CLAIMS

1. A recombinant fusion protein dimer comprising:

(a) a first recombinant fusion protein, in which the first recombinant fusion protein comprises a receptor tyrosine kinase-like orphan receptor 1 (ROR1) specific antigen binding molecule comprising an amino acid sequence represented by the formula (I):

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

in which:

CDR3 is a CDR sequence having an amino acid sequence selected from the group consisting of YPWGAGAPYNVQWY (SEQ ID NO: 23), YPWGAGAPYLVQWY (SEQ ID NO: 20), YPWGAGAPWNVQWY (SEQ ID NO: 24), YPSGAGAPRPVQWY (SEQ ID NO: 11), YPWGAGAPCLVQWY (SEQ ID NO: 12), YPWGAGAPRLVQWY (SEQ ID NO: 13), YPWGAGAPRQVQWY (SEQ ID NO: 14), YPWGAGAPRSVQWY (SEQ ID NO: 15), YPWGAGAPSLVQWY (SEQ ID NO: 16), YPWGAGAPSNVQWY (SEQ ID NO: 17), YPWGAGAPSQVQWY (SEQ ID NO: 18), YPWGAGAPSSVQWY (SEQ ID NO: 19), YPWGAGAPWQVQWY (SEQ ID NO: 21), YPWGAGAPWSVQWY (SEQ ID NO: 22), and YPWGAGAPWLVQWY (SEQ ID NO: 10);

CDR1 is a CDR sequence having an amino acid sequence selected from the group consisting of GANYGLAA (SEQ ID NO: 1), DANYGLAA (SEQ ID NO: 5), GANYDLSA (SEQ ID NO: 2), GANYGLSA (SEQ ID NO: 3), and GANYDLAA (SEQ ID NO: 4)

FW1 is a framework region;

FW2 is a framework region;

HV2 is a hypervariable sequence having an amino acid sequence selected from the group consisting of SSNQERISIS (SEQ ID NO: 6), and SSNKERISIS (SEQ ID NO: 7);

FW3a is a framework region;

HV4 is a hypervariable sequence having an amino acid sequence selected from the group consisting of NKRTM (SEQ ID NO: 8) and NKGTM (SEQ ID NO: 9);

FW3b is a framework region;

FW4 is a framework region;

in which if CDR3 is YPWGAGAPWLVQWY (SEQ ID NO: 10) then CDR1 is selected from the group consisting of DANYGLAA (SEQ ID NO: 5), GANYGLSA (SEQ ID NO: 3) and GANYDLAA (SEQ ID NO: 4),

and in which the first antigen binding molecule is fused to a first fragment of an immunoglobulin Fc region engineered to dimerize with a second fragment of an immunoglobulin Fc region;

and

(b) a second recombinant fusion protein, in which the second recombinant fusion protein comprises an epidermal growth factor receptor (EGFR) specific antigen binding molecule fused to a second fragment of an immunoglobulin Fc region engineered to dimerize with the first fragment of an immunoglobulin Fc region.

2. A recombinant fusion protein dimer according to Claim 1,

(a) in which:

(i) CDR1 is GANYGLAA (SEQ ID NO: 1), HV2 is SSNQERISIS (SEQ ID NO: 6), HV4 is NKRTM (SEQ ID NO: 8), and CDR3 is YPWGAGAPYNVQWY (SEQ ID NO: 23) or YPWGAGAPSSVQWY (SEQ ID NO: 19); and/or

(ii) CDR1 is DANYGLAA (SEQ IS NO: 5), HV2 is SSNKERISIS (SEQ ID NO: 7), HV4 is NKGTM (SEQ ID NO: 9), and CDR3 is YPWGAGAPYNVQWY (SEQ ID NO: 23);

or

(i) FW1 is ASVNQTPRTATKETGESLTINCVVT (SEQ ID NO: 40), FW2 is TYWYRKNPG (SEQ ID NO: 43), FW3a is GRYVESV (SEQ ID NO: 44), FW3b is SFSLRIKDLTVADSATYYCKA (SEQ ID NO: 84), and FW4 is DGAGTVLTVN (SEQ ID NO: 48), and/or

(ii) FW1 is TRVDQSPSSLSASVGDRVTITCVLT (SEQ ID NO: 41), FW2 is TYWYRKNPG (SEQ ID NO: 43), FW3a is GRYSESV (SEQ ID NO: 45), FW3b is SFTLTISSLQPEDSATYYCRA (SEQ ID NO: 46), and FW4 is DGAGTKVEIK (SEQ ID NO: 49); and/or

(b) in which the ROR1 specific antigen binding molecule comprises SEQ ID NO: 50, SEQ ID NO: 61 or SEQ ID NO: 71.

