

[DESCRIPTION]

[TITLE]

BI-SPECIFIC BINDING AGENTS TARGETING SYNDECAN-1 AND  
FIBROBLAST GROWTH FACTOR RECEPTOR

5 [TECHNICAL FIELD]

[0001]

Embodiments of the invention relate to a bi-specific binding agent comprising an antibody portion that binds specifically to CD138 (Syndecan-1), and a Fynomer portion that binds specifically to a Fibroblast Growth Factor Receptor 3 (FGFR3), as  
10 well as drug conjugates thereof, compositions thereof and uses thereof. The bi-specific binding agents disclosed herein can be used alone or in combination with other agents to effectively treat a neoplasm.

[BACKGROUND ART]

[0002]

15 The syndecan family includes four transmembrane heparan sulfate proteoglycans (HSPGs) mainly present on the cell surface. The structures of these different syndecans show high homology in vertebrates and invertebrates. All four syndecans are built up of a core protein decorated with varying number of glycosaminoglycan (GAG) side chains. Syndecans exert their function mainly through  
20 these GAG chains, but the different domains of the core protein have distinct roles as well. Syndecan-1 and syndecan-3 carry both heparan sulfate (HS) and chondroitin sulfate (CS) chains, whereas syndecan-2 and syndecan-4 carry only HS chains. Syndecans are involved in a wide range of biological processes including growth and differentiation, cell spreading, cell adhesion, cell migration, cytoskeletal organization,  
25 infiltration, and angiogenesis.

[0003]

Syndecan-1 is a transmembrane (Type 1) heparan sulfate proteoglycan comprising an N-terminal extracellular domain, a transmembrane domain and a C-terminal intracellular signaling domain. In humans syndecan-1 (CD138) comprises  
30 a core protein of 310 amino acids in length and is encoded by the SDC1 gene. The SDC1 gene consists of five exons and is located on human chromosome 2. The first

exon encodes a signal peptide, the second exon encodes attachment sites for heparan sulfate, the third and fourth exons encode a site for chondroitin sulfate binding and the fifth exon encodes the transmembrane and cytoplasmic domains.

[0004]

5           Syndecan-1 is expressed on the basolateral surface of epithelial cells in adult tissues, on mesenchymal cells during development, and on lymphoid cells during distinct stages of differentiation. Syndecan-1 can bind hepatocyte growth factor (HGF), can interact with various growth factors and act as a coreceptor resulting in the activation of multiple signaling pathways effecting cell migration, cell-matrix  
10 interactions, growth, proliferation and survival. Several studies have implicated syndecan-1, and/or its dysfunctional signaling activity or over-expression in the pathogenesis of various neoplasms.

[0005]

15           Fibroblast Growth Factor Receptors (FGFRs) are a family of highly conserved transmembrane tyrosine kinase receptors that are involved in various intracellular signaling pathways. FGFRs are composed of an extracellular ligand binding region comprising two to three immunoglobulin-like domains (D1, D2 and D3), a single-pass transmembrane region, and a cytoplasmic region having tyrosine kinase activity. There are 4 major FGF receptors (FGFR1-4), having multiple splice variants, most of  
20 which occur in exon III of the receptors (corresponding to domain D3) (e.g., see Holzmann et al. (2012) *J. of Nucleic Acids* 2012:950508). The D3 domain contains two parts encoded by 3 exons: IIIa, IIIb, and IIIc) and gene splicing events lead to a D3 domain transcribed from the invariant IIIa portion of the gene combined with either the IIIb or IIIc portions. These splice variations generate seven highly homologous human  
25 FGFRs: FGFR1b, FGFR1c, FGFR2b, FGFR2c, FGFR3b, FGFR3c, and FGFR4, which have distinct tissue distribution and ligand specificity.

[0006]

30           In humans, FGFRs can be activated by overexpression, or by binding one or more of the 22 known fibroblast growth factor (FGF) ligands. FGFR3 activation plays a critical role in embryogenesis, development, cell proliferation, cell survival, migration, differentiation and growth arrest. Activation of FGFR3 can lead to activation of several

key pathways implicated in oncogenic signaling, including the mitogen-activated protein kinase (MAPK) and PI3K-AKT pathways.

[0007]

Presented herein are bi-specific binding agents comprising an antibody portion  
5 that binds to syndecan-1 and a Fynomer portion that binds to an FGFR3, and uses thereof for the treatment of a neoplasm.

[SUMMARY OF THE INVENTION]

[0008]

In some aspects, presented herein is a bi-specific binding agent comprising an  
10 antibody portion (e.g., an antibody or antigen binding portion thereof) and one or more Fynomers, where the antibody portion binds specifically to syndecan-1 (CD138) and the one or more Fynomers bind specifically to a fibroblast growth factor receptor 3 (FGFR3). Accordingly, in certain embodiments, presented herein is a bi-specific binding agent that binds specifically to syndecan-1 and binds specifically to FGFR3, or  
15 one or more isoforms of FGFR3. In some embodiments, a Fynomer portion of a bi-specific binding agent binds specifically to FGFR3b and/or FGFR3c. In certain aspects, a bi-specific binding agent comprising an antibody portion (e.g., an antibody or antigen binding portion thereof) that binds specifically to syndecan-1 (CD138), a Fynomer that bind specifically to a fibroblast growth factor receptor 3 (FGFR3) and an  
20 antineoplastic agent or toxin.

[0009]

In some embodiments, an antibody portion of a bi-specific binding agent comprises one or more complementarity determining regions (CDRs) selected from (i) a CDR-L1 (light chain CDR1) comprising an amino acid sequence having at least 90%  
25 identity to an amino acid sequence selected from SEQ ID NOs:2-15, (ii) a CDR-L2 (light chain CDR2) comprising an amino acid sequence having at least 90% identity to an amino acid sequence selected from SEQ ID NOs:16-26, (iii) a CDR-L3 (light chain CDR3) comprising an amino acid sequence having at least 90% identity to an amino acid sequence selected from SEQ ID NOs:27-33, (iv) a CDR-H1 (heavy chain CDR1)  
30 comprising an amino acid sequence having at least 90% identity to an amino acid sequence selected from SEQ ID NOs:45-59, (v) a CDR-H2 (heavy chain CDR2)

comprising an amino acid sequence having at least 90% identity to an amino acid sequence selected from SEQ ID NOs:60-71, and (vi) a CDR-H3 (heavy chain CDR3) comprising an amino acid sequence having at least 90% identity to an amino acid sequence selected from SEQ ID NOs:72-81, wherein the antibody, or binding portion thereof, binds specifically to human syndecan-1. An antibody portion of a bi-specific binding agent may comprise any suitable CDR-L1 selected from Table 1, any suitable CDR-L2 selected from Table 2, any suitable CDR-L3 selected from Table 3, any suitable CDR-H1 selected from Table 6, any suitable CDR-H2 selected from Table 7 and any suitable CDR-H3 selected from Table 8, wherein the antibody, or binding portion thereof, binds specifically to human syndecan-1. In some embodiments, an antibody portion of a bi-specific binding agent comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO:2, a CDR-L2 comprising the amino acid sequence of SEQ ID NO:18, a CDR-L3 comprising the amino acid sequence of SEQ ID NO:28, a CDR-H1 comprising the amino acid sequence of SEQ ID NO:47, a CDR-H2 comprising the amino acid sequence of SEQ ID NO:60, and a CDR-H3 comprising the amino acid sequence of SEQ ID NO:73. In some embodiments, an antibody, or binding portion thereof, comprises a CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2 and CDR-H3 comprising the amino acid sequences of SEQ ID NOs:2, 17, 27, 47, 61 and 73, respectively. In some embodiments, an antibody, or binding portion thereof, comprises a CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2 and CDR-H3 comprising the amino acid sequences of SEQ ID NOs:2, 16, 27, 45, 60 and 72, respectively. In some embodiments, an antibody, or binding portion thereof, comprises a CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2 and CDR-H3 comprising the amino acid sequences of SEQ ID NOs:2, 16, 27, 45, 61 and 72, respectively. In some embodiments, an antibody, or binding portion thereof, comprises a CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2 and CDR-H3 comprising the amino acid sequences of SEQ ID NOs:2, 17, 27, 47, 61 and 73, respectively. In some embodiments, an antibody, or binding portion thereof, comprises a CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2 and CDR-H3 comprising the amino acid sequences of SEQ ID NOs:5, 21, 30, 50, 64 and 75 respectively. In some embodiments, an antibody, or binding portion thereof, comprises a CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2

and CDR-H3 comprising the amino acid sequences of SEQ ID NOs:4, 20, 29, 50, 63 and 75, respectively. In some embodiments, an antibody, or binding portion thereof, comprises a CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2 and CDR-H3 comprising the amino acid sequences of SEQ ID NOs: 4, 19, 29, 48, 63 and 74, respectively. In some embodiments, an antibody portion of a bi-specific binding agent comprises one or more constant regions of a human antibody (e.g., an IgG). In some embodiments, an antibody portion of a bi-specific binding agent comprises a humanized monoclonal antibody, or humanized antigen binding portion thereof, where the variable region sequences are humanized.

10 [0010]

In some embodiments, a Fynomer of a bi-specific binding agent comprises a polypeptide selected from a polypeptide having an amino acid sequence at least 80% identical to the amino acid sequence of (i) GVTLFVALYDYEYVGPTPMLSFHKGEKFQIL(X<sub>1</sub>)(X<sub>2</sub>)(X<sub>3</sub>)(X<sub>4</sub>)GPYWEARSL(X<sub>5</sub>) TGETG(X<sub>6</sub>)IPSNYVAPVDSIQ (SEQ ID NO:99), wherein the amino acids (X<sub>1</sub>), (X<sub>2</sub>), (X<sub>3</sub>), (X<sub>4</sub>), (X<sub>5</sub>), and (X<sub>6</sub>) are any amino acid, (ii) GVTLFVALYDYEVMSTTALSFKGEKFQILSQSPHGQYWEARSLTTGETG(X<sub>7</sub>)IPSNYVAPVDSIQ (SEQ ID NO:113), where (X<sub>7</sub>) is any amino acid, (iii) GVTLFVALYDYEYVGPTPMLSFHKGEKFQILNSSEGPGYWEARSLTTGETGLIPSNYVAPVDSIQ (SEQ ID NO:101; FF2L4C3), (iv) GVTLFVALYDYEYVGPTPMLSFHKGEKFQILRGGQGPYWEARSLTTGETGLIPSNYVAPVDSIQ (SEQ ID NO:103; FF44L65G12), (v) GVTLFVALYDYEYVGPTPMLSFHKGEKFQILRGGDGPYWEARSLTTGETGLIPSNYVAPVDSIQ (SEQ ID NO:105; FF44L65G7), (vi) GVTLFVALYDYEYVGPTPMLSFHKGEKFQILKGGSGPYWEARSLTTGETGLIPSNYVAPVDSIQ (SEQ ID NO:107; FF48L66G7; "G7"), (vii) GVTLFVALYDYEYVGPTPMLSFHKGEKFQILRKGGKGPYWEARSLATGETGLIPSNYVAPVDSIQ (SEQ ID NO:109; FF43L65D5), (viii) GVTLFVALYDYEYVGPTPMLSFHKGEKFQILRRGSGPYWEARSLTTGETGLIPSNYVAPVDSIQ (SEQ ID NO:111; FF44L65B7), and (ix)

GVTLFVALYDYEVMSSTALS~~FS~~HKGEKFQILSQSPHGQYWEARSLTTGETGWIPS  
 NYVAPVDSIQ (SEQ ID NO:116; FF40L54A5).

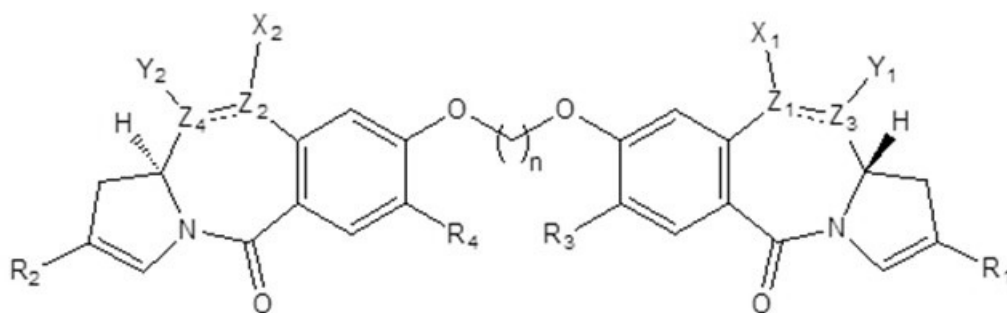
[0011]

In some embodiments, a Fynomer of a bi-specific binding agent comprises or  
 5 consists of a polypeptide having an amino acid sequence at least 80%, at least 85%, at  
 least 90%, at least 95% or 100% identical to the amino acid sequence of SEQ ID NO:  
 107. In some embodiments, a Fynomer of a bi-specific binding agent comprises or  
 consists of a polypeptide having the amino acid sequence of SEQ ID NO: 107.

[0012]

10 In some embodiments, a bi-specific agent comprises an anti-neoplastic agent  
 selected from the group consisting of a dolastatin, an auristatin, a maytansine, a  
 tubulysin, a calicheamicin, a pyrrolobenzodiazepine (PBD), a duocarmycin, a  
 doxorubicin, a pseudomonas exotoxin-A (PE38), an irinotecan and derivatives thereof.  
 In certain embodiments, a bi-specific binding agent comprises a pyrrolobenzodiazepine  
 15 toxin comprising the structure of chemical formula I:

[Chem. 1.]



( I )

wherein

Z<sub>1</sub> and Z<sub>2</sub> are both N;

20 Z<sub>3</sub> and Z<sub>4</sub> are both C;

[Chem. 2.]

the double-dash lines  $\equiv$  represent a single bond or a double bond;

n is 1 to 10;

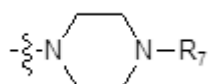
each of  $R_3$  and  $R_4$  are independently H, or a  $C_{1-4}$  alkoxy; and

each of  $R_1$  and  $R_2$  are independently selected from the group consisting of H,  $C_{1-5}$  alkyl,  $C_{3-6}$  cycloalkyl,  $C_{2-5}$  alkenyl, and a phenyl optionally substituted with  $R_5$ , wherein

$R_5$  is selected from the group consisting of  $-NH_2$ ,  $-NHR_6$ , and a piperazinyl substituted

5 with  $R_7$  having the structure

[Chem. 3.]



wherein  $R_6$  comprises the linking group, and

$R_7$  is H, or a  $C_{1-5}$  alkyl;

10  $X_1$  is null, a protecting group, or comprises the linking group;

$X_2$  is null, a protecting group, or comprises the linking group;

only one of  $X_1$ ,  $X_2$ ,  $R_1$ , and  $R_2$  comprises the linking group; and

each of  $Y_1$  and  $Y_2$  are independently either null, OH, or  $SO_3H$ ;

provided that:

15 [Chem. 4.]

(i) when  $X_1$  comprises the linking group,  $Z_1 \text{-----} Z_3$  is N-C,

(ii) when  $X_2$  comprises the linking group,  $Z_2 \text{-----} Z_4$  is N-C,

(iii) when  $X_1$  is the protecting group,  $Z_1 \text{-----} Z_3$  is N-C, and

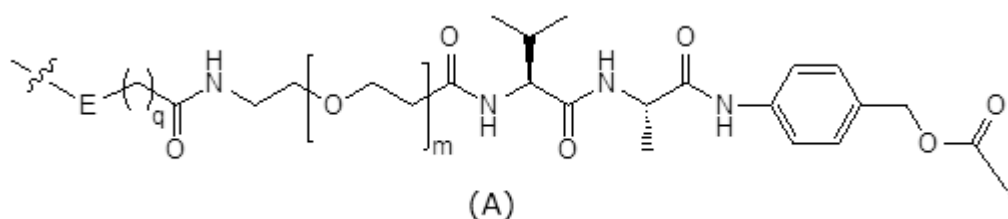
(iv) when  $X_2$  is the protecting group,  $Z_2 \text{-----} Z_4$  is N-C

wherein null means the absence of the moiety or the presence of one or more hydrogens to complete a required valence.

[0013]

20 In certain embodiments, an anti-neoplastic agent comprises the pyrrolobenzodiazepine toxin of formula I and a linking group, where the pyrrolobenzodiazepine toxin is attached to the linking group, and the linking group is attached to a bi-specific agent described herein. In certain embodiments, the linking group comprises a structure selected from the structure of chemical formula (A):

25 [Chem. 5.]



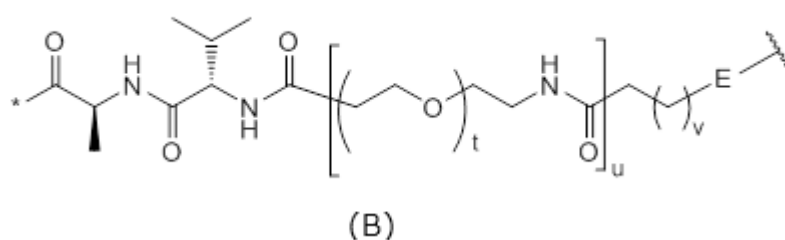
wherein the asterisk indicates the point of attachment to a pyrrolobenzodiazepine toxin;  
the wavy line indicates the point of attachment to the binding agent;

m is 1 to 20;

5 q is 1 to 10; and

E is a connecting group, and the structure of chemical formula (B):

[Chem. 6.]



wherein

10 the asterisk indicates the point of attachment to the pyrrolobenzodiazepine toxin;

the wavy line indicates the point of attachment to the binding agent;

E comprises a connecting group;

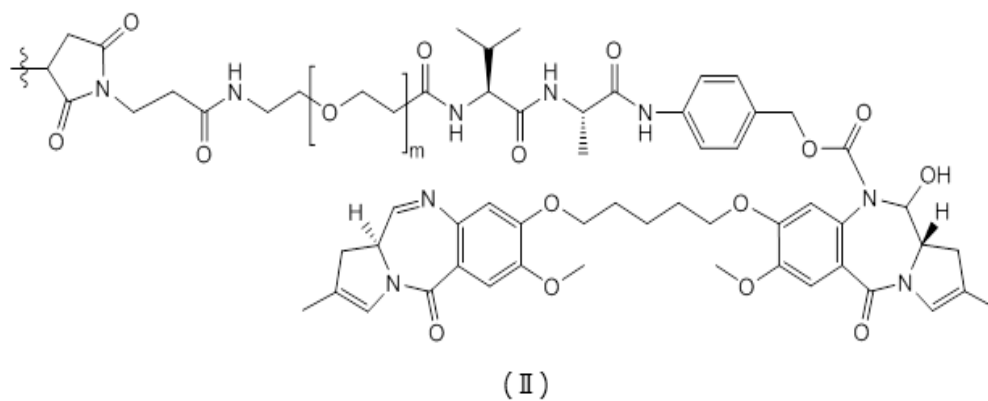
v is 0 to 10; and

u is 0 or 1; wherein when u is 1, t is 1 to 10.

15 [0014]

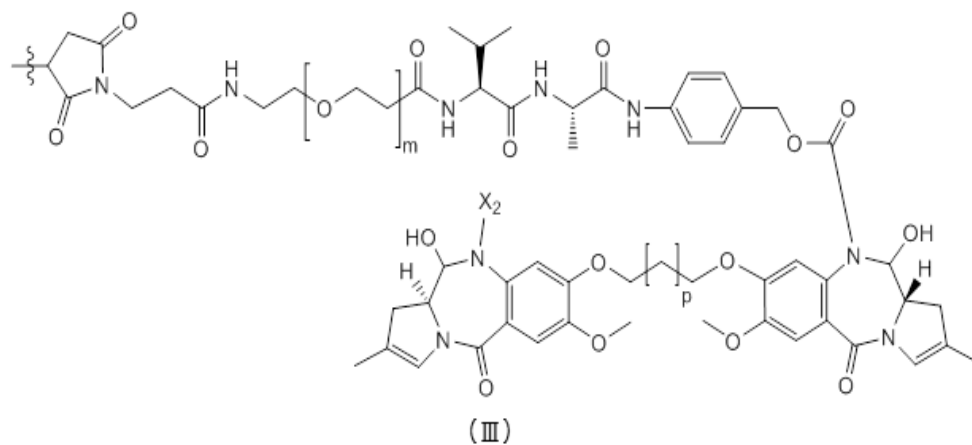
In some embodiments, an anti-neoplastic agent comprises a structure selected from the group consisting of formula II below;

[Chem. 7.]



wherein  $m$  is 8; formula III below:

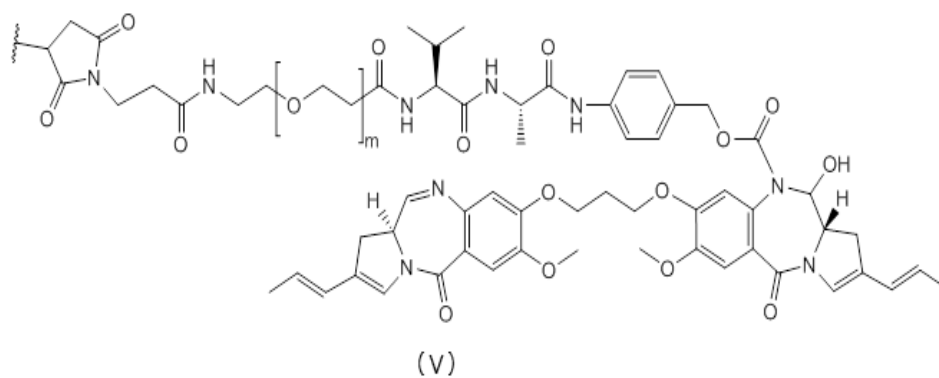
[Chem. 8.]



5

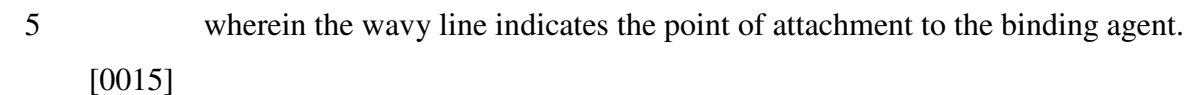
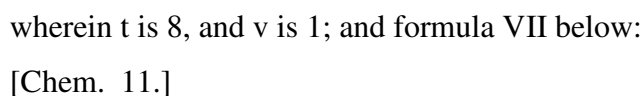
wherein  $m$  is 8,  $p$  is 3, and  $X_2$  is a protecting group; formula V below:

[Chem. 9.]



wherein  $m$  is 8, formula VI below:

[Chem. 10.]



10 [0016]

In some aspects, presented herein is a method of treating a subject having, or suspected of having, a neoplasm comprising administering to the subject a therapeutically effective amount of the bi-specific binding agent or pharmaceutical composition described herein. In certain embodiments, the neoplasm is selected from a carcinoma, sarcoma, neuroblastoma, glioblastoma, myeloma, lymphoma, melanoma or a solid or soft tissue tumor, where the neoplasm expresses syndecan-1 and/or an FGFR3. In some embodiments, a subject having, or suspected of having a neoplasm,

is a human having or harboring a neoplastic cell that expresses human syndecan-1 and/or human FGFR3 on the cell surface of the neoplastic cell.

[0017]

Certain aspects of the technology are described further in the following  
5 description, examples, claims and drawings.

[BRIEF DESCRIPTION OF THE DRAWINGS]

[0018]

The drawings illustrate embodiments of the technology and are not limiting.  
For clarity and ease of illustration, the drawings are not made to scale and, in some  
10 instances, various aspects may be shown exaggerated or enlarged to facilitate an understanding of particular embodiments.

[0019]

[Fig. 1]

Fig. 1 shows an alignment of syndecan-1 proteins derived from human,  
15 cynomolgus monkey (cyno) and mouse. Peptides used for immunization were derived from the boxed areas.

[0020]

[Fig. 2]

Fig. 2 shows Fluorescence-activated cell sorting (FACS) histograms for  
20 F12P16F6 (FIG. 2A) and another representative positive hybridoma clone F12P16G3 (FIG. 2B) binding to CD138 on H929 cells.

[0021]

[Fig. 3]

Fig. 3 shows the results of kinetic binding analysis (i.e., a SPR sensorgram) for  
25 a representative hybridoma F12P16F6 (indicated in the figure as “12P16F6”) for binding to human CD138 at 167 nM (L1A1) to 10.4 nM (L1A4).

[0022]

[Fig. 4]

Fig. 4 shows binding of representative chimeric antibodies 12P16F6 hIgG1  
30 (“chF6”, Fig. 4A) and 13P30A7 hIgG1 (“chP30A7”, Fig. 4B) to human CD138 expressing cells (human) and cynomolgus CD138 expressing cells (cyno). Control

antibody (“Sec”, i.e., Secukinumab) showed little or no specific binding to CD138. Y-axis represents the concentration of the antibodies chF6, 13P30A7 or Secukinumab

[0023]

[Fig. 5A]

5            Fig. 5A shows the humanized heavy chains compared to those of the parent mouse F12P16F6 (“P16F6 VH”). The designation cdr indicates the CDR grafting approach, the designation abb indicates the Grafting of abbreviated CDRs approach, the designation sdr indicates the SDR-transfer approach, the designation fra indicates The Frankenstein approach, and the designation ven indicates the Veneering approach.

10          The designation repair indicates that the variable regions were subjected to a second round of humanization.

[Fig. 5B]

            Fig. 5B shows the humanized light chains compared to those of the parent mouse F12P16F6 (“P16F6 VL”).

15          [0024]

[Fig. 6]

            Fig. 6 shows a picture of an SDS-PAGE gel ran under reducing conditions illustrating the molecular weight (kilodaltons, kDa) and purity of 11 representative humanized antibodies. Lane 1= 12P16F6 hIgG1 (chF6), Lane 2=F6 aka-rep, Lane

20          3=F6 aks-rep, Lane 4=F6 akf-rep, Lane 5=F6 cka-rep, Lane 6=F6 ckf-rep (hF6), Lane 7=F6 f2ka-rep, Lane 8=F6 f2ks-rep, Lane 9=F6 f2kf-rep, Lane 10=F6 f1ka-rep, Lane 11=F6 f1ks-rep, Lane 12=F6 f1kf-rep, and MW=molecular weight marker. Molecular weight markers are labeled to the right of the gel.

[0025]

25          [Fig. 7]

            Fig. 7 shows FACS analysis of cell-surface binding of eleven representative humanized antibodies to human CD138 on the surface of multiple myeloma cell line KMS-11 (Fig. 7B) and bladder cancer line RT112/84 (Fig. 7A). Secukinumab was used as a negative control.

30          [0026]

[Fig. 8]

Fig. 8 shows an illustration of an X-ray crystal structure derived from a human syndecan-1 peptide in complex with an antibody Fab fragment that was solved at 1.95 Å resolution. There is one copy each of the syndecan-1 peptide and Fab per asymmetric unit. Fig. 8 illustrates the syndecan-1-Fab binding interface. The Fab Heavy chain is shown in the form of ribbon side chain carbon atoms to the left of the figure. The Fab light chain is shown in the form of ribbon side chain carbon atoms to the right of the figure. The syndecan-1 peptide carbon atoms are shown sandwiched between the Fab heavy and light chains. Certain amino acids of the syndecan-1 peptide and certain side chains of the Fab fragment are labeled with their corresponding 3-letter amino acid abbreviation and positions.

[0027]

[Fig. 9]

Fig. 9 shows an alignment of the anti-FGFR3 Fynomer of SEQ ID NOs:101, 109, 103, 105, 107 and 111.

[0028]

[Fig. 10]

Fig. 10 shows internalization properties of anti-FGFR3 Fynomers designated as FF2L4C3 (SEQ ID NO:101); FF2L4D4; FF3L6G2; FF5L7D3; FF5L7D4; FF15L31B1 and FynSH3 (negative control).

[0029]

[Fig. 11]

Fig. 11 shows a FACS binding profile of Fynomer polypeptides specifically binding to FGFR3 on the surface of FGFR3 positive KMS-11 cells. Fig. 11A shows specific binding of FF2L4C3 (SEQ ID NO:101); FF43L65D5 (SEQ ID NO:109); FF44L65B7 (SEQ ID NO:111); FF44L65G7 (SEQ ID NO:105); FF44L65G12 (SEQ ID NO:103); FF48L66G7 (SEQ ID NO:107) and an anti-FGFR3 monoclonal antibody (positive control) to FGFR3-positive KMS-11 cells, while Fig. 11B shows no binding of the indicated Fynomers to an FGFR3 negative control cell line N87.

[0030]

[Fig. 12-1]

Fig. 12-1 shows an ELISA of FGFR3-Fynomers FF2L4C3-SEQ ID NO:101 (Fig. 12A) and FF43L65D5 - SEQ ID NO:109 (Fig. 12B) binding to plated coated human FGFR3b (huFGFR3b), human FGFR3c (huFGFR3c), cynomolgus monkey FGFR3c (cyFGFR3c), murine FGFR3c (muFGFR3c), human FGFR3 D1 domain (huFGFR3-D1), human FGFR3c D2 domain (huFGFR3-D2), human FGFR3 D1 and D2 domain (huFGFR3-D1D2), a negative control polyclonal antibody (IgG) and uncoated plates (PBS), as indicated on the x-axis.

[Fig. 12-2]

Fig. 12-2 shows an ELISA of FF44L65B7 - SEQ ID NO:111 (Fig. 12C) and FF44L65G7 - SEQ ID NO:105 (Fig. 12D) binding to plated coated human FGFR3b (huFGFR3b), human FGFR3c (huFGFR3c), cynomolgus monkey FGFR3c (cyFGFR3c), murine FGFR3c (muFGFR3c), human FGFR3 D1 domain (huFGFR3-D1), human FGFR3c D2 domain (huFGFR3-D2), human FGFR3 D1 and D2 domain (huFGFR3-D1D2), a negative control polyclonal antibody (IgG) and uncoated plates (PBS), as indicated on the x-axis.

[Fig. 12-3]

Fig. 12-3 shows an ELISA of FF44L65G12 - SEQ ID NO:103 (Fig. 12E), and FF48L66G7 - SEQ ID NO:107 (Fig. 12F) binding to plated coated human FGFR3b (huFGFR3b), human FGFR3c (huFGFR3c), cynomolgus monkey FGFR3c (cyFGFR3c), murine FGFR3c (muFGFR3c), human FGFR3 D1 domain (huFGFR3-D1), human FGFR3c D2 domain (huFGFR3-D2), human FGFR3 D1 and D2 domain (huFGFR3-D1D2), a negative control polyclonal antibody (IgG) and uncoated plates (PBS), as indicated on the x-axis.

[0031]

[Fig. 13]

Fig. 13 shows an internalization assay demonstrating the cytotoxic effect of toxin conjugated Fynomers FF2L4C3 (Fig. 13A, B and C), FF43L65D5 (Fig. 13B), FF44L65G7 (Fig. 13B), FF44L65G12 (Fig. 13B), FF48L66G7 (Fig. 13B) and FF44L65B7 (Fig. 13C). The mouse monoclonal antibody 9E10, which binds a cytosolic myc protein, FynSH3, and no agent (cells only) were included as negative controls.

[0032]

[Fig. 14]

Fig. 14 shows an illustration of four embodiments of a bi-specific binding agent, each comprising an antibody portion having two Ig heavy chain and two Ig light chain polypeptides, and a Fynomer portion, where the Fynomer (represented as a sphere) is attached to different parts of the antibody. Fig. 14A shows a Fynomer attached to the C-terminal end of the heavy chain of the antibody. Fig. 14B shows a Fynomer attached to the N-terminal end of the heavy chain of the antibody. Fig. 14C shows a Fynomer attached to the C-terminal end of the light chain of the antibody. Fig. 14D shows a Fynomer attached to the N-terminal end of the light chain of the antibody.

[0033]

[Fig. 15]

Fig. 15 shows four representative bi-specific binding agents that were made and subjected to SDS PAGE under reducing conditions. Lane 7 was loaded with the full length anti-CD138 monoclonal antibody P16F6, which binds specifically to syndecan-1. Lane 7 provides a reference for the molecular weight of the heavy chain (upper band) and light chain (lower band) in the absence of an attached Fynomer. Lanes 2-6 show an embodiment of a bi-specific binding agent comprising the P16F6 antibody and the FGFR3-binding Fynomer G7 (SEQ ID NO:107) attached to the N-terminus of the heavy chain of the antibody (Lanes 2 and 6), attached to the C-terminus of the heavy chain of the antibody (Lane 4), attached to the N-terminus of the light chain of the antibody (Lane 5) and attached to the C-terminus of the light chain of the antibody (Lane 3). The increase in molecular weight of the heavy chains in lanes 2, 4 and 6 is evidence of the presence of the attached Fynomer (compare to heavy chain in control lane 7). The increase in molecular weight of the light chains in lanes 3 and 5 is evidence of the presence of the attached Fynomer (compare to light chain in control lane 7).

[0034]

[Fig. 16]

Fig. 16 shows a FACs histogram showing cell surface binding of the representative bi-specific binding agent "hF6-HN-G7" and humanized monoclonal

antibody “hF6” to FGFR3c (Fig. 16A) and FGFR3b (Fig. 16B) expressed on the surface of transfected CHO cells. hF6 comprises the light chain of SEQ ID NO:44 and the heavy chain of SEQ ID NO:93. hF6-HN-G7 comprises the light chain of SEQ ID NO:44 and a fusion protein that includes the Fynomer of SEQ ID NO:107 (G7) and the heavy chain of SEQ ID NO:93, where the C-terminus of G7 is connected to the N-terminus of the heavy chain by a peptide bond.

[0035]

[Fig. 17]

Fig. 17 shows an in vitro cytotoxicity assay conducted on untransfected CHO cells (Fig. 17A), CHO cells expressing FGFR3b (Fig. 17B) and CHO cell expressing FGFR3c (Fig. 17C). Cell viability is shown on the y-axis and concentration of the binding agents is shown on the x-axis. The agents tested include Secukinumab (a monoclonal antibody that specifically binds to interleukin-17A) that was stochastically conjugated to the PBD toxin of formula II (Secukinumab-II), a Secukinumab fusion expressed with the G7 Fynomer at the heavy chain N-terminus and site specifically conjugated to the PBD toxin of formula II (Secukinumab-S119C-HN-G7-II), and the representative bi-specific binding agent hF6-S119C-HN-G7 that was site specifically conjugated to the PBD toxin of formula II (hF6-S119C-HN-G7-II). The hF6 antibody portion of the bi-specific binding agent hF6-S119C-HN-G7 comprises the light chain of SEQ ID NO:44 and the heavy chain of SEQ ID NO:93. Accordingly, hF6-S119C-HN-G7 is an hF6 antibody where the Fynomer of SEQ ID NO:107 (G7) and the heavy chain of SEQ ID NO:93 are expressed as a fusion protein where the C-terminus of G7 is connected to the N-terminus of the heavy chain by a peptide bond. S119C indicates that Serine at position 119 of the heavy chain constant region of hF6 was mutated to a Cysteine for covalent attachment of the PBD toxin (i.e., the anti-neoplastic agent). The representative PBD toxin used in this experiment was the toxin of formula II. The toxin was covalently attached to the sulfhydryl group of the cysteine at position 119 using maleimide chemistry.

[0036]

[Fig. 18]

Fig. 18 shows an exemplary anti-neoplastic agent of formula II.

[0037]

[Fig. 19]

Fig. 19 shows an in vitro cytotoxicity assay conducted on cell lines KMS-11 (Fig. 19A), OPM-2 (Fig. 19B) and ARH-77 (Fig. 19C) with a full length hF6 anti-CD138 monoclonal antibody conjugated stochastically to the anti-neoplastic agent of formula II (“hF6-II”), hF6-HN-G7 conjugated stochastically to the anti-neoplastic agent of formula II (hF6-HN-G7-II), and hF6-LN-G7 conjugated stochastically to the anti-neoplastic agent of formula II (hF6-LN-G7-II).

[0038]

10 [Fig. 20-1]

Fig. 20-2 shows an in vitro cytotoxicity assay conducted on the cell lines KMS-11 (Fig. 20A) and RT-112 (Fig. 20B) with hF6-T289C-HN-G7 site-specifically conjugated to the anti-neoplastic agent of formula II (hF6-T289C-HN-G7-II), hF6-S119C-HN-G7 site-specifically conjugated to the anti-neoplastic agent of formula II (hF6-S119C-HN-G7-II), hF6-V282C-HN-G7 site-specifically conjugated to the anti-neoplastic agent of formula II (hF6-V282C-HN-G7-II, Fig. 20C and 20D only), hF6-II (Fig. 20A and 20B only) and control antibody Secukinumab that was conjugated to the PBD toxin of formula II (Secukinumab-II).

[Fig. 20-2]

20 Fig. 20-2 shows an in vitro cytotoxicity assay conducted on the cell lines AN3CA (Fig. 20C) and HCC1806 (Fig. 20D) with hF6-T289C-HN-G7 site-specifically conjugated to the anti-neoplastic agent of formula II (hF6-T289C-HN-G7-II), hF6-S119C-HN-G7 site-specifically conjugated to the anti-neoplastic agent of formula II (hF6-S119C-HN-G7-II), hF6-V282C-HN-G7 site-specifically conjugated to the anti-neoplastic agent of formula II (hF6-V282C-HN-G7-II, Fig. 20C and 20D only), hF6-II (Fig. 20A and 20B only) and control antibody Secukinumab that was conjugated to the PBD toxin of formula II (Secukinumab-II).

[0039]

[Fig. 21A]

Fig. 21A shows the graphical results of an in vivo xenograft study testing the ability of the indicated bi-specific binding agents to kill or inhibit the growth of AN3CA cells (Fig.21A) implanted in a mouse.

[Fig. 21B]

5            Fig. 21B shows the graphical results of an in vivo xenograft study testing the ability of the indicated bi-specific binding agents to kill or inhibit the growth of HCC1807 cells implanted in a mouse.

#### [DESCRIPTION OF EMBODIMENTS]

10    [0040]

Many neoplastic cells express a Fibroblast Growth Factor Receptor 3 (FGFR3, including one or more isoforms thereof) and syndecan-1 (CD138) on their cells surface. Presented herein are bi-specific binding agents that bind to syndecan-1 and an FGFR3 with high affinity. It was determined that these bi-specific binding agents can bind  
15 with high affinity and high selectivity to neoplastic cells that express both of the target receptors. In some embodiments, the bi-specific binding agents described herein induce internalization of the binding agent upon binding to one or both of the target receptors (i.e., CD138 and FGFR3). Further, by conjugating the bi-specific binding agents to a toxic payload, the bi-specific binding agents can specifically kill neoplastic  
20 cells that express one or both of the target receptors. Also, off-target cytotoxicity can be significantly reduced by incorporating a protease cleavable linking group between the toxic payload and the bi-specific binding agent, such that the toxin is release from the binding agent only after internalization and cleavage of the linking group by a cytosolic protease. The bi-specific binding agents herein represent a next generation  
25 of biologics that offer more efficient and selective killing of neoplastic cells while reducing adverse events.

[0041]

In some embodiments, a bi-specific binding agent presented herein comprises an antibody portion (e.g., an antibody, or antigen binding portion thereof) and a  
30 Fynomer portion (i.e., a Fynomer), where the Fynomer is attached to the antibody portion. In some embodiments, the Fynomer is attached to the antibody portion by a

covalent bond. In some embodiments, the antibody portion comprises an antibody, or antigen binding portion thereof, that binds specifically to syndecan-1 (i.e., a syndecan-1 polypeptide; e.g., CD138), or an extracellular portion thereof. In certain embodiments, the Fynomer portion comprises a Fynomer that binds specifically to an FGFR3 (e.g.,  
 5 FGFR3, or one or more isoforms thereof). In some embodiments, the bi-specific binding agent comprises a cytotoxic payload.

[0042]

In some embodiments the bi-specific binding agents presented herein are used for the treatment, prevention and/or diagnosis of a neoplasm in a subject.

