIS (SEQ ID NO: 8)

FW3a is a framework region of from 6 to 10 amino acids

HV4 is a hypervariable sequence selected from NKRAK (SEQ ID NO: 9), NKRTM (SEQ ID NO: 10), NKGAK (SEQ ID NO: 11), or NKGTK (SEQ ID NO: 12)

FW3b is a framework region of from 17 to 24 amino acids

CDR3 is a CDR sequence selected from QSGMAISTGSGHGYNWY (SEQ ID NO: 13), QSGMAIDIGSGHGYNWY (SEQ ID NO: 14), YPWAMWQWY (SEQ ID NO: 15), VFMPQHWHPAAHWY (SEQ ID NO: 16), REARHPWLRQWY (SEQ ID NO: 17), or YPWGAGAPWLVQWY (SEQ ID NO: 18)

FW4 is a framework region of from 7 to 14 amino acids

or a functional variant thereof with at least 45% sequence identity thereto, optionally in which

(i) FW1 is selected from ASVNQTPRTATKETGESLTINCVLT (SEQ ID NO: 19), AKVDQTPRTATKETGESLTINCVLT (SEQ ID NO: 20), TRVDQTPRTATKETGESLTINCVVT (SEQ ID NO: 21), TRVDQTPRTATKETGESLTINCVLT (SEQ ID NO: 22), ASVNQTPRTATKETGESLTINCVVT (SEQ ID NO: 23), or TRVDQSPSSLSASVGDRVTITCVLT (SEQ ID NO: 24), FW2 is selected from TSWFRKNPG (SEQ ID NO: 25), or TYWYRKNPG (SEQ ID NO: 26), FW3a is selected from GRYVESV (SEQ ID NO: 27), or GRYSESV (SEQ ID NO: 28), FW3b is selected from SFSRLIKDLTVADSATYYCKA (SEQ ID NO: 29), SFTLTISLQPEDSATYYCRA (SEQ ID NO: 30), or SFTLTISLQPEDFATYYCKA (SEQ ID NO: 31), and FW4 is selected from DGAGTVLTVN (SEQ ID NO: 32), or DGAGTKVEIK (SEQ ID NO: 33), or functional variants thereof with a sequence identity of at least 45%, or

(ii) the ROR1-specific antigen binding molecule comprises an amino acid sequence selected from

ASVNQTPRTATKETGESLTINCVLTDTSYGLYSTSWFRKNPGTTDWERMSI
GGRYVESVNKRAKSFSRLIKDLTVADSATYYCKAQSGMAISTGSGHGYN
WYDGAGTVLTVN (SEQ ID NO: 39);

AKVDQTPRTATKETGESLTINCVLTDTSYGLYSTSWFRKNPGTTDWERMS
IGGRYVESVKNKRAKSFSRLIKDLTVADSATYYCKAQSGMAIDIGSGHGYN
WYDGAGTVLTVN (SEQ ID NO: 40);

5

TRVDQTPRTATKETGESLTINCVVTGAKYGLAATYWYRKNPGSSNQERISI
SGRYVESVKNKRTMSFSRLIKDLTVADSATYYCKAYPWAMWGQWYDGA
GTVLTVN (SEQ ID NO: 41);

TRVDQTPRTATKETGESLTINCVVTGAKYGLFATYWYRKNPGSSNQERISI
SGRYVESVKNKRTMSFSRLIKDLTVADSATYYCKAVFMPQHWHPPAAHWY
DGAGTVLTVN (SEQ ID NO: 42);

10

TRVDQTPRTATKETGESLTINCVLTDTSYGLYSTSWFRKNPGTTDWERMSI
GGRYVESVKNKGAKSFSRLIKDLTVADSATYYCKAREARHPWLRQWYDG
AGTVLTVN (SEQ ID NO: 43);

ASVNQTPRTATKETGESLTINCVVTGANYGLAATYWYRKNPGSSNQERISI
SGRYVESVKNKRTMSFSRLIKDLTVADSATYYCKAYPWGAGAPWLQWY
DGAGTVLTVN (SEQ ID NO: 44);