3. A recombinant fusion protein dimer comprising:

(a) a first recombinant fusion protein, in which the first recombinant fusion protein comprises a receptor tyrosine kinase-like orphan receptor 1 (ROR1) specific antigen binding molecule comprising an amino acid sequence represented by the formula (I):

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

in which:

CDR1 is a CDR sequence having an amino acid sequence selected from the group consisting of GTRYGLYS (SEQ ID NO: 25), GTRYGLYSS (SEQ ID NO: 26), DTRYALYS (SEQ ID NO: 27), DTRYALYSS (SEQ ID NO: 28), GTKYGLYA (SEQ ID NO: 29), GTKYGLYAS (SEQ ID NO: 30) and DTSYGLYS (SEQ ID NO:207);

FW1 is a framework region;

FW2 is a framework region;

HV2 is a hypervariable sequence having an amino acid sequence selected from the group consisting of SSDEERISIS (SEQ ID NO: 31), STDEERISIG (SEQ ID NO: 32), SPNKDRMIIG (SEQ ID NO: 33), STDKERIIIG (SEQ ID NO: 34) and TTDWERMSIG (SEQ ID NO:208);

FW3a is a framework region;

HV4 is a hypervariable sequence having an amino acid sequence selected from the group consisting of NKGTK (SEQ ID NO: 35), NKGSK (SEQ ID NO: 36), NNGTK (SEQ ID NO: 37), NNRSK (SEQ ID NO: 38) and NKGAK (SEQ ID NO:209);

FW3b is a framework region;

CDR3 is a CDR sequence having an amino acid sequence according to REARHPWLRQWY (SEQ ID NO: 39);

FW4 is a framework region,

and in which the first antigen binding molecule is fused to a first fragment of an immunoglobulin Fc region engineered to dimerize with a second fragment of an immunoglobulin Fc region;

and

(b) a second recombinant fusion protein, in which the second recombinant fusion protein comprises an epidermal growth factor receptor (EGFR) specific antigen binding molecule fused to a second fragment of an immunoglobulin Fc region engineered to dimerize with the first fragment of an immunoglobulin Fc region.

4. A recombinant fusion protein dimer according to Claim 3, in which:

(a) CDR1 is DTSYGLYS (SEQ ID NO: 207), HV2 is TTDWERMSIG (SEQ ID NO: 208), HV4 is NKGAK (SEQ ID NO: 209), and CDR3 is REARHPWLRQWY (SEQ ID NO: 39); and/or

(b) FW1 is TRVDQTPRTATKETGESLTINCVLT (SEQ ID NO:220), FW2 is TSWFRKNPG (SEQ ID NO:221), FW3a is GRYVESV (SEQ ID NO: 44), FW3b is SFSLRIKDLTVADSATYYCKA (SEQ ID NO: 84) and FW4 is DGAGTVLTVN (SEQ ID NO: 48); and/or

(c) the ROR1 specific antigen binding molecule comprises SEQ ID NO: 206.