10 [0043]

#### Syndecans

Human syndecan-1 (e.g., SEQ ID NO:1) generally comprises an immature polypeptide sequence of 310 amino acids which includes an N-terminal single sequence from amino acids 1-22, an extracellular domain from about amino acid 23-254, a  
 15 transmembrane domain from about amino acid 255 to 275 and a cytoplasmic domain from about amino acid 276 to 310, numbered from the N-terminus to the C-terminus. Methods of identifying leader sequences, extracellular domains, transmembrane domains, and cytoplasmic domains of a syndecan-1 receptor are known and any suitable method can be used to identify such domains or regions within a syndecan-1  
 20 polypeptide sequence derived from a suitable mammalian species.

[0044]

In some embodiments syndecan-1 is a mammalian syndecan-1. A syndecan-1 may be derived from any mammalian species. In some embodiments, a syndecan-1 polypeptide is a human syndecan-1. In certain embodiments, an extracellular domain  
 25 of syndecan-1 comprises an N-terminal portion of a syndecan-1 polypeptide that is typically expressed on the cell surface of an intact mammalian cell. In certain embodiments an extracellular domain of syndecan-1 is expressed in a soluble or a non-membrane bound form that lacks a cytoplasmic and/or transmembrane domain. In certain embodiments syndecan-1 and/or the extracellular domain of syndecan-1  
 30 comprises one or more amino acid additions, deletions or substitutions. A syndecan-1 polypeptide may comprise an amino acid sequence at least 80%, at least 85%, at least

90%, at least 95%, at least 98% or 100% identical to the syndecan-1 polypeptide of SEQ ID NO:1. In certain embodiments, a syndecan-1 polypeptide comprises a portion of (e.g., a sub-sequence of) a syndecan-1 protein. In some embodiments a portion of a syndecan-1 comprises an extracellular domain of syndecan-1, or a portion thereof.

5 [0045]

#### Antibodies & Ag Binding Portions Thereof

In some embodiments a bi-specific binding agent comprises an antibody, or a portion thereof (e.g., a binding portion thereof, or an antigen binding portion thereof) that binds specifically to syndecan-1 (CD138). An antibody, or a binding portion thereof, that binds specifically to syndecan-1 (CD138) is sometime referred to herein as an anti-CD138 antibody. Anti-CD138 antibodies and binding portions thereof that are contemplated for use in a bi-specific binding agent described herein are described in detail in International Patent Application No. PCT/JP2018/016847, which is incorporated herein by reference in its entirety.

15 [0046]

In some embodiments an antibody is a monoclonal antibody, or binding portion thereof. Certain non-limiting examples of an antibody include monoclonal antibodies, chimeric antibodies, CDR-grafted antibodies, humanized antibodies, and human antibodies. Human antibodies can be obtained by a suitable method. For example, human antibodies can be obtained from trans-chromosomal animals engineered to produce fully human antibodies. In certain embodiments, an antibody is not polyclonal, and/or is not a polyclonal antibody.

[0047]

An antibody, or binding portion thereof, can be generated, manufactured or produced by a suitable method. In some embodiments an antibody, or binding portion thereof, is derived, produced, obtained, isolated, and/or purified from a suitable species. In some embodiments an antibody, or binding portion thereof, is derived, produced, obtained, isolated, and/or purified from a rabbit, goat, horse, cow, rat, mouse, fish, bird, or llama, for example. In some embodiments an antibody is derived, produced, obtained, isolated, and/or purified from a bird (e.g., a chicken, or a bird egg). In some embodiments an antibody, or binding portion thereof, is derived, produced, obtained,

isolated, and/or purified from a plant (e.g., a recombinant antibody, or binding portion thereof, produced by a genetically engineered plant). In some embodiments an antibody, or binding portion thereof, is derived, produced, obtained, isolated, and/or purified from a suitable mammal. In certain embodiments a suitable mammal is a  
5 genetically altered mammal (e.g., a trans-chromosomal or transgenic mammal) engineered to produce antibodies comprising human heavy chains and/or human light chains or portions thereof. In some embodiments an antibody, or binding portion thereof, is produced, obtained, isolated, or purified from a prokaryotic or eukaryotic cell (e.g., a recombinant antibody, or binding portion thereof, produced by a genetically  
10 engineered cell). In some embodiments an antibody, or binding portion thereof, is produced, obtained, isolated, or purified from a virus (e.g., a recombinant antibody, or binding portion thereof, produced by a genetically engineered virus).

[0048]

An antibody, or binding portion thereof, or bi-specific agent can be expressed,  
15 isolated from and/or purified from a suitable expression system non-limiting examples of which include a suitable bacteria, phage, insect, virus, plant or mammalian expression system. For example, a nucleic acid encoding an antibody can be introduced into a suitable mammalian cell line that expresses and secretes the antibody into the cell culture media. Any suitable mammalian cell line can be used to generate  
20 an antibody or bi-specific binding agent. A method of producing an antibody or bi-specific binding agent, or a portion thereof, may comprise one or more of (i) introducing one or more nucleic acids into a suitable cell line wherein the nucleic acid directs the expression of the antibody, bi-specific binding agent or portion thereof; (ii) culturing the cell line using a suitable culturing method for a period of time that allows  
25 expression of the antibody or bi-specific binding agent; (iii) harvesting the cell line (e.g., by way of generating a lysate) or harvesting conditioned media produced from the cell line (e.g., where the antibody or bi-specific binding agent is secreted into the media); and (iv) isolating and/or purifying the antibody or bi-specific binding agent using a suitable method.

30 [0049]

The modifier “monoclonal” is not to be construed as requiring production of an antibody, or binding portion thereof, by any particular method. A monoclonal antibody, or binding portion thereof, can be produced by any suitable method. For example, in certain embodiments, a monoclonal antibody is made by a hybridoma method (e.g., as described by Kohler *et al.*, *Nature*, 256:495 (1975)), or a variation thereof. In some embodiments a monoclonal antibody, or binding portion thereof, is made by a recombinant DNA method. For example, a monoclonal antibody, or binding portion thereof, can be made by screening a recombinant library using a suitable expression system (e.g., a phage display expression system). In some embodiments a monoclonal antibody, or binding portion thereof, is isolated from a phage library, for example by using a technique described in Clackson *et al.*, *Nature*, 352:624-628 (1991) and/or Marks *et al.*, *J.Mol.Biol.*, 222:581-597 (1991), or a variation thereof.

[0050]

In certain embodiments, an antibody, or binding portion thereof, comprises one or more structural portions of a mammalian antibody. In certain embodiments an antibody, or binding portion thereof comprises one or more constant regions (e.g., constant regions derived from an antibody, e.g., a mammalian antibody). An antibody, or binding portion thereof, may comprise any suitable constant region of an antibody, or one or more portions thereof. In certain embodiments an antibody, or binding portion thereof, comprises a constant region of an antibody light chain and/or a constant region of an antibody heavy chain. In some embodiments an antibody, or binding portion thereof, comprises a lambda ( $\lambda$ ) light chain constant region, or a portion thereof. In some embodiments an antibody, or binding portion thereof, comprises a kappa ( $\kappa$ ) light chain constant region, or a portion thereof. In some embodiments an antibody, or binding portion thereof, comprises a polypeptide that is at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or at least 99% identical to a polypeptide sequence of a light chain constant region of a mammalian antibody, or portion thereof. In some embodiments an antibody, or binding portion thereof, comprises a polypeptide that is at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or at least 99% identical to a polypeptide sequence of a light chain constant region of a human antibody. In some embodiments a binding portion of an antibody does not include a light chain

constant region. In some embodiments an antibody, or binding portion thereof, does not include one or more portions of a light chain constant region.

[0051]

An antibody, or binding portion thereof, can include any suitable heavy chain  
5 constant region, or portion thereof. In mammals, an antibody can have at least five  
types/classes of Ig heavy chains denoted as IgA, IgD, IgE, IgG, and IgM, which are  
determined by the presence of distinct heavy chain constant regions, or portion thereof  
(e.g., CH1, CL, CH2, CH3 domains). In some embodiments an antibody, or binding  
portion thereof, comprises one or more heavy chain constant regions of an IgM, IgD,  
10 IgA, or IgE isotype, or a portion thereof. In some embodiments an antibody, or  
binding portion thereof, comprises a heavy chain constant region of an IgG<sub>1</sub>, IgG<sub>2</sub>, IgG<sub>3</sub>  
or IgG<sub>4</sub>, or one or more portions thereof. In some embodiments an antibody, or  
binding portion thereof, comprises a polypeptide that is at least 75%, at least 80%, at  
least 85%, at least 90%, at least 95%, at least 99% identical, or 100% identical to a  
15 polypeptide sequence of a heavy chain constant region of a mammalian antibody. In  
some embodiments an antibody, or binding portion thereof, comprises a polypeptide  
that is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%  
identical or 100% identical to a polypeptide sequence of a heavy chain constant region  
of a human antibody. In some embodiments an antibody, or binding portion thereof,  
20 comprises one or more additions, deletions and/or modification to a constant region.  
An antibody, or binding portion thereof, is sometimes modified to change the antibody  
class, or isotype of the antibody. In some embodiments an antibody, or binding  
portion thereof, comprises one or more additions, deletions and/or modification (one or  
more amino acid substitutions, deletions or additions) to modify one or more functions  
25 of the antibody, or binding portion thereof, for example to abolish, enhance or decrease  
serum half-life, Fc receptor binding, complement binding (e.g., C1q binding),  
glycosylation, sialylation, cellular toxicity, antibody-dependent cell-mediated  
phagocytosis (ADCP), antibody dependent cellular cytotoxicity (ADCC), and the like.  
In some embodiments a binding portion of an antibody does not include a heavy chain  
30 constant region. In some embodiments an antibody, or binding portion thereof, does  
not include one or more portions of a heavy chain constant region.

[0052]

In some embodiments an antibody, or binding portion thereof, comprises or consists of one or more variable regions of an antibody, or a portion thereof. In some embodiments an antibody, or binding portion thereof, comprises one or more light chain  
5 variable regions, or a portion thereof. In some embodiments an antibody, or binding portion thereof, comprises one or more heavy chain variable regions, or a portion thereof. In certain embodiments an antibody, or binding portion thereof, comprises at least one light chain variable region and at least one heavy chain variable region. A light chain variable region and heavy chain variable region can be on the same or  
10 different polypeptides.

[0053]

In mammals, the heavy chain variable region and light chain variable region of an antibody each contribute three CDRs (complementarity determining regions) commonly referred to as CDR1, CDR2 and CDR3, that are separated and/or flanked by  
15 framework regions (e.g., FR1, FR2, FR3 and FR4). The term “CDR” as used herein refers to an amino acid sequence of a polypeptide identified as a complementarity determining region. In certain embodiments, definitive delineation of a CDR polypeptide sequence and identification of residues comprising the binding site of antibody, or binding portion thereof, is accomplished by solving the structure of  
20 antibody, or binding portion thereof, and/or solving the structure of an antibody-antigen complex or the like. In certain embodiments, this is accomplished by any suitable method, such as X-ray crystallography and/or computer modeling. In certain embodiments, various methods of analysis are employed to identify or approximate the CDR sequences of an antibody or binding portion thereof. For example, the amino  
25 acid sequence and/or location of CDRs in a polypeptide sequence of an antibody, a binding portion thereof or variable region thereof, is identified using a suitable method, non-limiting examples of which include the Kabat or EU system (e.g., see Kabat, E. A., et al., 1991; Sequences of Proteins of Immunological Interest, Fifth Edition, NIH Publication No. 91-3242, as well as Johnson, G. and Wu, T. T. 2000, Nucleic Acids  
30 Research), and/or the Chothia Numbering Scheme (e.g., Chothia & Lesk, (1987) J.Mol.Biol., 196:901-917; Chothia et al., Nature, (1989) 342:878-883; and Al-Lazikani

et al., (1997) JMB 273,927-948). In some embodiments the amino sequence and/or location of CDRs of an antibody are identified using the AbM method and/or contact method. The “AbM” definition uses an integrated suite of computer programs produced by Oxford Molecular Group that model antibody structure (see e.g., Martin et al., Proc. Natl. Acad. Sci. (USA), 86:9268-9272 (1989); “AbM(Trademark), A Computer Program for Modeling Variable Regions of Antibodies,” Oxford, UK; Oxford Molecular, Ltd.). The AbM definition models the tertiary structure of an antibody from primary sequence using a combination of knowledge databases and ab initio methods, such as those described by Samudrala et al., “Ab Initio Protein Structure Prediction Using a Combined Hierarchical Approach,” in PROTEINS, Structure, Function and Genetics Suppl., 3:194-198 (1999). In certain embodiments, a contact definition is based on an analysis of the available complex crystal structures (see e.g., MacCallum et al., J.Mol.Biol, 5:732-45 (1996)).

[0054]

15 In some embodiments an antibody comprises at least 6 distinct CDRs (e.g., 3 distinct heavy chain CDRs and 3 distinct light chain CDRs). In certain embodiments, a binding portion of an antibody comprises at least 2, at least 3, at least 4, at least 5 or at least 6 distinct CDRs. In some embodiments a binding portion of an antibody comprises 3 to 6 distinct CDRs.

20 [0055]

In certain embodiments, an antibody or binding portion thereof, comprises one, two or three CDRs of a light chain variable region. In some embodiments a light chain variable region of an antibody, or binding portion thereof, comprises one or more CDRs (e.g., one, two, three, or more CDRs). The amino acid sequences representing a CDR in a light chain variable region of an antibody is referred to as CDR-L1 (light chain CDR1), CDR-L2 (light chain CDR2), and CDR-L3 (light chain CDR3), which are numbered sequentially (i.e., L1, L2 and L3) in the direction from the amino terminus (N-terminus) to the carboxy terminus (C-terminus) of a light chain variable region. For example, in a polypeptide representing a light chain variable region of an antibody, or binding portion thereof, CDR-L1, when present, is the most N-terminal light chain CDR; CDR-L3, when present, is the most C-terminal light chain CDR; and CDR-L2,

when present, is located (i) between CDR-L1 and CDR-L3, (ii) on the N-terminal side of CDR-L3 or (iii) on the C-terminal side of CDR-L1, of a light chain variable region or binding portion of an antibody. The terms “CDR-L1”, “CDR-L2” and “CDR-L3” refer to, in part, an amino acid sequence of a polypeptide identified as, or disclosed  
5 herein as, a complementarity determining region of an antibody (e.g., a CDR of a light chain variable region). Non-limiting examples of amino acid sequences of a CDR-L1, CDR-L2 and CDR-L3 are provided in Tables 1-3, respectively. A light chain variable region or antigen binding portion of an antibody may comprise any combination of a CDR-L1, a CDR-L2, and a CDR-L3 disclosed herein, wherein the binding portion of  
10 the antibody retains specific binding to syndecan-1, or a portion thereof. In certain embodiments, a light chain variable region or antigen binding portion of an antibody comprises a single light chain CDR comprising an amino acid sequence at least 70% identical to a CDR-L3 selected from Table 3. In certain embodiments, a light chain variable region or antigen binding portion of an antibody comprises an amino acid  
15 sequence at least 70% identical to a CDR-L3 selected from Table 3, and/or another suitable CDR-L2 and/or CDR-L1 polypeptide sequence, where the antibody, or binding portion thereof, retains specific binding to syndecan-1, or a portion thereof. In certain embodiments, the light chain CDRs of a light chain variable region or antigen binding portion of an antibody consists of a CDR-L3 and a CDR-L2, where the CDR-L3  
20 comprises an amino acid sequence at least 70% identical to a CDR-L3 selected from Table 3 and the CDR-L2 comprises an amino acid sequence at least 70% identical to a CDR-L2 selected from Table 2. In certain embodiments, a light chain variable region or antigen binding portion of an antibody comprises an amino acid sequence at least 70% identical to a CDR-L3 selected from Table 3 and an amino acid sequence at least  
25 70% identical to a CDR-L2 selected from Table 2, and any other suitable CDR-L1 polypeptide sequence, where the antibody, or binding portion thereof, retains specific binding to syndecan-1, or a portion thereof. In certain embodiments, a light chain variable region or antigen binding portion of an antibody comprises three light chain CDRs consisting of an amino acid sequence at least 70% identical to a CDR-L3 selected  
30 from Table 3, an amino acid sequence at least 70% identical to a CDR-L2 selected from Table 2 and an amino acid sequence selected at least 70% identical to a CDR-L1 of

Table 1. In certain embodiments, a light chain variable region or antigen binding portion of an antibody comprises an amino acid sequence at least 70% identical to a CDR-L3 selected from Table 3, an amino acid sequence at least 70% identical to a CDR-L2 selected from Table 2 and an amino acid sequence at least 70% identical to a CDR-L1 selected from Table 1, where the antibody, or binding portion thereof, retains specific binding to syndecan-1, or a portion thereof.

[0056]

In some embodiments an antibody, or binding portion thereof, comprises one or more light chain CDRs that are at least 70%, 75%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least 99% identical to any one of the CDR sequences listed in Tables 1, 2 or 3. In some embodiments an antibody, or binding portion thereof, comprises a CDR-L1 that is at least 70%, 75%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least 99% identical to any one of the sequences shown in Table 1. In some embodiments an antibody, or binding portion thereof, comprises a CDR-L1 of any one of the sequences shown in Table 1.

[TABLE 1]

## CDR-L1 Sequences

| SEQ ID       | Hybridoma Clone/<br>Antibody Name | Amino Acid Sequence |
|--------------|-----------------------------------|---------------------|
| SEQ ID NO:2  | F12P16F6                          | *KSSQSLLASDGKTYLN   |
| SEQ ID NO:3  | F12P16F6                          | QSLLASDGKTY         |
| SEQ ID NO:4  | F13P30A7                          | *KASENVGNYVS        |
| SEQ ID NO:5  | F13P30A7                          | ENVGNY              |
| SEQ ID NO:6  | F13P18D8                          | *KASENVGTYVS        |
| SEQ ID NO:7  | F13P18D8                          | ASENVGTY            |
| SEQ ID NO:8  | F12P7G11                          | *RASSSVNYMH         |
| SEQ ID NO:9  | F12P7G11                          | ASSSVNY             |
| SEQ ID NO:10 | F13P14D3                          | *KASENVGSYVS        |
| SEQ ID NO:11 | F13P14D3                          | ASENVGSY            |
| SEQ ID NO:12 | F11AP11E5                         | *KSGQSLLYSNGKTYLT   |
| SEQ ID NO:13 | F11AP11E5                         | KSGQSLLYSNG         |
| SEQ ID NO:14 | F12P18D4.a                        | *KSSQSLLYSNGKTYLN   |
| SEQ ID NO:15 | F12P18D4.a                        | KSSQSLLYSNG         |

\*Indicates that the CDR was defined by the Kabat Method.

[0057]

In some embodiments a syndecan-1 antibody, or binding portion thereof, comprises a CDR-L2 that is at least 70%, 75%, 85%, 86%, 87%, 88%, 89%, 90%, 91%,  
5 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least 99% identical to any one of the sequences shown in Table 2. In some embodiments a syndecan-1 antibody, or binding portion thereof, comprises a CDR-L2 of any one of the sequences shown in Table 2.

[TABLE 2]

## CDR-L2 Sequences

| SEQ ID       | Hybridoma Clone/<br>Antibody Name | Amino Acid Sequence |
|--------------|-----------------------------------|---------------------|
| SEQ ID NO:16 | F12P16F6                          | YLVSKLDS            |
| SEQ ID NO:17 | F12P16F6                          | *LVSKLDS            |
| SEQ ID NO:18 | F12P16F6                          | LVSKLD              |
| SEQ ID NO:19 | F13P30A7                          | YGASYRYT            |
| SEQ ID NO:20 | F13P30A7                          | *GASYRYT            |
| SEQ ID NO:21 | F13P30A7                          | GASYRY              |
| SEQ ID NO:22 | F13P18D8                          | *GASNRYT            |
| SEQ ID NO:23 | F12P7G11                          | *ATSYLAS            |
| SEQ ID NO:24 | F13P14D3                          | *GASNRNT            |
| SEQ ID NO:25 | F11AP11E5                         | *QVSKLDP            |
| SEQ ID NO:26 | F12P18D4.a                        | *LVSKVDS            |

\*Indicates that the CDR was defined by the Kabat Method.

[0058]

In some embodiments a syndecan-1 antibody, or binding portion thereof, comprises a CDR-L3 that is at least 70%, 75%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least 99% identical to any one of the sequences shown in Table 3. In some embodiments a syndecan-1 antibody, or binding portion thereof, comprises a CDR-L3 of any one of the sequences shown in Table 3.

[TABLE 3]

## CDR-L3 Sequences

| SEQ ID       | Hybridoma Clone/<br>Antibody Name | Amino Acid Sequence |
|--------------|-----------------------------------|---------------------|
| SEQ ID NO:27 | F12P16F6                          | *WQGAHFPFT          |
| SEQ ID NO:28 | F12P16F6                          | QGAHFPF             |
| SEQ ID NO:29 | F13P30A7/F13P14D3/<br>F13P18D8    | *GQSSRYPLT          |
| SEQ ID NO:30 | F13P30A7/F13P14D3/<br>F13P18D8    | QSSRYPL             |
| SEQ ID NO:31 | F12P7G11                          | *QQWSSDPLT          |
| SEQ ID NO:32 | F11AP11E5                         | *LQNTYYPHT          |
| SEQ ID NO:33 | F12P18D4.a                        | *VQGTHFPLT          |

\*Indicates that the CDR was defined by the Kabat Method.

10 [0059]

In some embodiments a syndecan-1 antibody, or binding portion thereof, comprises a light chain variable region having at least 70%, 75%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least 99% identity to an amino acid sequence of Table 4. In some embodiments a syndecan-1 antibody, or binding portion thereof, comprises a light chain variable region sequence of Table 4.

[TABLE 4]

MOUSE VARIABLE LIGHT CHAIN SEQUENCES

| SEQ ID       | Hybridoma Clone/<br>Antibody Name | Mouse Variable Light Chain Amino Acid Sequence                                                                            |
|--------------|-----------------------------------|---------------------------------------------------------------------------------------------------------------------------|
| SEQ ID NO:34 | F12P16F6                          | DVVMQTPTLTLSTIGQPASISCKSSQSLLASDGKTYLN<br>WLLQRPQGQSPKRLIYLVSKLDSGVPDRFTGSGSGTGFTL<br>QISRVEAEDLGIYYCWQGAHFPFTFGSGTKLEIKR |
| SEQ ID NO:35 | F13P30A7                          | NIIMTQSPKSMAMSVGERVTLSCKASENVGNYVSWYQ<br>QKPEQSPKLLIYGASYRYTGVPDRFTGSGSGTDFTLTISS<br>VQAEDLADYHCGQSSRYPLTFGAGTKLELKR      |
| SEQ ID NO:36 | F13P18D8                          | NIVMTQSPKSMMSVGERVTLSCKASENVGTIVSWYQ<br>QKSDQSPKLLIYGASNRYTGVPDRFTGSGSATDFTLTITS<br>VQSEDLADYHCGQSSRYPLTFGAGTKLELKR       |
| SEQ ID NO:37 | F12P7G11                          | QIVLSQSPAILASAPGEKVTMTCRASSSVNYMHYQQK<br>PGSSPKHWIYATSYLASGVPARFSGSGSGTSYSLTISRVE<br>AEDAATYYCQWSSDPLTFGAGTKLELKR         |
| SEQ ID NO:38 | F13P14D3                          | NIVMTQSPKSMMSVQSRVTLSCKASENVGSYVSWYQ<br>QKPEQSPKLLIYGASNRNTGVPDRFTGSGSATDFTLTISS<br>VQAEDLADYHCGQSSRYPLTFGGGKLELKR        |
| SEQ ID NO:39 | F11AP11E5                         | DVVMQTPTLSLSTIGQPASISCKSGQSLLYSNGKTYLT<br>WLQQRPGQAPKLLMYQVSKLDPGIPDRFSGSGSETDFTL<br>KISRVEAEDLGYYCLQNTYYPHTFGAGTKLELKR   |
| SEQ ID NO:40 | F12P18D4.a                        | DVVMQTPTLTLSTIGQSASISCKSSQSLLYSNGKTYLN<br>WLLQRPQGQSPKRLIYLVSKVDSGVPDRFTGSGSGTDFTL<br>SISRVEAEDLGYYCVQGTHFPLTFGVGKLELKR   |

[0060]

In some embodiments a syndecan-1 antibody, or binding portion thereof, comprises a humanized light chain variable region having at least 70%, 75%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least 99% identity to a sequence of Table 5. In some embodiments a syndecan-1 antibody, or

binding portion thereof, comprises a humanized light chain variable region sequence of Table 5.

[TABLE 5]

Humanized Light Chains Variable Regions

| SEQ ID       | Humanized Light Chain Name                            | Humanized Light Chain Variable Region Amino Acid Sequences 41-43 & Complete Humanized Light Chain Sequence 44                                                                                                                                 |
|--------------|-------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| SEQ ID NO:41 | hF6 (sdr/cdr/ven-rep)                                 | DVVMQTQPLSLSVTPGQPASISCKSSQSLLASDGKTYL<br>NWLLQRPQGSPKRLIYLVSKLDSGVPNRFSGSGSGTDF<br>TLQISRVEAEDVGLYYCWQGAHFPFTFGSGTKLEIKR                                                                                                                     |
| SEQ ID NO:42 | P16F6 abb-rep                                         | DVVMQTQPLSLSVTPGQPASISCKSSQSLLASDGKTYL<br>NWLLQRPQGSPKRLIYLVSKLDSGVPNRFSGSGSGTDF<br>TLQISRVEAEDVGLYYCMQGAHFPFTFGGGTKVEIKR                                                                                                                     |
| SEQ ID NO:43 | P16F6 fra-rep                                         | DVVMQTQPLSLSVTLGQPASISCKSSQSLLASDGKTYL<br>NWLLQRPQGSPRRLIYLVSKLDSGVPNRFSGSGSGTDF<br>TLQISRVEAEDVGLYYCWQGAHFPFTFGSGTKLEIKR                                                                                                                     |
| SEQ ID NO:44 | hF6 (sdr/cdr/ven-rep) (Complete Light Chain sequence) | DVVMQTQPLSLSVTPGQPASISCKSSQSLLASDGKTYL<br>NWLLQRPQGSPKRLIYLVSKLDSGVPNRFSGSGSGTDF<br>TLQISRVEAEDVGLYYCWQGAHFPFTFGSGTKLEIKRT<br>VAAPSVFIFPPSDEQLKSGTASVVCLLNFPYPREAKVQ<br>WKVDNALQSGNSQESVTEQDSKSTYLSSTLTLSKA<br>DYEKHKVYACEVTHQGLSSPVTKSFNRGEC |

5 [0061]

In certain embodiments, a syndecan-1 antibody, or binding portion thereof, comprises one, two or three CDRs of a heavy chain variable region. In some embodiments a heavy chain variable region comprises one or more distinct CDRs (e.g., one, two, three, or more distinct CDRs). The amino acid sequences representing a CDR in a heavy chain variable region of an antibody is referred to as CDR-H1 (heavy chain CDR1), CDR-H2 (heavy chain CDR2), and CDR-H3 (heavy chain CDR3), which are numbered sequentially (i.e., H1, H2 and H3) in the direction from the amino terminus (N-terminus) to the carboxy terminus (C-terminus) of a heavy chain variable region. For example, in a polypeptide representing a heavy chain variable region of a syndecan-1 antibody, or a binding portion thereof, CDR-H1, when present, is the most N-terminal CDR; CDR-H3, when present, is the most C-terminal CDR; and CDR-H2, when present, is located (i) between CDR-H1 and CDR-H3, (ii) on the N-terminal side of CDR-H3 or (iii) on the C-terminal side of CDR-H1, of a heavy chain variable region. The terms “CDR-H1”, “CDR-H2” and “CDR-H3” refer to, in part, an amino acid

sequence of a polypeptide identified as, or disclosed herein as, a complementarity determining region of a syndecan-1 antibody, or binding portion thereof (e.g., a CDR of a heavy chain variable region of a syndecan-1 antibody). Non-limiting examples of amino acid sequences of a CDR-H1, CDR-H2 and CDR-H3 are provided in Tables 6-8, respectively. A heavy chain variable region or antigen binding portion of a syndecan-1 antibody may comprise any combination of a CDR-H1, a CDR-H2, and a CDR-H3, where the syndecan-1 antibody, or binding portion thereof, retains specific binding to syndecan-1, or a portion thereof. In certain embodiments, a heavy chain variable region or antigen binding portion of a syndecan-1 antibody, or binding portion thereof, comprises a single heavy chain CDR consisting of an amino acid sequence at least 70% identical to a CDR-H3 selected from Table 8. In certain embodiments, a heavy chain variable region or antigen binding portion of a syndecan-1 antibody comprises an amino acid sequence at least 70% identical to a CDR-H3 selected from Table 8, and any other suitable CDR-H2 and/or CDR-H1 polypeptide sequence, where the syndecan-1 antibody, or binding portion thereof, retains specific binding to syndecan-1, or a portion thereof. In certain embodiments, the heavy chain CDRs of a heavy chain variable region or antigen binding portion of a syndecan-1 antibody consists of a CDR-H3 and a CDR-H2, where the CDR-H3 comprises an amino acid sequence at least 70% identical to a CDR-H3 selected from Table 8 and the CDR-H2 comprises an amino acid sequence at least 70% identical to a CDR-H2 selected from Table 7. In certain embodiments, a heavy chain variable region or antigen binding portion of a syndecan-1 antibody comprises an amino acid sequence at least 70% identical to a CDR-H3 selected from Table 8 and an amino acid sequence at least 70% identical to a CDR-H2 selected from Table 7, and any other suitable CDR-H1 polypeptide sequence, where the syndecan-1 antibody, or binding portion thereof, retains specific binding to syndecan-1 or a portion thereof. In certain embodiments, a heavy chain variable region or antigen binding portion of a syndecan-1 antibody comprises three heavy chain CDRs consisting of an amino acid sequence at least 70% identical to a CDR-H3 selected from Table 8, an amino acid sequence at least 70% identical to a CDR-H2 selected from Table 7 and an amino acid sequence selected at least 70% identical to a CDR-H1 of Table 6. In certain embodiments, a heavy chain variable region or antigen binding portion of a

syndecan-1 antibody comprises an amino acid sequence at least 70% identical to a CDR-H3 selected from Table 8, an amino acid sequence at least 70% identical to a CDR-H2 selected from Table 7 and an amino acid sequence at least 70% identical to a CDR-H1 selected from Table 6, where the syndecan-1 antibody retains specific binding  
5 to syndecan-1, or a portion thereof.

[0062]

In some embodiments a syndecan-1 antibody, or binding portion thereof, comprises one or more heavy chain CDRs with at least 70%, 75%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least 99% identity  
10 to any one of the CDRs of Tables 6, 7 or 8. In some embodiments a syndecan-1 antibody, or binding portion thereof, comprises a CDR-H1 that is at least 70%, 75%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least 99% identical to any one of the sequences shown in Table 6. In some embodiments a syndecan-1 antibody, or binding portion thereof, comprises a CDR-H1  
15 of any one of the sequences shown in Table 6.

[TABLE 6]

## CDR-H1 Sequences

| SEQ ID       | Hybridoma Clone/<br>Antibody Name | Amino Acid Sequence |
|--------------|-----------------------------------|---------------------|
| SEQ ID NO:45 | F12P16F6                          | KASGYTFTSYLY        |
| SEQ ID NO:46 | F12P16F6                          | GYTFTSYLY           |
| SEQ ID NO:47 | F12P16F6                          | *SYLY               |
| SEQ ID NO:48 | F13P30A7                          | AASGFTFNTYAMN       |
| SEQ ID NO:49 | F13P30A7                          | ASGFTFNTYAM         |
| SEQ ID NO:50 | F13P30A7/ F13P18D8/ F13P14D3      | *TYAMN              |
| SEQ ID NO:51 | F13P18D8                          | GFAFNTYAMN          |
| SEQ ID NO:52 | F12P7G11                          | GYTFSSHWMQ          |
| SEQ ID NO:53 | F12P7G11                          | *SHWMQ              |
| SEQ ID NO:54 | F13P14D3                          | GFTFNTYAMN          |
| SEQ ID NO:55 | F11AP11E5                         | KASGYTFTNYYMY       |
| SEQ ID NO:56 | F11AP11E5                         | *NYYMY              |
| SEQ ID NO:57 | F12P18D4.a                        | YTFAD               |
| SEQ ID NO:58 | F12P18D4.a                        | YTFADYYMK           |
| SEQ ID NO:59 | F12P18D4.a                        | *DYMK               |

\*Indicates that the CDR was defined by the Kabat Method.

[0063]

In some embodiments a syndecan-1 antibody, or binding portion thereof, comprises a CDR-H2 that is at least 70%, 75%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least 99% identical to any one of the sequences shown in Table 7. In some embodiments a syndecan-1 antibody, or binding portion thereof, comprises a CDR-H2 of any one of the sequences shown in Table 7.

[TABLE 7]

## CDR-H2 Sequences

| SEQ ID       | Hybridoma Clone/<br>Antibody Name  | Amino Acid Sequence  |
|--------------|------------------------------------|----------------------|
| SEQ ID NO:60 | F12P16F6                           | EIYPRSGGTN           |
| SEQ ID NO:61 | F12P16F6                           | *EIYPRSGGTNINEKFLS   |
| SEQ ID NO:62 | F13P30A7,<br>F13P18D8,<br>F13P14D3 | RIRSKSNNYATY         |
| SEQ ID NO:63 | F13P30A7,<br>F13P18D8              | *RIRSKSNNYATYYADSVKD |
| SEQ ID NO:64 | F13P30A7,<br>F13P18D8,<br>F13P14D3 | IRSKSNNYATY          |
| SEQ ID NO:65 | F12P7G11                           | *AIYPGDGDTRFTQKFKG   |
| SEQ ID NO:66 | F12P7G11                           | YPGDGDTRFTQK         |
| SEQ ID NO:67 | F13P14D3                           | *RIRSKSNNYATYYVDSVKD |
| SEQ ID NO:68 | F11AP11E5                          | *EINPGNGGTNFNEKFKN   |
| SEQ ID NO:69 | F11AP11E5                          | NPGNGGTNFNEKF        |
| SEQ ID NO:70 | F12P18D4.a                         | DINPNSGDTF           |
| SEQ ID NO:71 | F12P18D4.a                         | *DINPNSGDTFYNHKFKG   |

\*Indicates that the CDR was defined by the Kabat Method.

[0064]

In some embodiments a syndecan-1 antibody, or binding portion thereof, comprises a CDR-H3 that is at least 70%, 75%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least 99% identical to any one of the sequences shown in Table 8. In some embodiments a syndecan-1 antibody, or binding portion thereof, comprises a CDR-H3 of any one of the sequences shown in Table 8.

[TABLE 8]

## CDR-H3 Sequences

| SEQ ID       | Hybridoma Clone/<br>Antibody Name | Amino Acid Sequence |
|--------------|-----------------------------------|---------------------|
| SEQ ID NO:72 | F12P16F6                          | TRSLLY              |
| SEQ ID NO:73 | F12P16F6                          | *SLLY               |
| SEQ ID NO:74 | F13P30A7                          | VTDYGYVYFDA         |
| SEQ ID NO:75 | F13P30A7                          | *DYGyVYFDA          |
| SEQ ID NO:76 | F13P18D8                          | *DYyYVYFDV          |
| SEQ ID NO:77 | F12P7G11                          | *GIYYDRSRAMDY       |
| SEQ ID NO:78 | F13P14D3                          | VTDYGHVYFDV         |
| SEQ ID NO:79 | F13P14D3                          | *DYGHVYFDV          |
| SEQ ID NO:80 | F11AP11E5                         | *RFAY               |
| SEQ ID NO:81 | F12P18D4.a                        | *TYYDY              |

\*Indicates that the CDR was defined by the Kabat Method.

[0065]

In some embodiments, an antibody, or binding portion thereof, comprises, or consists of, a CDR-L1 comprising an amino acid sequence at least 90%, at least 95%, or 100% identical to any one of the amino acid sequences of Table 1, a CDR-L2 comprising an amino acid sequence at least 90%, at least 95%, or 100% identical to any one of the amino acid sequences of Table 2, a CDR-L3 comprising an amino acid sequence at least 90%, at least 95%, or 100% identical to any one of the amino acid sequences of Table 3, a CDR-H1 comprising an amino acid sequence at least 90%, at least 95%, or 100% identical to any one of the amino acid sequences of Table 6, a CDR-H2 comprising an amino acid sequence at least 90%, at least 95%, or 100% identical to any one of the amino acid sequences of Table 7, and a CDR-H3 comprising an amino acid sequence at least 90%, at least 95%, or 100% identical to any one of the amino acid sequences of Table 8.

[0066]

In some embodiments an antibody, or binding portion thereof, comprises, or consists of, a CDR-L1 comprising an amino acid sequence at least 70%, at least 75%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to the amino acid sequence of SEQ ID NO:2 or 3, a CDR-L2 comprising an amino acid sequence at least 70%, at least 75%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at

least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to the amino acid sequence of SEQ ID NO:16, 17 or 18, a CDR-L3 comprising an amino acid sequence at least 70%, at least 75%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to the amino acid sequence of SEQ ID NO:27 or 28, a CDR-H1 comprising an amino acid sequence at least 70%, at least 75%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to the amino acid sequence of SEQ ID NO:45, 46 or 47, a CDR-H2 comprising an amino acid sequence at least 70%, at least 75%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to the amino acid sequence of SEQ ID NO:60 or 61, and a CDR-H3 comprising an amino acid sequence at least 70%, at least 75%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to the amino acid sequence of SEQ ID NO:72 or 73.

[0067]

In some embodiments an antibody, or binding portion thereof, comprises, or consists of, a CDR-L1 comprising an amino acid sequence at least 80%, at least 85%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:3, a CDR-L2 comprising an amino acid sequence at least 80%, at least 85%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:18, a CDR-L3 comprising an amino acid sequence at least 80%, at least 85%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:28, a CDR-H1 comprising an amino acid sequence at least 80%, at least 85%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:47, a CDR-H2 comprising an amino acid sequence at least 80%, at least 85%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:60, and

a CDR-H3 comprising an amino acid sequence at least 80%, at least 85%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:73.

[0068]

In some embodiments an antibody, or binding portion thereof, comprises a  
 5 CDR-L1 comprising an amino acid sequence at least 80%, at least 85%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:2, a CDR-L2 comprising an amino acid sequence at least 80%, at least 85%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:17, a CDR-L3 comprising an amino acid sequence at least 80%, at least 85%, at least 90%, or 100% identical to the amino acid sequence of  
 10 SEQ ID NO:27, a CDR-H1 comprising an amino acid sequence at least 80%, at least 85%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:47, a CDR-H2 comprising an amino acid sequence at least 80%, at least 85%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:61, and a CDR-H3 comprising an amino acid sequence at least 80%, at least 85%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:73. In some embodiments an antibody, or binding portion thereof, comprises a CDR-L1 comprising an amino acid sequence at least 80%, at least 85%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:2, a CDR-L2 comprising an amino acid sequence at least 80%, at least 85%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID  
 20 NO:16, a CDR-L3 comprising an amino acid sequence at least 80%, at least 85%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:27, a CDR-H1 comprising an amino acid sequence at least 80%, at least 85%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:45, a CDR-H2 comprising an amino acid sequence at least 80%, at least 85%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:60, and a CDR-H3 comprising an amino acid sequence at least 80%, at least 85%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:72. In some embodiments, an antibody, or binding portion thereof, comprises a CDR-L1, CDR-L2 and a CDR-L3 comprising the amino acid sequences of SEQ ID NOs:2, 16 and 27, respectively, and a CDR-H1, CDR-H2 and a  
 25 amino acid sequence of SEQ ID NO:60, and a CDR-H3 comprising an amino acid sequence at least 80%, at least 85%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:72. In some embodiments, an antibody, or binding portion thereof, comprises a CDR-L1, CDR-L2 and a CDR-L3 comprising the amino acid sequences of SEQ ID NOs:2, 16 and 27, respectively, and a CDR-H1, CDR-H2 and a  
 30 CDR-H3 comprising the amino acid sequences of SEQ ID NOs:45, 60 and 72, respectively. In some embodiments, an antibody, or binding portion thereof,

comprises a CDR-L1, CDR-L2 and a CDR-L3 comprising the amino acid sequences of SEQ ID NOs:2, 16 and 27, respectively, and a CDR-H1, CDR-H2 and a CDR-H3 comprising the amino acid sequences of SEQ ID NOs:46, 61 and 72, respectively. In some embodiments, an antibody, or binding portion thereof, comprises a CDR-L1, CDR-L2 and a CDR-L3 comprising the amino acid sequences of SEQ ID NOs:2, 16 and 27, respectively, and a CDR-H1, CDR-H2 and a CDR-H3 comprising the amino acid sequences of SEQ ID NOs:45, 61 and 72, respectively.