15

TRVDQSPSSLSASVGDRVTITCVLTGANYGLASTYWYRKNPGSSNKEQISI
SGRYSESVNKGTKSFTLTISLQPEDSATYYCRAYPWGAGAPWLQWYD
GAGTKVEIK (SEQ ID NO: 45);

TRVDQSPSSLSASVGDRVTITCVLTGANYGLASTYWYRKNPGSSNQERISI
SGRYSESVNKRRTMSFTLTISLQPEDSATYYCRAYPWGAGAPWLQWYD
GAGTKVEIK (SEQ ID NO: 46);

20

TRVDQSPSSLSASVGDRVTITCVLTDTSYGLYSTSWFRKNPGTTDWERMSI
GGRYVESVKNKGAKSFTLTISLQPEDFATYYCKAREARHPWLRQWYDGA
GTKVEIK (SEQ ID NO: 47);

25

TRVDQSPSSLSASVGDRVTITCVLTDTSYGLYSTYWYRKNPGSSNKEQISIS
GRYSESVNKGTKSFTLTISLQPEDSATYYCRAREARHPWLRQWYDGA
KVEIK (SEQ ID NO: 48);

30

TRVDQSPSSLSASVGDRVTITCVLTDTSYGLYSTYWYRKNPGTTDWERMS
IGGRYSESVNKGAKSFTLTISLQPEDSATYYCRAREARHPWLRQWYDGA
GTKVEIK (SEQ ID NO: 49); or a functional variant thereof with a sequence
identity of at least 45%.

28. A bi-specific antigen binding molecule according to Claim 26 or 27, in which the ROR1-specific antigen binding molecule is humanized or the ROR1-specific antigen binding molecule is de-immunized.

- 5 29. A bi-specific antigen binding molecule according to any of Claims 23 to 28, in which the EGFR-specific antigen binding molecule is selected from the group comprising an immunoglobulin, an immunoglobulin Fab region, a single chain Fv (scFv), a diabody, a triabody, a tetrabody, a bispecific t-cell engager (BiTE), an intein, a VNAR domain, a single domain antibody (sdAb), a VH domain, or a scaffold protein.

CLAIMS

1. A bi-specific antigen binding molecule comprising:

(i) a receptor tyrosine kinase-like orphan receptor 1 (ROR1) specific antigen binding molecule comprising an amino acid sequence represented by the formula (I):

5 FW1-CDR1-FW2-HV2-FW3a-HV4-FW3b-CDR3-FW4 (I)

in which

FW1 is a framework region of from 20 to 28 amino acids

CDR1 is DTSYGLYS (SEQ ID NO: 1)

FW2 is a framework region of from 6 to 14 amino acids

10 HV2 is TTDWERMSIG (SEQ ID NO: 6)

FW3a is a framework region of from 6 to 10 amino acids

HV4 is NKGAK (SEQ ID NO: 11)

FW3b is a framework region of from 17 to 24 amino acids

CDR3 is REARHPWLRQWY (SEQ ID NO: 17)

15 FW4 is a framework region of from 7 to 14 amino acids; and

(ii) an epidermal growth factor receptor (EGFR) specific antigen binding molecule;
or a functional variant thereof with at least 45% sequence identity thereto.

2. A bi-specific antigen binding molecule according to Claim 1, in which

20 (i) the ROR1-specific antigen binding molecule does not bind to receptor tyrosine kinase-like orphan receptor 2 (ROR2),

(ii) the ROR1-specific antigen binding molecule binds to both human ROR1 and murine ROR1 (mROR1),

(iii) the ROR1-specific antigen binding molecule binds to deglycosylated ROR1, and/or

25 (iv) the ROR1-specific antigen binding molecule does not bind to a linear peptide sequence selected from:

YMESLHMQGEIENQI (SEQ ID NO: 34)

CQPWNSQYPHTHTFTALRFP (SEQ ID NO: 35)

RSTIYGSRLRIRNLDTTDTGYFQ (SEQ ID NO: 36)

QCVATNGKEVVSSSTGVLFVKFGPPPTASPGYSDEYE (SEQ ID NO: 37)

- 5 3. A bi-specific antigen binding molecule according to Claim 1 or 2 comprising a ROR1-specific antigen binding molecule in which