5. A recombinant fusion protein dimer according to any preceding claim,

(i) in which:

(a) the EGFR-specific antigen binding molecule comprises a single domain antibody; and/or

(b) the EGFR-specific antigen binding molecule comprises any one of SEQ ID NO: 210 to SEQ ID NO: 215, or for each sequence an amino acid sequence with

(i) at least 85% identity thereto, and/or

(ii) one, two, or three amino acid substitutions relative thereto; and/or

(c) the EGFR-specific antigen binding molecule comprises any one of SEQ ID NO: 210, SEQ ID NO: 212, SEQ ID NO: 214 or SEQ ID NO: 215; and/or

(d) the EGFR-specific antigen binding molecule comprises any one of SEQ ID NO: 210, SEQ ID NO: 212 or SEQ ID NO: 214; and/or

(e) the EGFR-specific antigen binding molecule is humanized; or

(ii) in which:

(a) the EGFR-specific antigen binding molecule comprises any one or more of SEQ ID NO: 357 to SEQ ID NO: 362; and/or

(b) the EGFR-specific antigen binding molecule comprises

(i) SEQ ID NO: 357 and SEQ ID NO: 358,

(ii) SEQ ID NO: 359,

(iii) SEQ ID NO: 360 and SEQ ID NO: 361, or

(iv) SEQ ID NO: 362.

6. A target-binding molecule-drug conjugate, comprising:

(a) a recombinant fusion protein dimer according to any preceding claim, and

(b) at least one toxin selected from the group consisting of:

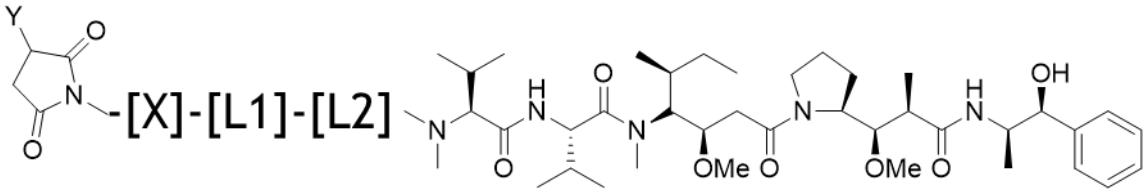
- auristatins,
- anthracyclines, preferably PNU-derived anthracyclines
- maytansinoids,
- amanitin derivatives, preferably α-amanitin derivatives
- calicheamicins,
- tubulysins
- duocarmycins
- radioisotopes - such as an alpha-emitting radionuclide, such as 227 Th and 225 Ac label
- liposomes comprising a toxic payload,
- protein toxins
- taxanes
- pyrrolbenzodiazepines and dimers thereof
- indolinobenzodiazepine pseudodimers
- spliceosome inhibitors

- CDK11 inhibitors
- nicotinamide phosphoribosyltransferase inhibitors (NAMPTi)
- Pyridinobenzodiazepines and dimers thereof
- Cyclopropapyrroloindole (CPI), cyclopropabenzindole (CBI) or cyclopropathienoindole (CTI) and optionally dimers thereof
- Irinotecan or exatecan and their derivatives.

7. A target-binding molecule-drug conjugate according to Claim 6, in which:

(i) the toxin is an aurastatin and (b) is an a MMAE derivative,

in which the target-binding molecule-drug conjugate has the structure of formula (VI):



(VI)

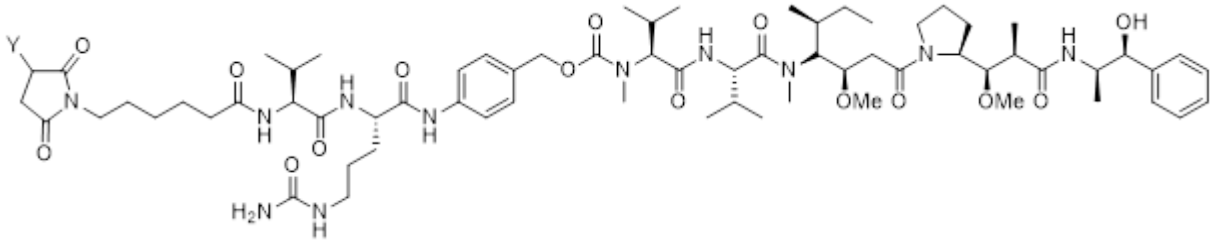
[X] is an optional spacer selected from the group comprising substituted or unsubstituted alkyl groups, substituted or unsubstituted heteroalkyl groups, substituted or unsubstituted aryl groups, substituted or unsubstituted heteroaryl groups, one or more heteroatoms, polyethylene glycol, or a combination thereof;