[0069]

In some embodiments an antibody, or binding portion thereof, comprises, or consists of, a CDR-L1 comprising an amino acid sequence at least 70%, at least 75%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to the amino acid sequence of SEQ ID NO:4 or 5, a CDR-L2 comprising an amino acid sequence at least 70%, at least 75%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to the amino acid sequence of SEQ ID NO:19, 20 or 21, a CDR-L3 comprising an amino acid sequence at least 70%, at least 75%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to the amino acid sequence of SEQ ID NO:29 or 30, a CDR-H1 comprising an amino acid sequence at least 70%, at least 75%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to the amino acid sequence of SEQ ID NO:48, 49 or 50, a CDR-H2 comprising an amino acid sequence at least 70%, at least 75%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to the amino acid sequence of SEQ ID NO:62, 63 or 64, and a CDR-H3 comprising an amino acid sequence at least 70%, at least 75%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least

91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to the amino acid sequence of SEQ ID NO:74 or 75.

[0070]

5 In some embodiments an antibody, or binding portion thereof, comprises, or consists of, a CDR-L1 comprising an amino acid sequence at least 80%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:5, a CDR-L2 comprising an amino acid sequence at least 80%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:21, a CDR-L3 comprising an amino acid sequence at least  
10 80%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:30, a CDR-H1 comprising an amino acid sequence at least 80%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:50, a CDR-H2 comprising an amino acid sequence at least 80%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:62 or 64, and a CDR-H3 comprising an amino acid sequence  
15 at least 80%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:75.

[0071]

In some embodiments an antibody, or binding portion thereof, comprises a CDR-L1 comprising an amino acid sequence at least 80%, at least 90%, or 100%  
20 identical to the amino acid sequence of SEQ ID NO:4, a CDR-L2 comprising an amino acid sequence at least 80%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:20, a CDR-L3 comprising an amino acid sequence at least 80%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:29, a CDR-H1 comprising an amino acid sequence at least 80%, at least 90%, or 100% identical to the  
25 amino acid sequence of SEQ ID NO:50, a CDR-H2 comprising an amino acid sequence at least 80%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:63, and a CDR-H3 comprising an amino acid sequence at least 80%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:75. In some embodiments, an antibody, or binding portion thereof, comprises a CDR-L1, CDR-L2  
30 and a CDR-L3 comprising the amino acid sequences of SEQ ID NOs:4, 19 and 29, respectively, and a CDR-H1, CDR-H2 and a CDR-H3 comprising the amino acid

sequences of SEQ ID NOs:48, 63 and 75, respectively. In some embodiments, an antibody, or binding portion thereof, comprises a CDR-L1, CDR-L2 and a CDR-L3 comprising the amino acid sequences of SEQ ID NOs:4, 19 and 29, respectively, and a CDR-H1, CDR-H2 and a CDR-H3 comprising the amino acid sequences of SEQ ID NOs:49, 63 and 75, respectively. In some embodiments, an antibody, or binding portion thereof, comprises a CDR-L1, CDR-L2 and a CDR-L3 comprising the amino acid sequences of SEQ ID NOs:4, 19 and 29, respectively, and a CDR-H1, CDR-H2 and a CDR-H3 comprising the amino acid sequences of SEQ ID NOs:49, 63 and 74, respectively. In some embodiments, an antibody, or binding portion thereof, comprises a CDR-L1, CDR-L2 and a CDR-L3 comprising the amino acid sequences of SEQ ID NOs:4, 19 and 29, respectively, and a CDR-H1, CDR-H2 and a CDR-H3 comprising the amino acid sequences of SEQ ID NOs:48, 63 and 74, respectively.

[0072]

In some embodiments an antibody, or binding portion thereof, comprises, or consists of, a CDR-L1 comprising an amino acid sequence at least 70%, at least 75%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to the amino acid sequence of SEQ ID NO:6 or 7, a CDR-L2 comprising an amino acid sequence at least 70%, at least 75%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to the amino acid sequence of SEQ ID NO:22, a CDR-L3 comprising an amino acid sequence at least 70%, at least 75%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to the amino acid sequence of SEQ ID NO:29 or 30, a CDR-H1 comprising an amino acid sequence at least 70%, at least 75%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to the amino acid sequence of SEQ ID NO:50 or 51, a CDR-H2 comprising an amino acid sequence at least 70%, at least 75%, at least 85%, at

least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to the amino acid sequence of SEQ ID NO:62, 63 or 64, and a CDR-H3 comprising an amino acid sequence at least 70%, at least 75%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to the amino acid sequence of SEQ ID NO:76.  
[0073]

In some embodiments an antibody, or binding portion thereof, comprises, or consists of, a CDR-L1 comprising an amino acid sequence at least 80%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:7, a CDR-L2 comprising an amino acid sequence at least 80%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:22, a CDR-L3 comprising an amino acid sequence at least 80%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:30, a CDR-H1 comprising an amino acid sequence at least 80%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:50, a CDR-H2 comprising an amino acid sequence at least 80%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:63, and a CDR-H3 comprising an amino acid sequence at least 80%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:76.  
[0074]

In some embodiments an antibody, or binding portion thereof, comprises a CDR-L1 comprising an amino acid sequence at least 80%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:6, a CDR-L2 comprising an amino acid sequence at least 80%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:22, a CDR-L3 comprising an amino acid sequence at least 80%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:29, a CDR-H1 comprising an amino acid sequence at least 80%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:50, a CDR-H2 comprising an amino acid sequence at least 80%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:63, and a CDR-H3 comprising an amino acid sequence at least 80%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:76. In some

embodiments, an antibody, or binding portion thereof, comprises a CDR-L1, CDR-L2 and a CDR-L3 comprising the amino acid sequences of SEQ ID NOs:6, 22 and 29, respectively, and a CDR-H1, CDR-H2 and a CDR-H3 comprising the amino acid sequences of SEQ ID NOs:51, 63 and 76, respectively.

5 [0075]

In some embodiments an antibody, or binding portion thereof, comprises, or consists of, a CDR-L1 comprising an amino acid sequence at least 70%, at least 75%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to the amino acid sequence of SEQ ID NO:8 or 9, a CDR-L2 comprising an amino acid sequence at least 70%, at least 75%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to the amino acid sequence of SEQ ID NO:23, a CDR-L3 comprising an amino acid sequence at least 70%, at least 75%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to the amino acid sequence of SEQ ID NO:31, a CDR-H1 comprising an amino acid sequence at least 70%, at least 75%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to the amino acid sequence of SEQ ID NO:52 or 53, a CDR-H2 comprising an amino acid sequence at least 70%, at least 75%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to the amino acid sequence of SEQ ID NO:65 or 66, and a CDR-H3 comprising an amino acid sequence at least 70%, at least 75%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to the amino acid sequence of SEQ ID NO:77.

[0076]

In some embodiments an antibody, or binding portion thereof, comprises, or consists of, a CDR-L1 comprising an amino acid sequence at least 80%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:9, a CDR-L2 comprising an amino acid sequence at least 80%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:23, a CDR-L3 comprising an amino acid sequence at least 80%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:31, a CDR-H1 comprising an amino acid sequence at least 80%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:53, a CDR-H2 comprising an amino acid sequence at least 80%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:66, and a CDR-H3 comprising an amino acid sequence at least 80%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:77.

[0077]

In some embodiments an antibody, or binding portion thereof, comprises a CDR-L1 comprising an amino acid sequence at least 80%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:8, a CDR-L2 comprising an amino acid sequence at least 80%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:23, a CDR-L3 comprising an amino acid sequence at least 80%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:31, a CDR-H1 comprising an amino acid sequence at least 80%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:53, a CDR-H2 comprising an amino acid sequence at least 80%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:65, and a CDR-H3 comprising an amino acid sequence at least 80%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:77. In some embodiments, an antibody, or binding portion thereof, comprises a CDR-L1, CDR-L2 and a CDR-L3 comprising the amino acid sequences of SEQ ID NOs:8, 23 and 31, respectively, and a CDR-H1, CDR-H2 and a CDR-H3 comprising the amino acid sequences of SEQ ID NOs:52, 65 and 77, respectively.

[0078]

In some embodiments an antibody, or binding portion thereof, comprises, or consists of, a CDR-L1 comprising an amino acid sequence at least 70%, at least 75%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least

91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to the amino acid sequence of SEQ ID NO:10 or 11, a CDR-L2 comprising an amino acid sequence at least 70%, at least 75%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to the amino acid sequence of SEQ ID NO:24, a CDR-L3 comprising an amino acid sequence at least 70%, at least 75%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to the amino acid sequence of SEQ ID NO:29 or 30, a CDR-H1 comprising an amino acid sequence at least 70%, at least 75%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to the amino acid sequence of SEQ ID NO:50 or 54, a CDR-H2 comprising an amino acid sequence at least 70%, at least 75%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to the amino acid sequence of SEQ ID NO:62, 64 or 67, and a CDR-H3 comprising an amino acid sequence at least 70%, at least 75%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to the amino acid sequence of SEQ ID NO:78 or 79.

[0079]

In some embodiments an antibody, or binding portion thereof, comprises, or consists of, a CDR-L1 comprising an amino acid sequence at least 80%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:11, a CDR-L2 comprising an amino acid sequence at least 80%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:24, a CDR-L3 comprising an amino acid sequence at least 80%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:30, a CDR-H1 comprising an amino acid sequence at least 80%, at least 90%, or 100%

identical to the amino acid sequence of SEQ ID NO:50, a CDR-H2 comprising an amino acid sequence at least 80%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:62 or 64, and a CDR-H3 comprising an amino acid sequence at least 80%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID  
 5 NO:79.  
 [0080]

In some embodiments an antibody, or binding portion thereof, comprises a CDR-L1 comprising an amino acid sequence at least 80%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:10, a CDR-L2 comprising an amino  
 10 acid sequence at least 80%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:24, a CDR-L3 comprising an amino acid sequence at least 80%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:29, a CDR-H1 comprising an amino acid sequence at least 80%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:50, a CDR-H2 comprising an amino acid sequence  
 15 at least 80%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:67, and a CDR-H3 comprising an amino acid sequence at least 80%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:79. In some embodiments, an antibody, or binding portion thereof, comprises a CDR-L1, CDR-L2 and a CDR-L3 comprising the amino acid sequences of SEQ ID NOs:10, 24 and 29,  
 20 respectively, and a CDR-H1, CDR-H2 and a CDR-H3 comprising the amino acid sequences of SEQ ID NOs:54, 67 and 78, respectively.  
 [0081]

In some embodiments an antibody, or binding portion thereof, comprises, or consists of, a CDR-L1 comprising an amino acid sequence at least 70%, at least 75%, at  
 25 least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to the amino acid sequence of SEQ ID NO:12 or 13, a CDR-L2 comprising an amino acid sequence at least 70%, at least 75%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at  
 30 least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to the amino acid sequence of SEQ ID NO:25, a

CDR-L3 comprising an amino acid sequence at least 70%, at least 75%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to the amino acid sequence of SEQ ID NO:32, a CDR-H1  
 5 comprising an amino acid sequence at least 70%, at least 75%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to the amino acid sequence of SEQ ID NO:55 or 56, a CDR-H2 comprising an amino acid sequence at least 70%, at least 75%, at least 85%, at least  
 10 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to the amino acid sequence of SEQ ID NO:68 or 69, and a CDR-H3 comprising an amino acid sequence at least 70%, at least 75%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least  
 15 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to the amino acid sequence of SEQ ID NO:80.

[0082]

In some embodiments an antibody, or binding portion thereof, comprises, or consists of, a CDR-L1 comprising an amino acid sequence at least 80%, at least 90%, or  
 20 100% identical to the amino acid sequence of SEQ ID NO:13, a CDR-L2 comprising an amino acid sequence at least 80%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:25, a CDR-L3 comprising an amino acid sequence at least 80%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:32, a CDR-H1 comprising an amino acid sequence at least 80%, at least 90%, or 100%  
 25 identical to the amino acid sequence of SEQ ID NO:56, a CDR-H2 comprising an amino acid sequence at least 80%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:69, and a CDR-H3 comprising an amino acid sequence at least 80%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:80.

[0083]

30 In some embodiments an antibody, or binding portion thereof, comprises a CDR-L1 comprising an amino acid sequence at least 80%, at least 90%, or 100%

identical to the amino acid sequence of SEQ ID NO:12, a CDR-L2 comprising an amino acid sequence at least 80%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:25, a CDR-L3 comprising an amino acid sequence at least 80%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:32, a CDR-H1  
 5 comprising an amino acid sequence at least 80%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:56, a CDR-H2 comprising an amino acid sequence at least 80%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:68, and a CDR-H3 comprising an amino acid sequence at least 80%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:80. In some  
 10 embodiments, an antibody, or binding portion thereof, comprises a CDR-L1, CDR-L2 and a CDR-L3 comprising the amino acid sequences of SEQ ID NOs:12, 25 and 32, respectively, and a CDR-H1, CDR-H2 and a CDR-H3 comprising the amino acid sequences of SEQ ID NOs:55, 68 and 80, respectively.

[0084]

15 In some embodiments an antibody, or binding portion thereof, comprises, or consists of, a CDR-L1 comprising an amino acid sequence at least 70%, at least 75%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to the amino acid sequence of SEQ ID NO:14  
 20 or 15, a CDR-L2 comprising an amino acid sequence at least 70%, at least 75%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to the amino acid sequence of SEQ ID NO:26, a CDR-L3 comprising an amino acid sequence at least 70%, at least 75%, at least 85%, at  
 25 least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to the amino acid sequence of SEQ ID NO:33, a CDR-H1 comprising an amino acid sequence at least 70%, at least 75%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at  
 30 least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to the amino acid sequence of SEQ ID NO:57, 58 or 59, a

CDR-H2 comprising an amino acid sequence at least 70%, at least 75%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to the amino acid sequence of SEQ ID NO:70 or 71, and a  
 5 CDR-H3 comprising an amino acid sequence at least 70%, at least 75%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to the amino acid sequence of SEQ ID NO:81  
 [0085]

10 In some embodiments an antibody, or binding portion thereof, comprises, or consists of, a CDR-L1 comprising an amino acid sequence at least 80%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:15, a CDR-L2 comprising an amino acid sequence at least 80%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:26, a CDR-L3 comprising an amino acid sequence at least  
 15 80%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:33, a CDR-H1 comprising an amino acid sequence at least 80%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:59, a CDR-H2 comprising an amino acid sequence at least 80%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:70, and a CDR-H3 comprising an amino acid sequence at least  
 20 80%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:81. In some embodiments an antibody, or binding portion thereof, comprises, or consists of, a CDR-L1, a CDR-L2, a CDR-L3, a CDR-H1, a CDR-H2 and a CDR-H3 comprising an amino acid sequence at least 80%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NOs:15, 26, 33, 57, 70, and 81, respectively.

25 [0086]

In some embodiments an antibody, or binding portion thereof, comprises a CDR-L1 comprising an amino acid sequence at least 80%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:14, a CDR-L2 comprising an amino acid sequence at least 80%, at least 90%, or 100% identical to the amino acid sequence  
 30 of SEQ ID NO:26, a CDR-L3 comprising an amino acid sequence at least 80%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:33, a CDR-H1

comprising an amino acid sequence at least 80%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:59, a CDR-H2 comprising an amino acid sequence at least 80%, at least 90%, or 100% identical to the amino acid sequence of SEQ ID NO:71, and a CDR-H3 comprising an amino acid sequence at least 80%, at least 90%,  
5 or 100% identical to the amino acid sequence of SEQ ID NO:81. In some embodiments, an antibody, or binding portion thereof, comprises a CDR-L1, CDR-L2 and a CDR-L3 comprising the amino acid sequences of SEQ ID NOs:14, 26 and 33, respectively, and a CDR-H1, CDR-H2 and a CDR-H3 comprising the amino acid sequences of SEQ ID NOs:58, 71 and 81, respectively. In some embodiments, an  
10 antibody, or binding portion thereof, comprises a CDR-L1, CDR-L2 and a CDR-L3 comprising the amino acid sequences of SEQ ID NOs:14, 26 and 33, respectively, and a CDR-H1, CDR-H2 and a CDR-H3 comprising the amino acid sequences of SEQ ID NOs:57, 71 and 81, respectively.

[0087]

15 In some embodiments a syndecan-1 antibody, or binding portion thereof, comprises a heavy chain variable region having at least 70%, 75%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least 99% identity to a sequence of Table 9. In some embodiments a syndecan-1 antibody, or binding portion thereof, comprises a heavy chain variable region sequence of Table 9.

20 [TABLE 9]

## MOUSE VARIABLE HEAVY CHAIN SEQUENCES

| SEQ ID       | Hybridoma Clone/<br>Antibody Name | Mouse Variable Heavy Chain Amino Acid Sequence                                                                                        |
|--------------|-----------------------------------|---------------------------------------------------------------------------------------------------------------------------------------|
| SEQ ID NO:82 | F12P16F6                          | QVQLQQSGAEVVKPGASVKLSCKASGYTFTSYLYWV<br>KKGPGQGLDWIGEIYPRSGGTNINEKFLSKATLTAD<br>STAYLQLSSLTSEDSAVYYCTRSLLYWGQGTTLIVSS                 |
| SEQ ID NO:83 | F13P30A7                          | EVQLVESGGGLVQPKGSLKLSCAASGFTFNTYAMNWV<br>RQAPGKGLEWIARIRSKSNNYATYYADSVKDRFTISR<br>DSQSLLYLQMNNLKTEDTAIFYCVTDYGYVYFDAWGA<br>GTTVTVSS   |
| SEQ ID NO:84 | F13P18D8                          | EVQLLES GGGLVQPEGSLKLSCAASGFAFNTYAMNWV<br>RQAPGKGLEWLARIRSKSNNYATYYADSVKDRFTISR<br>DSQGM LYLQMNNLKTEDTAMYYCVTDY YVYFDVW<br>GAGTTVTVSS |
| SEQ ID NO:85 | F12P7G11                          | QVQLQQSGAELARPGASVKLSCKASGYTFSSHWMQW<br>VKQRPGQGLEWIGAIYPGDGDRFTQKFKGKATLTAD<br>KSSNTAYMQLSSLASEDSAVYYCARGIYYDRSRAMDY<br>WGQGTSVTVSS  |
| SEQ ID NO:86 | F13P14D3                          | EVQLVESGGGLVQPKGSLKLSCATSGFTFNTYAMNWV<br>RQAPGKGLEWVARIRSKSNNYATYYVDSVKDRFTISR<br>DSQSTVHLQMNNLKTEDTAIYYCVTDYGHVYFDVWG<br>AGTTVTVSS   |
| SEQ ID NO:87 | F11AP11E5                         | QVQLQQSGAELVKPGASVKLSCKASGYTFTNYYMYW<br>VKQRPGQGLEWIGEINPGNGGTNFNEKFKNKATLTVD<br>KSSSTAYMQLSSLTSEDSAVYYCTTRFAYWGQGTLLIV<br>SA         |
| SEQ ID NO:88 | F12P18D4.a                        | EVQLQQSGPELVKPGASVKMSCKASGYTFADYYMKW<br>VKQSHGKSLEWIGDINPNSGDTFYNHKFKGKATLTVD<br>KSSSTAYMQLNSLTSEDSAVYYCARTYYDYWGQGTLL<br>TVSS        |

[0088]

In some embodiments a syndecan-1 antibody, or binding portion thereof, comprises a humanized heavy chain variable region having at least 70%, 75%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least 99% identity to a sequence of Table 10. In some embodiments a syndecan-1 antibody, or binding portion thereof, comprises a humanized heavy chain variable region sequence of Table 10.

[TABLE 10]

## HUMANIZED HEAVY CHAINS

| SEQ ID       | Heavy Chain Name                                              | Humanized Heavy Chain Variable Region Amino Acid Sequences 89-92 & Complete Humanized Heavy Chain Sequence 93                                                                                                                                                                                                                                                                                                                                                                                     |
|--------------|---------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| SEQ ID NO:89 | P16F6 abb/sdr-rep                                             | QVQLVQSGAEVVKPGASVKLSCKASGYTFTSYLYWVKKA<br>PGQGLDWIGEIYPRSGGTNYAEKFQGRVTLTADTSTSTAYL<br>ELSSLTSEDТАVYYCTRSLLYWGQGTTLTVSS                                                                                                                                                                                                                                                                                                                                                                          |
| SEQ ID NO:90 | hF6 (cdr/ven-rep)                                             | QVQLVQSGAEVVKPGASVKLSCKASGYTFTSYLYWVKKA<br>PGQGLDWIGEIYPRSGGTNINEKFLSRVTLTADTSTSTAYLEL<br>SSLTSEDТАVYYCTRSLLYWGQGTTLTVSS                                                                                                                                                                                                                                                                                                                                                                          |
| SEQ ID NO:91 | P16F6 fra1-rep                                                | QVQLQQSGAEVVKPGASVKLSCKASGYTFTSYLYWVKKA<br>PGQGLDWIGEIYPRSGGTNINEKFLSRVTLTADTSTSTAYMD<br>LSSLTSEDТАVYYCTRSLLYWGQGTTLTVSS                                                                                                                                                                                                                                                                                                                                                                          |
| SEQ ID NO:92 | P16F6 fra2-rep                                                | QVQLQQSGAEVVKPGASVKLSCKASGYTFTSYLYWVKKA<br>PGQGLDWIGEIYPRSGGTNINEKFLSRVTITADESTSTVYMQ<br>LSSLTSEDSAVYYCTRSLLYWGQGTTLTVSS                                                                                                                                                                                                                                                                                                                                                                          |
| SEQ ID NO:93 | hF6 heavy chain (cdr/ven-rep) (complete heavy chain sequence) | QVQLVQSGAEVVKPGASVKLSCKASGYTFTSYLYWVKKA<br>PGQGLDWIGEIYPRSGGTNINEKFLSRVTLTADTSTSTAYLEL<br>SSLTSEDТАVYYCTRSLLYWGQGTTLTVSSASTKGPSVFPLA<br>PSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF<br>PAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVD<br>KKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR<br>TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ<br>YNSTYRVVSVLTVHLHQDWLNGKEYKCKVSNKALPAPIEKTI<br>SKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIA<br>VEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQ<br>QGNVFSCSVMEALHNHYTQKSLSLSPGK |

[0089]

In some embodiments an antibody, or binding portion thereof, comprises, or consists of, a heavy chain variable region comprising an amino acid sequence at least 70%, at least 75%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to any one of the amino acid sequences of SEQ ID NOs:82 to 92, and/or a light chain variable region comprising an amino acid sequence at least 70%, at least 75%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% identical to any one of the amino acid sequences of SEQ ID NOs:34 to 43. In some embodiments an antibody, or binding portion thereof, comprises a heavy chain variable region comprising an amino acid sequence at least 80%, at least 85%, at least 90%, or

100% identical to any one of the amino acid sequences of SEQ ID NOs:89-92, and a light chain variable region comprising an amino acid sequence at least 80%, at least 85%, at least 90%, or 100% identical to any one of the amino acid sequences of SEQ ID NOs:41 to 43. In some embodiments an antibody, or binding portion thereof,  
5 comprises a heavy chain variable region comprising an amino acid sequence at least 80%, at least 85%, at least 90%, or 100% identical to the amino acid sequences of SEQ ID NO:90, and a light chain variable region comprising an amino acid sequence at least 80%, at least 85%, at least 90%, or 100% identical to the amino acid sequences of SEQ ID NO:41.

10 [0090]

In some embodiments an antibody, or binding portion thereof, comprises one or more CDRs selected from a light chain variable region of Tables 4 and 5. In some embodiments an antibody, or binding portion thereof, comprises one or more CDRs selected from a heavy chain variable region of Tables 9 and 10. In some embodiments  
15 an antibody, or binding portion thereof, comprises one or more CDRs selected from a light chain variable region of Tables 4 and 5 and one or more CDRs selected from a heavy chain variable region of Tables 9 and 10. In certain embodiments, an antibody, or binding portion thereof, comprises a CDR-L1, a CDR-L2, and a CDR-L3, each selected from any one of the light chain variable regions of Tables 4 and 5, and a  
20 CDR-H1, a CDR-H2, and a CDR-H3, each selected from any one of the heavy chain variable regions of Tables 9 and 10. An amino acid sequence of a CDR (e.g., a CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2, and CDR-H3) can be identified within a heavy chain or light chain variable region disclosed herein by any suitable method described herein or known to those skilled in the art.

25 [0091]

In some embodiments an antibody comprises a heavy chain comprising an amino acid sequence at least 80%, at least 85%, at least 90%, or 100% identical to the amino acid sequences of SEQ ID NO:93, and a light chain comprising an amino acid sequence at least 80%, at least 85%, at least 90%, or 100% identical to the amino acid  
30 sequences of SEQ ID NO:44.

[0092]

In some embodiments an antibody, or binding portion thereof, comprises one or more suitable sequences selected from Tables 1-10 wherein the selected polypeptide sequence comprises 0 to 5, 1 to 5, 0 to 10, 1 to 10, 0 to 15, or 1 to 12 amino acid modifications, additions, deletions and/or substitutions. In some embodiments, an amino acid substitution is a conservative substitution where one amino acid is replaced with another amino acid of similar structure or having similar biochemical characteristics. Non-limiting examples of a conservative substitution include substituting a hydrophilic amino acid with another hydrophilic amino acid, substituting a hydrophobic amino acid with another hydrophobic amino acid, substituting an acidic amino acid with another acidic amino acid, substituting a basic amino acid with another basic amino acid, and substituting a neutral charged amino acid with another neutral charged amino acid. In some embodiments, an antibody, or binding portion thereof, comprises one or more amino acid analogues, non-native amino acids or amino acid derivatives.

15 [0093]

In certain embodiments, an antibody, or binding portion thereof, comprises one or more framework regions (FR). Framework regions are often located between CDRs and/or flank CDR sequences of a heavy or light chain variable region of an antibody, or binding portion thereof. In mammals, a heavy chain variable region often comprises four framework regions and a light chain variable region often comprises four framework regions. Any suitable method can be used to identify one or more framework regions in an antibody, in a variable region of an antibody, or binding portion thereof. An antibody, or binding portion thereof, may comprise synthetic or naturally occurring framework regions which are unmodified or modified (e.g., optimized) as discussed below.

25

[0094]

In some embodiments an antibody, or binding portion thereof, is chimeric, grafted and/or humanized. Chimeric, grafted and/or humanized antibodies often comprise modified or substituted constant regions and/or framework regions while maintaining binding specificity to syndecan-1, or a portion thereof. In some embodiments an antibody, or binding portion thereof, comprises constant regions,

30

framework regions, or portions thereof, derived from a human antibody. In some embodiments an antibody, or binding portion thereof, comprises fully synthetic portions, one or more amino acids, or sequences of amino acids that are not found in native antibody molecules.

5 [0095]

Naturally occurring framework regions, or portions thereof may be obtained from any suitable species. In certain embodiments the complementarity determining regions (CDRs) of the light and heavy chain variable regions of an antibody, or binding portion thereof, is grafted into framework regions from the same, or another, species.

10 For example, one or more framework regions of an antibody, or binding portion thereof, may be derived from a rodent species (e.g., a mouse or rat) or a primate species (e.g., a human).

[0096]

In certain embodiments, the CDRs of the light and/or heavy chain variable

15 regions of an antibody, or binding portion thereof, are grafted to consensus human framework regions. To create consensus human framework regions, in certain embodiments, framework regions from several human heavy chain or light chain amino acid sequences are aligned to identify a consensus sequence. In certain embodiments, the heavy chain or light chain framework regions of an antibody, or binding portion

20 thereof, are replaced with one or more framework regions, or portions thereof, from a different heavy chain or light chain variable region. In some embodiments an antibody, or binding portion thereof, comprises one or more human framework regions. In certain embodiments an antibody, or binding portion thereof, comprises at least 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 human framework regions. In some embodiments an antibody, or

25 binding portion thereof, comprises one or more mouse framework regions. In certain embodiments an antibody, or binding portion thereof, comprises at least 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 mouse framework regions. In certain embodiments an antibody, or binding portion thereof, comprises one or more human framework regions and one or more mouse framework regions.

30 [0097]

Methods of generating chimeric, humanized and/or optimized antibodies, or binding portions thereof, for example by modifying, substituting or deleting framework regions, or portions thereof, are known. Non-limiting examples of CDR grafting are described, e.g., in U.S. Patent Nos. 6, 180,370, 6,054,297, 5,693,762, 5,859,205, 5,693,761, 5,565,332, 5,585,089, and 5,530, 101, and in Jones et al., Nature, 321 :522-525 (1986); Verhoeven et al., Science, 239:1534-1536 (1988), and Winter, FEBS Letts., 430:92-94 (1998). Additional non-limiting examples of generating chimeric, grafted and/or humanized antibodies include U.S. patent No. 5,530,101; U.S. patent No. 5,707,622; U.S. patent No. 5,994,524; U.S. patent No. 6,245,894; Queen et al., (1988) PNAS 86:10029-10033; Riechmann et al., Nature (1988) 332:323-327; Antibody Engineering: Methods and Protocols, Vol. 248 of Methods in molecular biology, edited by Benny K. C. Lo, Springer Science & Business Media, (2004); and Antibody Engineering, Vol. 1, Roland E. Kontermann, Stefan Dübel, Edition 2, Publisher Springer Science & Business Media, (2010). In some embodiments an antibody, or binding portion thereof, is humanized by exchanging one or more framework regions, or portions thereof (e.g., one or more amino acids), with one or more framework regions, or portions thereof from a human antibody (e.g., see "Humanization of Antibodies" by Eduardo A. Padlan Publ. Landes BioScience 2002). In certain embodiments, an antibody, or binding portion thereof, is humanized or grafted by transferring one or more CDRs (e.g., 1, 2, 3, 4, 5 or all 6 CDRs) from a donor antibody (e.g., a mouse monoclonal antibody) to an acceptor antibody (e.g., a human antibody) while retaining the binding specificity of the donor antibody. In certain embodiments, the process of making a chimeric, grafted or humanized antibodies comprises making one or more amino acid substitutions, additions or deletions in a constant region or framework region of an antibody, or binding portion thereof. In certain embodiments, techniques such as "reshaping", "hyperchimerization", or "veneering/resurfacing" are used to produce a humanized antibody, or binding portion thereof. (e.g., see Vaswami et al., Annals of Allergy, Asthma, & Immunol. 81 :105 (1998); Roguska et al., Prot. Engin., 9:895-904 (1996); and U.S. Patent No. 6,072,035). In some aspects, an antibody, or binding portion thereof, is modified by a method

discussed above, or by another suitable method, to reduce immunogenicity (e.g., see Gilliland et al., J. Immunol, 62(6):3663-71 (1999)).

[0098]

In certain embodiments, an amino acid sequence of an antibody, or binding  
 5 portion thereof, is modified to optimize binding affinity for a target (e.g., syndecan-1),  
 species cross-reactivity, solubility and/or function (e.g., agonist activity, or lack thereof).  
 In some embodiments a specific combination of CDRs disclosed herein is optimized for  
 binding to syndecan-1, and/or to optimize a function or characteristic of an antibody, or  
 binding portion thereof. For example, a characterized light chain variable region  
 10 disclosed herein (e.g., a light chain variable region of any one of SEQ ID NOs:34-43)  
 can be co-expressed, using a suitable expression system, with a library of heavy chain  
 variable regions comprising a CDR-H1 and CDR-H2 of a characterized heavy chain  
 variable region (e.g., a heavy chain variable region selected from Tables 6 or 7), where  
 the CDR-H3 is replaced with a library of CDR-H3 sequences, which may include one or  
 15 more CDR-H3 regions of Table 8, for example. The resulting light chain/heavy chain  
 antibody can be screened for binding to syndecan-1 and/or for a specific function.  
 Optimized antibodies can be identified and the amino acid sequence of the CDR-H3 can  
 be identified by a suitable method. The above screening method can be used to  
 identify antibodies, or binding portions thereof, comprising specific combinations of  
 20 CDRs, or specific optimized CDR sequences (e.g., CDR sequences comprising amino  
 acid substitutions, additions or deletions) that provide an antibody, or binding portion  
 thereof, with improved binding specificity, binding affinity and/or function. Such  
 methods of screening and optimizing an antibody, or binding portion thereof, are known  
 (e.g., see Portolano et al., (1993) Journal of Immunology 150:880-887; and Clarkson et  
 25 al., (1991) Nature 352:624-628). Such references teach methods of producing  
 antibodies that bind a specific antigen by using known variable light chain, known  
 variable heavy chains, or portion thereof (e.g., CDRs thereof) by screening a library of  
 complementarity variable regions.

[0099]

30 In certain embodiments, an antibody, or binding portion thereof, is modified to  
 eliminate or add glycosylation sites in order to optimize affinity and/or function of an

antibody, or binding portion thereof (e.g., see Co et al., Mol. Immunol, 30:1361-1367 (1993)). In some embodiments the number and/or type of glycosylation sites in an antibody, or binding portion thereof, are modified or altered. An N-linked glycosylation site is often characterized by the sequence Asn-X-Ser or Asn-X-Thr, where the amino acid residue designated as X are any amino acid residue except proline. The substitution of amino acid residues to create this sequence provides a potential new site for the addition of an N-linked carbohydrate chain. Alternatively, substitutions which eliminate this sequence will remove an existing N-linked carbohydrate chain. Also provided in certain embodiments is a rearrangement of N-linked carbohydrate chains where one or more N-linked glycosylation sites (typically those that are naturally occurring) are eliminated and one or more new N-linked sites are created. In some embodiments an antibody, or binding portion thereof, is modified by deleting one or more cysteine residues or substituting one or more cysteine residues for another amino acid (e.g., serine) as compared to an unmodified antibody, or binding portion thereof. In certain embodiments cysteine variants are useful for optimizing expression, secretion, and/or solubility.

[0100]

In certain embodiments an antibody, or binding portion thereof, is modified to include certain amino acid additions, substitutions, or deletions designed or intended, for example, to reduce susceptibility of an antibody, or binding portion thereof, to proteolysis, reduce susceptibility of an antibody, or binding portion thereof, to oxidation, increase serum half-life and/or confer or modify other physicochemical, pharmacokinetic or functional properties of an antibody, or binding portion thereof.

[0101]

The antibody portion of a bi-specific binding agent described herein may comprise an antigen binding portion of an antibody (e.g., binding portion) that binds specifically to CD138. A binding portion of an antibody refers to the antigen binding portion of an antibody. In certain embodiments, a binding portion of an antibody comprises, at least, the minimal portion of an antibody that is sufficient to specifically bind to an antigen (e.g., syndecan-1, or a portion thereof). In certain embodiments, a binding portion of an antibody comprises one or more complementarity determining

regions (CDRs) of an antibody that are necessary and sufficient to direct specific binding to CD138. In certain embodiments, a binding portion of an antibody comprises the heavy and light chain variable regions of an antibody. In certain embodiments a binding portion of antibody comprises or consists of a single polypeptide (e.g., single chain antibody). A single chain antibody may comprise one or more CDRs from a heavy and/or light chain of an antibody. In some embodiments a binding portion of antibody comprises or consists of two polypeptides (e.g., a heavy chain variable region and a light chain variable region). In some embodiments a binding portion of antibody comprises one or more structural portions (e.g., scaffolds, structural polypeptides, constant regions and/or framework regions). In some embodiments an antibody, or a binding portion of antibody is attached to a carrier or substrate (e.g., a polymer, a non-organic material, silicon, a bead, a particle or the like).  
[0102]

A bi-specific binding agent may comprise one binding portion of an antibody or multiple binding portions of an antibody. When a bi-specific binding agent comprises multiple binding portions of an antibody, each binding portion binds specifically to the same antigen (e.g., syndecan-1, or a portion thereof). In some embodiments a bi-specific binding agent comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more binding portions of an antibody.

[0103]

Non-limiting examples of a binding portion of an antibody include a single-chain antibody, Fab, Fab', F(ab')<sub>2</sub>, Fv fragment, single-chain Fv (scFv), scFv-Fc, (scFv)<sub>2</sub>-Fc, disulfide-linked Fvs (sdFv), VL (variable light chain), VH (variable heavy chain), diabody (Dab), triabody (trivalent), tetrabody (tetravalent), minibody ((scFV-CH<sub>3</sub>)<sub>2</sub>), IgGdeltaCH<sub>2</sub>, nanobodies, scFv-Igs, SVD-Igs, the like, and combinations thereof.

[0104]

Nucleic acids, or portions thereof, that encode one or more polypeptides of a bi-specific binding agent, antibody, or binding portions thereof, may be cloned, subcloned, rearranged or modified for recombinant expression using a suitable cloning procedure and subsequently expressed using a suitable expression system by a method

known to those skilled in the art (e.g., see Maniatis et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, 1982; Antibody Engineering: Methods and Protocols, Vol. 248 of Methods in molecular biology, edited by Benny K. C. Lo, Springer Science & Business Media, 2004; Antibody Engineering, Vol. 1, Roland E. Kontermann, Stefan Dübel, Edition 2, Publisher Springer Science & Business Media, 2010; Antibody Phage Display: Methods and Protocols, Biomed Protocols, Vol. 178 of Methods in molecular biology, Editors Philippa M. O'Brien, Robert Aitken, Springer Science & Business Media, 2004).

[0105]

10           In some embodiments an antibody, or binding portion thereof, binds specifically to a mammalian syndecan-1, or portion thereof. In certain embodiments, an antibody, or binding portion thereof, binds specifically to a mammalian syndecan-1, or portion thereof, with a binding affinity (KD) of  $10^{-5}$  M or less,  $10^{-6}$  M or less,  $10^{-7}$  M or less,  $10^{-8}$  M or less, 50 nM or less, 10 nM or less, or 1 nM or less. In certain  
15           embodiments, an antibody, or binding portion thereof, binds specifically to a mammalian syndecan-1, or portion thereof, with a binding affinity (KD) from about  $10^{-5}$  to  $10^{-15}$  M,  $10^{-6}$  to  $10^{-15}$  M,  $10^{-7}$  to  $10^{-15}$  M,  $10^{-9}$  to  $10^{-15}$  M,  $10^{-9}$  to  $10^{-14}$  M,  $10^{-9}$  to  $10^{-13}$  M, or  $10^{-9}$  to about  $10^{-12}$  M. In some embodiments an antibody, or binding portion thereof, binds specifically to an extracellular domain or extracellular region of a  
20           mammalian syndecan-1, or a portion thereof. In certain aspects, an antibody, or binding portion thereof, binds specifically to a wild-type syndecan-1 produced by a cell of an unaltered (non-genetically modified) mammal found in nature. In certain aspects an antibody, or binding portion thereof, binds specifically to a naturally occurring syndecan-1 variant. In certain aspects an antibody, or binding portion thereof, binds  
25           specifically to a syndecan-1 comprising one or more amino acid substitutions, additions or deletions. In certain embodiments an antibody, or binding portion thereof, binds specifically to a syndecan-1 produced and/or expressed on the surface of a cell of a human, non-human primate, dog, cat, or rodent (e.g., a mouse or rat). In certain embodiments, an antibody, or binding portion thereof, binds specifically to one or more  
30           syndecan-1 polypeptides, or a portion thereof (e.g., an extracellular domain), comprising an amino acid sequence of any one of SEQ ID NOs: 1 and 126 to 130. In

certain embodiments, an antibody, or binding portion thereof, binds specifically to one or more syndecan-1 polypeptides, or a portion thereof, having an amino acid sequence of any one of SEQ ID NOs: 1 and 126 to 130 with a binding affinity (KD) of 50 nM or less, 10 nM or less, or 1 nM or less. In certain embodiments, an antibody, or binding portion thereof, binds specifically to a human syndecan-1. In certain embodiments, an antibody, or binding portion thereof, binds specifically to an extracellular domain of human syndecan-1. In certain embodiments, an antibody, or binding portion thereof, binds specifically to a human syndecan-1, and/or an extracellular domain thereof.