FW1 is TRVDQTPRTATKETGESLTINCVL (SEQ ID NO: 22), FW2 is
 TSWFRKNPG (SEQ ID NO: 25), FW3a is GRYVESV (SEQ ID NO: 27), FW3b is
 SFSRLRIKDLTVADSATYYCKA (SEQ ID NO: 29) and FW4 is DGAGTVLTVN (SEQ
 10 ID NO: 32), or functional variants thereof with a sequence identity of at least 45%.
4. A bi-specific antigen binding molecule according to Claim 1, 2 or 3, in which the ROR1-specific antigen binding molecule comprises the amino acid
 TRVDQTPRTATKETGESLTINCVLTDTSYGLYSTSWFRKNPGTTDWERMSIGGRYVES
 VNKGAKSFSRLRIKDLTVADSATYYCKAREARHPWLRQWYDGAGTVLTVN (SEQ ID
 15 NO: 43); or a functional variant thereof with a sequence identity of at least 45%.
5. A bi-specific antigen binding molecule according to any preceding claim, in which the ROR1-specific antigen binding molecule is humanized, or in which the ROR1-specific antigen binding molecule is de-immunized.
6. A bi-specific antigen binding molecule according to any preceding claim, in which the
 20 bi-specific antigen binding molecule is conjugated to a detectable label, dye, toxin, drug, pro-drug, radionuclide or biologically active molecule.
7. A bi-specific antigen binding molecule according to any preceding claim, in which the ROR1-specific antigen binding molecule selectively interacts with ROR1 protein with an affinity constant of 0.01 to 50 nM, preferably 0.1 to 30 nM, even more preferably 0.1 to 10
 25 nM.
8. A bi-specific antigen binding molecule according to any preceding claim, in which the bi-specific antigen binding molecule is capable of
 - (i) mediating killing of ROR1-expressing tumour cells,
 - (ii) inhibiting cancer cell proliferation,

- (iii) being endocytosed upon binding to ROR1,
- (iv) down regulating ROR1 or EGFR upon binding, or
- (v) down regulating ROR1 or EGFR signalling.

9. A recombinant fusion protein comprising a bi-specific antigen binding molecule
5 according to any preceding claim.
10. A recombinant fusion protein according to Claim 9, in which the bi-specific antigen binding molecule is fused to one or more biologically active proteins, optionally in which
 - (i) the specific antigen binding molecule is fused to one or more biologically active proteins via one or more linker domains, and/or
 - 10 (ii) at least one biologically active protein is an immunoglobulin, an immunoglobulin Fc region, an immunoglobulin Fab region, a single chain Fv (scFv), a diabody, a triabody, a tetrabody, a bispecific t-cell engager (BiTE), an intein, a VNAR domain, a single domain antibody (sdAb), a VH domain, or a scaffold protein.
11. A chimeric antigen receptor (CAR), comprising at least one bi-specific antigen binding
15 molecule as defined in any of Claims 1 to 5, fused or conjugated to at least one transmembrane region and at least one intracellular domain.
12. A cell comprising a chimeric antigen receptor according to Claim 11, which cell is preferably an engineered T-cell.
13. A nucleic acid sequence comprising a polynucleotide sequence that encodes a bi-
20 specific antigen binding molecule, recombinant fusion protein or chimeric antigen receptor according to any of Claims 1 to 11, a vector comprising the nucleic acid sequence and optionally further comprising one or more regulatory sequences, or a host cell comprising the vector.
14. A method for preparing a bi-specific antigen binding molecule, recombinant fusion
25 protein or chimeric antigen receptor, comprising cultivating or maintaining a host cell comprising a polynucleotide according to Claim 13 under conditions such that the host cell produces the binding molecule, optionally further comprising isolating the binding molecule.
15. A pharmaceutical composition comprising a specific antigen binding molecule, recombinant fusion protein or chimeric antigen receptor according to any of Claims 1 to 11.

16. A bi-specific antigen binding molecule, recombinant fusion protein or chimeric antigen receptor according to any of Claims 1 to 11, for use in therapy.