[L1] and [L2] are optional linkers selected from the group consisting of valine (Val), citrulline (Cit), alanine (Ala), asparagine (Asn), a peptide, -(CH2)n-, -(CH2CH2O)n-, p-aminobenzyloxycarbonyl (PAB), Val-Cit-PAB, Val-Ala-PAB, Ala-Ala-Asn-PAB, Val-Ala, Asn-Ala, any amino acid except glycine, and combinations thereof; and

Y comprises a bi-specific antigen binding molecule according to any of Claims 1 to 5; or

(ii) the toxin is an aurastatin and (b) is an a MMAE derivative,

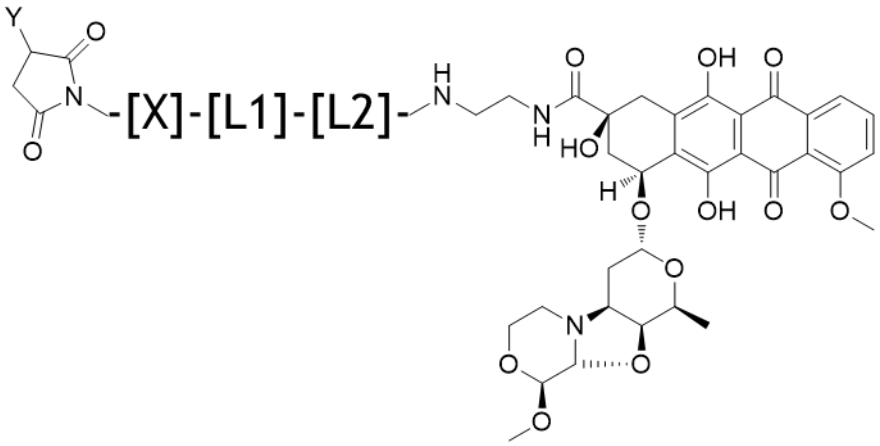
in which the target-binding molecule-drug conjugate has the structure of formula (VIII):



(VIII); or

(iii) (b) is an anthracycline (PNU) derivative, and

in which the target-binding molecule-drug conjugate has the structure of formula (III):