[0106]

10 In certain embodiments, an antibody, or binding portion thereof, binds specifically to a polypeptide sequence comprising or consisting of the amino acid sequence of AGE<sub>1</sub>GPKEGEAVVLP (SEQ ID NO:94) or GPKEGEAVVLP (SEQ ID NO:95). In certain embodiments, an antibody, or binding portion thereof, binds specifically to a polypeptide sequence comprising or consisting of the amino acid sequence of AGE<sub>1</sub>GPKEGEAVVLP (SEQ ID NO:94) or GPKEGEAVVLP (SEQ ID NO:95) with a binding affinity (KD) of  $10^{-5}$  M or less,  $10^{-6}$  M or less,  $10^{-7}$  M or less,  $10^{-8}$  M or less, 50 nM or less, 10 nM or less, or 1 nM or less. In certain embodiments, an antibody, or binding portion thereof, binds specifically to a polypeptide sequence comprising or consisting of the amino acid sequence of GX<sub>1</sub>KEX<sub>2</sub>EAX<sub>3</sub>VLP (SEQ ID NO:96), wherein X<sub>1</sub>, X<sub>2</sub> and X<sub>3</sub> are selected from any amino acid. In some embodiments X<sub>1</sub> is selected from proline, alanine, cysteine, glycine, serine, threonine, and valine, and/or X<sub>2</sub> is selected from proline, alanine, cysteine, glycine, serine, threonine, and valine, and/or X<sub>3</sub> is selected from proline, alanine, cysteine, glycine, serine, threonine, valine, methionine, leucine, isoleucine and phenylalanine. In certain embodiments, an antibody, or binding portion thereof, binds specifically to a polypeptide sequence comprising or consisting of the amino acid sequence of GX<sub>1</sub>KEX<sub>2</sub>EAX<sub>3</sub>VLP (SEQ ID NO:96) with a binding affinity (KD) of 50 nM or less, 10 nM or less, or 1 nM or less, where X<sub>1</sub> is selected from proline, alanine, cysteine, glycine, serine, threonine, and valine, X<sub>2</sub> is selected from proline, alanine, cysteine, glycine, serine, threonine, and valine, and X<sub>3</sub> is selected from proline, alanine, cysteine, glycine, serine, threonine, valine, methionine, leucine, isoleucine and phenylalanine. In certain

embodiments, X<sub>1</sub> is proline, X<sub>2</sub> is selected from alanine, glycine, or serine and X<sub>3</sub> is selected from alanine, glycine, and valine.

[0107]

#### FGFRs

5 Aberrantly activated FGFRs have been implicated in several human malignancies and overexpression of FGFR3 is sufficient to induce oncogenic transformation in several animal models. Several attempts to generate antibodies and antibody drug conjugates (ADCs) that target FGFRs have been made. However, these antibodies often recognize only one isoform of the FGFR or display a significant  
10 difference in binding affinity among the different FGFR isoforms.

[0108]

Presented herein are bi-specific binding agents that comprise a Fynomer that binds one or more splice forms of FGFR3 with high affinity and specificity. In some embodiments, the bi-specific binding agents are capable of being internalized into a  
15 target cell upon binding.

[0109]

#### Fynomers

In some embodiments, a Fynomer is a small antigen-binding polypeptide (e.g., from 5-10 kDa, in some embodiments about 7 kDa) comprising a non-immunoglobulin  
20 scaffold derived from an SH3 domain of the human proto-oncogene tyrosine-protein kinase Fyn (p59-FYN, Slk, Syn, MGC45350, Gene ID 2534). Accordingly, in certain embodiments a Fynomer is a single chain polypeptide comprising an N-terminus (N-terminal amino acid) and a C-terminus (C-terminal amino acid) that can be expressed from a nucleic acid sequence using recombinant technology or can be  
25 chemically synthesized (e.g., by using a suitable solid phase chemistry). Fyn SH3-derived Fynomers are known in the art and have been described e.g. in Grabulovski et al. (2007) JBC, 282, p. 3196-3204; WO 2008/022759; Bertschinger et al. (2007) Protein Eng. Des. Sel. 20(2):57-68; and Gebauer and Skerra (2009) Curr. Opinion in Chemical Biology 13:245-255. A Fynomer can comprise a polypeptide of  
30 50 to 80 amino acids in length. In some embodiments, a Fynomer comprises a polypeptide of 50 to 70, or 60 to 70 amino acids in length. In some embodiments, a

Fynomer comprises a polypeptide of 60, 61, 62, 63, 64, 65, 66, 67, 68, 69 or 70 amino acids in length. Fynomers can be engineered to bind with high affinity and specificity to an antigen target of choice often through random mutation of two variable loops (RT-loop and src-loop), while the surrounding sequences of the Fynomer provide a structural scaffold that is substantially conserved among Fynomer sequences. It is believed that the amino acid sequence of these two variable loops, and sometimes amino acids immediately adjacent to the variable loops, substantially contribute to the specificity and binding affinity of a Fynomer to a selected antigen target. Although Fynomer sequences outside of the variable loops primarily provide for the structural scaffold, it is understood that certain amino acids within the scaffold region can be substituted without substantial loss of structure or binding specificity.

[0110]

The amino acid sequences of the Fynomers presented herein bind specifically to an FGFR3, or one or more specific isoforms, or variants thereof. In some embodiments, a Fynomer described herein binds specifically to FGFR3, or one or more specific isoforms, or variants thereof. In some embodiments, a Fynomer described herein binds specifically to fibroblast growth factor receptor 3, isoforms 3b (FGFR3b). In some embodiments, a Fynomer described herein binds specifically to fibroblast growth factor receptor 3, isoform 3c (FGFR3c). In some embodiments, a Fynomer described herein binds specifically to both FGFR3b and FGFR3c. In some embodiments, an FGFR3 is a mammalian FGFR3. In some embodiments, an FGFR3 is a human, mouse, rat or monkey FGFR3 protein, or an isoform thereof. In some embodiments, an FGFR3 is a human FGFR3, or an isoform thereof. The amino acid sequence of human FGFR3b is shown in SEQ ID NO:97 and the amino acid sequence of human FGFR3c is shown in SEQ ID NO:98. In some embodiments, a Fynomer described herein binds specifically to human FGFR3b and/or human FGFR3c.

[0111]

In some embodiment, a Fynomer (e.g., a Fynomer portion of a bi-specific binding agent disclosed herein) binds to an FGFR3, or isoform thereof, with a  $K_D$  of  $1 \times 10^{-7}$  M or less, a  $K_D$  of  $1 \times 10^{-8}$  M or less, a  $K_D$  of  $1 \times 10^{-9}$  M or less, or with a  $K_D$  of  $1 \times 10^{-10}$  M or less. In some embodiments, a Fynomer (e.g., a Fynomer portion of a

bi-specific binding agent disclosed herein) binds to one or both isoforms FGFR3b and/or FGFR3c with a  $K_D$  of  $1 \times 10^{-7}$  M or less, a  $K_D$  of  $1 \times 10^{-8}$  M or less, a  $K_D$  of  $1 \times 10^{-9}$  M or less, or with a  $K_D$  of  $1 \times 10^{-10}$  M or less. In some embodiment, a Fynomer binds to both isoforms, FGFR3b and FGFR3c with a  $K_D$  of  $10^{-7}$  to  $10^{-12}$  M, a  $K_D$  of  $10^{-8}$  to  $10^{-12}$  M, or a  $K_D$  of  $10^{-9}$  to  $10^{-12}$  M. In some embodiments, a Fynomer binds specifically to one or both isoforms FGFR3b and/or FGFR3c, and does not bind substantially to other related proteins such as FGFR1, FGFR2, or FGFR4. In some embodiments, a Fynomer that does not substantially bind to an FGFR, is a Fynomer that does not demonstrate any detectable specific binding, or binds to an FGFR with a  $K_D$  of greater than  $5 \times 10^{-6}$  M.

[0112]

In some embodiments, a Fynomer portion of a bi-specific binding agent disclosed herein, binds to an FGFR3, or isoform thereof, in the presence of receptor bound ligand (e.g., FGF1). Accordingly, in some embodiments, a Fynomer portion of a bi-specific binding agent disclosed herein does not block, abrogate, or inhibit binding of an FGFR3 ligand (e.g., FGF1) to an FGFR3. In some embodiments, a Fynomer portion of a bi-specific binding agent disclosed herein does not block, abrogate, or inhibit binding of an FGFR3 ligand (e.g., FGF1) to an FGFR3. In some embodiments, a bi-specific binding agent disclosed herein does not block, abrogate, or inhibit binding of an FGFR3 ligand (e.g., FGF1) to an FGFR3. In some embodiments, a Fynomer portion of a bi-specific binding agent disclosed herein, and/or a bi-specific binding agent disclosed herein, binds to an FGFR3, or isoform thereof, in the presence of receptor bound ligand (e.g., FGF1).

[0113]

In certain embodiments, a Fynomer comprises a polypeptide having an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to the amino acid sequence GVTLFVALYDYEVYGPTPMLS FHKGEKFQIL(X<sub>1</sub>)(X<sub>2</sub>)(X<sub>3</sub>)(X<sub>4</sub>)GPYWEARSL(X<sub>5</sub>)TGETG(X<sub>6</sub>)IPSNYVAPVDSIQ (SEQ ID NO:99), where amino acids X<sub>1</sub> to X<sub>6</sub> are independently selected from any amino acid, and the Fynomer binds specifically to an FGFR3. In some embodiment, a Fynomer having a sequence at least 80%, at least

85%, at least 90%, at least 95% or 100% identical to the amino acid sequence of SEQ ID NO:99 binds specifically to an FGFR3 (e.g., human FGFR3). In some embodiment, a Fynomer having a sequence at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to the amino acid sequence of SEQ ID NO:99 binds specifically to both  
 5 FGFR3b and FGFR3c. The N-terminal RT loop and C-terminal src-loop of the Fynomer sequences provided herein are often underlined solely for purposes of illustration. In certain embodiments, a Fynomer comprises a polypeptide having an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to the amino acid sequence of SEQ ID NO:99, where (i) amino acids X<sub>1</sub> to X<sub>6</sub>  
 10 are independently selected from any amino acid, and (ii) the amino acid sequence EVYGPTPM (SEQ ID NO:100) at amino acid positions 12 to 19 of SEQ ID NO:99 and amino acids P and Y at amino acid positions 37 and 38 of SEQ ID NO:99 are conserved.

[0114]

15 In certain embodiments X<sub>1</sub> is selected from N, R, H and K. In certain embodiments X<sub>1</sub> is N, R or K. In certain embodiments X<sub>2</sub> is selected from S, G, A, V, and P. In certain embodiments X<sub>2</sub> is selected from S, G, A, V, P, and any basic amino acid. In certain embodiments X<sub>2</sub> is S, G, K or R. In certain embodiments X<sub>3</sub> is S, G, A, V, or P. In certain embodiments X<sub>3</sub> is S or G. In certain embodiments X<sub>4</sub> is  
 20 selected from any charged, basic or acidic amino acid. In certain embodiments X<sub>4</sub> is selected from S, G, A, V and P. In certain embodiments X<sub>4</sub> is E, Q, D, S or K. In certain embodiments X<sub>5</sub> is selected from S, G, A, V, P, S and T. In certain embodiments X<sub>5</sub> is T or A. In certain embodiments X<sub>6</sub> is selected from any hydrophobic amino acid. In certain embodiments X<sub>6</sub> is selected from any polar amino  
 25 acid. In certain embodiments X<sub>6</sub> is selected from Q, N, H, S, T, Y, C, W, A, L, V and I. In certain embodiments X<sub>6</sub> is Y, W or L.

[0115]

In certain embodiments X<sub>1</sub> is N, R or K; X<sub>2</sub> is S, G, K or R; X<sub>3</sub> is S or G; X<sub>4</sub> is E, Q, D, S or K; X<sub>5</sub> is T or A; and/or X<sub>6</sub> is Y, W or L.

30 [0116]

In some embodiments, a Fynomer comprises an amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO:99, where (i) amino acid positions X<sub>1</sub> to X<sub>6</sub> may be any amino acid sequence, (ii) the identity determination excludes amino acid positions X<sub>1</sub> to X<sub>6</sub>, (iii) and the amino acid sequence EVYGPTPM (SEQ ID NO:100) in amino acid positions 12 to 19 of SEQ ID NO:99 and amino acids P and Y in amino acid positions 37 and 38 of SEQ ID NO:99 are conserved, and (iv) the Fynomer binds specifically to an FGFR3.

[0117]

In some embodiments, a Fynomer comprises an amino acid sequence at least 95% identical to the amino acid sequence of SEQ ID NO:99, where (i) X<sub>1</sub> is N, R or K; X<sub>2</sub> is S, G, K or R; X<sub>3</sub> is S or G; X<sub>4</sub> is E, Q, D, S or K; X<sub>5</sub> is T or A; and X<sub>6</sub> is Y, W or L, (ii) the identity determination excludes amino acid positions X<sub>1</sub> to X<sub>6</sub>, (iii) and the amino acid sequence EVYGPTPM (SEQ ID NO:100) in amino acid positions 12 to 19 of SEQ ID NO:99 and amino acids P and Y in amino acid positions 37 and 38 of SEQ ID NO:99 are conserved, and (iv) the Fynomer binds specifically to an FGFR3.

[0118]

In certain embodiments, a Fynomer comprises a polypeptide having an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to the amino acid sequence of GVTLFVALYDYEEVYGPTPMLSFHKGEKFQILNSSEGPYWEARSLTTGETGLIPSNYVAPVDSIQ (SEQ ID NO:101; sometimes referred to herein as FF2L4C3), where the Fynomer binds specifically to an FGFR3. In certain embodiments, a Fynomer comprises a polypeptide having an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to the amino acid sequence of SEQ ID NO:101, where the amino acid sequence EVYGPTPM (SEQ ID NO:100) at amino acid positions 12 to 19 of SEQ ID NO:101 and amino acids P and Y at amino acid positions 37 and 38 of SEQ ID NO:101 are conserved, and the Fynomer binds specifically to an FGFR3. In certain embodiments, a Fynomer comprises a polypeptide having an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to the amino acid sequence of SEQ ID NO:101, where the amino acid sequence EVYGPTPM (SEQ ID NO:100) at amino acid positions 12 to 19 of SEQ ID NO:101,

and the amino acid sequence NSSEGPY (SEQ ID NO:102) at amino acid positions 32 to 38 of SEQ ID NO:101 are conserved, and the Fynomer binds specifically to an FGFR3. In some embodiments, a Fynomer having an amino acid sequence at least 80%, at least 85%, at least 90%, or at least 95% identical to the amino acid sequence of SEQ ID NO:101, where the Fynomer binds specifically to an FGFR3 (e.g., FGFR3b and/or FGFR3c), is a Fynomer comprising 0 to 12, 0 to 8, 0 to 5, or 1 to 2 amino acid substitutions, additions and/or deletions that do not ablate or significantly reduce the ability of the Fynomer to specifically bind to an FGFR3. A significant reduction in specific binding is a reduction of binding affinity (i.e.,  $K_D$ ) in excess of 20% of the binding affinity of the Fynomer of SEQ ID NO:101 to human FGFR3b or FGFR3c. One of skill in the art can readily determine the binding affinity of a Fynomer to FGFR3b or FGFR3c using routine methods and such a determination would not require undue experimentation. Accordingly, one of skill in the art can readily determine how to make a Fynomer having an amino acid sequence at least 80%, at least 85%, at least 90%, or at least 95% identical to the amino acid sequence of SEQ ID NO:101, where the Fynomer binds specifically to an FGFR3. In certain embodiments, a Fynomer comprises or consists of the amino acid sequence of SEQ ID NO:101.

[0119]

In certain embodiments, a Fynomer comprises a polypeptide having an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to the amino acid sequence of GVTFLFVALYDYEEVYGPTPMLSFHKGEKFQILRGGQGPYWEARSLTTGETGLIPSNYVAPVDSIQ (SEQ ID NO:103); sometimes referred to herein as FF44L65G12) and the Fynomer binds specifically to an FGFR3. In certain embodiments, a Fynomer comprises a polypeptide having an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to the amino acid sequence of SEQ ID NO:103, where the amino acid sequence EVYGPTPM (SEQ ID NO:100) at amino acid positions 12 to 19 of SEQ ID NO:103 and amino acids P and Y at amino acid positions 37 and 38 of SEQ ID NO:103 are conserved, and the Fynomer binds specifically to an FGFR3. In certain embodiments, a Fynomer comprises a polypeptide having an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to

the amino acid sequence of SEQ ID NO:103, where the amino acid sequence EVYGPTPM (SEQ ID NO:100) at amino acid positions 12 to 19 of SEQ ID NO:103, and the amino acid sequence RGGQGPY (SEQ ID NO:104) at amino acid positions 32 to 38 of SEQ ID NO:103 are conserved, and the Fynomer binds specifically to an FGFR3. In some embodiments, a Fynomer having an amino acid sequence at least 80%, at least 85%, at least 90%, or at least 95% identical to the amino acid sequence of SEQ ID NO:103, where the Fynomer binds specifically to an FGFR3 (e.g., FGFR3b and/or FGFR3c), is a Fynomer comprising 0 to 12, 0 to 8, 0 to 5, or 1 to 2 amino acid substitutions, additions and/or deletions that do not ablate or significantly reduce the ability of the Fynomer to specifically bind to an FGFR3. A significant reduction in specific binding is a reduction of binding affinity (i.e.,  $K_D$ ) in excess of 20% of the binding affinity of the Fynomer of SEQ ID NO:103 to human FGFR3b or FGFR3c. One of skill in the art can readily determine the binding affinity of a Fynomer to FGFR3b or FGFR3c using routine methods and such a determination would not require undue experimentation. Accordingly, one of skill in the art can readily determine how to make a Fynomer having an amino acid sequence at least 80%, at least 85%, at least 90%, or at least 95% identical to the amino acid sequence of SEQ ID NO:103, where the Fynomer binds specifically to an FGFR3. In certain embodiments, a Fynomer comprises or consists of the amino acid sequence of SEQ ID NO:103.

[0120]

In certain embodiments, a Fynomer comprises a polypeptide having an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to the amino acid sequence of GVTLFVALYDYEEVYGPTPMLSFHKGEKFQILRGGDGPYWEARSLTTGETGLIPS NYVAPVDSIQ (SEQ ID NO:105; sometimes referred to herein as FF44L65G7) and the Fynomer binds specifically to an FGFR3. In certain embodiments, a Fynomer comprises a polypeptide having an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to the amino acid sequence of SEQ ID NO:105, where the amino acid sequence EVYGPTPM (SEQ ID NO:100) at amino acid positions 12 to 19 of SEQ ID NO:105 and amino acids P and Y at amino acid positions 37 and 38 of SEQ ID NO:105 are conserved, and the Fynomer binds specifically to an

FGFR3. In certain embodiments, a Fynomer comprises a polypeptide having an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to the amino acid sequence of SEQ ID NO:105, where the amino acid sequence EVYGPTPM (SEQ ID NO:100) at amino acid positions 12 to 19 of SEQ ID NO:105, and amino acids RGGDGPY (SEQ ID NO:106) at amino acid positions 32 to 38 of SEQ ID NO:105 are conserved, and the Fynomer binds specifically to an FGFR3. In some embodiments, a Fynomer having an amino acid sequence at least 80%, at least 85%, at least 90%, or at least 95% identical to the amino acid sequence of SEQ ID NO:105, where the Fynomer binds specifically to an FGFR3 (e.g., FGFR3b and/or FGFR3c), is a Fynomer comprising 0 to 12, 0 to 8, 0 to 5, or 1 to 2 amino acid substitutions, additions and/or deletions that do not ablate or significantly reduce the ability of the Fynomer to specifically bind to an FGFR3. A significant reduction in specific binding is a reduction of binding affinity (i.e.,  $K_D$ ) in excess of 20% of the binding affinity of the Fynomer of SEQ ID NO:105 to human FGFR3b or FGFR3c. One of skill in the art can readily determine the binding affinity of a Fynomer to FGFR3b or FGFR3c using routine methods and such a determination would not require undue experimentation. Accordingly, one of skill in the art can readily determine how to make a Fynomer having an amino acid sequence at least 80%, at least 85%, at least 90%, or at least 95% identical to the amino acid sequence of SEQ ID NO:105, where the Fynomer binds specifically to an FGFR3. In certain embodiments, a Fynomer comprises or consists of the amino acid sequence of SEQ ID NO:105).

[0121]

In certain embodiments, a Fynomer comprises a polypeptide having an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to the amino acid sequence of GVTLFVALYDYEEVYGPTPMLSFHKGEKFQILKGGSGPYWEARSLTTGETGLIPSNYVAPVDSIQ (SEQ ID NO:107; sometimes referred to herein as FF48L66G7 or “G7”) and the Fynomer binds specifically to an FGFR3. In certain embodiments, a Fynomer comprises a polypeptide having an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to the amino acid sequence of SEQ ID NO:107, where the amino acid sequence EVYGPTPM (SEQ ID NO:100) at amino

acid positions 12 to 19 of SEQ ID NO:107 and amino acids P and Y at amino acid positions 37 and 38 of SEQ ID NO:107 are conserved, and the Fynomer binds specifically to an FGFR3. In certain embodiments, a Fynomer comprises a polypeptide having an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to the amino acid sequence of SEQ ID NO:107, where the amino acid sequence EVYGPTPM (SEQ ID NO:100) at amino acid positions 12 to 19 of SEQ ID NO:107, and amino acids KGGSGPY (SEQ ID NO:108) at amino acid positions 32 to 38 of SEQ ID NO:107 are conserved, and the Fynomer binds specifically to an FGFR3. In some embodiments, a Fynomer having an amino acid sequence at least 80%, at least 85%, at least 90%, or at least 95% identical to the amino acid sequence of SEQ ID NO:107, where the Fynomer binds specifically to an FGFR3 (e.g., FGFR3b and/or FGFR3c), is a Fynomer comprising 0 to 12, 0 to 8, 0 to 5, or 1 to 2 amino acid substitutions, additions and/or deletions that do not ablate or significantly reduce the ability of the Fynomer to specifically bind to an FGFR3. A significant reduction in specific binding is a reduction of binding affinity (i.e.,  $K_D$ ) in excess of 20% of the binding affinity of the Fynomer of SEQ ID NO:107 to human FGFR3b or FGFR3c. One of skill in the art can readily determine the binding affinity of a Fynomer to FGFR3b or FGFR3c using routine methods and such a determination would not require undue experimentation. Accordingly, one of skill in the art can readily determine how to make a Fynomer having an amino acid sequence at least 80%, at least 85%, at least 90%, or at least 95% identical to the amino acid sequence of SEQ ID NO:107, where the Fynomer binds specifically to an FGFR3. In certain embodiments, a Fynomer comprises or consists of the amino acid sequence of SEQ ID NO:107.

[0122]

In certain embodiments, a Fynomer comprises a polypeptide having an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to the amino acid sequence of GVTLFVALYDYEEVYGPTPMLSFKGEKFQILRKGKGPYWEARSLATGETGLIPSNYVAPVDSIQ (SEQ ID NO:109; sometimes referred to herein as FF43L65D5) and the Fynomer binds specifically to an FGFR3. In certain embodiments, a Fynomer comprises a polypeptide having an amino acid sequence at least 80%, at least 85%, at

least 90%, at least 95% or 100% identical to the amino acid sequence of SEQ ID NO:109, where the amino acid sequence EVYGPTPM (SEQ ID NO:100) at amino acid positions 12 to 19 of SEQ ID NO:109 and amino acids P and Y at amino acid positions 37 and 38 of SEQ ID NO:109 are conserved, and the Fynomer binds specifically to an FGFR3. In certain embodiments, a Fynomer comprises a polypeptide having an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to the amino acid sequence of SEQ ID NO:109, where the amino acid sequence EVYGPTPM (SEQ ID NO:100) at amino acid positions 12 to 19 of SEQ ID NO:109 and amino acids RKGKGPY (SEQ ID NO:110) at amino acid positions 32 to 38 of SEQ ID NO:109 are conserved, and the Fynomer binds specifically to an FGFR3. In some embodiments, a Fynomer having an amino acid sequence at least 80%, at least 85%, at least 90%, or at least 95% identical to the amino acid sequence of SEQ ID NO:109, where the Fynomer binds specifically to an FGFR3 (e.g., FGFR3b and/or FGFR3c), is a Fynomer comprising 0 to 12, 0 to 8, 0 to 5, or 1 to 2 amino acid substitutions, additions and/or deletions that do not ablate or significantly reduce the ability of the Fynomer to specifically bind to an FGFR3. A significant reduction in specific binding is a reduction of binding affinity (i.e.,  $K_D$ ) in excess of 20% of the binding affinity of the Fynomer of SEQ ID NO:109 to human FGFR3b or FGFR3c. One of skill in the art can readily determine the binding affinity of a Fynomer to FGFR3b or FGFR3c using routine methods and such a determination would not require undue experimentation. Accordingly, one of skill in the art can readily determine how to make a Fynomer having an amino acid sequence at least 80%, at least 85%, at least 90%, or at least 95% identical to the amino acid sequence of SEQ ID NO:109, where the Fynomer binds specifically to an FGFR3. In certain embodiments, a Fynomer comprises or consists of the amino acid sequence of SEQ ID NO:109.

[0123]

In certain embodiments, a Fynomer comprises a polypeptide having an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to the amino acid sequence of

GVTLFVALYDYEEVYGPTPMLSFHKGEKFQILRRGSGPYWEARSLTTGETGLIPS  
 NYVAPVDSIQ (SEQ ID NO:111; sometimes referred to herein as FF44L65B7) and

the Fynomer binds specifically to an FGFR3, or an isoform thereof (e.g., FGFR3b and/or FGFR3c). In certain embodiments, a Fynomer comprises a polypeptide having an amino acid sequence at least 80%, at least 85%, at least 90%, or at least 95% identical to the amino acid sequence of SEQ ID NO:111, where the amino acid sequence EVYGPTPM (SEQ ID NO:100) at amino acid positions 12 to 19 of SEQ ID NO:111 and amino acids P and Y at amino acid positions 37 and 38 of SEQ ID NO:111 are conserved, and the Fynomer binds specifically to an FGFR3 (e.g., FGFR3b and/or FGFR3c). In certain embodiments, a Fynomer comprises a polypeptide having an amino acid sequence at least 80%, at least 85%, at least 90%, or at least 95% identical to the amino acid sequence of SEQ ID NO:111, where the amino acid sequence EVYGPTPM (SEQ ID NO:100) at amino acid positions 12 to 19 of SEQ ID NO:111 and amino acids RRGSGPY (SEQ ID NO:112) at amino acid positions 32 to 38 of SEQ ID NO:111 are conserved, and the Fynomer binds specifically to an FGFR3 (e.g., FGFR3b and/or FGFR3c). In some embodiments, a Fynomer having an amino acid sequence at least 80%, at least 85%, at least 90%, or at least 95% identical to the amino acid sequence of SEQ ID NO:111, where the Fynomer binds specifically to an FGFR3 (e.g., FGFR3b and/or FGFR3c), is a Fynomer comprising 0 to 12, 0 to 8, 0 to 5, or 1 to 2 amino acid substitutions, additions and/or deletions that do not ablate or significantly reduce the ability of the Fynomer to specifically bind to an FGFR3. A significant reduction in specific binding is a reduction of binding affinity (i.e.,  $K_D$ ) in excess of 20% of the binding affinity of the Fynomer of SEQ ID NO:111 to human FGFR3b or FGFR3c. One of skill in the art can readily determine the binding affinity of a Fynomer to FGFR3b or FGFR3c using routine methods and such a determination would not require undue experimentation. Accordingly, one of skill in the art can readily determine how to make a Fynomer having an amino acid sequence at least 80%, at least 85%, at least 90%, or at least 95% identical to the amino acid sequence of SEQ ID NO:111, where the Fynomer binds specifically to an FGFR3. In certain embodiments, a Fynomer comprises or consists of the amino acid sequence of SEQ ID NO:111.

[0124]

In certain embodiments, a Fynomer comprises a polypeptide having an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to

the amino acid sequence of  
 GVTLFVALYDYEEVMSTTALSFSHKGEKFQILSQSPHGQYWEARSLTTGETG(X<sub>7</sub>)I

PSNYVAPVDSIQ (SEQ ID NO:113), wherein the amino acid (X<sub>7</sub>) is selected from any amino acid. In certain embodiments X<sub>7</sub> is selected from any hydrophobic amino acid.

- 5 In certain embodiments X<sub>7</sub> is selected from any polar amino acid. In certain embodiments X<sub>7</sub> is selected from Q, N, H, S, T, Y, C, W, A, L, V and I. In certain embodiments X<sub>7</sub> is Y, W or L. In certain embodiments X<sub>7</sub> is W.

[0125]

- In certain embodiments, a Fynomer comprises a polypeptide having an amino  
 10 acid sequence at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to the amino acid sequence of SEQ ID NO:113 where (i) the amino acid at position X<sub>7</sub> may be any amino acid; (ii) the identity determination excludes the amino acid position X<sub>7</sub>, (iii) the amino acid sequence EVMSTTA (SEQ ID NO:114) at amino acid positions 12 to 18 of SEQ ID NO:113 and SQSPH (SEQ ID NO:115) at amino acid positions 31  
 15 to 35 of SEQ ID NO:113 are conserved, (iv) the amino acids Q and Y at amino acid positions 37 and 38 of SEQ ID NO:113 are conserved, and (v) the Fynomer binds specifically to an FGFR3 (e.g., FGFR3b, and/or FGFR3c).

[0126]

- In certain embodiments, a Fynomer comprises a polypeptide having an amino  
 20 acid sequence at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to the amino acid sequence of  
 GVTLFVALYDYEEVMSTTALSFSHKGEKFQILSQSPHGQYWEARSLTTGETGWIPS  
 NYVAPVDSIQ (SEQ ID NO:116; sometimes referred to herein as FF40L54A5) and the Fynomer binds specifically to an FGFR3. In certain embodiments, a Fynomer  
 25 comprises a polypeptide having an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to the amino acid sequence of SEQ ID NO:116, where (i) the amino acid sequence EVMSTTA (SEQ ID NO:114) at amino acid positions 12 to 18 of SEQ ID NO:116 are conserved, (ii) the amino acid sequence SQSPH (SEQ ID NO:115) at amino acid positions 31 to 35 of SEQ ID NO:116 are  
 30 conserved, (iii) the amino acids Q and Y at amino acid positions 37 and 38 of SEQ ID NO:116 are conserved, and the Fynomer binds specifically to an FGFR3. In certain

embodiments, a Fynomer comprises a polypeptide having an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to the amino acid sequence of SEQ ID NO:116, where (i) the amino acid sequence EVMSTTA (SEQ ID NO:114) at amino acid positions 12 to 18 of SEQ ID NO:116 is conserved, (ii) the amino acid sequence SQSPHGQY (SEQ ID NO:117) at amino acid positions 31 to 38 of SEQ ID NO:116 are conserved, and the Fynomer binds specifically to an FGFR3. In some embodiments, a Fynomer having an amino acid sequence at least 80%, at least 85%, at least 90%, or at least 95% identical to the amino acid sequence of SEQ ID NO:116, where the Fynomer binds specifically to an FGFR3 (e.g., FGFR3b and/or FGFR3c), is a Fynomer comprising 0 to 12, 0 to 8, 0 to 5, or 1 to 2 amino acid substitutions, additions and/or deletions that do not ablate or significantly reduce the ability of the Fynomer to specifically bind to an FGFR3. A significant reduction in specific binding is a reduction of binding affinity (i.e.,  $K_D$ ) in excess of 20% of the binding affinity of the Fynomer of SEQ ID NO:116 to human FGFR3b or FGFR3c. One of skill in the art can readily determine the binding affinity of a Fynomer to FGFR3b or FGFR3c using routine methods and such a determination would not require undue experimentation. Accordingly, one of skill in the art can readily determine how to make a Fynomer having an amino acid sequence at least 80%, at least 85%, at least 90%, or at least 95% identical to the amino acid sequence of SEQ ID NO:116, where the Fynomer binds specifically to an FGFR3. In certain embodiments, a Fynomer comprises or consists of a polypeptide having an amino acid sequence of SEQ ID NO:116.

[0127]

The Fynomer portion of a bi-specific binding agent can be attached to the antibody portion of a bi-specific binding agent at any suitable location using any suitable method. A Fynomer can be attached covalently or non-covalently to an antibody, or binding portion thereof. A Fynomer can be attached to the N-terminus (N-terminal amino acid) and/or C-terminus (C-terminal amino acid) of an antibody, or antigen binding portion thereof. In some embodiments, a Fynomer is attached to the N-terminus of a heavy chain and/or to the N-terminus of a light chain of an antibody, or antigen binding portion thereof. In some embodiments, a Fynomer is attached to the

C-terminus of a heavy chain and/or to the C-terminus of a light chain of an antibody, or antigen binding portion thereof. In some embodiments, a Fynomer is attached to suitable location within a constant domain of an antibody, or antigen binding portion thereof.

5 [0128]

In certain embodiments, a Fynomer is attached to an antibody, or within an antibody, by a peptide bond. In some embodiments, a bi-specific binding agent comprises a fusion protein comprising a Fynomer and a polypeptide of an antibody (e.g., a heavy chain, light chain, or single chain), where the Fynomer and the antibody polypeptide are joined by a peptide bond. Therefore, in some embodiments, a bi-specific binding agent is made using recombinant technology where a nucleic acid is configured to express a polypeptide comprising a Fynomer and an antibody, or portion thereof (e.g., a light chain or heavy chain) as a single polypeptide. In some embodiments, a bi-specific binding agent comprises a Fynomer and an antibody, or antigen binding portion thereof, where the C-terminal amino acid of the Fynomer is linked to an N-terminal amino acid of the antibody (e.g., an N-terminal amino acid of the heavy chain, or N-terminal amino acid of the light chain) by a peptide bond. In some embodiments, a bi-specific binding agent comprises a Fynomer and an antibody, or antigen binding portion thereof, where the N-terminal amino acid of the Fynomer is linked to a C-terminal amino acid of the antibody (e.g., a C-terminal amino acid of a heavy chain, or C-terminal amino acid of a light chain) by a peptide bond. In certain embodiments, a Fynomer peptide is integrated within a polypeptide of an antibody.

[0129]

In some embodiments, a bi-specific binding agent comprises one or more Fynomers that bind specifically to an FGFR3. For example, a bi-specific binding agent may comprise an antibody having two heavy chains and two light chains and a Fynomer attached to the N-terminus of one or both heavy chains, a Fynomer attached to the N-terminus of one or both light chains, a Fynomer attached to the C-terminus of one or both heavy chains and/or a Fynomer attached to the C-terminus of one or both light chains of the antibody. Accordingly, in certain embodiments, a bi-specific binding

agent comprises 1 to 12, 1 to 8 or 1 to 4 Fynomers. In some embodiments, a bi-specific binding agent comprise 1, 2, 3, 4, 5, 6, 7 or 8 Fynomers.

[0130]

In some embodiments, a bi-specific binding agent comprises a linker between a Fynomer and an antibody. Non-limiting examples of a suitable linker include amino acids, peptides (e.g., 2 or more amino acids), an optionally substituted C1-C50 alkyl, optionally substituted C2-C50 alkenyl, alkynyl, acyl, acyloxy, alkoxy, aryloxy, cycloalkyl, cycloalkenyl, cycloalkoxy, aryl, aminocarbonyl, azido, carboxy, silanes, thiols, sulfoxide, sulfones, sulfonate ester, cyano, amide, amino, ester, phosphonic acid, polyethylene glycol (PEG), the like, derivatives thereof, polymers thereof and combinations thereof. Methods of attaching two or more molecules using a linker are known to those skilled in the art and such methods are sometimes referred to as “crosslinking”.

[0131]

In some embodiments, a linker comprises a peptide comprising two or more amino acids, 2 to 100 amino acids, 5 to 100 amino acids, 2 to 50 amino acids, 5 to 50 amino acids, 2 to 25 amino acids, 5 to 25 amino acids, 2 to 20 amino acids, 5 to 20 amino acids, 2 to 10 amino acids or 5 to 10 amino acids. In some embodiments, a linker comprises a peptide comprising 2, 3, 4 or 5 amino acids. In some embodiments, a linker comprises a motif of (GGGGS)<sub>X</sub> (X=1, 2, 3, 4, 5, 6, 7, 8, 9 or 10) or of GGGGSGGGGSGGGGS.

[0132]

The term “percent identical” or “percent identity” refers to sequence identity between two amino acid sequences. In some embodiments, identity is determined by comparing a position in each sequence which may be aligned for purposes of comparison. When an equivalent position in the compared sequences is occupied by the same amino acid, then the molecules are identical at that position. When the equivalent site is occupied by the same or a similar amino acid residue (e.g., similar in steric and/or electronic nature), then the molecules can be referred to as homologous (similar) at that position. Expression as a percentage of homology, similarity, or identity refers to a function of the number of identical or similar amino acids at

positions shared by the compared sequences. Expression as a percentage of homology, similarity, or identity refers to a function of the number of identical or similar amino acids at positions shared by the compared sequences. Various alignment algorithms and/or programs may be used, including FASTA, BLAST, or ENTREZ. FASTA and BLAST are available as a part of the GCG sequence analysis package (University of Wisconsin, Madison, Wis.), and can be used with, e.g., default settings. ENTREZ is available through the National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health, Bethesda, Md. In one embodiment, the percent identity of two sequences can be determined by the GCG program with a gap weight of 1, e.g., each amino acid gap is weighted as if it were a single amino acid or nucleotide mismatch between the two sequences.

[0133]

Other techniques for alignment are described in Methods in Enzymology, vol. 266: Computer Methods for Macromolecular Sequence Analysis (1996), ed. Doolittle, Academic Press, Inc., a division of Harcourt Brace & Co., San Diego, Calif., USA. In some embodiments an alignment program that permits gaps in the sequence is utilized to align the sequences. The Smith-Waterman is one type of algorithm that permits gaps in sequence alignments. See Meth. Mol. Biol. 70:173-187 (1997). Also, the GAP program using the Needleman and Wunsch alignment method can be utilized to align sequences. An alternative search strategy uses MPSRCH software, which runs on a MASPAC computer. MPSRCH uses a Smith-Waterman algorithm to score sequences on a massively parallel computer. This approach improves ability to pick up distantly related matches, and is especially tolerant of small gaps and nucleotide sequence errors. Nucleic acid-encoded amino acid sequences can be used to search both protein and DNA databases.

[0134]

The term “binds specifically” refers to a bi-specific binding agent, Fynomer, antibody, or portion thereof, that binds to a target protein, peptide or epitope in preference to binding other molecules or other peptides as determined by, for example, a suitable in vitro assay (e.g., an ELISA, Immunoblot, Flow cytometry, and the like). A specific binding interaction discriminates over non-specific binding interactions by

about 2-fold or more, often about 10-fold or more, and sometimes about 100-fold or more, 1000-fold or more, 10,000-fold or more, 100,000-fold or more, or 1,000,000-fold or more.