17. A bi-specific antigen binding molecule, recombinant fusion protein or chimeric antigen receptor according to any of Claims 1 to 11, for use in the treatment of cancer, optionally in
5 which the cancer is a ROR1-positive cancer type or the cancer is selected from the group comprising blood cancers such as lymphomas and leukemias, chronic lymphocytic leukaemia (CLL), mantle cell lymphoma (MCL), B-cell acute lymphoblastic leukaemia (B-ALL), marginal zone lymphoma (MZL), non-Hodgkin lymphomas (NHL), acute myeloid leukemia (AML) and solid tumours including neuroblastoma, renal cancer, lung cancer, colon cancer,
10 ovarian cancer, pancreatic cancer, breast cancer, skin cancer, uterine cancer, prostate cancer, thyroid cancer, Head and Neck cancer, bladder cancer, stomach cancer or liver cancer.

18. A method of assaying for the presence of a target analyte in a sample, comprising the addition of a detectably labelled bi-specific antigen binding molecule according to any of Claims 1 to 8 or a recombinant fusion protein according to Claim 9 or 10 to the sample and
15 detecting the binding of the molecule to the target analyte.

19. A method of imaging a site of disease in a subject, comprising administration of a detectably labelled bi-specific antigen binding molecule according to any of Claims 1 to 8 or a detectably labelled recombinant fusion protein according to Claim 9 or 10 to a subject.

20. A method of diagnosis of a disease or medical condition in a subject comprising
20 administration of a detectably labelled bi-specific antigen binding molecule according to any of Claims 1 to 8 or a recombinant fusion protein according to Claim 9 or 10 to the subject and detecting the molecule.

21. A ROR1 and EGFR bi-specific antibody fragment or ROR1 and EGFR bi-specific antigen-binding molecule that competes for binding to ROR1 and/or EGFR with a bi-specific
25 antigen binding molecule according to any of Claims 1 to 8.

22. A kit for diagnosing a subject suffering from cancer, or a pre-disposition thereto, or for providing a prognosis of the subject's condition, the kit comprising detection means for detecting the concentration of antigen present in a sample from a test subject, in which the detection means comprises a bi-specific antigen binding molecule as defined in any of Claims
30 1 to 8, a recombinant fusion protein as defined in Claim 9 or 10, a CAR as defined in Claim

11, or a nucleic acid as defined in Claim 13, each being optionally derivatized, in which presence of antigen in the sample suggests that the subject suffers from cancer, optionally in which

(i) the antigen comprises ROR1 protein, more preferably an extracellular domain thereof,

(ii) the kit is used to identify the presence or absence of ROR1-positive cells in the sample, or determine the concentration thereof in the sample,

(iii) the kit comprises a positive control and/or a negative control against which the assay is compared, or

(iv) the kit further comprises a label which may be detected.

23. A bi-specific antigen binding molecule comprising an amino acid sequence represented by the formula (II):

Xb-X-Xa-FW1-CDR1-FW2-HV2-FW3a-HV4-FW3b-CDR3-FW4-Ya-Y-Yb (II)

in which

FW1 is a framework region of from 20 to 28 amino acids

CDR1 is DTSYGLYS (SEQ ID NO: 1)

FW2 is a framework region of from 6 to 14 amino acids

HV2 is TTDWERMSIG (SEQ ID NO: 6)

FW3a is a framework region of from 6 to 10 amino acids

HV4 is NKGAK (SEQ ID NO: 11)

FW3b is a framework region of from 17 to 24 amino acids

CDR3 is REARHPWLRQWY (SEQ ID NO: 17)

FW4 is a framework region of from 7 to 14 amino acids

in which Xa, Xb, Ya and Yb are either absent or an EGFR-specific binding molecule,

in which at least one of Xa, Xb, Ya and Yb is an EGFR-specific binding molecule,
X and Y are optional amino acid sequences,

in which the bi-specific antigen binding molecule is conjugated to a second moiety, or a functional variant thereof with at least 45% sequence identity thereto.