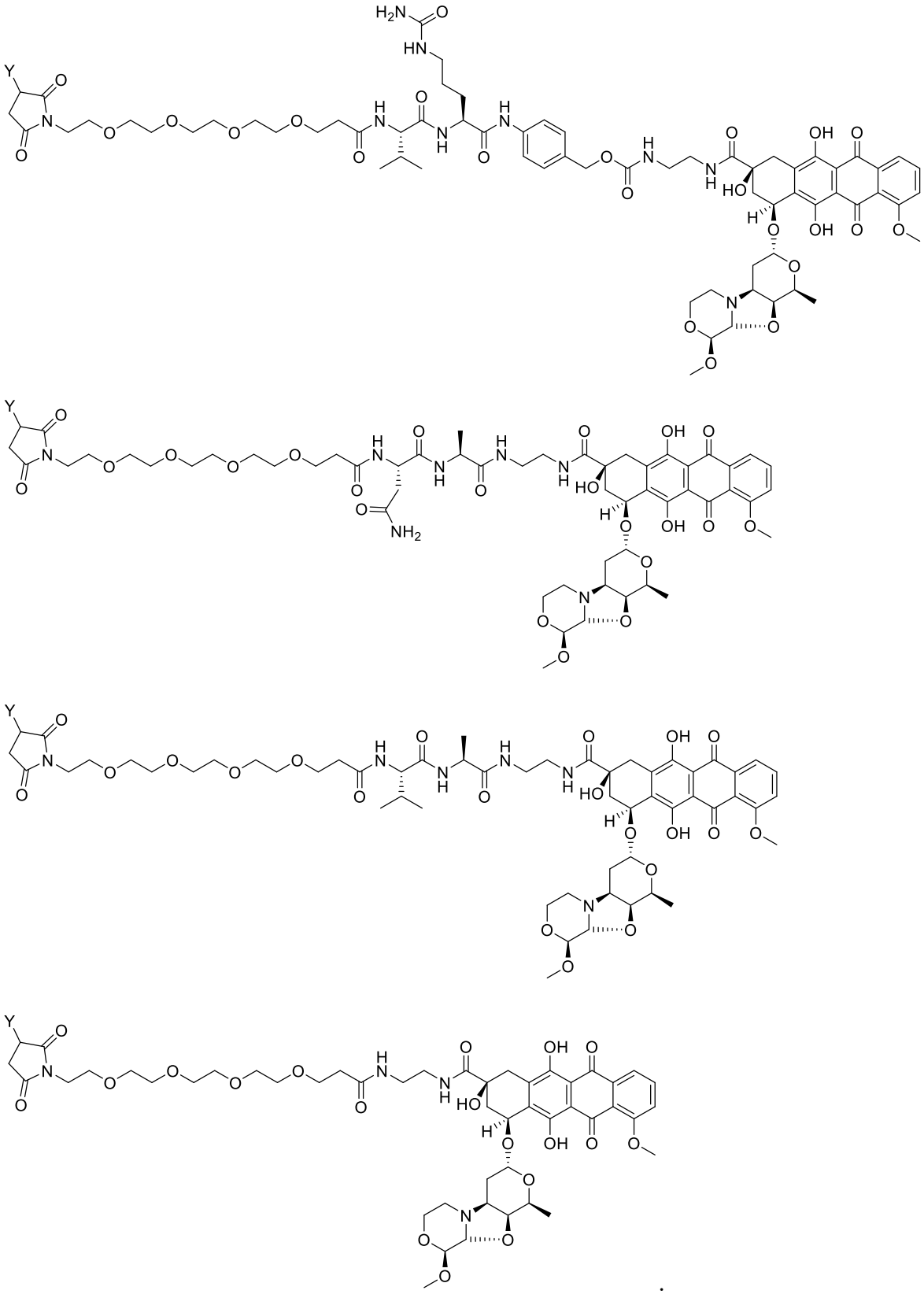


(III)

in which [X] is an optional spacer selected from the group comprising substituted or unsubstituted alkyl groups, substituted or unsubstituted heteroalkyl groups, substituted or unsubstituted aryl groups, substituted or unsubstituted heteroaryl groups, one or more heteroatoms, polyethylene glycol, or a combination thereof;

[L1] and [L2] are optional linkers selected from the group consisting of valine (Val), citrulline (Cit), alanine (Ala), asparagine (Asn), a peptide, $-(CH_2)_n-$, $-(CH_2CH_2O)_n-$, p-aminobenzyloxycarbonyl (PAB), Val-Cit-PAB, Val-Ala-PAB, Ala-Ala-Asn-PAB, Val-Ala, Asn-Ala, any amino acid except glycine, and combinations thereof; and

Y comprises a bi-specific antigen binding molecule according to any of Claims 1 to 5, optionally in which the target-binding molecule-drug conjugate has a structure selected from:



8. A recombinant fusion protein dimer according to any of Claims 1 to 5, or a target-binding molecule-drug conjugate according to Claim 6 or 7, in which:

(a) one or more residues of the first fragment of an immunoglobulin Fc region comprise one or more amino acid substitutions suitable for knobs-in-holes (KIH) dimerization with the second fragment of an immunoglobulin Fc region, and in which one or more residues of the second fragment of an immunoglobulin Fc region comprise one or more amino acid substitutions suitable for knobs-in-holes (KIH) dimerization with the first fragment of an immunoglobulin Fc region; and/or

(b)

(i) the first fragment of an immunoglobulin Fc region comprises a T366Y substitution and the second fragment of an immunoglobulin Fc region comprises a Y407T substitution, or

(ii) the first fragment of an immunoglobulin Fc region comprises a Y407T substitution and the second fragment of an immunoglobulin Fc region comprises a T366Y substitution; and/or

(c) the first and/or the second fragment of an immunoglobulin Fc region each individually comprise an S239C and/or an S442C substitution; and/or

(d) the ROR1-specific binding molecule and/or the EGFR-specific binding molecule is fused to the hFc region via a $[G_4S]_3$ linker or a G_4S linker.