[0135]

5           In some embodiments an antibody, or binding portion thereof, that binds specifically to syndecan-1, or a portion thereof, is an antibody, or binding portion thereof, that binds syndecan-1, or a portion thereof (e.g., an extracellular domain of syndecan-1), with a binding affinity constant (KD) equal to or less than 100 nM, equal to or less than 50 nM, equal to or less than 25 nM, equal to or less than 10 nM, equal to or less than 5 nM, equal to or less than 1 nM, equal to or less than 900 pM, equal to or less than 800 pM, equal to or less than 750 pM, equal to or less than 700 pM, equal to or less than 600 pM, equal to or less than 500 pM, equal to or less than 400 pM, equal to or less than 300 pM, equal to or less than 200 pM, or equal to or less than 100 pM. In some embodiments an antibody, or binding portion thereof, that binds specifically to syndecan-1, or a portion thereof, is an antibody, or binding portion thereof, that binds human syndecan-1, or a portion thereof (e.g., an extracellular domain of human syndecan-1), with a binding affinity constant (KD) equal to or less than 100 nM, equal to or less than 50 nM, equal to or less than 25 nM, equal to or less than 10 nM, equal to or less than 5 nM, equal to or less than 1 nM, equal to or less than 900 pM, equal to or less than 800 pM, equal to or less than 750 pM, equal to or less than 700 pM, equal to or less than 600 pM, equal to or less than 500 pM, equal to or less than 400 pM, equal to or less than 300 pM, equal to or less than 200 pM, or equal to or less than 100 pM. In some embodiments an antibody, or binding portion thereof, that binds specifically to syndecan-1, or a portion thereof, is an antibody, or binding portion thereof, that binds specifically to syndecan-1, or a portion thereof, derived from a non-human species (e.g., a non-human primate, or rodent; e.g., a mouse or rat), with a binding affinity constant (KD) equal to or less than 100 nM, equal to or less than 50 nM, equal to or less than 25 nM, equal to or less than 10 nM, equal to or less than 5 nM, equal to or less than 1 nM, equal to or less than 900 pM, equal to or less than 800 pM, equal to or less than 750 pM, equal to or less than 700 pM, equal to or less than 600 pM, equal to or less than 500 pM, equal to or less than 400 pM, equal to or less than 300 pM, equal to or less than 200 pM,

or equal to or less than 100 pM. In certain embodiments, an antibody, or binding portion thereof, disclosed herein binds specifically to human syndecan-1, or a portion thereof, and binds specifically to syndecan-1, or a portion thereof, derived from a non-human primate. In certain embodiments, an antibody, or binding portion thereof,  
5 disclosed herein binds specifically to human syndecan-1, or a portion thereof, and binds specifically to syndecan-1, or a portion thereof, derived from a rodent (e.g., a mouse or rat). In certain embodiments, an antibody, or binding portion thereof, (i) binds specifically to a human syndecan-1, or portion thereof (e.g., an extracellular domain of human syndecan-1) with a binding affinity (KD) of 10 nM or less, or 1 nM or less, and  
10 (ii) binds specifically to a rat or mouse syndecan-1, or portion thereof (e.g., an extracellular domain of rat or mouse syndecan-1) with a binding affinity (KD) of 100 nM or less, 90 nM or less, 80 nM or less, 70 nM or less, 60 nM or less, 50 nM or less, 40 nM or less, 30 nM or less, 20 nM or less or 10 nM or less.

[0136]

15 In certain embodiments, a bi-specific binding agent comprises an antibody, or binding portion thereof that competes for binding with an anti-syndecan-1 antibody described herein to syndecan-1, or to a polypeptide comprising the amino acid sequence of SEQ ID NO:94, 95 or 96. In certain embodiments, a bi-specific binding agent comprises an antibody portion that competes for binding with an anti-syndecan-1  
20 antibody described herein to syndecan-1, where the anti-syndecan-1 antibody described herein comprises one or more CDRs shown in Tables 1-10, or one or more CDRs that are substantially similar to those shown in Tables 1-10. In certain embodiments, a bi-specific binding agent comprises an antibody portion that competes for binding with an anti-syndecan-1 antibody described herein to syndecan-1, where the anti-syndecan-1  
25 antibody described herein comprises a CDR-L1 selected from Table 1, a CDR-L2 selected from Table 2, a CDR-L3 selected from Table 3, a CDR-H1 selected from Table 6, a CDR-H2 selected from Table 7, and a CDR-H3 selected from Table 8. In certain embodiments, a bi-specific binding agent comprises an antibody portion that competes for binding with an anti-syndecan-1 antibody described herein to syndecan-1, where the  
30 anti-syndecan-1 antibody described herein comprises a CDR-L1, a CDR-L2 a CDR-L3, a CDR-H1, a CDR-H2, and a CDR-H3 comprising the amino acid sequences of SEQ ID

NOs:2, 17, 27, 47, 61 and 73, respectively. In certain embodiments, a bi-specific binding agent comprises an antibody, or antibody portion that binds to the same epitope of syndecan-1 as an anti-syndecan-1 antibody described herein. In certain embodiments, a bi-specific binding agent comprises an antibody, or antibody portion  
5 that binds specifically to the same epitope of syndecan-1 as an anti-syndecan-1 antibody described herein. In certain embodiments, a bi-specific binding agent comprises an antibody, or antibody portion that binds specifically to the amino acid sequence of SEQ ID NO:94, 95 or 96.

[0137]

10 In certain embodiments, a bi-specific agent comprises an antibody portion having one or more CDR sequences that are distinct and/or different from an anti-syndecan-1 antibody described herein, where the bi-specific agent competes for binding to syndecan-1 with an anti-syndecan-1 antibody described herein.

[0138]

15 Methods of identifying antibodies that compete for binding to an antigen are known. Any suitable method can be used to determine if a bi-specific agent, or antibody portion thereof competes for binding to syndecan-1 with an anti-syndecan-1 antibody described herein. For example, ELISA-based methods can be used where a syndecan-1 antigen, or portion thereof, is coated on a 96-well plate. A bi-specific  
20 agent is added and allowed to bind to the coated antigen. The plate is then washed and an anti-syndecan-1 antibody described herein is added to the plate and allowed to bind. The amount of binding of the anti-syndecan-1 antibody described herein is measured in the presence or absence of the bi-specific agent to determine if the bi-specific agent competes for binding with the anti-syndecan-1 antibody described herein. Other  
25 suitable methods known in the art can also be used.

[0139]

In some embodiments a bi-specific binding agent comprises a label. As used herein, the terms “label” or “labeled” refers to incorporation of a detectable marker, e.g., by incorporation of a labeled amino acid or attachment to a polypeptide of biotin  
30 moieties that can be detected by labeled avidin (e.g., streptavidin containing a fluorescent marker or enzymatic activity that can be detected by optical or colorimetric

methods). In certain embodiments, a label or marker can be attached to a bi-specific binding agent to generate a diagnostic agent. A bi-specific binding agent can be attached covalently or non-covalently to any suitable label or marker. Various methods of labeling polypeptides and glycoproteins are known to those skilled in the art and can be used. Non-limiting examples of labels for polypeptides include, but are not limited to fluorescent labels, enzymatic labels (e.g., horseradish peroxidase,  $\beta$ -galactosidase, luciferase, alkaline phosphatase), chemiluminescent labels, a metallic label, a chromophore, an electro-chemiluminescent label, a phosphorescent label, a quencher (e.g., a fluorophore quencher), a fluorescence resonance energy transfer (FRET) pair (e.g., donor and acceptor), a dye, an enzyme substrate, a small molecule, a mass tag, quantum dots, nanoparticles, biotinyl groups, predetermined polypeptide epitopes recognized by a secondary reporter (e.g., leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags), the like or combinations thereof.

15 [0140]

In some embodiments a bi-specific binding agent comprises a suitable carrier. A bi-specific binding agent can be attached covalently or non-covalently to a suitable carrier. In some embodiments, a Fynomer portion of a bi-specific agent is attached to a carrier. In some embodiments an antibody portion of a bi-specific binding agent is attached to a carrier. Non-limiting examples of a carrier include agents or molecules that alter or extend the in vivo half-life of a bi-specific binding agent include polyethylene glycol, glycogen and/or carbohydrates (e.g., as introduced by glycosylation of a polypeptide), a dextran, a carrier or vehicle described in U.S. Patent No. 6,660,843, the like or combinations thereof. In certain embodiments, a bi-specific agent, or portion thereof, is glycosylated. In some embodiments a label or carrier is bound to a bi-specific binding agent by use of a suitable linker.

[0141]

In some embodiments a label, carrier, anti-neoplastic agent, toxin or linker is attached to a suitable thiol group of a bi-specific binding agent (e.g., a thiol group of a cysteine residue). In some embodiments a label, carrier, anti-neoplastic agent, toxin or linker is attached to a suitable reactive nitrogen of an amino group of a bi-specific

binding agent. Any suitable amino acid residue of bi-specific binding agent can be substituted with an amino acid residue containing a thiol group (e.g., cysteine) or reactive nitrogen (e.g., lysine) for the purpose of attaching a label, carrier, anti-neoplastic agent, toxin or linker. Non-limiting examples of amino acids in an antibody portion of a bi-specific binding agent that can be substituted with a thiol containing amino acid residue or free amino group include A118, S119, S239, V282, T289, N361, and V422 of an IgG<sub>2</sub> or an IgG<sub>1</sub> (corresponding to the EU numbering system), or a corresponding position in an IgG<sub>3</sub> or IgG<sub>4</sub>. Accordingly, in some embodiments, a bi-specific binding agent described herein comprises an antibody comprising a human heavy chain constant region, wherein the heavy chain constant region of the IgG heavy chain of the antibody comprises an A118C (alanine at position 118 to cysteine), S119C (serine at position 119 to cysteine), S239C (serine at position 239 to cysteine), V282C (valine at position 282 to cysteine), T289C (threonine at position 289 to cysteine), N361C (asparagine at position 361 to cysteine), and/or a V422C (valine at position 422 to cysteine) substitution, where an anti-neoplastic agent or toxin is covalently attached to the thiol group of the indicated cysteine residue. In some embodiments, a bi-specific binding agent described herein comprises an antibody comprising a human heavy chain constant region, wherein the constant region of an IgG heavy chain of the antibody comprises an A118K (alanine at position 118 to lysine), S119K (serine at position 119 to lysine), S239K (serine at position 239 to lysine), V282K (valine at position 282 to lysine), T289K (threonine at position 289 to lysine), N361K (asparagine at position 361 to lysine), and/or a V422K (valine at position 422 to lysine) substitution, where an anti-neoplastic agent or toxin is covalently attached to the reactive nitrogen of the free amino group of the indicated lysine residue. Other non-limiting examples of attaching a label, carrier, anti-neoplastic agent, toxin and/or a linker to a bi-specific binding agent include reacting an amine with a succinimidyl ester (e.g., an N-hydroxysuccinimide (NHS) ester), an imidoester, a pentafluorophenyl (PFP) ester, a hydroxymethyl phosphine, an oxirane, an isothiocyanate, a sulfonyl halide, a haloacetyl derivative or any other carbonyl compound; reacting a carboxyl with a carbodiimide; reacting a sulfhydryl with a maleimide, a haloacetyl derivative, a pyridyldisulfide, or a vinyl sulfone; reacting an aldehyde with a hydrazine; reacting any

non-selective group with diazirine or an aryl azide; reacting a hydroxyl with an isocyanate; reacting a hydroxylamine with a carbonyl compound; the like and combinations thereof.

[0142]

5 Anti-Neoplastic Agents, Toxins and Linking Groups

In certain embodiments, a bi-specific binding agent disclosed herein comprises an anti-neoplastic agent. In some embodiments, an antibody, or binding portion thereof comprises an anti-neoplastic agent. In some embodiments, a Fynomer comprises an anti-neoplastic agent. In some embodiments, a bi-specific binding agent, antibody or  
10 Fynomer comprises one or more (e.g., 1 to 20, 1 to 10 or 1 to 5) anti-neoplastic agents. In some embodiments, a bi-specific binding agent, antibody or Fynomer comprises 1, 2, 3, 4 or 5 anti-neoplastic agents.

[0143]

Any suitable anti-neoplastic agent can be attached to a bi-specific binding  
15 agent, antibody or Fynomer disclosed herein. In some embodiments, an anti-neoplastic agent is an agent that is toxic to a neoplastic cell. In some embodiments, an anti-neoplastic agent comprises a toxic compound, a toxic molecule or toxic payload. Non-limiting examples of an anti-neoplastic agent include a dolastatin, an auristatin, a maytansine, a tubulysin, a calicheamicin, a pyrrolobenzodiazepine  
20 (PBD), a duocarmycin, a doxorubicin, a pseudomonas exotoxin-A (PE38), an irinotecan and analogs or derivatives thereof. In some embodiments, an anti-neoplastic agent comprises monomethyl auristatin E (MMAE) or monomethyl auristatin F (MMAF). In some embodiments, an anti-neoplastic agent comprises a pyrrolobenzodiazepine (PBD) toxin and/or a linking group.

25 [0144]

In some embodiments, an anti-neoplastic agent is attached to an antibody portion or a Fynomer portion of a bi-specific binding agent. An anti-neoplastic agent may be attached covalently or non-covalently to a bi-specific binding agent, antibody or Fynomer. An anti-neoplastic agent may be attached directly to, or indirectly to (e.g.,  
30 by means of a linker or linking group) to a bi-specific binding agent, antibody or Fynomer. For example, in some embodiments an anti-neoplastic agent is covalently

attached to a bi-specific binding agent, antibody or Fynomer by a linker or a linking group.

[0145]

An anti-neoplastic agent can be attached to a bi-specific binding agent at any  
 5 suitable position of a bi-specific binding agent. In some embodiment, an  
 anti-neoplastic agent is attached to a Fynomer portion of a bi-specific binding agent  
 and/or to an antibody portion of a bi-specific binding agent. In some embodiments, an  
 anti-neoplastic agent is attached to a constant region of an antibody portion (e.g., a  
 constant region of an antibody) of a bi-specific binding agent. In some embodiments,  
 10 an anti-neoplastic agent is directly or indirectly attached (e.g., by means of a linker or  
 linking group) to suitable cysteine residue of a constant region of an antibody portion of  
 a bi-specific binding agent.

[0146]

In some embodiments, an anti-neoplastic agent comprises a  
 15 pyrrolobenzodiazepine (PBD) toxin. In some embodiments, an anti-neoplastic agent  
 comprises a linking group or a suitable linker. In some embodiments, an  
 anti-neoplastic agent comprises a pyrrolobenzodiazepine (PBD) toxin and a linking  
 group. In certain embodiments a pyrrolobenzodiazepine toxin is covalently linked to a  
 linking group, and the linking group is covalently linked to a bi-specific binding agent  
 20 described herein.

[0147]

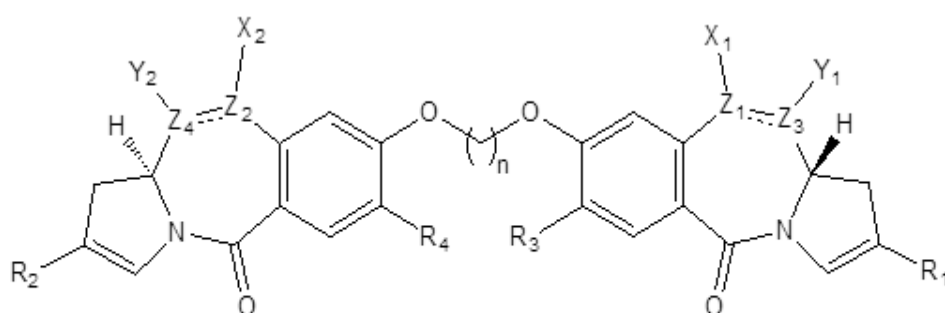
Non-limiting examples of PBD toxins and methods of making PBD toxins are  
 described in the following patent application publications: US2011/0256157,  
 WO2015/052322, US2016/0106861, US2007/0072846, US2011/0201803,  
 25 US2010/0113425, US2008/0167293, US2014/0127239, US2015/0158869,  
 US2015/0344482, US2015/0111880, US2015/0315196, US2016/0015828,  
 US2014/0088089, US2013/0035484, US2011/0196148, US2013/0028919,  
 US2013/0059800, US2014/0274907, US2014/0275522, US2014/0234346,  
 US2013/0266595, US2014/0302066, US2014/0286970, US2014/0294868,  
 30 US2016/0144052, US2016/0031887, US2014/0120118, US2016/0250344,

WO2017/137553, WO2017/137555 and WO2017/186894, the entire contents of which are incorporated herein by reference in their entirety.

[0148]

In some embodiments, a pyrrolobenzodiazepine toxin comprises the structure of chemical formula I:

[Chem. 12.]



( I )

[0149]

where  $Z_1$  and  $Z_2$  are both N;  $Z_3$  and  $Z_4$  are both C;

10

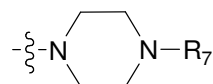
[Chem. 13.]

the double-dash lines ----- represent a single bond or a double bond;

$n$  is 1 to 12; each of  $R_3$  and  $R_4$  are independently H, or a C1-4 alkoxy; and each of  $R_1$  and  $R_2$  are independently selected from the group consisting of H, C1-5 alkyl, C3-6 cycloalkyl, C2-5 alkenyl, and a phenyl optionally substituted with  $R_5$ , where  $R_5$  is selected from the group consisting of  $-NH_2$ ,  $-NHR_6$ , and a piperazinyl substituted with  $R_7$  having the structure,

15

[Chem. 14.]



where  $R_6$  comprises a linking group, and  $R_7$  is null, or a C1-5 alkyl;  $X_1$  is null, a protecting group, or comprises a linking group;  $X_2$  is null, a protecting group, or comprises a linking group; only one of  $X_1$ ,  $X_2$ ,  $R_1$ , and  $R_2$  comprises a linking group; and each of  $Y_1$  and  $Y_2$  are independently either null, OH, or  $SO_3$ ; provided that:

20

[Chem. 15.]

- (i) when  $X_1$  comprises a linking group,  $Z_1 \text{---} Z_3$  is N-C, (ii) when  $X_2$  comprises a linking group,  $Z_2 \text{---} Z_4$  is N-C, (iii) when  $X_1$  comprises the protecting group,  $Z_1 \text{---} Z_3$  is N-C, and (iv) when  $X_2$  comprises the protecting group,  $Z_2 \text{---} Z_4$  is N-C.

wherein null means the absence of the moiety or the presence of one or more hydrogens to complete a required valence.

5 [0150]

In certain embodiments, a PBD toxin comprises only one linking group. For example, in chemical formula I, only one of  $X_1$ ,  $X_2$ ,  $R_1$ , and  $R_2$  may comprise a linking group. For example, where  $X_1$  comprises a linking group,  $X_2$ ,  $R_1$ , and  $R_2$  do not comprise a linking group.

10 [0151]

In certain embodiments of the PBD toxin of chemical formula I,  $n$  is 1-12. In certain embodiments of the PBD toxin of chemical formula I,  $n$  is 1-10, 1-9, 1-7, 1-5, or 1-3. In certain embodiments of the PBD toxin of chemical formula I,  $n$  is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12. In some embodiments,  $n$  is 1, 3 or 5. In some

15

[0152]

In certain embodiments of the PBD toxin of chemical formula I,  $R_3$  and  $R_4$  are independently  $C_{1-4}$  alkoxyl. In certain embodiments of the PBD toxin of chemical formula I,  $R_3$  and  $R_4$  are independently selected from  $-O-CH_2CH_3$  or  $-O-CH_3$ . In

20

[0153]

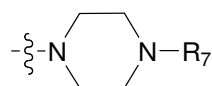
In certain embodiments of the PBD toxin of chemical formula I,  $R_1$  and  $R_2$  are independently selected from the group consisting of H,  $C_{1-5}$  alkyl,  $C_3-C_6$  cycloalkyl, and  $C_{2-5}$  alkenyl.  $R_1$  and  $R_2$  can be the same or different. In some embodiments,  $R_1$  and  $R_2$  are independently selected from a  $C_1-C_3$  alkyl and a  $C_2-C_3$  alkenyl. In certain embodiments,  $R_1$  and  $R_2$  are independently selected from  $-CH_2CH_2CH_3$  and  $-CH_3$ . In certain embodiments, both  $R_1$  and  $R_2$  are  $-CH_2CH_2CH_3$  or  $-CH_3$ .

25

[0154]

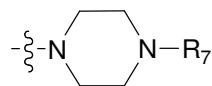
In certain embodiments of the PBD toxin of chemical formula I, R<sub>1</sub> and R<sub>2</sub> are independently selected from a C3-C6 cycloalkyl, and a phenyl optionally substituted with R<sub>5</sub>, where R<sub>5</sub> is selected from the group consisting of -NH<sub>2</sub>, -NHR<sub>6</sub>, and a  
 5 piperazinyl substituted with R<sub>7</sub> having the structure

[Chem. 16.]



where R<sub>6</sub> comprises a linking group, and R<sub>7</sub> is null, or a C<sub>1-5</sub> alkyl. In certain  
 embodiments, R<sub>1</sub> and R<sub>2</sub> are different and independently selected from a (i) a C3-C6  
 10 cycloalkyl, and (ii) a phenyl optionally substituted with R<sub>5</sub>, where R<sub>5</sub> is selected  
 from -NH<sub>2</sub>, and -NHR<sub>6</sub>, where R<sub>6</sub> comprises a linking group. In certain  
 embodiments, R<sub>1</sub> and R<sub>2</sub> are different and independently selected from a (i) a C3  
 cycloalkyl, and (ii) a phenyl substituted with -NH<sub>2</sub>, or -NHR<sub>6</sub>, where R<sub>6</sub> comprises  
 a linking group. In certain embodiments, R<sub>1</sub> and R<sub>2</sub> are different and  
 15 independently selected from a (i) a phenyl optionally substituted with R<sub>5</sub>, where R<sub>5</sub>  
 is selected from -NH<sub>2</sub>, and -NHR<sub>6</sub>, where R<sub>6</sub> comprises a linking group and (ii) a  
 piperazinyl substituted with R<sub>7</sub> having the structure, where R<sub>7</sub> is null, or a C<sub>1-2</sub>  
 alkyl. In certain embodiments, R<sub>1</sub> and R<sub>2</sub> are different and independently selected  
 from a (i) a phenyl substituted with R<sub>5</sub>, where R<sub>5</sub> is -NH<sub>2</sub>, and -NHR<sub>6</sub>, where R<sub>6</sub>  
 20 comprises a linking group and (ii) a piperazinyl substituted with R<sub>7</sub> having the  
 structure

[Chem. 17.]



where R<sub>7</sub> is -CH<sub>3</sub>. In certain embodiments, R<sub>2</sub> is phenyl substituted with  
 25 4-methylpiperazin-1-yl.

[0155]

In certain embodiments of the PBD toxin of chemical formula I, X<sub>1</sub> is null, Y<sub>1</sub> is null,

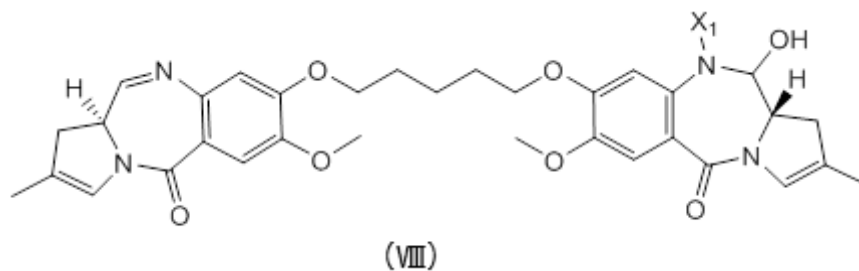
[Chem. 18.]

In some embodiments, a PBD toxin comprises the structure of chemical formula VII shown below:

(VII)

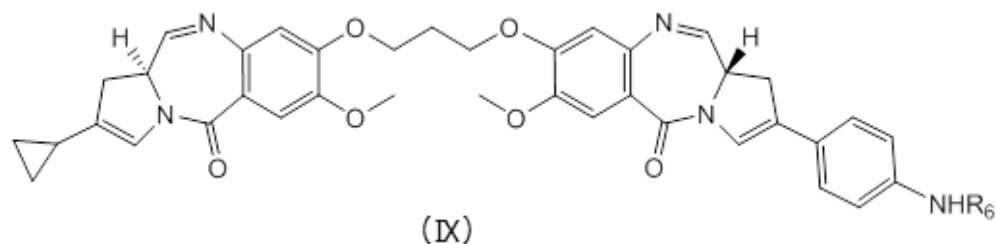
[0157]

[Chem. 20.]



[0158]

[Chem. 21.]

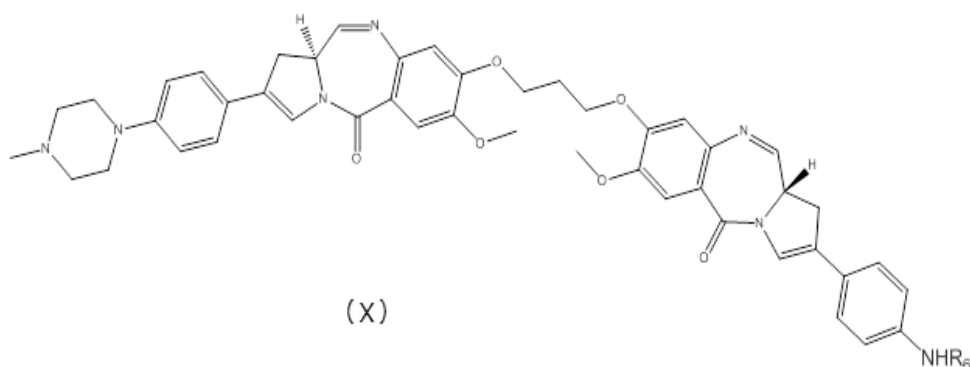


where  $R_6$  comprises the linking group.

[0159]

In some embodiments, a PBD toxin comprises the structure of chemical  
5 formula X shown below:

[Chem. 22.]



where  $R_6$  comprises the linking group.

[0160]

10 In some embodiments, a PBD toxin is attached (e.g., covalently linked) to a linking group by a suitable bond, moiety or group. In some embodiments, a PBD toxin is attached (e.g., covalently linked) to a linking group by a carbonyl linkage or an amide linkage. In some embodiments, a PBD toxin is attached (e.g., covalently linked) to a linking group by a carbamate group. In some embodiments, a PBD toxin is attached  
15 (e.g., covalently linked) to a linking group by an amide group. Non-limiting examples of attaching PBD toxin to a linking group are described in US2017/0002096, US2016/0331842, US2015/0250896, US2017/0080103, US2016/0136300, US2017/0152274, US2015/0209444, US2013/0274091, US2017/0095570, US2017/0157264, US2015/0125474, US2011/0256157, WO2015/052322,

US2016/0106861, US2007/0072846, US2011/0201803, US2010/0113425,  
 US2008/0167293, US2014/0127239, US2015/0158869, US2015/0344482,  
 US2015/0111880, US2015/0315196, US2016/0015828, US2014/0088089,  
 US2013/0035484, US2011/0196148, US2013/0028919, US2013/0059800,  
 5 US2014/0274907, US2014/0275522, US2014/0234346, US2013/0266595,  
 US2014/0302066, US2014/0286970, US2014/0294868, US2016/0144052,  
 US2016/0031887, US2014/0120118, US2016/0250344, WO2017/137553,  
 WO2017/137555 and WO2017/186894, the entire contents of which are incorporated  
 herein by reference in their entirety.

10 [0161]

The term “null” as used herein means that an indicated moiety is absent from a  
 structure, however, the indicated moiety may be replaced or occupied by one or more  
 hydrogen atoms to complete a required valence. Further, in reference to any structure  
 shown herein, one or more hydrogens may be present to complete a required valence of  
 15 a carbon, nitrogen or oxygen atom shown in a structure. Accordingly, where not  
 explicitly indicated, one or more hydrogen atoms may be present.

[0162]

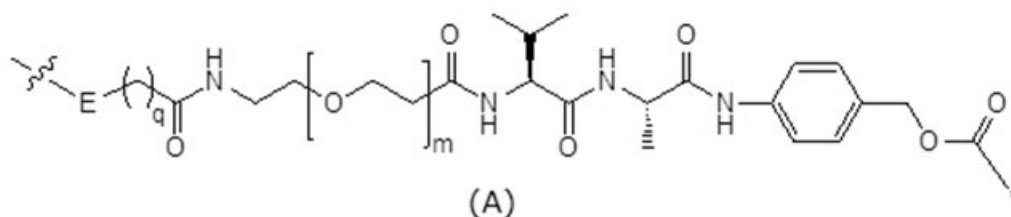
In some embodiments, an anti-neoplastic agent comprises a suitable linking  
 group. In some embodiments a linking group facilitates a linkage between a  
 20 bi-specific binding agent and a toxin (e.g., a PBD toxin). In some embodiments a  
 linking group is cleavable. For example, in certain embodiments a linking group  
 comprises an endopeptidase cleavage site which is recognized by an intracellular  
 peptidase. An endopeptidase cleavage site provides a means of detaching and/or  
 releasing an anti-neoplastic agent from a bi-specific binding agent after the bi-specific  
 25 binding agent is internalized into a neoplastic cell. Non-limiting examples of linking  
 groups and methods a making linking groups are described in WO2015/052322,  
 US2015/0158869, US2015/0344482, US2014/0127239, US2017/0002096,  
 US2016/0331842, US2015/0250896, US2017/0080103, US2016/0136300,  
 US2017/0152274, US2015/0209444, US2013/0274091, US2017/0095570,  
 30 US2017/0157264 and US2015/0125474, which are incorporated herein by reference in  
 their entirety. In some embodiments, a linking group comprises a C1-C20 alkyl, a

C1-C20 alkenyl, a C1-C20 alkoxy, one or more amino acids or amino acid derivatives, a peptide comprising 1 to 20 amino acids, a phenyl group, a suitable polymer (e.g., polyethylene glycol), or a combination thereof.

[0163]

- 5 In some embodiments, a linking group comprises the structure of chemical formula A:

[Chem. 23.]

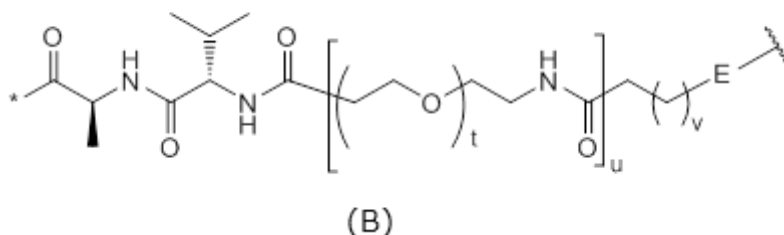


- wherein the asterisk indicates the point of attachment of the linking group to a  
 10 pyrrolobenzodiazepine toxin, the wavy line indicates the point of attachment of the  
 linking group to a bi-specific binding agent, m is 0 to 20, q is 0 to 10 and E is a  
 connecting group. In some embodiments of the linking group of chemical formula A,  
 m is 1 to 20, 1 to 10, 1 to 8, 1 to 6, 1 to 4, 2 to 8 or 4 or 8. In some embodiments of the  
 linking group of chemical formula A, m is selected from 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10.  
 15 In some embodiments of the linking group of chemical formula A, q is 1 to 10, 1 to 8, 1  
 to 6, or 1 to 4. In some embodiments of the linking group of chemical formula A, q is  
 selected from 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10. In some embodiments of the linking  
 group of chemical formula A, q is 0, 1 or 2. In some embodiments of the linking  
 group of chemical formula A, m is 8 and q is 2.

- 20 [0164]

In some embodiments, a linking group comprises the structure of chemical formula B:

[Chem. 24.]



wherein the asterisk indicates the point of attachment of the linking group to a pyrrolobenzodiazepine toxin, the wavy line indicates the point of attachment of the linking group to a bi-specific binding agent, v is 0 to 10, and u is 0 or 1, wherein when u is 1, t is 1 to 10, and E is a connecting group. In some embodiments of the linking group of chemical formula B, v is 1 to 10, 1 to 8, 1 to 4, or 0 to 4. In some embodiments of the linking group of chemical formula B, v is selected from 0, 1, 2, 3, 4, 5, 6, 7 or 8. In some embodiments of the linking group of chemical formula B, when u is 1, t is 1 to 8, 1 to 5, 1 to 4, or 2 to 5. In some embodiments of the linking group of chemical formula B, when u is 1, t is selected from 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10. In some embodiments of the linking group of chemical formula B, t is 8, u is 1, and v is 2. In some embodiments of the linking group of chemical formula B, u is 0, and v is 4.

[0165]

The connecting group E of chemical formulas A and B can comprise any suitable bond, linker or moiety non-limiting examples of which include a disulfide bond, a thioether bond, a thioester bond, an amide bond, an amine, a ketone, a carboxylate ether, a carbamate, an ester, a thioester, the like, or a combination thereof. In certain embodiments, E comprises a covalent linkage between the linking group and the bi-specific binding agent. In some embodiments, E comprises a covalent bond. In some embodiments, E comprises a reacted moiety that remains after a suitable conjugation reaction is conducted. A multitude of conjugation reactions are known in the art, any one of which can be used to covalently link a linking group disclosed herein to a bi-specific binding agent disclosed herein. Any suitable conjugation chemistry can be used to covalently attach a linking group to a bi-specific binding agent, either stochastically or site-specifically, non-limiting examples of which include a conjugation

reaction described in Shan S. Wong (Published June 18, 1991) Chemistry of Protein Conjugation and Cross-Linking, CRC Press; Greg T. Hermanson (Copyright 2013) Bioconjugate Techniques, Third Edition, Elsevier Inc.; and Thiol-X Chemistries in Polymer and Materials Science, RSC Polymer Chemistry Series No. 6 (2013) Edited by

5 Andrew B. Lowe and Christopher N. Bowman, RCS Publishing, WO2015/052322, US2015/0158869, US2015/0344482, US2014/0127239, US2017/0002096, US2016/0331842, US2015/0250896, US2017/0080103, US2016/0136300, US2017/0152274, US2015/0209444, US2013/0274091, US2017/0095570, US2017/0157264 and US2015/0125474, the entire contents of which are incorporated

10 herein by reference in their entirety. Other non-limiting examples of conjugating an anti-neoplastic agent or linking group to a bi-specific binding agent include reacting an amine or amino group with an N-hydroxysuccinimide (NHS) ester, succinimidyl succinate, succinimidyl succinamide, succinimidyl propionate, succinimidyl carbonate, oxycarbonylimidazole, nitrophenyl carbonates, trichlorophenyl carbonate, tresylate,

15 maleic anhydride, methylmaleic anhydride, an imidoester, a pentafluorophenyl (PFP) ester, a hydroxymethyl phosphine, an oxirane or any other carbonyl moiety; reacting a carboxyl moiety with a carbodiimide; reacting a sulfhydryl moiety with a maleimide, a haloacetyl, a pyridyldisulfide, orthopyridyldisulfide and/or a vinyl sulfone; reacting an aldehyde moiety with a hydrazine or hydrazide; reacting any non-selective group with

20 diazirine and/or aryl azide; reacting a hydroxyl moiety with isocyanate; reacting a hydroxylamine moiety with a carbonyl moiety; the like and combinations thereof.

[0166]

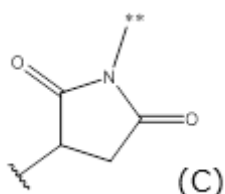
Accordingly, E is often defined by a chemistry used to conjugate a linking group to a bi-specific binding agent. In some embodiments, E comprises a suitable

25 moiety configured to attach a linking group to a bi-specific binding agent. In some embodiments, a linking group is covalently linked to a bi-specific binding agent by means of a suitable sulfhydryl-sulfhydryl reaction, for example by use of a maleimide or pyridyldithiol reactive group that reacts with a reduced cysteine to form stable thioether bond. Additional non-limiting examples of reactive sulfhydryl reactive moieties

30 include a haloacetyls, aziridines, acryloyls, arylating agents, vinylsulfones, a pyridyl disulfide, and TNB-thiol. In certain embodiments, a bi-specific binding agent is

connected to E by a thioether bond formed between a cysteine thiol residue (e.g., a thiol) of the bi-specific binding agent and E. Accordingly, in certain embodiments, E comprises a disulfide bond or thioether bond. In some embodiments, for example where a maleimide reaction is used to covalently link a bi-specific binding agent to a linking group, E comprises the structure of chemical formula C:

[Chem. 25.]



wherein the wavy line indicates the point of attachment to the binding agent and the double asterisk (\*\*) indicates the point of attachment to the linking group. In certain embodiments the double asterisk of chemical formula C represents a thioether bond.

[0167]

An anti-neoplastic agent, toxin, linking group or connecting group can be conjugated stochastically or site-specifically to any suitable amino acid of a bi-specific binding agent. In some embodiments, anti-neoplastic agent, toxin, linking group or connecting group is conjugated to one or more suitable cysteine residues of a bi-specific binding agent. In some embodiments, anti-neoplastic agent, toxin, linking group or connecting group is conjugated to one or more suitable lysine residues of a bi-specific binding agent. In certain embodiments, one or more amino acids of a bi-specific binding agent are substituted with an amino acid that is suitable for conjugation to anti-neoplastic agent, toxin, linking group or connecting group. Non-limiting examples of amino acids that can be substituted with a thiol containing amino acid residue or a lysine residue include A118, S119, S239, V282, T289, N361, and V422 of an IgG1 or IgG2 constant domain (corresponding to the EU numbering system), or a corresponding position in an IgG3 or IgG4 constant domain. Incorporation of a cysteine into a bi-specific binding agent or antibody by mutagenesis allows for direct conjugation of anti-neoplastic agent, toxin, linking group or connecting group to

specific sites on the bi-specific binding agent or antibody, for example via a disulfide bond or thioether bond. For example, one or more amino acids of a bi-specific binding agent can be substituted with a cysteine, where the cysteine can be used for site-specific conjugation of an anti-neoplastic agent, toxin, linking group or connecting group using a suitable chemical reaction. Any suitable amino acid of a constant region of an antibody can be mutated to a cysteine or lysine for site-specific conjugation to an anti-neoplastic agent, toxin, linking group or connecting group.

[0168]

In some embodiments, a linking group comprises a suitable enzyme cleavage site. In certain embodiments, an enzyme cleavage site comprises an enzyme recognition site of a mammalian protease. Accordingly, in some embodiments, a linking group, or portion thereof, is cleavable by a mammalian protease. A linking group may be cleaved by an enzyme present at or near a target site (e.g., at or near an FGFR3 or CD138 protein). An enzyme present at or near a target site may be intracellular, membrane bound, membrane associated or extracellular (e.g., secreted). For example, a linking group may be configured to be cleaved by a cell surface protease, a secreted protease, or an intracellular protease (e.g., a lysosomal protease). Non-limiting examples of enzyme cleavage sites include a protease recognition site of a lysosomal cysteine protease and/or a lysosomal aspartic protease. Non-limiting examples of lysosomal proteases include cathepsin B, C, H, I, J, K, L, M, N, O, P, S, T and X, and cathepsin D, E, F, G, and/or cathepsin A (carboxypeptidase A).

[0169]

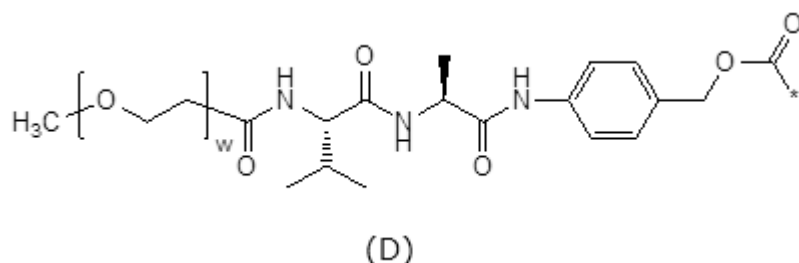
#### Protecting groups

In some embodiments, a PBD toxin comprises a suitable protecting group. Non-limiting examples of protecting groups and method of making protecting groups are described in the following patent application publications: US2011/0256157, WO2015/052322, US2011/0201803, US2008/0167293, US2014/0127239, US2015/0158869, US2015/0344482, US2015/0315196, US2015/0315196, US2014/0302066, US2006/0264622 and US2015/0133435, the entire contents of which are incorporated herein by reference in their entirety.

[0170]

In some embodiments, a protecting group comprises the structure of chemical formula D below:

[Chem. 26.]



- 5 wherein the asterisk indicates a point of attachment to a pyrrolobenzodiazepine toxin; and w is 0 to 10. In some embodiments, w is 0 to 8, 0 to 6, 0 to 4, 1 to 10, 1 to 8, 1 to 5, or 1 to 4. In certain embodiments, w is selected from 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10. In some embodiments, w is 2.