- 5 24. A bi-specific antigen binding molecule according to Claim 23, in which X or Y are individually either absent or selected from the group comprising an immunoglobulin, an immunoglobulin Fc region, an immunoglobulin Fab region, a single chain Fv (scFv), a diabody, a triabody, a tetrabody, a bispecific t-cell engager (BiTE), an intein, a VNAR domain, a single domain antibody (sdAb), a VH domain, or a scaffold protein.
- 10 25. A bi-specific antigen binding molecule according to Claim 23 or 24, in which
- (i) the conjugation is via a cysteine residue in the amino acid sequence of the specific antigen binding molecule,
- (ii) the conjugation is via a thiol, aminoxy or hydrazinyl moiety incorporated at the N-terminus or C-terminus of the amino acid sequence of the specific antigen binding molecule, and/or
- 15 (iii) the second moiety is selected from the group comprising an immunoglobulin, an immunoglobulin Fc region, an immunoglobulin Fab region, a single chain Fv (scFv), a diabody, a triabody, a tetrabody, a bispecific t-cell engager (BiTE), an intein, a VNAR domain, a single domain antibody (sdAb), a VH domain, a scaffold protein, detectable label, dye, toxin, drug, pro-drug, radionuclide or biologically active molecule, or at least
- 20 one toxin selected from the group comprising:
- maytansinoids,
 - auristatins,
 - anthracyclins, preferably PNU-derived anthracyclins
 - 25 • amanitin derivatives, preferably α -amanitin derivatives
 - calicheamicins,
 - tubulysins
 - duocarmycins

- radioisotopes - such as an alpha-emitting radionuclide, such as ²²⁷Th and ²²⁵Ac label

- liposomes comprising a toxic payload,

- protein toxins

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- taxanes

- pyrrolbenzodiazepines and/or

- indolinobenzodiazepine pseudodimers and/or

- spliceosome inhibitors

- CDK11 inhibitors

10

- Pyridinobenzodiazepines

26. A bi-specific antigen binding molecule according to Claim 23, 24 or 25, in which the specific antigen binding molecule is a receptor tyrosine kinase-like orphan receptor 1 (ROR1) specific antigen binding molecule, optionally in which

(i) the ROR1-specific antigen binding molecule does not bind to receptor tyrosine kinase-like orphan receptor 2 (ROR2),

15

(ii) the ROR1-specific antigen binding molecule binds to both human ROR1 and murine ROR1 (mROR1),

(iii) the ROR1-specific antigen binding molecule binds to deglycosylated ROR1,

(iv) the ROR1-specific antigen binding molecule does not bind to a linear peptide sequence selected from:

20

YMESLHMQGEIENQI (SEQ ID NO: 34)

CQPWNSQYPHTHTFTALRFP (SEQ ID NO: 35)

RSTIYGSRLRIRNLDTTDTGYFQ (SEQ ID NO: 36)

QCVATNGKEVVSSSTGVLFVKFGPPPTASPGYSDEYE (SEQ ID NO: 37)

25

27. A bi-specific antigen binding molecule according to Claim 26, in which:

(i) FW1 is TRVDQTPRTATKETGESLTINCVLT (SEQ ID NO: 22), FW2 is TSWFRKNPG (SEQ ID NO: 25), FW3a is GRYVESV (SEQ ID NO: 27), FW3b

is SFSLRIKDLTVADSATYYCKA (SEQ ID NO: 29) and FW4 is DGAGTVLTVN (SEQ ID NO: 32), or functional variants thereof with a sequence identity of at least 45%, or

(ii) the ROR1-specific antigen binding molecule comprises the amino acid sequence

TRVDQTPRTATKETGESLTINCVLTDTSYGLYSTSWFRKNPGTTDWERMSI
GGRYVESVNKGAKSFSLRIKDLTVADSATYYCKAREARHPWLRQWYDG
AGTVLTVN (SEQ ID NO: 43); or a functional variant thereof with a sequence identity of at least 45%.

- 10 28. A bi-specific antigen binding molecule according to Claim 26 or 27, in which the ROR1-specific antigen binding molecule is humanized or the ROR1-specific antigen binding molecule is de-immunized.
- 15 29. A bi-specific antigen binding molecule according to any of Claims 23 to 28, in which the EGFR-specific antigen binding molecule is selected from the group comprising an immunoglobulin, an immunoglobulin Fab region, a single chain Fv (scFv), a diabody, a triabody, a tetrabody, a bispecific t-cell engager (BiTE), an intein, a VNAR domain, a single domain antibody (sdAb), a VH domain, or a scaffold protein.