9. A recombinant fusion protein dimer or a target-binding molecule-drug conjugate according to any preceding claim, in which the first recombinant fusion protein comprises any one of SEQ ID NO: 148, 167, 188, 189, 191, 192, 197 to 199, 203 to 205, 259 to 264, 302 to 307, 315 to 320, 327 to 332, 550 to 555 and 568 to 573 and the second recombinant fusion protein comprises any one of SEQ ID NO: 265 to 276, 289 to 292, 333 to 344, 371, 372, 375, 376, 379, 380, 398, 399, 402, 403, 406, 407 and 590 to 593.

10. A recombinant fusion protein dimer or a target-binding molecule-drug conjugate according to any of Claims 1 to 8, in which the first recombinant fusion protein comprises any one of SEQ ID NO: 146, 147, 165, 166, 190, 193 to 196, 200 to 202, 253 to 258, 297 to 301, 308 to 314 321 to 326 and 556 to 567 and the second recombinant fusion protein comprises any one of SEQ ID NO 277 to 288, 293 to 296, 345 to 356, 369, 370, 373, 374, 377, 378, 396, 397, 400, 401, 404, 405, and 594 to 597.

11. A recombinant fusion protein dimer or a target-binding molecule-drug conjugate according to any preceding claim, in which the recombinant fusion protein dimer or target-binding molecule-drug conjugate comprises any of SEQ ID NO: 369, 373, 377, 396, 400, 404, 371, 375, 379, 398, 402 and 406 and the recombinant fusion protein dimer or target-binding molecule-drug conjugate further comprises SEQ ID NO: 360.

12. A recombinant fusion protein dimer or a target-binding molecule-drug conjugate according to any of

(a) Claims 1 to 9, in which:

(i) the first recombinant fusion protein comprises any one of SEQ ID NO: 148, 191, 192, 197, 198, 199, 302, 303, 304, 305, 306 and 307 and the second recombinant fusion protein comprises any one of SEQ ID NO: 265 to 268 and 333 to 336; or

(ii) the first recombinant fusion protein comprises any one of SEQ ID NOs: 327 to 332, and 550 to 555 and the second recombinant fusion protein comprises any one of SEQ ID NOs: 269, 270, 271, 272, 337, 338, 339, 340, 379, 380, 406 and 407; or

(iii) the first recombinant fusion protein comprises any one of SEQ ID NO: 259 to 264 and 315 to 320 and the second recombinant fusion protein comprises any one of SEQ ID NO: 273 to 276 and 341 to 344; or

(b) Claims 1 to 8 and 11, in which:

(i) the first recombinant fusion protein comprises any one of SEQ ID NO: 146, 147, 193, 194, 195, 196, 297, 298, 299, 300, 301 and 308 and the second recombinant fusion protein comprises any one of SEQ ID NO: 277 to 280 and 345 to 348; or

(ii) the first recombinant fusion protein comprises any one of SEQ ID NOs: 321 to 326, and 556 to 561 and the second recombinant fusion protein comprises any one of SEQ ID NOs: 281, 282, 283, 284, 349, 350, 351, 352, 377, 378, 404 and 405; or

(iii) the first recombinant fusion protein comprises any one of SEQ ID NO: 253 to 258 and 309 to 314 and the second recombinant fusion protein comprises any one of SEQ ID NO: 285 to 288 and 353 to 356.

13. A pharmaceutical composition comprising a recombinant fusion protein dimer or a target-binding molecule-drug conjugate according to any preceding claim.

14. A recombinant fusion protein dimer or a target-binding molecule-drug conjugate according to any of Claims 1 to 12, or a pharmaceutical composition according to Claim 13, for use in therapy.

15. A recombinant fusion protein dimer or a target-binding molecule-drug conjugate according to any of Claims 1 to 12, or a pharmaceutical composition according to Claim 13, for use in the treatment of cancer, optionally in which the cancer is:

(a) a ROR1-positive cancer type and/or an EGFR-positive cancer type, or

(b) selected from the group consisting of 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, oesophageal cancer, stomach cancer or liver cancer.