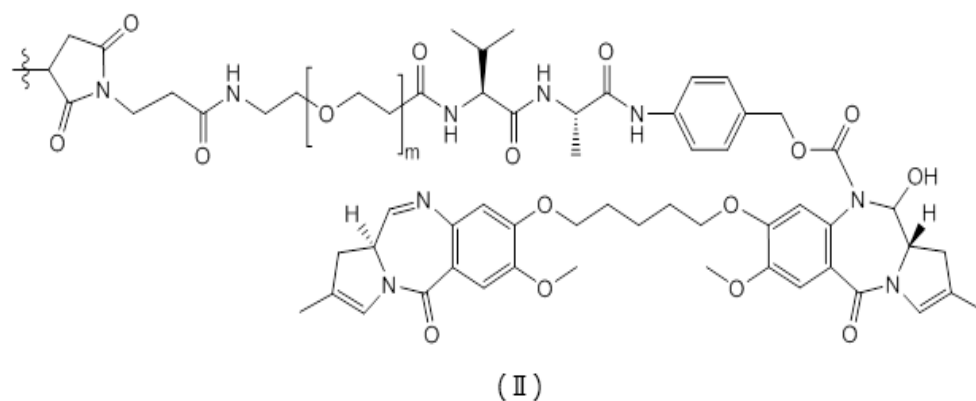
[0171]

- 10 In some embodiments, a protecting group is removable. In certain embodiments, a protecting group is cleavable using a suitable chemistry.

[0172]

In some embodiments, an anti-neoplastic agent comprises a structure of chemical formula II:

- 15 [Chem. 27.]

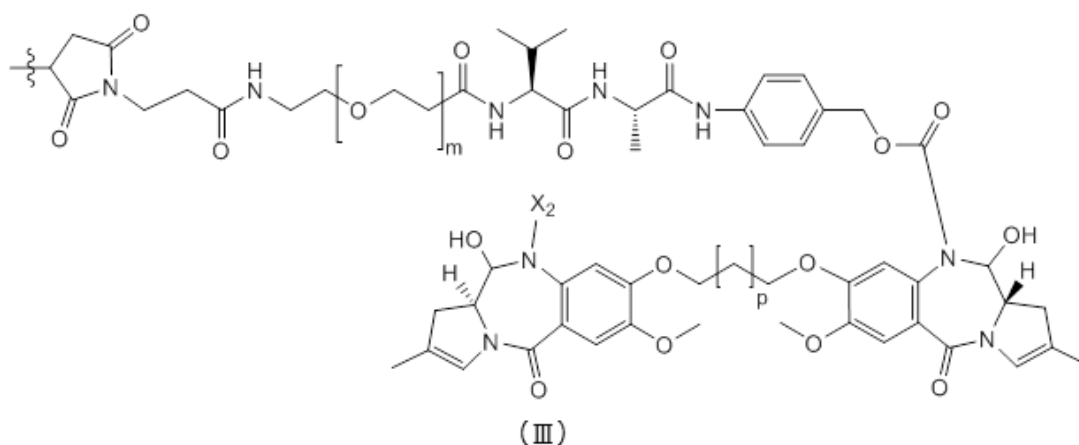


wherein m is 8 and the wavy line indicates the point of attachment to the binding agent.

[0173]

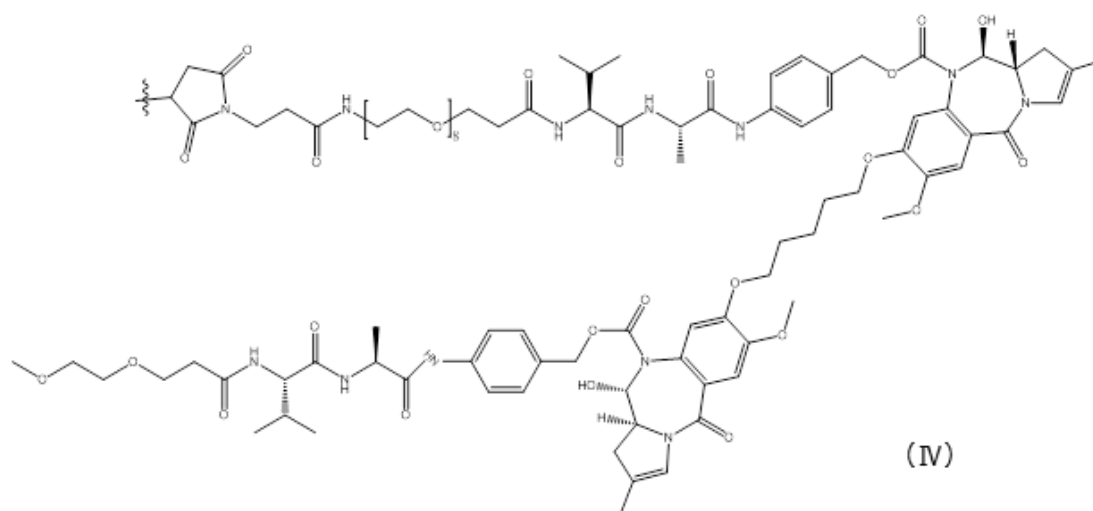
In some embodiments, an anti-neoplastic agent comprises a structure of chemical formula III:

[Chem. 28.]



- 5 wherein m is 8, p is 1 or 3, X<sub>2</sub> is null, or is a protecting group and the wavy line indicates the point of attachment to the binding agent. In certain embodiments, an anti-neoplastic agent comprises a structure of chemical formula IV:

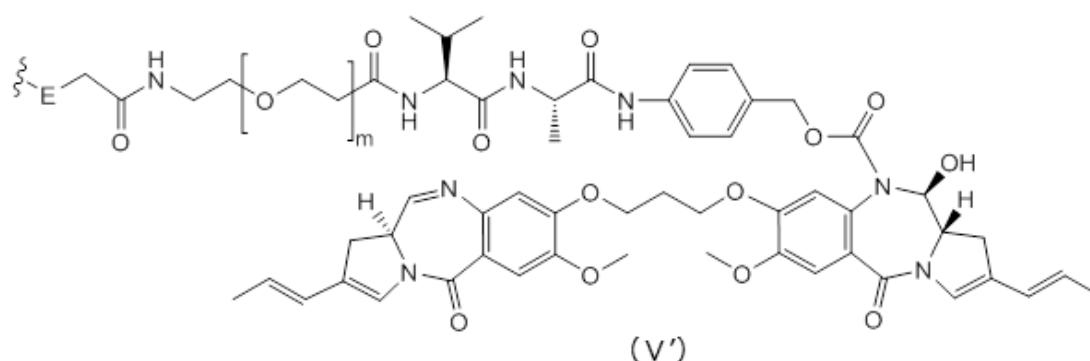
[Chem. 29.]



- 10 where the wavy line indicates the point of attachment to the binding agent.  
[0174]

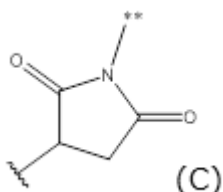
In some embodiments, an anti-neoplastic agent comprises a structure of chemical formula V'

[Chem. 30.]



wherein m is 8, E is a suitable connecting group and the wavy line indicates the point of attachment to the binding agent. In some embodiments, E comprises a succinamide moiety of the structure C:

[Chem. 31.]



wherein the wavy line indicates the point of attachment to the binding agent and the double asterisk indicates the point of attachment to the anti-neoplastic agent of chemical formula V. The anti-neoplastic agent of chemical formula V comprising the connecting group of structure C is sometimes referred to herein as chemical formula XI.

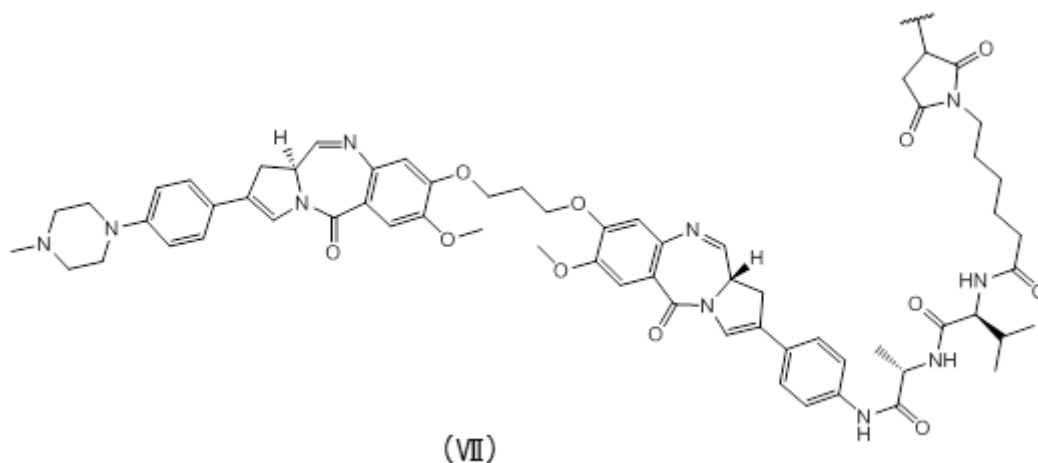
[0175]

In some embodiments, an anti-neoplastic agent comprises a structure of chemical formula VI:

[Chem. 32.]

[0176]

- [Chem. 33.]



10 [0177]

[0178]

In some embodiments, a composition or pharmaceutical composition comprises a bi-specific binding agent described herein (e.g., a bi-specific binding agent comprising an anti-neoplastic agent). In some embodiments, a pharmaceutical

composition comprises a bi-specific binding agent and a pharmaceutically acceptable excipient, diluent, additive or carrier.

[0179]

A pharmaceutical composition can be formulated for a suitable route of administration. In some embodiments a pharmaceutical composition is formulated for subcutaneous (s.c.), intradermal, intramuscular, intraperitoneal and/or intravenous (i.v.) administration. In certain embodiments, a pharmaceutical composition can contain formulation materials for modifying, maintaining, or preserving, for example, the pH, osmolarity, viscosity, clarity, color, isotonicity, odor, sterility, stability, rate of dissolution or release, adsorption or penetration of the composition. In certain embodiments, suitable formulation materials include, but are not limited to, amino acids (such as glycine, glutamine, asparagine, arginine or lysine); antimicrobials; antioxidants (such as ascorbic acid, sodium sulfite or sodium hydrogen-sulfite); buffers (such as borate, bicarbonate, Tris-HCl, citrates, phosphates (e.g., phosphate buffered saline) or suitable organic acids); bulking agents (such as mannitol or glycine); chelating agents (such as ethylenediamine tetraacetic acid (EDTA)); complexing agents (such as caffeine, polyvinylpyrrolidone, beta-cyclodextrin or hydroxypropyl-beta- cyclodextrin); proteins (such as serum albumin, gelatin or immunoglobulins); coloring, flavoring and diluting agents; emulsifying agents; hydrophilic polymers (such as polyvinylpyrrolidone); low molecular weight polypeptides; salt-forming counter ions (such as sodium); solvents (such as glycerin, propylene glycol or polyethylene glycol); diluents; excipients and/or pharmaceutical adjuvants. In particular, pharmaceutical compositions can comprise any suitable carrier, formulation, or ingredient, the like or combinations thereof as listed in “Remington: The Science And Practice Of Pharmacy” Mack Publishing Co., Easton, PA, 19<sup>th</sup> Edition, (1995) (hereafter, “Remington ’95”), or “Remington: The Science And Practice Of Pharmacy”, Pharmaceutical Press, Easton, PA, 22<sup>nd</sup> Edition, (2013) (hereafter, “Remington 2013”), the contents of which are incorporated herein by reference in their entirety. The various materials listed herein, alone or in combination, can be incorporated into or used with the materials described in Remington ’95 or Remington 2013. Any suitable techniques, carriers, and excipients can be used,

including those understood in the art; e.g., as described in Remington '95 or Remington 2013.

[0180]

In certain embodiments, a pharmaceutical composition comprises a suitable  
5 excipient, non-limiting example of which include anti-adherents (e.g., magnesium stearate), a binder, fillers, monosaccharides, disaccharides, other carbohydrates (e.g., glucose, mannose or dextrins), sugar alcohols (e.g., mannitol or sorbitol), coatings (e.g., cellulose, hydroxypropyl methylcellulose (HPMC), microcrystalline cellulose, synthetic polymers, shellac, gelatin, corn protein zein, enterics or other polysaccharides), starch  
10 (e.g., potato, maize or wheat starch), silica, colors, disintegrants, flavors, lubricants, preservatives, sorbents, sweeteners, vehicles, suspending agents, surfactants and/or wetting agents (such as pluronics, PEG, sorbitan esters, polysorbates such as polysorbate 20, polysorbate 80, triton, tromethamine, lecithin, cholesterol, tyloxapal), stability enhancing agents (such as sucrose or sorbitol), and tonicity enhancing agents  
15 (such as alkali metal halides, sodium or potassium chloride, mannitol, sorbitol), and/or any excipient disclosed in Remington '95 or Remington 2013. The term "binder" as used herein refers to a compound or ingredient that helps keeps a pharmaceutical mixture combined. Suitable binders for making pharmaceutical formulations and are often used in the preparation of pharmaceutical tablets, capsules and granules are known  
20 to those skilled in the art.

[0181]

In some embodiments a pharmaceutical composition comprises a suitable pharmaceutically acceptable additive and/or carrier. Non-limiting examples of suitable additives include a suitable pH adjuster, a soothing agent, a buffer, a sulfur-containing  
25 reducing agent, an antioxidant and the like. Non-limiting examples of a sulfur-containing reducing agent includes those having a sulfhydryl group such as N-acetylcysteine, N-acetylhomocysteine, thiocetic acid, thiodiglycol, thioethanolamine, thioglycerol, thiosorbitol, thioglycolic acid and a salt thereof, sodium thiosulfate, glutathione, and a C1-C7 thioalkanoic acid. Non-limiting examples of an antioxidant  
30 include erythorbic acid, dibutylhydroxytoluene, butylhydroxyanisole, alpha -tocopherol, tocopherol acetate, L-ascorbic acid and a salt thereof, L-ascorbyl palmitate, L-ascorbyl

stearate, sodium bisulfite, sodium sulfite, triamyl gallate and propyl gallate, as well as chelating agents such as disodium ethylenediaminetetraacetate (EDTA), sodium pyrophosphate and sodium metaphosphate. Furthermore, diluents, additives and excipients may comprise other commonly used ingredients, for example, inorganic salts  
 5 such as sodium chloride, potassium chloride, calcium chloride, sodium phosphate, potassium phosphate and sodium bicarbonate, as well as organic salts such as sodium citrate, potassium citrate and sodium acetate.

[0182]

The pharmaceutical compositions used herein can be stable over an extended  
 10 period of time, for example on the order of months or years. In some embodiments a pharmaceutical composition comprises one or more suitable preservatives. Non limiting examples of preservatives include benzalkonium chloride, benzoic acid, salicylic acid, thimerosal, phenethyl alcohol, methylparaben, propylparaben, chlorhexidine, sorbic acid, hydrogen peroxide, the like and/or combinations thereof. A  
 15 preservative can comprise a quaternary ammonium compound, such as benzalkonium chloride, benzoxonium chloride, benzethonium chloride, cetrimide, sepazonium chloride, cetylpyridinium chloride, or domiphen bromide (BRADOSOL(registered trademark)). A preservative can comprise an alkyl-mercury salt of thiosalicylic acid, such as thimerosal, phenylmercuric nitrate, phenylmercuric acetate or phenylmercuric  
 20 borate. A preservative can comprise a paraben, such as methylparaben or propylparaben. A preservative can comprise an alcohol, such as chlorobutanol, benzyl alcohol or phenyl ethyl alcohol. A preservative can comprise a biguanide derivative, such as chlorhexidine or polyhexamethylene biguanide. A preservative can comprise sodium perborate, imidazolidinyl urea, and/or sorbic acid. A preservative can  
 25 comprise stabilized oxychloro complexes, such as known and commercially available under the trade name PURITE(registered trademark). A preservative can comprise polyglycol-polyamine condensation resins, such as known and commercially available under the trade name POLYQUART(registered trademark) from Henkel KGaA. A preservative can comprise stabilized hydrogen peroxide. A preservative can be  
 30 benzalkonium chloride. In some embodiments a pharmaceutical composition is free of preservatives.

[0183]

In some embodiments a composition, pharmaceutical composition or bi-specific binding agent is substantially free of blood, or a blood product contaminant (e.g., blood cells, platelets, polypeptides, minerals, blood borne compounds or chemicals, and the like). In some embodiments a composition, pharmaceutical composition or bi-specific binding agent is substantially free of serum and serum contaminants (e.g., serum proteins, serum lipids, serum carbohydrates, serum antigens and the like). In some embodiments a composition, pharmaceutical composition or bi-specific binding agent is substantially free a pathogen (e.g., a virus, parasite or bacteria). In some embodiments a composition, pharmaceutical composition or bi-specific binding agent is substantially free of endotoxin. In some embodiments a composition, pharmaceutical composition or bi-specific binding agent is sterile. In certain embodiments, a composition or pharmaceutical composition comprises a bi-specific binding agent and a diluent (e.g., phosphate buffered saline (PBS)). In certain embodiments, a composition or pharmaceutical composition comprises a bi-specific binding agent and an excipient, (e.g., sodium citrate dehydrate, or polyoxyethylene-sorbitan-20 mono-oleate (polysorbate 80)).

[0184]

The pharmaceutical compositions described herein may be configured for administration to a subject in any suitable form and/or amount according to the therapy in which they are employed. For example, a pharmaceutical composition configured for parenteral administration (e.g., by injection or infusion), may take the form of a suspension, solution or emulsion in an oily or aqueous vehicle and it may contain formulation agents, excipients, additives and/or diluents such as aqueous or non-aqueous solvents, co-solvents, suspending solutions, preservatives, stabilizing agents and or dispersing agents. In some embodiments a pharmaceutical composition suitable for parental administration may contain one or more excipients. In some embodiments a pharmaceutical composition is lyophilized to a dry powder form. In some embodiments a pharmaceutical composition is lyophilized to a dry powder form, which is suitable for reconstitution with a suitable pharmaceutical solvent (e.g., water, saline, an isotonic buffer solution (e.g., PBS), and the like). In certain embodiments,

reconstituted forms of a lyophilized pharmaceutical composition are suitable for parental administration (e.g., intravenous administration) to a mammal.

[0185]

In certain embodiments, a pharmaceutical composition is configured for oral administration and may be formulated as a tablet, microtablet, minitables, micropellets, 5 powders granules, capsules (e.g., capsules filled with microtablets, micropellets, powders or granules), emulsions or solutions. Pharmaceutical compositions configured for oral administration may comprise suitable coatings to delay or sustain release of the active ingredient (e.g., a bi-specific binding agent), non-limiting examples 10 of which include enteric coatings such as fatty acids, waxes, shellac, plastics, methyl acrylate-methacrylic acid copolymers, cellulose acetate phthalate (CAP), cellulose acetate succinate, hydroxypropyl methyl cellulose phthalate, hydroxypropyl methyl cellulose acetate succinate (hypromellose acetate succinate), polyvinyl acetate phthalate (PVAP), methyl methacrylate-methacrylic acid copolymers, cellulose acetate 15 trimellitate, sodium alginate, zein, plant fibers, the like and combinations thereof.

[0186]

In some embodiments a pharmaceutical compositions described herein may be configured for topical administration and may include one or more of a binding and/or lubricating agent, polymeric glycols, gelatins, cocoa-butter or other suitable waxes or 20 fats. In some embodiments a pharmaceutical composition described herein is incorporated into a topical formulation containing a topical carrier that is generally suited to topical drug administration and comprising any suitable material known to those skilled in the art. In certain embodiments, a topical formulation of a pharmaceutical composition is formulated for administration of a bi-specific binding 25 agent from a topical patch.

[0187]

In certain embodiments, an optimal pharmaceutical composition will be determined by one skilled in the art depending upon, for example, the intended route of administration, delivery format and desired dosage (see e.g., Remington '95 or 30 Remington 2013, *supra*). In certain embodiments, such compositions may influence the physical state, stability, rate of in vivo release and rate of in vivo clearance of the

antibody drug conjugates of the invention. A pharmaceutical composition can be manufactured by any suitable manner, including, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or tableting processes (e.g., see methods described in Remington '95 or

5 Remington 2013).

[0188]

In some embodiments, presented herein is a composition or pharmaceutical composition for use as a medicament for the treatment of a neoplasm in a subject, wherein the composition or pharmaceutical composition comprises a bi-specific binding agent described herein (e.g., a bi-specific binding agent comprising an anti-neoplastic agent). In some embodiments, presented herein is a composition or pharmaceutical composition comprising a bi-specific binding agent, or a bi-specific binding agent conjugated (e.g., covalently attached) to an anti-neoplastic agent or PBD toxin for use in the treatment of a neoplasm.

15 [0189]

In some embodiments a composition, pharmaceutical composition or bi-specific binding agent (e.g., a bi-specific binding agent comprising an anti-neoplastic agent) described herein is used to treat a subject having or suspected of having a neoplasm. In some embodiments a composition, pharmaceutical composition or bi-specific binding agent (e.g., a bi-specific binding agent comprising an anti-neoplastic agent) described herein is administered to a subject having or suspected of having a neoplasm. In some embodiments, presented herein is a method of treating a subject having or suspected of having a neoplasm. In certain embodiments a method of treating a subject having or suspected of having a neoplasm comprises administering to the subject a therapeutically effective amount of a composition or bi-specific binding agent described herein. In certain embodiments a bi-specific binding agent, or pharmaceutical composition comprising a bi-specific binding agent, is administered to a subject, wherein the bi-specific binding agent binds specifically to an extracellular domain of human syndecan-1 and/or to an FGFR3 (e.g., an FGFR3, FGFR3b and/or FGFR3c).

30 [0190]

In certain embodiments, a method of treating a subject comprises contacting a cell (e.g., one or more cells) of a subject with a therapeutically effective amount of a composition, pharmaceutical composition or bi-specific binding agent described herein. In certain embodiments, a method of treatment comprises contacting a cell (e.g., one or  
 5 more cells) of a subject with a therapeutically effective amount of a bi-specific binding agent that specifically binding to an extracellular portion of human syndecan-1, or variant thereof, and/or to an FGFR3 (e.g., an FGFR3, FGFR3b and/or FGFR3c). The cell of a subject is often a cell that expresses an extracellular portion of syndecan-1. A cell of a subject may be found inside a subject (e.g., in vivo) or outside the subject (e.g.,  
 10 in vitro or ex vivo).  
 [0191]

Non-limiting examples of a neoplasm that can be treated by a method described herein include a carcinoma, sarcoma, nervous system neoplasia (neoplasia of the nervous system), lymphoma, myeloma, leukemia, melanoma, mesothelioma, solid  
 15 or soft tissue tumors, and secondary cancers (e.g., derived from a primary site)). Non-limiting examples of a carcinoma include respiratory system carcinomas, gastrointestinal system carcinomas, genitourinary system carcinomas, testicular carcinomas, prostatic carcinomas, endocrine system carcinomas, basal cell carcinoma of the skin, carcinoma of unknown primary, cholangiocarcinoma, ductal carcinoma in situ  
 20 (DCIS), Merkel cell carcinoma, lung carcinoma, thymoma and thymic carcinoma, midline tract carcinoma, lung small cell carcinoma, thyroid carcinoma, liver hepatocellular carcinoma, squamous cell carcinoma, head and neck squamous carcinoma, breast carcinoma, epithelial carcinoma, adrenocortical carcinoma, ovarian surface epithelial carcinoma, and the like, further including carcinomas of the uterus,  
 25 cervix, colon, pancreas, kidney, esophagus, stomach and ovary. Non-limiting examples of a sarcoma include Ewing sarcoma, lymphosarcoma, liposarcoma, osteosarcoma, breast sarcoma, soft tissue sarcoma, Kaposi sarcoma, rhabdomyosarcoma, uterine sarcoma, chondrosarcoma, leiomyosarcoma, fibrosarcoma and the like. Non-limiting examples of a nervous system neoplasia include glioma, glioblastoma,  
 30 meningioma, neuroblastoma, retinoblastoma, astrocytoma, oligodendrocytoma and the like. Non-limiting examples of lymphomas, myelomas, and leukemia include acute

and chronic lymphoblastic leukemia, myeloblastic leukemia, multiple myeloma, poorly differentiated acute leukemias (e.g., erythroblastic leukemia and acute megakaryoblastic leukemia), acute promyeloid leukemia (APML), acute myelogenous leukemia (AML), chronic myelogenous leukemia (CML), acute lymphoblastic leukemia (ALL), which

5 includes B-lineage ALL and T-lineage ALL, chronic lymphocytic leukemia (CLL), prolymphocytic leukemia (PLL), hairy cell leukemia (HLL), Waldenstrom's macroglobulinemia (WM), non-Hodgkin lymphoma and variants, peripheral T-cell lymphomas, adult T-cell leukemia/lymphoma (ATL), cutaneous T-cell lymphoma (CTCL), large granular lymphocytic leukemia (LGF), Hodgkin's disease and

10 Reed-Sternberg disease. Non-limiting examples of soft or solid tissue tumors include visceral tumors, seminomas, hepatomas, and other tumors of the breast, liver, lung, pancreas, uterus, ovary, testicle, head, neck, eye, brain, mouth, pharynx, vocal cord, ear, nose, esophagus, stomach, intestine, colon, adrenal gland, kidney, bone, bladder, urethra, carcinomas, lung, muscle, skin, feet, hands, and soft tissue. In some embodiments, a

15 neoplasm that can be treated by a pharmaceutical composition or bi-specific binding agent disclosed herein is selected from a bladder cancer, breast cancer, colorectal cancer, cervical cancer, gastric cancer, liver cancer, hepatocellular cancer, hypopharynx cancer, lung cancer, adenocarcinoma, ovarian cancer and renal cancer. In some embodiments, a neoplasm that can be treated by a pharmaceutical composition or bi-specific binding

20 agent disclosed herein is selected from a pancreatic cancer (e.g., a pancreatic adenocarcinoma, exocrine pancreatic cancer or pancreatic neuroendocrine cancer), a colorectal cancer (e.g., a colorectal adenocarcinoma), small intestinal malignancy, cholangiocarcinoma, non-small cell lung cancer (NSCLC), thyroid carcinoma, esophageal or esophagogastric junction (EGJ) cancer, gastric adenocarcinoma, liver

25 hepatocellular carcinoma, head and neck squamous carcinoma, female genital tract malignancy, breast carcinoma, lung small cell carcinoma, ovarian surface epithelial carcinoma, retroperitoneal or peritoneal sarcoma, prostatic adenocarcinoma, neuroendocrine tumor, gastrointestinal stromal tumor, glioblastoma or non-epithelial ovarian cancer. In some embodiments, a neoplasm that can be treated by a

30 pharmaceutical composition or bi-specific binding agent disclosed herein is a breast cancer, non-limiting examples of which include ductal carcinoma in situ (DCIS),

invasive ductal carcinoma (IDC)(e.g., tubular carcinoma of the breast, medullary carcinoma of the breast, mucinous carcinoma of the breast, papillary carcinoma of the breast, and cribriform carcinoma of the breast), invasive lobular carcinoma (ILC), inflammatory breast cancer, lobular carcinoma in situ (LCIS), male breast cancer, 5 molecular subtypes of breast cancer (e.g., Luminal B breast cancer or hormone-receptor positive breast cancer, Triple-negative breast cancer, HER2-enriched breast cancer, and normal-like breast cancer), Paget's disease of the nipple, phyllodes tumors of the breast, and metastatic breast cancer. In some embodiments a neoplasm that can be treated by a pharmaceutical composition or bi-specific binding agent disclosed herein is a triple 10 negative breast cancer.

[0192]

In some embodiments, the effectiveness of a treatment described herein can be determined or predicted, in part, by an amount of CD138 and/or FGFR3 (e.g., FGFR3, or an isoform thereof) that a neoplasm or neoplastic cell expresses. For example, 15 without being limited to theory, a subject having a neoplasm or neoplastic cells expressing high levels of CD138 and/or FGFR3 may respond better to therapy with a bi-specific binding agent described herein than another subject having a neoplasm or neoplastic cells that express little or no CD138 or FGFR3. A neoplastic cell or cancer cell can be quickly assayed to determine an expression level of CD138 using a suitable 20 anti-CD138 antibody and a suitable immunoassay (e.g., whole-cell ELISA, FACs, and the like). Likewise, a neoplastic cell or cancer cell can be quickly assayed for expression of an FGFR3 using a suitable anti-FGFR3 antibody and a suitable immunoassay. Accordingly, in some embodiments, a method of treating a subject having or suspected of having a neoplasm comprises administering a therapeutically 25 effective amount of a bi-specific binding agent described herein, wherein the neoplasm, or neoplastic cells thereof, express a detectable level of CD138 and/or an FGFR3 (e.g., FGFR3, or an isoform thereof). In certain embodiments, a neoplasm that expresses detectable levels of CD138 and/or an FGFR3 is a neoplasm that is known or reported to express CD138 and/or an FGFR3. In certain embodiments, a neoplasm is suspected of 30 expressing CD138 and/or an FGFR3 (e.g., by having a similar genotype or phenotype to another neoplastic cell type that is known to express CD138 and/or an FGFR3). In

some embodiments, a neoplasm that expresses, or is suspected of expressing CD138 and/or an FGFR3 is a neoplasm that expresses an RNA transcript that encodes CD138 and/or an FGFR3, or a portion thereof. In some embodiments, a neoplasm that expresses, or is suspected of expressing CD138 and/or an FGFR3, is a neoplasm that  
 5 expresses CD138 and/or an FGFR3 on its cell surface. Non-limiting examples of cancers with confirmed expression of CD138 and/or FGFR3 include adrenocortical carcinoma, bladder urothelial carcinoma, breast invasive carcinoma, cervical squamous cell carcinoma, endocervical adenocarcinoma, cholangiocarcinoma, colon adenocarcinoma, colorectal adenocarcinoma, lymphoid neoplasm diffuse large B-cell  
 10 lymphoma, esophageal carcinoma, glioblastoma multiforme, glioma, head and neck squamous cell carcinoma, kidney chromophobe, pan-kidney cohort (KICH+KIRC+KIR), kidney renal clear cell carcinoma, kidney renal papillary cell carcinoma, acute myeloid leukemia, brain lower grade glioma, liver hepatocellular carcinoma, lung adenocarcinoma, lung squamous cell carcinoma, mesothelioma,  
 15 ovarian serous cystadenocarcinoma, pancreatic adenocarcinoma, pheochromocytoma and paraganglioma, prostate adenocarcinoma, rectum adenocarcinoma, sarcoma, skin cutaneous melanoma, stomach adenocarcinoma, stomach and esophageal carcinoma, testicular germ cell tumors, thyroid carcinoma, thymoma, uterine corpus endometrial carcinoma, uterine carcinosarcoma, and uveal melanoma.

20 [0193]

In some embodiments, a method of treating a subject having or suspected of having a neoplasm comprises administering a therapeutically effective amount of a bi-specific binding agent described herein in combination with another anti-cancer therapy, non-limiting examples of which include a T-cell activating agent, an adjuvant,  
 25 an anti-cancer vaccine, a radiation treatment, an immunotherapy (e.g., anti-HER2, or anti-CD20), a chemotherapy, and the like or combinations thereof. In some embodiments, a T-cell activating agent is an antibody that binds to CD3, OX40, GITR, CD137 (41BB), CD27, HVEM, LAG-3, TIM3, VISTA or BTLA.

[0194]

30 Any suitable chemotherapeutic agent can be used for a method described herein. In some embodiments a chemotherapeutic agent comprises or consists of an

alkylating agent, an anthracycline, cytoskeletal disruptors, epothilones (e.g., epothilone), histone deacetylase inhibitors (e.g., vorinostat, romidepsin), inhibitors of topoisomerase I (e.g., irinotecan, topotecan), inhibitors of topoisomerase II (e.g., etoposide, teniposide, tafluposidean), kinase inhibitors, peptide antibiotics (e.g., bleomycin, actinomycin), platinum-based agents (e.g., carboplatin, cisplatin, oxaliplatin), compounds targeting DNA repair enzyme poly-ADP ribose polymerase-1 (e.g., Parp inhibitors), retinoids (e.g., tretinoin, alitretinoin, bexarotene), vinca alkaloids and compounds (e.g., vinblastine, vincristine, vindesine, vinorelbine), anti-metabolites, plant extracts, plant alkaloids, nitrosourea, hormone, nucleoside or nucleotide analog and combinations thereof. Non-limiting examples of alkylating anti-neoplastic agents include Altretamine (hexamethylmelamine, HEXALEN(registered trademark)), Busulfan, Carmustine (BCNU), Chlorambucil, Cyclophosphamide, Dacarbazine (DTIC), Fotemustine, Ifosfamide, Lomustine (CCNU), Mechlorethamine, Melphalan, Procarbazine, semustine (MeCCNU), Streptozotocin, Temozolomide, Thiotepea (triethylenethio-phosphoramidate), Carboplatin, Cisplatin, Oxaliplatin, monofunctional alkylators, nitrosoureas, temozolomide, the like or combinations thereof. Non-limiting examples of a DNA intercalating agent include acrolein, anthracycline, phosphoramidate, Actinomycin D, bleomycin, idarubicin, daunorubicin, doxorubicin, elsamycin A, epirubicin, ethidium, m-AMSA, mitoxantrone, doxorubicin (Adriamycin, Doxil, Myocet, hydroxydaunorubicin, hydroxydaunomycin), Epirubicin, Idarubicin, Valrubicin, TAS-103, MLN944 (XR5944), Obatoclax, mechlorethamine, methotrexate, 6-mercaptopurine, thioguanine, 5-fluorouracil, cytosine arabinoside, 5-azacytidine (5-AZC) and 5-azacytidine related compounds, mithramycin, mitomycin C, hydroxyurea, carboplatin, oxiplatin, mitotane, a taxane, vinblastine, vincristine, dibromomannitol, gemcitabine, pemetrexed, the like or a combination thereof. Non-limiting examples of cytoskeletal disruptors (e.g., taxanes) include paclitaxel, taxol, and docetaxel. Non-limiting examples of kinase inhibitors include bortezomib, erlotinib, gefitinib, imatinib, vemurafenib, vismodegib, the like, analogs and compounds thereof. Non-limiting examples of nucleotide analogs include azacitidine, azathioprine, capecitabine, cytarabine, doxifluridine, fluorouracil, gemcitabine, hydroxyurea, mercaptopurine, methotrexate, tioguanine (formerly thioguanine), the like, analogs and

compounds thereof. Non-limiting examples of PARP inhibitors are olaparib, rucaparib, niraparib, veliparib, talazoparib and the like, analogs and compounds thereof.

[0195]

The term “subject” refers to a mammal. Any suitable mammal can be treated  
 5 by a method or composition described herein. Non-limiting examples of mammals include humans, non-human primates (e.g., apes, gibbons, chimpanzees, orangutans, monkeys, macaques, and the like), domestic animals (e.g., dogs and cats), farm animals (e.g., horses, cows, goats, sheep, and pigs) and experimental animals (e.g., mouse, rat, rabbit, and guinea pig). In some embodiments a mammal is a human. A mammal  
 10 can be any age or at any stage of development (e.g., an adult, teen, child, infant, or a mammal in utero). A mammal can be male or female. In some embodiments a subject in need is a subject who has or is suspected of having a neoplasm.

[0196]

Any suitable method of administering a composition, pharmaceutical  
 15 composition or bi-specific binding agent to a subject can be used. The exact formulation and route of administration for a composition for use according to the methods of the invention described herein can be chosen by the individual physician in view of the patient’s condition. See, e.g., Fingl et al. 1975, in “The Pharmacological Basis of Therapeutics,” Ch. 1, p. 1; which is incorporated herein by reference in its  
 20 entirety. Any suitable route of administration can be used for administration of a pharmaceutical composition or a bi-specific binding agent described herein. Non-limiting examples of routes of administration include topical or local (e.g., subcutaneously, transdermally or cutaneously, (e.g., on the skin or epidermis), in or on the eye, intranasally, transmucosally, in the ear, inside the ear (e.g., behind the ear  
 25 drum)), enteral (e.g., delivered through the gastrointestinal tract, e.g., orally (e.g., as a tablet, capsule, granule, liquid, emulsification, lozenge, or combination thereof), sublingual, by gastric feeding tube, rectally, and the like), by parenteral administration (e.g., parenterally, e.g., intravenously, intra-arterially, intramuscularly, intraperitoneally, intradermally, subcutaneously, intracavity, intracranial, intra-articular, into a joint space,  
 30 intracardiac (into the heart), intracavernous injection, intralesional (into a skin lesion), intraosseous infusion (into the bone marrow), intrathecal (into the spinal canal),

intrauterine, intravaginal, intravesical infusion, intravitreal), the like or combinations thereof.

[0197]

In some embodiments a composition herein is provided to a subject. A  
5 composition that is provided to a subject is sometimes provided to a subject for self-administration or for administration to a subject by another (e.g., a non-medical professional). For example a composition described herein can be provided with an instruction written by a medical practitioner that authorizes a patient to be provided a composition or treatment described herein (e.g., a prescription). In another example, a  
10 composition can be provided to a subject where the subject self-administers a composition orally, intravenously or by way of an inhaler, for example.

[0198]

Alternately, one can administer compositions for use according to the methods of the invention in a local rather than systemic manner, for example, via direct  
15 application to the skin, mucous membrane or region of interest for treating, including using a depot or sustained release formulation.

[0199]

In some embodiments a pharmaceutical composition comprising a bi-specific binding agent is administered alone (e.g., as a single active ingredient (AI or e.g., as a  
20 single active pharmaceutical ingredient (API)). In other embodiments, a pharmaceutical composition comprising a bi-specific binding agent is administered in combination with one or more additional AIs/APIs, for example, as two separate compositions or as a single composition where the one or more additional AIs/APIs are mixed or formulated together with the bi-specific binding agent in a pharmaceutical  
25 composition.

[0200]

In certain embodiments, a bi-specific binding agent is delivered to a cell (e.g., a mammalian cell). A bi-specific binding agent can be delivered to a cell using any suitable method. In certain embodiments, delivering a bi-specific binding agent to a  
30 cell comprises contacting a mammalian cell, in vitro or in vivo, with a composition

comprising a bi-specific binding agent under conditions that allow the bi-specific binding agent to bind to the cell.

[0201]

A pharmaceutical composition can be manufactured by any suitable manner, including, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or tableting processes.

[0202]

In some embodiments a method of treating a neoplasm in a subject comprising administering a therapeutically effective amount of a bi-specific binding agent, or a therapeutically effective amount of a pharmaceutical composition comprising a bi-specific binding agent, to the subject. A “therapeutically effective amount” means an amount sufficient to obtain an effective therapeutic outcome and/or an amount necessary and/or sufficient to prevent, terminate, block, inhibit, ameliorate, abrogate, slow, suppress, kill or reduce the growth, viability, metastasis, severity, onset, or a symptom of a neoplasm. In some embodiments, an effective therapeutic outcome can be determined by measuring and/or monitoring the number, size, viability, growth, mitosis, or metastasis of a neoplasm or neoplastic cells in a subject before and/or after treatment. Accordingly, in some embodiments, administering an therapeutically effective amount of a bi-specific binding agent, or a therapeutically effective amount of a pharmaceutical composition comprising a bi-specific binding agent, to a subject prevents, terminates, blocks, inhibits, ameliorates, abrogates, slows, suppresses, kills or reduces the growth, viability, metastasis, severity, onset, or a symptom of a neoplasm. In certain embodiments, administering a therapeutically effective amount of a bi-specific binding agent, or a therapeutically effective amount of a pharmaceutical composition comprising a bi-specific binding agent, to a subject induces death, necrosis, or apoptosis of some or all of the cancerous cells of a neoplasm. Determination of a therapeutically effective amount is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

[0203]

In some embodiments, an amount of a bi-specific binding agent in a composition is an amount that is at least a therapeutically effective amount and an amount low enough to minimize unwanted adverse reactions. The exact amount of a bi-specific binding agent will vary from subject to subject, depending on age, weight, and general condition of a subject, the severity of the condition being treated, and/or the amount of other therapeutic drugs administered. Thus, it is not always possible to specify an exact therapeutically effective amount of a bi-specific binding agent that can be administered to treat a neoplasm in a diverse group of subjects. As is well known, the specific dosage for a given patient under specific conditions and for a specific disease will routinely vary, but determination of the optimum amount in each case can readily be accomplished by simple routine procedures. Thus, a therapeutically effective amount of a bi-specific binding agent used to treat a neoplasm may be determined by one of ordinary skill in the art using routine experimentation.

[0204]

In certain embodiments, an therapeutically effective amount of a bi-specific binding agent in a composition comprises a dose from about 0.01 mg/kg (e.g., per kg body weight of a subject) to 500 mg/kg, 0.1 mg/kg to 500 mg/kg, 0.1 mg/kg to 400 mg/kg, 0.01 mg/kg to 300 mg/kg, 0.1 mg/kg to 300 mg/kg, 0.1 mg/kg to 200 mg/kg, 0.1 mg/kg to 150 mg/kg, 0.1 mg/kg to 100 mg/kg, 0.1 mg/kg to 75 mg/kg, 0.1 mg/kg to 50 mg/kg, 0.1 mg/kg to 25 mg/kg, 0.1 mg/kg to 10 mg/kg, 0.1 mg/kg to 5 mg/kg or 0.1 mg/kg to 1 mg/kg. In some aspects the amount of a bi-specific binding agent can be about 10 mg/kg, 9 mg/kg, 8 mg/kg, 7 mg/kg, 6 mg/kg, 5 mg/kg, 4 mg/kg, 3 mg/kg, 2 mg/kg, 1 mg/kg, 0.9 mg/kg, 0.8 mg/kg, 0.7 mg/kg, 0.6 mg/kg, 0.5 mg/kg, 0.4 mg/kg, 0.3 mg/kg, 0.2 mg/kg, or 0.1 mg/kg. In some embodiments a therapeutically effective amount of a bi-specific binding agent is between about 0.1 mg/kg to 500 mg/kg, or between about 1 mg/kg and about 300 mg/kg. Volumes suitable for various routes of administration are known in the art.

[0205]

In some embodiments a bi-specific binding agent or a pharmaceutical composition comprising a bi-specific binding agent is administered at a suitable frequency and/or interval as needed to obtain an effective therapeutic outcome. In

some embodiments, a pharmaceutical composition comprising a bi-specific binding agent is administered hourly, once a day, twice a day, three times a day, four times a day, five times a day, and/or at regular intervals, for example, every day, every other day, three times a week, weekly, every other week, once a month and/or simply at a frequency or interval as needed or recommended by a medical professional.

[0206]

#### Kits

In some embodiments, a pharmaceutical composition comprising an amount or dose of a bi-specific binding agent is provided in a kit, pack or dispensing device, which can contain one or more doses of a bi-specific binding agent. The kit or pack can for example comprise one or more suitable containers, vials, or blister packs and one or more suitable dispensing devices. In some embodiments, a kit or pack is accompanied by instructions for administration and/or a notice prescribed by a governmental agency regulating the manufacture, use, or sale of pharmaceuticals, which notice is reflective of approval by the agency of the form of the drug for human or veterinary administration. Such notice, for example, can be the labeling approved by the U.S. Food and Drug Administration for prescription drugs, or the approved product insert.

[0207]

In some embodiments a kit or pack comprises an amount of a bi-specific binding agent sufficient to treat a patient for 1 day to 1 year, 1 day to 180 days, 1 day to 120 days, 1 day to 90 days, 1 day to 60 days, 1 day to 30 days, or any day or number of days there between, 1-4 hours, 1-12 hours, or 1-24 hours.

[0208]

A kit optionally includes a product label or packaging inserts including a description of the components or instructions for use in vitro, in vivo, or ex vivo, of the components therein. Exemplary instructions include instructions for a diagnostic method, treatment protocol or therapeutic regimen. In certain embodiments, a kit comprises packaging material, which refers to a physical structure housing components of the kit. The packaging material can maintain the components sterily, and can be made of material commonly used for such purposes (e.g., paper, corrugated fiber, glass, plastic, foil, ampules, vials, tubes, etc.). Product labels or inserts include “printed

matter,” e.g., paper or cardboard, or separate or affixed to a component, a kit or packing material (e.g., a box), or attached to an ampule, tube or vial containing a kit component. Labels or inserts can additionally include a computer readable medium, optical disk such as CD- or DVD-ROM/RAM, DVD, MP3, magnetic tape, or an electrical storage media such as RAM and ROM or hybrids of these such as magnetic/optical storage media, FLASH media or memory type cards. Product labels or inserts can include identifying information of one or more components therein, dose amounts, clinical pharmacology of the active ingredient(s) including mechanism of action, pharmacokinetics (PK) and pharmacodynamics (PD). Product labels or inserts can include information identifying manufacturer information, lot numbers, manufacturer location, date, information on an indicated condition, disorder, disease or symptom for which a kit component may be used. Product labels or inserts can include instructions for the clinician or for a subject for using one or more of the kit components in a method, treatment protocol or therapeutic regimen. Instructions can include dosage amounts, frequency or duration, and instructions for practicing any of the methods, treatment protocols or therapeutic regimes set forth herein. Kits of the invention therefore can additionally include labels or instructions for practicing any of the methods and uses of the invention described herein. Product labels or inserts can include information on potential adverse side effects and/or warnings.

[0209]

In certain embodiments, a kit comprises one or more controls having a known amount of syndecan-1 and/or an FGFR3. In some embodiments, a kit comprises cells expressing syndecan-1 and/or an FGFR3. The cells in the kit can be maintained under appropriate storage conditions until the cells are ready to be used.

[0210]

In some embodiments, a kit is a diagnostic kit comprising a bi-specific binding agent. A bi-specific binding agent comprised in a diagnostic kit can take any suitable form. In some embodiments, a diagnostic kit comprises a bi-specific binding agent and a detectable label. In certain embodiments, for example, a diagnostic kit comprises or consists of a stick test, including necessary reagents to perform the method of the invention and to produce, for example, a colorimetric result which can be

compared against a color chart or standard curve. A diagnostic kit can also comprise components necessary for detecting a bi-specific binding agent that binds specifically to a cell, for example a secondary antibody.

#### EXAMPLES

5 [0211]

##### Example 1 – Generation of anti-CD138 antibodies

Monoclonal antibodies were generated against human CD138 that (i) bind with high affinity and specificity, (ii) display rapid internalization, and/or (iii) display cross-reactivity with cynomolgus monkey derived CD138. To generate antibodies,  
10 mice (Balb/C mice; female 6-8 weeks old) were immunized and boosted with a mix of CD138 peptides 1-3 (Fusion 12) or peptides 4-6 (Fusion 13), see Table 11.

[0212]

These peptides were designed to be distal to glycosylation sites, and in regions that were poorly conserved between human and mouse, but strongly conserved between  
15 human and cynomolgus species. Mice were immunized with the indicated peptides which were conjugated to KLH carrier protein following an immunization schedule using Complete Freund's Adjuvant (CFA) for the primary injection and Incomplete Freund's Adjuvant for all the subsequent boosts. Antibody serum titers of the immunized mice were assessed for binding by human CD138 and CD138-Fc binding  
20 ELISA. Mice with high titers were selected for fusion. Hybridomas were produced by electrofusion of mouse B cells and SP2/O myeloma cells using an optimized method.  
[TABLE 11]

CD138 peptides used for immunization.

|           |                          |               |
|-----------|--------------------------|---------------|
| Peptide 1 | AGEGPKEGEAVVLPEVEPG      | SEQ ID NO:118 |
| Peptide 2 | KEGEAVVLPEVEPGLTARE      | SEQ ID NO:119 |
| Peptide 3 | VVLPEVEPGLTAREQEATP      | SEQ ID NO:120 |
| Peptide 4 | PEPTGLEATTASTSTLP        | SEQ ID NO:121 |
| Peptide 5 | ETTQLPTTHQA              | SEQ ID NO:122 |
| Peptide 6 | ATTAQEPATSHPHRDMQPGHHETS | SEQ ID NO:123 |

[0213]

Unlike in traditional methods, cloning of hybridomas was performed simultaneously in a single step wherein fused cells were selected by HAT selection, and single cell colonies were transferred into 96 well plates containing HT media, grown under limited selection and supernatants screened for antigen binding. Positive hybridomas were expanded into larger volumes and the cells were frozen for storage. [0214]

Primary Hybridoma FACS screen on CD138 positive cells (H929)

Hybridoma supernatants from Fusion 12 (plates 12-19) were screened by Fluorescence-activated cell sorting (FACS) using 96-well plates containing 20,000 H929 cells per well. H929 cells are a human B-lymphocyte cell line that expresses CD138 on its cell surface. Hybridoma supernatants were added to H929 cells for 1 hour at 4°C. The cells were washed followed by addition of an AlexaFluro 647 anti-mouse antibody and washed again to remove unbound antibody. The cells were analyzed by FACS to detect binding. FACS Data was analyzed by Flowjo software. If supernatant antibodies bound cells at a level  $\geq 3$  standard deviations above the average signal of the plate, they were selected for confirmation FACS and further characterization. A total of 5013 hybridomas from Fusion 12 underwent this primary FACS screen with 134 positive hits; a total hit rate of 2.7% in primary screening. A sample of the positive hybridoma clones from fusion 12 plate 16 is shown in Table 12 below. A FACS histogram of two representative positive hybridoma clones is shown in Fig. 2.

[TABLE 12]

Table from representative H929 FACS screen showing representative positive hybridomas from fusion 12 plate 16.

| Hybridoma<br>Clone/Antibody<br>Name | plate | Well ID | %Q1  | Q1<br>Geom.<br>Mean | %Q4   | Q4<br>Geom.<br>Mean | Cells   Geom.<br>Mean (FL4-A) |
|-------------------------------------|-------|---------|------|---------------------|-------|---------------------|-------------------------------|
| F12P16A4                            | 16    | A4      | 58.3 | 3971                | 41.6  | 1139                | 2363                          |
| F12P16B10                           | 16    | B10     | 72   | 5051                | 27.8  | 1219                | 3409                          |
| F12P16F6                            | 16    | F6      | 99.6 | 7.07E+05            | 0.223 | 146                 | 6.94E+05                      |
| F12P16G3                            | 16    | G3      | 64.1 | 4245                | 35.8  | 1136                | 2652                          |
| F12P16H6                            | 16    | H6      | 58   | 3733                | 41.7  | 1027                | 2182                          |

\* Geom. Mean indicates the average fluorescence intensity of cells within the indicated quadrant shown in Fig. 2; Q1 indicates positive binding of antibody to H929 cells, while Q4 indicates unbound cells.

### Secondary FACS screen of Fusion 12 hybridomas

Hybridomas demonstrating positive binding in the primary FACS screening were selected and further characterized. For secondary screening, hybridoma supernatants were assayed by FACS for positive binding to H929 (CD138 expressing) and negative binding to ARH-77 (negative CD138 expression) cells. Negative cells were CFSE stained to help distinguish better positive and negative cell lines. The supernatants were then compared with their non-specific binding versus specific CD138 binding. The results of the representative secondary FACS screen are summarized in Table 13.

10 [TABLE 13]

FACS data summary for representative hybridoma clone F12P16F6 (12P16F6) showing positive binding on CD138-positive H292 cells and negative binding on CD138-negative ARH77 cells.

| Hybridoma<br>Clone/Antibody<br>Name | H929 Q1% | H929<br>GeoMean | % ARH77 (-) | ARH77<br>Geom. Mean |
|-------------------------------------|----------|-----------------|-------------|---------------------|
| F12P16F6                            | 99.32%   | 6.46E+05        | 21.85%      | 1.12E+04            |

\* Geom. Mean indicates the average fluorescence intensity of cells; Q1% indicates the percentage of H929 cells bound by antibody.

[0216]

### Secondary ELISA screen on Fusion 12 hybridomas

CD138 and IgG1 isotype ELISAs were also conducted after the secondary screens to help confirm binding specificity and IgG type. CD138 binding ELISA was performed using recombinant CD138-Flag. Data indicated that all FACS-positive hybridomas also bound CD138 by ELISA. The IgG ELISA identified IgG positive antibodies. IgM antibodies were eliminated from further studies. A summary of the selection process to this point is shown in Table 14.

20 [TABLE 14]

## Summary of primary and secondary screening results.

| Screening Characterization | number of hybridoma clones |
|----------------------------|----------------------------|
| Total Hybridomas Screened  | 5013                       |
| Primary Screen hits        | 134                        |
| No Expression              | 39                         |
| IgM positive               | 23                         |
| No Secondary Binding       | 60                         |
| IgG positive               | 72                         |
| Secondary FACS binders     | 12                         |

[0217]

Kinetic binding of representative antibodies by SPR

Murine IgGs were purified from the hybridoma supernatants and the IgGs were subject to SPR (surface plasmon resonance) for kinetic binding measurements. Human or mouse CD138 His was immobilized on a GLM chip at 50 µg/mL on a BioRad Proteon. The antibodies were flowed over the bound chip at a rate of 30 µL/min at a concentration range of 167 nM to 10.4 nM to detect kinetic binding. KDs were measured using bivalent analyte fit. Kinetic results are shown in Table 15 and Fig. 3.

10 [TABLE 15]

SPR kinetic measurement for representative antibodies.

| Antibody Name | hCD138 ka (1/Ms)     | hCD138 kd (1/s)       | hCD138 K <sub>D</sub> | hCD138 K <sub>D</sub> (nM) | mCD138 K <sub>D</sub> (nM) |
|---------------|----------------------|-----------------------|-----------------------|----------------------------|----------------------------|
| mBT062        | 3.42x10 <sup>5</sup> | 6.66x10 <sup>-4</sup> | 1.95x10 <sup>-9</sup> | 2.0                        | NB                         |
| F13P30A7      | 2.38x10 <sup>5</sup> | 1.27x10 <sup>-3</sup> | 5.32x10 <sup>-9</sup> | 5.3                        | NA                         |
| F12P16F6      | 5.57x10 <sup>4</sup> | 1.17x10 <sup>-3</sup> | 2.10x10 <sup>-8</sup> | 21.0                       | NB                         |
| F13P18D8      | 2.78x10 <sup>5</sup> | 5.23x10 <sup>-4</sup> | 1.88x10 <sup>-9</sup> | 1.9                        | NA                         |
| F12P7G11      | 3.81x10 <sup>6</sup> | 3.30x10 <sup>-2</sup> | 8.66x10 <sup>-9</sup> | 8.6                        | NB                         |
| F13P14D3      | 1.07x10 <sup>5</sup> | 1.04x10 <sup>-3</sup> | 9.67x10 <sup>-9</sup> | 9.7                        | NA                         |
| F11AP11E5     | 1.28x10 <sup>5</sup> | 1.17x10 <sup>-2</sup> | 9.16x10 <sup>-8</sup> | 91.6                       | NB                         |
| F12P18D4.a    | 6.31x10 <sup>5</sup> | 1.8x10 <sup>-2</sup>  | 2.86x10 <sup>-8</sup> | 28.6                       | 672                        |

\* NB = No binding; NA = Not Analyzed.

\*\*Those marked NA were not analyzed by SPR but were shown to not cross-react with mouse CD138 via an ELISA binding assay (data not shown).

[0218]

### Antibody Expression

Expression of two representative chimeric antibodies were assessed to determine the potential for scale-up production. Expi293 cells (250 mL) were transiently transfected with a vector directing the expression of 12P16F6 hIgG1 (also referred to as “chF6”) or 13P30A7 hIgG1 (also referred to as “chP30a7”). The chimeric antibody 12P16F6 hIgG1 includes the murine heavy chain variable region (SEQ ID NO:82) and light chain variable region (SEQ ID NO:34) of F12P16F6 and constant regions of human IgG1, kappa isotype. The chimeric antibody 13P30A7 hIgG1 includes the murine heavy chain variable region (SEQ ID NO:83) and light chain variable region (SEQ ID NO:35) of F13P30A7 and constant regions of human IgG1, kappa isotype. The results are summarized in Table 16 below. The expressed antibodies were also analyzed by SDS-PAGE and size exclusion chromatography (data not shown).

[TABLE 16]

| Name          | Transfection Volume (mL) | Purification Lot # | [Conc] (mg/mL) | Volume (mL) | Total Yield (mg) | Endotoxin (Eu/mg) |
|---------------|--------------------------|--------------------|----------------|-------------|------------------|-------------------|
| 12P16F6 hIgG1 | 250 mL                   | AB150616-F6        | 2.21           | 3.2         | 7.07             | <4.5              |
| 13P30A7 hIgG1 | 250 mL                   | AB150616-A7        | 1.02           | 1.05        | 1.07             | <9.8              |

[0219]

### Cynomolgus cross-reactivity of representative antibodies.

12P16F6 hIgG1 or 13P30A7 hIgG1 were tested for cross-reactivity to cynomolgus monkey CD138. Antibodies that cross-react with CD138 from cynomolgus monkey (cyno) have an advantage that they can be tested for toxicity in this strain of non-human primate prior to conducting efficacy trials. Briefly, a vector directing the cell-surface expression of human CD138 or cyno CD138 was transfected in Expi293 cells. Binding of 12P16F6 hIgG1 and 13P30A7 hIgG1 to transfected Expi293 cells was tested at 3-fold dilutions starting at 33.3 µg/mL. Secukinumab (Sec) was used as a negative control to ensure the transfected cells did not have any background binding. Representative antibodies 12P16F6 hIgG1 and 13P30A7 hIgG1 showed specific binding to both human and cyno CD138 (Fig. 4).

[TABLE 17]

Summary of data for representative CD138 hybridoma-derived antibodies.

| Antibody Name | SPR mCD138 KD (nM) | SPR hCD138 KD (nM) |
|---------------|--------------------|--------------------|
| mBT062        | NB                 | 2                  |
| IgG2a         | NB                 | NB                 |
| F13P30A7      | NA                 | 5                  |
| F12P16F6      | NB                 | 21                 |
| F13P18D8      | NA                 | 2                  |
| F12P7G11      | NB                 | 9                  |
| F13P14D3      | NA                 | 10                 |
| F11AP11E5     | NB                 | 92                 |
| F12P18D4.a    | 672                | 29                 |

\*NB=No detectable binding. NA=Not analyzed.

[0220]

#### Definitions of Certain Reagents and Materials used in Example 1

- 5           Note that the name of a hybridoma clone here can refer to either the hybridoma cells or the antibody produced from the hybridoma cells, depending on the context in which the name is used. The name of a hybridoma clone often refers to the fusion (e.g., fusion #12 or #13, abbreviated F12 and F13 respectively), followed by the plate number preceded by the letter “P”, and the well number. For example, the hybridoma clone
- 10 F12P16F6 (also referred to herein as 12P16F6 or P16F6), refers to an antibody obtained from a hybridoma derived from Fusion 12, plate 16, and well F6. mBT-062 is an IgG1, CD138 binding control antibody.

[0221]

#### Example 2 - Humanization

- 15           A strategy was developed to design and create humanized versions of the murine anti-CD138 (anti-syndecan-1) antibodies described herein where the humanized version of the antibody retains the properties of the parental monoclonal antibody. Provided herein are examples of humanizing the chimeric monoclonal anti-CD138 antibody designated as chF6, which includes the human constant regions of a human
- 20 IgG1/kappa isotype and the mouse variable regions of F12P16F6.

[0222]

The humanized versions of chF6 generated herein were often benchmarked against the parent chF6 chimeric antibody. Other positive and negative controls were also used where appropriate.

5 [0223]

Five humanization strategies were employed in parallel which resulted in the generation of three humanized F12P16F6 light chain sequences and four humanized F12P16F6 heavy chain sequences. In certain embodiments, the methods involve grafting of the murine complementarity determining regions (CDRs) onto human  
10 framework and constant regions. Each of the resulting three humanized light chains and four humanized heavy chains were expressed in combination with each other, and purified, which resulted in a total of twelve humanized anti-CD138 monoclonal antibodies. The humanized antibodies were analyzed for their expression/purification profiles, biophysical properties, binding to a CD138 peptide antigen, binding to native  
15 CD138, and specificity. Representative humanized antibodies were also evaluated for other biophysical properties.

[0224]

## Methods

### Expression and purification of chP16F6

20 A vector directing the expression of the chimeric antibody chP16F6 was transfected in a volume of 250 ml into Expi293 cells using EXPIFECTAMINE(Trademark) 293 Transfection Kit. The supernatant was purified utilizing pH dependent, protein A purification. The chimeric antibodies were purified using HiTrap MabSelect SuRe 5 ml. After purification, the antibodies were buffer  
25 exchanged into 1x DPBS using Zeba spin columns. The recovery of chP16F6 was 7.1 mg at 2.21 mg/mL.

[0225]

### Humanization of F12P16F6.

Humanization of the heavy and light chain variable domains was performed  
30 using a method selected from (i) CDR grafting (designated as cdr) which was performed according to Jones et al. (1986) "Replacing the complementarity determining regions in

a human antibody with those from a mouse" Nature 321:522-525 and Verhoeyen *et al.* (1988) "Reshaping human antibodies: grafting an anti-lysozyme activity" Science 239:1534-1536, where the CDRs as defined by Kabat *et al.* (1991) "Sequences of Proteins of Immunological Interest" 5th ed. US Department of Health and Human Services, Public Health Service, National Institutes of Health (NIH Publication No 91-3242), are grafted onto an appropriate human scaffold, while the critical framework residues are preserved; (ii) Grafting of abbreviated CDRs (designated as abb) which was performed according to Padlan *et al.* (1995) "Identification of specificity-determining residues in antibodies" FASEB J 9:133-139 where abbreviated CDRs, defined as residues 27D-34, 50-55, and 89-96 in the light chain, and 31- 35B, 50-58, and 95-101 in the heavy chain, are grafted onto an appropriate human scaffold while the critical framework residues are preserved; (iii) SDR-transfer (designated as sdr) which was performed according to Padlan *et al.* (1995) "Identification of specificity determining residues in antibodies" FASEB J 9:133-139 where the residues that could be involved in antigen binding, are transplanted into an appropriate human sequence while the critical framework residues are preserved; (iv) The Frankenstein approach (designated as fra) which was performed according to Wu and Kabat (1992) "Possible use of similar framework region amino acid sequences between human and mouse immunoglobulins for humanizing mouse antibodies" Mol. Immunol. 29:1141-1146 where the CDRs are grafted onto a human scaffold made up of individual framework regions coming from appropriate human antibodies while the critical framework residues are preserved; and (v) Veneering (designated as ven) which was performed according to Padlan (1991) "A possible procedure for reducing the immunogenicity of antibody variable domains while preserving their ligand-binding properties" Mol. Immunol. 28:489-498 where the residues which are exposed in the nonhuman antibody, if the structure is known, or in a homologous molecule, if the structure is not known, are changed to the corresponding residues from an appropriate human antibody while the CDRs and the critical framework residues are preserved. In all of the methods described, 'an appropriate human antibody' is used to denote the closest human sequence (available in GenBank).

The term "critical framework residue" is used to denote a residue that is deemed essential for the maintenance of three-dimensional structure (from the analysis of

relevant high-resolution X-ray structures in the PDB). Sometimes a second “repaired” round of humanization was performed to improve the SEC profile of the antibody. Humanized antibodies produced in a second round are indicated by the designation rep or repair. The amino acid sequences of the resulting humanized heavy and light chain variable regions are shown in Figs. 10A and 10B, respectively.

[0226]

#### Expression and Purification of Humanized P16F6 repaired constructs

Each of the four humanized heavy chains was paired with each of the 3 light chains to yield 12 different antibodies (Table 18). The 12 humanized P16F6 antibodies were expressed in Expi293 cells using EXPIFECTAMINE(Trademark) 293 Transfection Kit. All constructs were transfected in a volume of 125 ml except F6 cks-rep (more was needed for additional studies) and 375 ml of F6 f2ka-rep due to low protein expression. The supernatants were filtered through a 0.22  $\mu$ m filter and treated with a protease inhibitor. Antibodies providing an expression level of >5 mg/L after buffer exchange and the ability to concentrate above  $\geq 1$  mg/mL were selected for further analysis.

[TABLE 18]

| Antibody Name    | Heavy Chain Variable Region Name (SEQ ID) | Light Chain Variable Region Name (SEQ ID) |
|------------------|-------------------------------------------|-------------------------------------------|
| F6 aka-rep       | P16F6 abb/sdr-rep (SEQ ID NO:89)          | P16F6 abb-rep (SEQ ID NO:42)              |
| F6 akf-rep       | P16F6 abb/sdr-rep (SEQ ID NO:89)          | P16F6 fra-rep (SEQ ID NO:43)              |
| F6 aks-rep       | P16F6 abb/sdr-rep (SEQ ID NO:89)          | P16F6 sdr/cdr/ven-rep (SEQ ID NO:41)      |
| F6 cka-rep       | P16F6 cdr/ven-rep (SEQ ID NO:90)          | P16F6 abb-rep (SEQ ID NO:42)              |
| F6 ckf-rep       | P16F6 cdr/ven-rep (SEQ ID NO:90)          | P16F6 fra-rep (SEQ ID NO:43)              |
| F6 cks-rep (hF6) | P16F6 cdr/ven-rep (SEQ ID NO:90)          | P16F6 sdr/cdr/ven-rep (SEQ ID NO:41)      |
| F6 flka-rep      | P16F6 fra1-rep (SEQ ID NO:91)             | P16F6 abb-rep (SEQ ID NO:42)              |
| F6 flkf-rep      | P16F6 fra1-rep (SEQ ID NO:91)             | P16F6 fra-rep (SEQ ID NO:43)              |
| F6 flks-rep      | P16F6 fra1-rep (SEQ ID NO:91)             | P16F6 sdr/cdr/ven-rep (SEQ ID NO:41)      |
| F6 f2ka-rep      | P16F6 fra2-rep (SEQ ID NO:92)             | P16F6 abb-rep (SEQ ID NO:42)              |
| F6 f2kf-rep      | P16F6 fra2-rep (SEQ ID NO:92)             | P16F6 fra-rep (SEQ ID NO:43)              |
| F6 f2ks-rep      | P16F6 fra2-rep (SEQ ID NO:92)             | P16F6 sdr/cdr/ven-rep (SEQ ID NO:41)      |

\*F6 and P16F6 indicates that the humanized antibody chains were derived from F12P16F6.

[0227]

The antibodies were purified utilizing pH dependent, protein A purification (HiTrap MabSelect SuRe 5 mL). After purification, the antibodies were buffer

exchanged into 1x DPBS using zeba spin columns. The recovery and relative stability as determined by size exclusion chromatography (SEC) analysis varied between the humanized antibodies (SEC profiles not shown). Table 19 summarizes the recovery, concentration and the percent monomer as determined by SEC. An SDS-PAGE analysis of eleven representative humanized antibodies is shown in Fig. 6. The nomenclature sometimes takes the form of hF6 xky-rep where h stands for humanized, F6 stands for F12P16F6-derived, x stands for the first letter of the first procedure used to generate the humanized heavy chain sequence (e.g., x can be a=abb, s=sdr, f=fra, or c=cdr), k stands for kappa light chain, and y stands for the first letter of the first procedure used to generate the humanized light chain sequence (a=abb, s=sdr, f=fra, c=cdr). The term “rep” stands for “repaired” indicating that at least a second round of humanization was performed, often using a different method.

[TABLE 19]

| Antibody Name       | Final Recovery (mg) | Concentration (mg/mL) | Recovery (mg/mL) | SEC % Monomer |
|---------------------|---------------------|-----------------------|------------------|---------------|
| F6 aka-rep          | 1.2                 | 1.44                  | 9.6              | 75.2          |
| F6 akf-rep          | 3.28                | 1.13                  | 26.24            | 95.4          |
| F6 aks-rep          | 4.89                | 1.63                  | 39.12            | 95.2          |
| F6 cka-rep          | 1.72                | 1.23                  | 13.76            | 88.8          |
| F6 ckf-rep          | 8.4                 | 1.05                  | 67.2             | 95.3          |
| F6 cks-rep (hF6)    | 15.25               | 1.22                  | 68.8             | 99.3          |
| F6 flka-rep         | 0.79                | 1.08                  | 6.32             | 92.9          |
| F6 flkf-rep         | 2.86                | 1.1                   | 22.88            | 94.3          |
| F6 flks-rep         | 3.5                 | 1.25                  | 28               | 95.6          |
| F6 f2ka-rep (Lot 1) | 0.37                | 1.06                  | 2.96             | 79.1          |
| F6 f2ka-rep (Lot 2) | 0.71                | 1.23                  | 2.84             | 95            |
| F6 f2kf-rep         | 1.8                 | 1.8                   | 14.4             | 95.1          |
| F6 f2ks-rep         | 2.81                | 1.34                  | 22.48            | 77.6          |

15 [0228]

#### CD138 binding of humanized P16F6 repaired constructs by FACS

Analysis of cell-surface binding to human CD138 was performed on 9 representative humanized anti-CD138 antibodies by FACS (Fig. 7). Secukinumab was used as a negative control. Two cell lines expressing moderate levels of CD138 were used to test binding; multiple myeloma cell line KMS-11 and bladder cancer line RT112/84. In previous experiments, 12P16F6 hIgG1 showed an EC<sub>50</sub> of approximately 9 nM in RT- 122/84 cells and ~3 nM in KMS-11 cells. EC<sub>50</sub>s were calculated using four parameter fit curves (Table 20). The constructs were also tested against ARH-77 cells, which are CD138 negative lymphoblasts. NB indicates no specific binding observed.

10 [TABLE 20]

Calculated EC<sub>50</sub> values of binding to endogenous CD138

| Antibody Name    | KMS-11 (nM) | RT-112 (nM) | ARH-77 (nM) |
|------------------|-------------|-------------|-------------|
| F6 akf-rep       | 1.09        | 1.88        | NB          |
| F6 aks-rep       | 1.36        | 2.13        | NB          |
| F6 cka-rep       | 1.30        | 2.44        | NB          |
| F6 ckf-rep       | 1.64        | 2.09        | NB          |
| F6 cks-rep (hF6) | 1.2         | 2.2         | NB          |
| F6 flka-rep      | 0.83        | 1.39        | NB          |
| F6 flkf-rep      | 0.94        | 1.93        | NB          |
| F6 flks-rep      | 0.97        | 2.73        | NB          |
| F6 f2kf-rep      | 1.81        | 2.46        | NB          |
| secukinumab      | NB          | NB          | -           |

\*NB=No detectable binding.

[0229]

#### CD138 binding ELISA of humanized P16F6 repaired constructs.

A CD138 binding ELISA was performed with 9 representative humanized antibodies to determine binding to portion of the linear CD138 peptide used for immunization (Fig. 7). Plates were coated with the hCD138 peptide (AGEGPKEGEAVVLP; SEQ ID NO:94) and a negative control peptide (QAAVTSHPHGGMQPGLHETSA; SEQ ID NO:124), or a mouse CD138 peptide for which F12P16F6 does not bind. Coated plates were incubated with various dilutions of each of the 9 representative antibodies overnight and binding was detected with a goat anti-human IgG (H+L)-HRP. EC<sub>50</sub>s were determined using four parameter fit curves (Table 21). An ELISA was also performed to detect binding to plate-coated human CD138-Fc protein (Table 21). The analysis and results were similar.

[TABLE 21]

| Antibody Name        | hCD138<br>peptide EC50 (nM) | hCD138-Fc EC50 (nM) |
|----------------------|-----------------------------|---------------------|
| 12P16F6 hIgG1 (chF6) | 0.599                       | 0.4715              |
| F6 akf-rep           | 1.54                        | 0.8791              |
| F6 aks-rep           | 1.285                       | 0.9694              |
| F6 cka-rep           | 1.654                       | 0.8549              |
| F6 ckf-rep           | 0.9812                      | 0.5354              |
| F6 cks-rep (hF6)     | 0.3299                      | 0.1974              |
| F6 flka-rep          | 0.7443                      | 0.433               |
| F6 flkf-rep          | 0.4133                      | 0.2247              |
| F6 flks-rep          | 1.314                       | 0.7616              |
| F6 f2kf-rep          | 0.7257                      | 0.4051              |

[0230]

#### Summary of Selected Study Results

- 5 Table 22 shows a summary of the analytic results for 13 representative humanized anti-CD138 monoclonal antibodies.

[TABLE 22]

Summary of biophysical characteristics of representative humanized antibodies.

| <b>Antibody Name</b> | <b>Recovery (mg/L)</b> | <b>SEC % monomer</b> | <b>FACS binding (KMS11)</b> | <b>ELISA CD138 Fc binding</b> |
|----------------------|------------------------|----------------------|-----------------------------|-------------------------------|
| F6 aka-rep           | 9.6                    | 75.2                 | -                           | -                             |
| F6 akf-rep           | 26.24                  | 95.4                 | 1.09                        | 0.8791                        |
| F6 aks-rep           | 39.12                  | 95.2                 | 1.36                        | 0.9694                        |
| F6 cka-rep           | 13.76                  | 88.8                 | 1.30                        | 0.8549                        |
| F6 ckf-rep           | 67.2                   | 95.3                 | 1.64                        | 0.5354                        |
| F6 cks-rep (hF6)     | 68.8                   | 99.3                 | 1.20                        | 0.1974                        |
| F6 flka-rep          | 6.32                   | 92.9                 | 0.83                        | 0.433                         |
| F6 flkf-rep          | 22.88                  | 94.3                 | 0.94                        | 0.2247                        |
| F6 flks-rep          | 28                     | 95.6                 | 0.97                        | 0.7616                        |
| F6 f2ka-rep (lot 1)  | 2.96                   | 79.1                 | -                           | -                             |
| F6 f2ka-rep (lot 2)  | 2.84                   | 95                   | -                           | -                             |
| F6 f2kf-rep          | 14.4                   | 95.1                 | 1.81                        | 0.4051                        |
| F6 f2ks-rep          | 22.48                  | 77.6                 | -                           | -                             |

[0231]

#### Example 3 – Determination of Crystal structure

The X-ray crystal structure of a human syndecan-1 peptide in complex with a humanized anti-CD138 antibody Fab fragment was solved at 1.95 Å resolution. The structure included one copy each of syndecan-1 peptide and Fab per asymmetric unit (FIG. 8).

[0232]

#### Structure Description

The humanized antibody Fab comprises the humanized heavy chain variable region (SEQ ID NO:90) and the humanized light chain variable region (SEQ ID NO:41).

The CDR canonical structures were analyzed in accordance with the PyIgClassify database. The heavy chain CDRs were classified as follows: H1-13-1 (CDR-length-cluster), H2-10-1 and H3-6-1. The light chain CDRs were classified as follows: L1-16-1, L2-8-1 and L3-9-cis7-1. Syndecan-1 peptide binds to a single Fab, and complex formation buries 540 Å<sup>2</sup> of the solvent-accessible surface areas of syndecan-1 peptide and Fab (313.6 Å<sup>2</sup> chains A and H; 226.4 Å<sup>2</sup> chains A and L).

[0233]

All visible syndecan-1 peptide residues from 98-108 participate in direct contacts with Fab (Fig. 8). The specific Fab residues involved in the interface are 31-33, 35, 47, 50, 52, 58, 94-96 and 101-102 from chain H and 27-28, 32, 34, 46, 49-50, 89-94 and 96 from chain L. This means that four residues from CDR H1 participate in the interface, along with three residues from CDR H2 and five residues from CDR H3. In addition, four residues from CDR L1, two residues from CDR L2 and seven residues from CDR L3 participate in the interface.

[0234]

A 1 mL aliquot of Fab at 5.88 mg/mL (approx. 125 µM) was mixed with 250 µM syndecan-1 peptide (AGEGPKEGEAVVLP; SEQ ID NO:94) and incubated at 4 °C for two hours. The complex was fractionated on an S200 size exclusion column which had been pre-equilibrated with buffer containing 20 mM Tris pH 7.5 and 150 mM NaCl. Peak fractions were pooled and concentrated for crystallization. The final protein concentration as determined by Bradford assay was 3 mg/mL.

[0235]

Approximately 400 crystallization conditions were screened by the hanging drop method of vapour diffusion in 96 well format using a mosquito robot (TTP Labtech). Crystal growth was observed at 20 °C in two conditions: 2.1 M DL-malic acid pH 7.0, and 60% Tacsimate pH 7.0. Crystallization was optimized further in 24 well format.

[0236]

#### Crystal Cooling and Data Collection

The crystal described was grown using the hanging drop method of vapour diffusion in a 24 well plate with a precipitant solution containing 1.7 M DL-Malic acid,

pH 7.0. In house X-ray diffraction screening indicated that resolution could be improved by pre-soaking crystals in a solution containing 3.0 M DL-Malic acid, pH 7.0 for 24 hours. The crystal was cryo-cooled by capturing it in a loop directly from the soaking drop and plunging it into liquid nitrogen. A synchrotron data set was collected at ESRF beamline ID30A-1.

[0237]

### Structure Solution and Refinement

Data processing in MOSFLM (CCP4) and AIMLESS (CCP4) indicated that the most likely space group was P212121 with unit cell dimensions  $a = 60.6 \text{ \AA}$ ,  $b = 132.9 \text{ \AA}$  and  $c = 51.2 \text{ \AA}$ , giving a total cell volume of  $411706.34 \text{ \AA}^3$ . Calculation of the Matthews coefficient ( $2.14 \text{ \AA}^3/\text{Da}$  and 42.7% solvent content) indicated that there was most probably one complete Fab-syndecan-1 complex per asymmetric unit. Models for use in molecular replacement (MR) were chosen by BLAST searching the sequences of each component (Fab heavy and light chains) against the PDB. Models with highest sequence identity were 3sqo (Fab heavy chain) and 4ojf (Fab light chain). The large number of Fab crystal structures deposited in the PDB has revealed a wide variety in elbow angles present between variable and constant domains. This variation in elbow angles can cause the overall tertiary structure of two otherwise highly homologous Fab fragments to be significantly different, which in turn causes MR to fail. For this reason the hinge regions between the variable and constant domains of the heavy and light chains were removed to create four separate MR search ensembles (VH, VL, CH and CL). Amino acid residues were trimmed from the CDRs of the heavy and light variable domain models after visual inspection in COOT to prevent any potential clashes that might also cause MR to fail. All four of the input search ensembles (VH, VL, CH and CL) that were required to build a complete Fab were correctly located by MR using PHASER (McCoy *et al.*, 2007) (CCP4). The MR output model was given 20 cycles of jelly body refinement using REFMAC5 (CCP4). The protein sequence was mutated to match that of Fab using CHAINSAW (CCP4). The model was improved iteratively through successive cycles of model building and refinement until all of the ordered regions of Fab visible in the electron density maps were complete. The heavy and light chain amino acids were renumbered in accordance with the Kabat antibody

numbering convention. Electron density corresponding to the syndecan-1 peptide was clearly visible. Syndecan-1 amino acid residues were added by hand in COOT and the correct numbering was applied. Water molecules were added using the water placement option in COOT and the complete model was refined using REFMAC5 (CCP4). The final Fab model contained heavy chain residues 1-216 (chain H) and light chain residues 1-212 (chain L) with no breaks in either chain. The final syndecan-1 model contained residues 98-108 (chain A). The final model also contained 205 water molecules. Final Rwork = 21.2%, Rfree = 26.1%.

[TABLE 23]

***Data Collection, Processing, and Refinement Statistics***

| <b><i>Data collection and processing statistics</i></b> |                      |
|---------------------------------------------------------|----------------------|
| Synchrotron, Beam line                                  | ESRF, ID30A-1        |
| Date and time of data collection                        | 28/01/2017; 04:13:17 |
| Wavelength (Å)                                          | 0.966                |
| Detector type                                           | Dectris Pilatus3 2M  |
| Transmission (%)                                        | 100                  |
| Temperature (K)                                         | 100                  |
| Exposure time (s)                                       | 0.1                  |
| Oscillation range per frame (°)                         | 0.2                  |
| Overall rotation (°)                                    | 180                  |
| Resolution range (Å)                                    | 44.76 – 1.95         |
| Number of observed reflections                          | 197864               |
| Number of unique reflections                            | 30915                |
| Multiplicity (overall and last shell)                   | 6.4 (6.1)            |
| Completeness (%) (overall and last shell)               | 99.9 (99.9)          |
| Rmerge (%) (overall and last shell)                     | 12.0 (92.1)          |
| Mean I/sigma (overall and last shell)                   | 12.1 (1.1)           |
| CC(1/2) (overall and last shell)                        | 0.996 (0.592)        |
| Space group                                             | <i>P212121</i>       |
| Unit cell parameters (Å), (°)                           | 60.57 132.87 51.16   |
| 90.00 90.00 90.00                                       |                      |
| <b><i>Refinement statistics</i></b>                     |                      |
| Refinement program                                      | REFMAC5              |
| Resolution range (Å)                                    | 66.43 – 1.95         |
| Number of reflections (working/test)                    | 29294 / 1561         |
| Rwork (%)                                               | 21.2                 |
| Rfree (%)                                               | 26.1                 |
| Protein residues modelled                               | 435                  |
| Number of protein atoms modelled                        | 3324                 |
| Number of water atoms modelled                          | 205                  |
| RMSD Bond lengths (Å)                                   | 0.007                |
| RMSD Bond angles (°)                                    | 1.289                |
| Mean overall B value (Å <sup>2</sup> )                  | 28.9                 |
| Ramachandran plot favoured (%)                          | 96.8                 |
| Ramachandran plot allowed (%)                           | 3.2                  |
| Ramachandran plot outlier region (%)                    | 0.0                  |

[0238]

**Example 4 – FGFR3 Fynomer Production**

- 5           The SH3 domain of the human Fyn kinase was successfully used as a scaffold to engineer Fynomers polypeptides that bind with high affinity and specificity to FGFR3 target proteins. A proprietary phage display library containing more than 8.5 x

10<sup>10</sup> individual Fynomer clones was used for selections on different recombinant FGFR3 antigens. Different selection and screening strategies were used. In one strategy, recombinant FGFR3 isoform 3B and FGFR3 isoform 3C derived from human and/or monkey (e.g., Cynomolgus monkey, *Macaca fascicularis*) were used for selection  
5 of phage clones expressing a Fynomer that specifically binds to at least two FGFR3 splice isoforms (e.g., 3B and 3C). Several rounds of selection were conducted using different FGFR3 antigens and/or combinations of FGFR3 antigens. One goal of the Fynomer selection process was to isolate a Fynomer with (i) selective binding to both FGFR3b and FGFR3c, (ii) cross-reactivity to human and monkey, and possibly mouse,  
10 and (iii) the ability to internalize bound receptors. This representative example describes a process of selecting such a Fynomer.

[0239]

Using recombinant human FGFR3b-Fc and FGFR3c-Fc as targets, we successfully selected and isolated several families of Fyn SH3-derived binding proteins  
15 that are capable of binding to both splice variants of human FGFR3 (FGFR3b and FGFR3c). We continued with the most promising candidate family for further studies.

[0240]

Interestingly, a Fyn SH3-derived polypeptide referred to as FF2L4C3 (SEQ ID NO:101), carrying the RT-loop sequence “EVYGPTPM” (SEQ ID NO:100), was  
20 enriched during the selection process and showed very promising internalization properties among 29 tested anti-FGFR3 Fynomers (see Fig. 10). In more detail, 5 other sequence families were excluded from further analysis. The Fynomers belonging to the most promising sequence family showed the best affinities and internalization properties.

25 [0241]

In order to obtain Fyn SH3-derived FGFR3 binders with higher affinities and improved internalization properties, FF2L4C3 (SEQ ID NO:101) was used as template for affinity maturation. The RT-loop sequence “EVYGPTP” (SEQ ID NO:131) was kept constant and was combined with a randomized n-src-loop repertoire (where a  
30 stretch of 4 to 6 randomized amino acid residues were introduced at the positions (X<sup>1</sup>) to (X<sup>4</sup>) in SEQ ID NO:99). The process of affinity maturation library generation was

essentially the same as described for cloning of the naïve library with a randomized n-src-loop (“library 0” as described in [25]).

[0242]

After naïve and affinity maturation selections, enriched Fyn SH3-derived polypeptides were screened for binding to FGFR3 by lysate ELISA. DNAs encoding the Fyn SH3-derived binding proteins were cloned into the bacterial expression vector pQE12 (Qiagen) so that the resulting constructs carried a C-terminal myc-hexahistidine tag as described in Grabulovski *et al.* [26]. The polypeptides were expressed in the cytosol of *E. coli* bacteria in a 96-well format and 200 µL of cleared lysate per well was prepared as described in Bertschinger *et al.* [27]. Briefly, transformed bacterial colonies were picked from the agar plate and grown in a round bottom 96-well plate (Nunc, cat. no. 163320) in 200 µL 2xYT medium containing 100 µg/mL ampicillin and 0.1% (w/v) glucose. Protein expression was induced after growth for 3 h at 37°C and rotational shaking at 200 r.p.m. by adding 1 mM IPTG (Applichem, Germany). Proteins were expressed overnight in a rotary shaker (200 r.p.m., 30°C). Subsequently, the 96-well plate was centrifuged at 1800 g for 10 min and the supernatant was discarded. Bacterial pellets were lysed using BugBuster(registered trademark) plus Benzonase(registered trademark) (Millipore 70750-3) and lysates were subsequently cleared by centrifugation for 10 min at 1800 x g. 60 µL lysate were mixed with 170 µL PBS and filtered through a 0.45 µm Multiscreen filter plate (Millipore MSHVN4510), in order to eliminate any residual bacterial debris.

[0243]

Monoclonal bacterial lysates were used for ELISA. For the ELISA, Maxisorp plates were coated overnight with either 5 µg/mL huFGFR3b-Fc, 5 µg/mL huFGFR3c-Fc or 5 µg/mL poly IgG and blocked for at least 1 h with 2% MPBS. Cleared lysates containing soluble Fynomer with a C-terminal myc- and hexahistidine peptide tag were added in 2% MPBS containing murine monoclonal anti-myc tag antibody, clone 9E10 (Roche Applied Science 11 667 203 001) to the maxisorp plates. Bound Fynomer was detected via 9E10 by an anti-mouse IgG-horse radish peroxidase conjugate (Sigma-Aldrich A2554). The detection of peroxidase activity was done by

adding BM blue POD substrate (Roche) and the reaction was stopped by adding 1 M H<sub>2</sub>SO<sub>4</sub>.

[0244]

The DNA sequence of the specific binders was verified by DNA sequencing.

5 [0245]

### Results

The amino acid sequences of representative ELISA positive Fyn SH3-derived polypeptides that bind specifically to FGFR3b and FGFR3c are presented in SEQ ID NOs: 101, 103, 105, 107, 109 and 111. The Fyn-SH3 derived polypeptides SEQ ID NOs: 103, 105, 107 and 109 are a selection of binders from a large pool of molecules that were obtained after affinity maturation of FF2L4C3 (SEQ ID NO:101), and are presented here because of their improved affinities and internalization properties (as shown herein).

[0246]

15 More than 80 Fynomers derived from SEQ ID NO:101 were obtained and characterized, each demonstrating specific binding to FGFR3b and FGFR3c and demonstrating desirable biophysical properties, affinities and internalization properties. Fynomer having the amino acid sequences of SEQ ID NOs:103, 105, 107, 109, and 111 are representative of these binders.

20 [0247]

Example 5: Fyn SH3-derived polypeptides of the invention bind to human FGFR3b and FGFR3c with high affinities.

This example shows the characterization of the preferred Fyn SH3-derived FGFR3-binding polypeptides by surface plasmon resonance and flow cytometry experiments.

25

[0248]

### Methods

#### a) Affinity measurements by BIAcore

Affinities were measured using a BIAcore T200 instrument. One flow cell on a CM5 series S chip (GE Healthcare BR-1005-30) was coated with the anti – myc

30

antibody 9E10 (Roche 11 667 203 001; coating density ranging between 6000 and 8000 RU) using the amine coupling kit (GE Healthcare BR100633).

[0249]

The parental Fynomer FF2L4C3 (SEQ ID NO:101), at a concentration of 500 nM, and the Fynomers with SEQ ID NOs:103, 105, 107, 109 and 111, at a concentration of 100 nM, were captured on the 9E10 surface followed by injections of different concentrations of huFGFR3b-Fc, huFGFR3c-Fc or cynoFGFR3c-Fc (0 nM, 3.9 nM, 7.8 nM, 15.6 nM, 31.25 nM, 62.5 nM, 125 nM, 250 nM and 500 nM for the measurements of the parental Fynomer FF2L4C3, 0 nM, 0.046 nM, 0.14 nM, 0.41 nM, 1.2 nM, 3.7 nM, 11.1 nM, 33.3 nM and 100 nM for Fynomers with SEQ ID NOs: 103, 105, 107, 109 and 111). Sensograms were recorded and apparent kinetics constants were determined by curve fitting using the 1.1 Langmuir interaction model in the BIAevaluation 2.1 software.

[0250]

#### 15 b) Affinity measurements by Flow Cytometry

Binding of Fynomers to huFGFR3 on cells was analyzed by flow cytometry using KMS-11 cells (JCRB1179) as FGFR3-positive cells and N87 (ATCC, CRL-5822) as a FGFR3-negative control cell line. Both KMS-11 and N87 cells were maintained in RPMI1640 medium (Invitrogen 52400-25). All media were supplemented with 25 U/mL penicillin, 25 µg/mL streptomycin and 10% FCS. To harvest the semi-adherent KMS-11 cells from a T150 flask, the supernatant was removed into a 50 mL falcon tube, and the cells were washed with 10 ml PBS, which was also added to the falcon tube. 2 ml of Accutase (Sigma A6964) was added to the flask, and incubated for 10 min at 37°C. The Accutase was inactivated with the addition of 10 ml medium and added to the falcon tube, which was then centrifuged (250 x g, 5 min) to pellet the cells. The cells were resuspended in FACS buffer (PBS + 1% FCS + 0.2% sodium azide) to a cell concentration of  $1 \times 10^6$  cells/mL and 100 µL was used per well ( $1 \times 10^5$  cells/well) for the flow cytometry staining in a 96-well round bottomed plate (Nunc 163320). For adherent N87 cells the supernatant and wash were discarded, and only the Accutase-detached cells were collected and prepared.

[0251]

Fynomers were co-incubated with the mouse anti-myc antibody (clone 9E10; Roche 11667149001) to allow cross linking of myc-tagged Fynomers prior to cell binding. Fynomers were diluted to 1  $\mu$ M and co-incubated with 667 nM 9E10 anti-myc antibody (3:2 molar ratio) in FACS buffer, for approximately 10 minutes on ice.

5 [0252]

This mixture was serially diluted 1 in 4 down to a Fynomer concentration of 0.06 nM (8 concentrations in total). Controls included the secondary antibody 9E10 only (no Fynomer; 667 nM 9E10), cells only (FACS buffer only) and an anti-FGFR3 antibody (R&D systems; cat. No. MAB766) at a concentration of 10 nM. Cells were  
 10 centrifuged in the 96-well plate (250 x g, 5 min) and were resuspended with the samples indicated above, before incubation on ice for 1 hr. The plate was centrifuged and washed (PBS + 0.2% sodium azide), before centrifuging again. Then 50  $\mu$ L of the secondary antibody anti-mIgG Alexa488 (Life Technologies A21202) was added to the cells at a concentration of 4  $\mu$ g/mL, before incubating in the dark, on ice, for 45 min.  
 15 The plate was centrifuged and washed twice with PBS + 0.2% sodium azide, before resuspending in FACS buffer and FACS analysis (Millipore Guava easyCyte 8HT).

[0253]

FACS data analysis was performed using Prism 6. The data was transformed ( $X = \log X$ ), and analyzed using a non-linear fit, log(agonist) vs. response – Variable  
 20 slope (4 parameters).

[0254]

## Results

### a) Affinity Measurements by BIAcore

The binding properties were analyzed by real-time interaction analysis on a  
 25 BIAcore chip revealing the following dissociation constants (KD) for selected FGFR3-binding polypeptides:

[TABLE 24]

Apparent kinetics constants of the binding of FGFR3-binding Fynomers to recombinant human FGFR3b, human FGFR3c and cynomolgus FGFR3c.

| <b>Fynomer</b> | <b>SEQ ID NO.</b> | <b>huFGFR3b KD<sub>app</sub> (pM)</b> | <b>huFGFR3c KD<sub>app</sub> (pM)</b> | <b>cyFGFR3c KD<sub>app</sub> (pM)</b> |
|----------------|-------------------|---------------------------------------|---------------------------------------|---------------------------------------|
| FF2L4C3        | 101               | 4700                                  | 4600                                  | 5900                                  |
| FF44L65G12     | 103               | 690                                   | 685                                   | 110                                   |
| FF44L65G7      | 105               | 470                                   | 335                                   | 280                                   |
| FF48L66G7 (G7) | 107               | 260                                   | 190                                   | 210                                   |
| FF43L65D5      | 109               | 335                                   | 230                                   | 160                                   |
| FF44L65B7      | 111               | 100                                   | 250                                   | 260                                   |

[0255]

The measured apparent affinities (Table 24) of the Fyn SH3-derived polypeptides (SEQ ID NOs:101, 103, 105, 107, 109 and 111) binding to FGFR3b and FGFR3c are surprisingly high considering the fact that sub-nanomolar values were obtained after only one round of affinity maturation. Moreover, these measurements confirmed the comparable binding properties of the Fyn SH3 derived polypeptides (SEQ ID NOs: 101, 103, 105, 107, 109 and 111) to both human isoforms of FGFR3 (FGFR3b and FGFR3c), and to cynomolgus FGFR3c (binding to cynomolgus FGFR3b was not tested).

[0256]

#### b) Affinity measurements by flow cytometry

The binding properties were analyzed by flow cytometry using FGFR3-positive KMS-11 cells and FGFR3-negative N87 cells as negative control. The following EC50 values for selected FGFR3-binding polypeptides were measured as shown in Table 25 and Fig. 11.

[TABLE 25]

EC50 values determined on FGFR3-positive KMS-11 cells for Fyn SH3-derived FGFR3-binding polypeptides.

| <b>Fynomer</b> | <b>SEQ ID NO</b> | <b>EC50 (nM)</b> |
|----------------|------------------|------------------|
| FF2L4C3        | 101              | 5.9              |
| FF44L65G12     | 103              | 2.2              |
| FF44L65G7      | 105              | 4.2              |
| FF48L66G7 (G7) | 107              | 2.3              |
| FF43L65D5      | 109              | 1.2              |
| FF44L65B7      | 111              | 0.6              |

[0257]

EC50 values, in the low nanomolar range (Table 25), measured on a cell line expressing FGFR3 (Fig. 11A, KMS-11) confirmed the high apparent affinities measured by surface plasmon resonance (Table 24), and demonstrate binding to FGFR3 in the natural context of a cell surface. All the Fyn SH3-derived polypeptides (SEQ ID NOs: 101, 103, 105, 107, 109 and 111) binding to FGFR3 did not show unspecific binding on a cell line not expressing FGFR3 (Fig. 11B).

[0258]

- 10 Example 6: Fyn SH3-derived polypeptides of the invention specific to FGFR3 do not interfere with ligand binding

It would be preferred if the Fyn SH3-derived polypeptides for binding both isoforms FGFR3b and FGFR3c to not interfere with ligand (e.g. FGF1) binding, as the ligand binding site is located in proximity to the splice site give rise to either FGFR3b or FGFR3c.

[0259]

- 20 For the purpose of verifying the ability of the Fyn SH3-derived polypeptides to bind to FGFR3 in presence of one of its ligands, a BIAcore experiment was set up to measure the affinity of the Fynomers to FGFR3 in presence or absence of FGF1 (Fibroblast Growth Factor 1 is one of the major ligands of FGFR3).

[0260]

In analogy to the method used for measuring the affinities (as described in Example 5), Fynomers at concentration of 100 nM (with the exception of FF2L4C3 – SEQ ID NO:101 used at a concentration of 500 nM) were captured on the 9E10 surface followed by injections of different concentrations of huFGFR3c-Fc (0 nM, 11 nM, 33 nM, 100 nM) in presence or absence of 200 nM FGF1 (R&D systems 232-FA-025/CF). Sensograms were recorded and apparent kinetics constants were calculated using the BIAevaluation 2.1 software.

[0261]

## 10 Results

Independently of the presence or absence of 200 nM FGF1 in solution binding of the Fyn SH3-derived polypeptides to huFGFR3c was unchanged, showing that binding of the Fynomers to FGFR3 did not interfere with ligand binding. Table 27 shows the kinetics constants obtained in presence or absence of 200 nM FGF1.

15 [TABLE 26]

| <b>Fynomer</b> | <b>SEQ ID NO.</b> | <b>KD<sub>app</sub> (pM) to huFGFR3c-Fc (SEQ ID NO:13)</b> | <b>KD<sub>app</sub> (pM) to huFGFR3c-Fc (SEQ ID NO:13) in presence of 200 nM FGF1</b> |
|----------------|-------------------|------------------------------------------------------------|---------------------------------------------------------------------------------------|
| FF2L4C3        | 101               | 4000                                                       | 3800                                                                                  |
| FF44L65G12     | 103               | 140                                                        | 120                                                                                   |
| FF44L65G7      | 105               | 320                                                        | 230                                                                                   |
| FF48L66G7 (G7) | 107               | 170                                                        | 130                                                                                   |
| FF43L65D5      | 109               | 60                                                         | 70                                                                                    |
| FF44L65B7      | 111               | 170                                                        | 130                                                                                   |

[0262]

Even though, due to assay variability the values for  $KD_{app}$  in absence of FGF1 are slightly different than the values obtained in the experiment shown in Example 2 (Table 24), this experiment shows that the Fyn SH3-derived polypeptides are able bind to FGFR3 even if the ligand (in this case FGF1) is bound to the ligand binding site.

5 From this we conclude that the epitope bound by the Fyn SH3-derived polypeptides described here is located in a constant region of FGFR3.

[0263]

Example 7: Fyn SH3-derived polypeptides of the invention bind to the domains D1-D2 of FGFR3.

10 Specificity of Fyn-SH3 derived polypeptides binding to FGFR3 was tested by ELISA.

[0264]

Different antigens were coated on the plate (Maxisorp plate; Nunc 439454): huFGFR3b-Fc, huFGFR3c-Fc, cyFGFR3c-Fc, muFGFR3c-His, huD1-Fc, huD2-Fc,

15 huD1-D2-Fc.

[0265]

The plate was coated with 100  $\mu$ L antigen at 5  $\mu$ g/mL (0.5  $\mu$ g/well), and incubated at 4 °C overnight. The wells were washed 3x with PBS before being blocked with 200  $\mu$ L 4% MPBS for 1 hr at RT. The wells were washed again, and 20  $\mu$ L 10% MPBS containing 15  $\mu$ g/mL 9E10 was added, before the addition of 80  $\mu$ L Fynomer at 250 nM (200 nM final Fynomer concentration). The wells were incubated for 45 min at RT, before washing and the addition of 100  $\mu$ L anti-mouse IgG-HRP (Sigma A2554) diluted 1:1000 in 2% MPBS. The wells were incubated for 30 min at RT, before washing 3x with 0.1% Tween-20 in PBS, and then 3x PBS. 100  $\mu$ L BM POD Blue

20 substrate (Roche 11 484 281 001) was added to each well followed by 50  $\mu$ L 1M  $H_2SO_4$  to stop the reaction. The absorbance 450 nm – 650 nm was recorded using a Tecan M1000 instrument.

[0266]

### Results

30 As shown in Fig. 12 A-F, the Fyn SH3-derived polypeptides all are cross-reactive to cynomolgus and murine FGFR3c. Interestingly all binders are specific

for an epitope present only when the domains D1 and D2 are physically linked (see Fig. 12 A-F bar huFGFR3-D1D2), in fact no binding is observed if the single domains D1 or D2 (hFGFR3-D1 or huFGFR3-D2) are immobilized on the ELISA plate.

[0267]

- 5 Example 8: Fyn SH3-derived polypeptides of the invention cause efficient internalization of FGFR3

Internalization is a central feature of the Fyn SH3-derived polypeptides described here, and provides the opportunity to use these binders to deliver toxic payloads and/or fused proteins such as antibodies intracellularly.

10 [0268]

In order to assess the ability of the Fyn SH3-derived polypeptides binding to FGFR3 to internalize upon binding to the target, an internalization assay based on the intracellular delivery of a cytotoxic agent was established.

[0269]

- 15 The assay measures the cytotoxic effect of anti-FGFR3 Fynomers cross-linked with MMAF (Monomethyl auristatin F)-conjugated 9E10, on KMS-11 cells. MMAF is an antimitotic agent (blocks tubulin polymerization) and is active only upon internalization into the cells. Therefore, this assay indicates how well the Fynomers facilitate internalization of MMAF. 50  $\mu$ L of KMS-11 cells at  $2 \times 10^5$  cells/mL were
- 20 seeded into a 96-well flat bottomed plate (Corning Costar 3610), to give 10,000 cell per well. The cells were incubated for 4 hours to allow the cells to adhere (37 °C, 5% CO<sub>2</sub>). Fynomers and 9E10-MMAF were mixed at a 3:1 ratio. A 4x stock of Fynomer (4  $\mu$ M) and a 4x stock of 9E10-MMAF (1.33  $\mu$ M) were prepared in RPMI media (see section 5.4.1) and mixed 1:1 (40  $\mu$ L + 40  $\mu$ L). This mixture was then serially diluted 1 in 3, to
- 25 give a concentration range 1000 nM – 50 pM. 50  $\mu$ L of the sample was added to the 50  $\mu$ L of cells (as seeded above), and incubated for 5 days (37 °C, 5% CO<sub>2</sub>). Appropriate controls, the wild-type Fynomer FynSH3, MMAF-9E10 without Fynomer and also cells without addition of any reagents, were included. All samples were prepared in duplicate. After 5 days, 100  $\mu$ L Cell titer glo (Promega G7573) was added to each well and
- 30 incubated with gentle shaking for 10 min in the dark. As a read-out for cell viability, luminescence was measured using a Tecan M1000 instrument. Analysis was performed

using Prism 6. The data was transformed ( $X = \log X$ ), and analyzed using a non-linear fit,  $\log(\text{inhibitor})$  vs. response – Variable slope (4 parameters).

[0270]

### Results

- 5 All Fyn SH3-derived FGFR3-binding polypeptides described here show increased cytotoxicity (e.g. internalization) compared to the cells treated with the MMAF-labeled secondary antibody only (9E10 in Fig. 13A) or the wild-type Fynomer FynSH3 in combination with MMAF-labeled 9E10 shown in all 3 experiments (Fig. 13 A-C indicated as FynSH3), that show cytotoxicity only at the highest concentration
- 10 tested, probably due to the toxicity of MMAF itself. Fig. 13 shows the cytotoxicity profiles obtained in different experiments, and Table 27 shows the EC50 obtained for the different Fyn SH3-derived FGFR3-binding polypeptides.

[TABLE 27]

EC50 values determined in internalization assays using  
FGFR3+ KMS-11 cells for Fyn SH3-derived FGFR3-binding polypeptides.

| <b>Fynomer</b> | <b>EC50 (nM)</b> |
|----------------|------------------|
| FF2L4C3        | 28.5/21/26.4     |
| FF44L65G12     | 2.5              |
| FF44L65G7      | 2.6              |
| FF48L66G7 (G7) | 2.4              |
| FF43L65D5      | 0.8              |
| FF44L65B7      | 1.8              |

\*Note: for FF2L4C3 the 3 values obtained in the 3 experiments shown in Fig. 13 are indicated.

- 15 [0271]

The data shown in Fig. 13 and Table 27 show that increased affinity also leads to more efficient internalization.

[0272]

Example 9: Alternative Fyn-SH3 derived polypeptide that shows excellent binding and internalization properties and which are derived of a different family

In addition to the family of sequences derived from SEQ ID NO:99, we  
5 identified an alternative Fynomer, FF40L54A5  
(GVTLFVALYDYEVMSSTALS FHKGEKFQILSQSPHGQYWEARSLTTGETGWIP  
SNYVAPVDSIQ; SEQ ID NO:116), that surprisingly also shows excellent binding and  
internalization properties and shares manufacturability and cross-reactivity properties  
with the Fynomers derived from SEQ ID NO:99 (see Table 28). Fynomer FF40L54A5  
10 was not expected to have excellent internalization properties as its sequence was derived  
from a Fynomer that showed only very poor internalization properties.

[0273]

Table 28 summarizes the properties of Fynomer FF40L54A5.

[TABLE 28]

-part 1

| Fynomer   | SEQ ID NO. | Yield (mg/L) | Affinities measured by BIAcore     |                                    |                                    |
|-----------|------------|--------------|------------------------------------|------------------------------------|------------------------------------|
|           |            |              | huFGFR3b<br>KD <sub>app</sub> (pM) | huFGFR3c<br>KD <sub>app</sub> (pM) | cyFGFR3c<br>KD <sub>app</sub> (pM) |
| FF40L54A5 | 116        | 5.4          | 170                                | 170*                               | 160                                |

- part 2

| Fynomer   | SEQ ID NO. | EC50 (nM)<br>for binding to<br>FGFR3 <sup>+</sup><br>KMS-11 cells | Affinities measured by BIAcore<br>(competition with FGF1) |                                                                           | EC50 (nM) in<br>internalization<br>assay |
|-----------|------------|-------------------------------------------------------------------|-----------------------------------------------------------|---------------------------------------------------------------------------|------------------------------------------|
|           |            |                                                                   | KD <sub>app</sub> (pM)<br>to<br>huFGFR3c-<br>Fc           | KD <sub>app</sub> (pM) to<br>huFGFR3c-Fc in<br>presence of 200<br>nM FGF1 |                                          |
| FF40L54A5 | 116        | 6.6                                                               | 210*                                                      | 230                                                                       | 4.7                                      |

\*: differences due to experimental variability

- part 3

| Fynomer   | SEQ ID NO. | Specificity ELISA               |                                 |                                          |                                          |                                               |
|-----------|------------|---------------------------------|---------------------------------|------------------------------------------|------------------------------------------|-----------------------------------------------|
|           |            | Cross-reactivity to<br>cyFGFR3c | Cross-reactivity to<br>muFGFR3c | Binding to<br>domain D1<br>of<br>huFGFR3 | Binding to<br>domain D2<br>of<br>huFGFR3 | Binding to<br>domains D1-<br>D2 of<br>huFGFR3 |
| FF40L54A5 | 116        | +++                             | +++                             | -                                        | -                                        | +++                                           |

[0274]

## Example 10 – Fynomer-Antibody Bi-specific binding agents (Fynomabs)

The following provides a non-limiting example of making a bi-specific binding agent that comprises one embodiment of an FGFR3 binding Fynomer and one

embodiment of an anti-CD138 monoclonal antibody. Any embodiment of a Fynomer disclosed herein can be attached to any embodiment of an anti-CD138 antibody, or binding portion thereof that is disclosed herein to generate a bi-specific binding agent that binds specifically to an FGFR3 and to CD138. The FGFR3 binding Fynomer used in this example is designated as FF48L66G7 (“G7”, (SEQ ID NO:107)) which is attached to the humanized anti-CD138 monoclonal antibody designated as hF6. The hF6 antibody is a humanized IgG1/kappa antibody comprising the heavy chain variable region of SEQ ID NO:93 and the light chain variable region of SEQ ID NO:44. The G7 Fynomer binds specifically to both mouse and human FGFR3. The G7 Fynomer also binds specifically to both FGFR3b and FGFR3c. The G7 Fynomer was covalently attached by a linker GGGGSGGGGSGGGGS at four different positions on the hF6 monoclonal antibody using recombinant technology. The positions include the heavy chain or light chain N-terminus (indicated as HN and LN, respectively), and the heavy chain or light chain C-terminus (indicated as HC and LC, respectively)(e.g., see Fig. 14). Briefly, for expression of hF6 comprising G7 fused to the N-terminus of the heavy chain, a nucleic acid construct was generated comprising a coding region directing the expression of G7 located 5’ of, and in-frame with, a sequence encoding the linker and a coding region directing the expression of the hF6 heavy chain. The hF6-HN-G7 construct was then co-transfected into CHO cells with a nucleic acid construct directing the expression of the hF6 light chain. The resulting bi-specific binding agent comprising the full length hF6 antibody of IgG1/kappa isotype and the G7 Fynomer (hF6-HN-G7) was isolated and purified from the cell culture supernatant.

[0275]

Similarly, for expression of hF6 comprising G7 fused to the C-terminus of the heavy chain, a nucleic acid construct was generated comprising a coding region directing the expression of the hF6 heavy chain located 5’ of, and in-frame with, a sequence encoding the linker and a coding region directing the expression of G7. For expression of hF6 comprising G7 fused to the N-terminus of the light chain, a nucleic acid construct was generated comprising a coding region directing the expression of G7 located 5’ of, and in-frame with, a sequence encoding the linker and a coding region directing the expression of the hF6 light chain. For expression of hF6 comprising G7

fused to the C-terminus of the light chain, a nucleic acid construct was generated comprising a coding region directing the expression of the hF6 light chain located 5' of, and in-frame with, a sequence encoding the linker and a coding region directing the expression of G7. The indicated constructs were then co-transfected into CHO cells with a nucleic acid construct directing the expression of the corresponding heavy or light chain of hF6. The resulting bi-specific binding agents comprising the full length hF6 antibody of IgG1/kappa isotype and the G7 Fynomer (i.e., hF6-HC-G7, hF6-LC-G7, and hF6-LN-G7) were isolated and purified from the cell culture supernatants. Each of the resulting bi-specific binding agents were assayed for binding to CD138 and FGFR3 by ELISA, the results of which are shown in Table 29.

[TABLE 29]

| Construct        | mFGFR3c ELISA, EC50 (nM) | hCD138 ELISA, EC50 (nM) |
|------------------|--------------------------|-------------------------|
| hF6 (no Fynomer) | NB                       | 0.56                    |
| hF6-LC-G7        | 0.94                     | 0.98                    |
| hF6-LN-G7        | 0.49                     | 1.07                    |
| hF6-HC-G7        | 0.91                     | 0.55                    |
| hF6-HN-G7        | 0.53                     | 0.69                    |

\*NB=No binding.

[0276]

In Table 28, the bi-specific construct designated hF6-LC-G7 indicates attachment of the G7 Fynomer to the C-terminal end of the light chain of the hF6 antibody (e.g., see embodiment of Fig. 14C), hF6-LN-G7 indicates attachment of the G7 Fynomer to the N-terminal end of the light chain of the hF6 antibody (e.g., see embodiment of Fig. 14D), hF6-HC-G7 indicates attachment of the G7 Fynomer to the C-terminal end of the heavy chain of the hF6 antibody (e.g., see embodiment of Fig. 14A), and hF6-HN-G7 indicates attachment of the G7 Fynomer to the N-terminal end of the heavy chain of the hF6 antibody (e.g., see embodiment of Fig. 14B). Table 28 show that the resulting bi-specific binding agents retained specific binding affinity to mouse FGFR3c and human CD138 as determined by ELISA.

[0277]

CHO cells (Chinese Hamster Ovary cells) were transfected to express the human FGFR3c (Fig. 16A) or human FGFR3b (Fig. 16B) on the cell surface. The representative bi-specific binding agent hF6-HN-G7 was then shown to specifically bind both human FGFR3b (Fig. 16B) and human FGFR3c (Fig. 16A) expressed on the cell surface of the transfected CHO cells by FACs analysis.

[0278]

The representative binding data of Table 28 and Figure 16 demonstrates that the bi-specific binding agents retain the binding specificity of the G7 Fynomer, as well as the binding specificity of the hF6 anti-CD138 antibody.

[0279]

#### Example 11 – Fynomer/Antibody (Fynomab) Drug Conjugates

The representative bi-specific binding agent hF6-S119C-HN-G7 was site specifically conjugated to the PBD toxin of formula II to generate a representative drug conjugate of a bi-specific binding agent designated as hF6-S119C-HN-G7-II. hF6-S119C-HN-G7-II demonstrated specific cytotoxicity to CHO cells expressing human FGFR3b (Fig. 17B) and human FGFR3c (Fig. 17C) but did not demonstrate specific killing of untransfected CHO cells (Fig. 17A) at concentrations less than 10000 pM. The cytotoxicity observed at high concentrations of drug conjugated binding agents (i.e., > 10000 pM) is most likely due to non-specific killing by the high concentration of toxin itself, independent of binding.

[0280]

The representative bi-specific binding agent drug conjugates hF6-HN-G7-II and hF6-LN-G7-II also demonstrated specific cytotoxicity against the cell lines KMS-11 (a high CD138 expressing cell line), and OPM-2 (a medium CD138 expressing cell line), but not ARH-77 (a negative control cell line)(e.g., see Fig. 19).

[0281]

The antibody portion (i.e., hF6) of the bi-specific binding agent hF6-HN-G7 was engineered to incorporate a cysteine amino acid substitution at one of five positions (i.e., S119, V282, T289, N361, and V422) in the hF6 antibody heavy chain constant region thereby providing the cysteine substituted antibodies designated as hF6-S119C, hF6-V282C, hF6-T289C, hF6-N361C and hF6-V422C. The corresponding bi-specific

binding agents were designated as hF6-S119C-HN-G7, hF6-V282C-HN-G7, hF6-T289C-HN-G7, hF6-N361C-HN-G7 and hF6-V422C-HN-G7, respectively, wherein “G7” indicates the presence of the FF48L66G7 Fynomer that is attached to the N-terminus of the heavy chain of hF6. The engineered cysteine residues provided a  
5 conjugation point for site-specific conjugation (SSC) of an anti-neoplastic agent to each of the bi-specific binding agents. A representative sequence of the parent hF6-HN-G7 is shown below. The dotted underline indicates a signaling sequence, the single underline represents the Fynomer portion, the double underline represents an optional linker region which is followed by the heavy chain of the hF6 antibody. Each of the  
10 amino acids that were independently mutated to a cysteine (i.e., A118, S119, S239, V282, T289, N361 and V422) are indicated.

[0282]

SEQ ID NO:125

[Chem. 34.]

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      M...E...W...S...W...V...F...L...F...F...L...S...V...T...T...G...V...H...S...G
1  ATGGAG TGGTCC TGGGTG TTTCTG TTCTTC CTCAGC GTGACC ACGGGA GTGCAT AGCGGC
   TACCTC ACCAGG ACCCAC AAAGAC AAGAAG GAGTCG CACTGG TGCCCT CACGTA TCGCCG
   V T L F V A L Y D Y E V Y G P T P M L S
61 GTGACC CTGTTT GTGGCC CTGTAC GACTAC GAGGTG TACGGC CCCACA CCCATG CTGTCC
   CACTGG GACAAA CACCGG GACATG CTGATG TCCCAC ATGCCG GGGTGT GGGTAC GACAGG
   F H K G E K F Q I L K G G S G P Y W E A
121 TTCCAC AAGGGC GAGAAG TTCCAG ATCCTG AAGGGC GGCTCC GGCCCC TACTGG GAGGCC
   AAGGTG TTCCCG CTCTTC AAGGTC TAGGAC TTCCCG CCGAGG CCGGGG ATGACC CTCGGG
   R S L T T G E T G L I P S N Y V A P V D
181 AGATCC CTGACC ACAGGC GAGACA GGCCTG ATCCCC TCCAAC TACGTG GCCCCC GTGGAC
   TCTAGG GACTGG TGTCCG CTCTGT CCGGAC TAGGGG AGGTTG ATGCAC CGGGGG CACCTG
   S I Q G G G G S G G S G G G S Q V
241 TCCATT CAGGGC GGC CGA GATCC GGC CGA GAGGA AGTGGC GGAGGA GGAAGT CAAGTC
   AGGTAA GTCCCG CCGCCT CTAGG CCGCCT CCTCCT TCACCG CCTCCT CCTTCA GTTCAG
   Q L V Q S G A E V V K P G A S V K L S C
301 CAACTG GTCCAA TCAGGT GCTGAG GTCGTG AAGCCA GGC GCG TCAGTT AAGCTC TCCTGC
   GTTGAC CAGGTT AGTCCA CGACTC CAGCAC TTCGGT CCGCGC AGTCAA TTCGAG AGGACG
   K A S G Y T F T S Y Y L Y W V K K A P G
361 AAGGCT TCCGGA TACACC TTCACA AGTTAT TATCTT TACTGG GTCAAA AAAGCA CCCGGT
   TTCCGA AGGCCT ATGTGG AAGTGT TCAATA ATAGAA ATGACC CAGTTT TTTCGT GGGCCA
   Q G L D W I G E I Y P R S G G T N I N E
421 CAGGGC CTCGAT TGGATC GGTGAA ATTTAC CCCC GC TCCGGA GGGACT AATATT AACGAA
   GTCCCG GAGCTA ACCTAG CCACTT TAAATG GGGGCG AGGCCT CCCTGA TTATAA TTGCTT
   K F L S R V T L T A D T S T S T A Y L E
481 AAGTTC CTGAGT CGAGTG ACACCT ACTGCT GATACT TCCACC TCCACC GCATAC CTCGAG
   TTCAAG GACTCA GCTCAC TGTGAA TGACGA CTATGA AGGTGG AGGTGG CGTATG GAGCTC
   L S S L T S E D T A V Y Y C T R S L L Y
541 TTGTCC TCCCTC ACATCC GAGGAT ACCGCC GTGTAC TACTGC ACAAGG AGCCTG TTGTAT
   AACAGG AGGGAG TGTAGG CTCCTA TGGCGG CACATG ATGACG TGTTC TCGGAC AACATA
   W G Q G T T L T V S S A S T K G P S V F
601 TGGGGC CAAGGC ACTACA CTGACA GTGTCT TCAGCT TCCACC AAAGGT CCATCC GTGTTT
   ACCCCG GTTCCG TGATGT GACTGT CACAGA AGTCGA AGGTGG TTTCCA GGTAGG CACAAA
   P L A P S S K S T S G G T A A L G C L V
661 CCACTG GCACCC TCATCA AAAAGC ACTAGC GGC GCG ACCGCT GCTCTG GGGTGT CTGGTC
   GGTGAC CGTGGG AGTAGT TTTTCG TGATCG CCGCCG TGGCGA CGAGAC CCCACA GACCAG
   K D Y F P E P V T V S W N S G A L T S G
721 AAGGAC TATTTT CCTGAG CCTGTG ACAGTT AGCTGG AACAGC GGC GCC CTTACT AGCGGC
   TTCTTG ATAAAA GGACTC GGACAC TGTCAA TCGACC TTGTCT CCGCGG GAATGA TCGCCG
   V H T F P A V L Q S S G L Y S L S S V V
781 GTCCAC ACCTTT CCCGCC GTGCTT CAATCC TCTGGC CTCTAC TCCCTT TCAAGC GTGGTC
   CAGGTG TGA AAA GGC CGG CACGAA GTTAGG AGACCG GAGATG AGGGAA AGTTCC CACCAG
   T V P S S S L G T Q T Y I C N V N H K P
841 ACAGTC CCCAGC TCTTCA CTCGGT ACCCAG ACTTAT ATATGT AATGTT AATCAC AAGCCT
   TGTCAG GGTTCG AGAAGT GAGCCA TGGGTC TGAATA TATACA TTACAA TTAGTG TTCGGA
   S N T K V D K K V E P K S C D K T H T C
901 AGCAAC ACTAAG GTTGAT AAAAAG GTGGAG CCCAAA AGCTGT GACAAG ACGCAT ACATGC
   TCGTTG TGATT CAACTA TTTTTC CACCTG GGGTTT TCGACA CTGTTC TCGTA TGTACG
   P P C P A P E L L G G P S V F L F P P K
961 CCTCCT TGCCCC GCCCCC GAGCTC CTGGGC GGCCCT TCCGTC TTTCTG TTCCCA CCCAAA
   GGAGGA ACGGGG CGGGGG CTCGAG GACCCG CCGGGA AGGCAG AAAGAC AAGGGT GGGTTT

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SEQ ID NO:125 (continued)

5 [Chem. 35.]

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      P K   D T   L M   I S   R T   P E   V T   C V   V V   D V
1021 CCCAAA GATACC CTCATG ATCTCC AGAACG CCAGAA GTTACC TGC GTT TGTGTA GATGTG
      GGGTTT CTATGG GAGTAC TAGAGG TCTTGC GGTCTT CAATGG ACGCAA CAACAT CTACAC
      S H   E D   P E   V K   F N   W Y   V D   G V   E V   H N
1081 TCTCAC GAGGAC CCCGAA GTGAAG TTAAAC TGGTAT GTGGAT GGAGTG GAGGTT CATAAC
      AGAGTG CTCCTG GGGCTT CACTTC AAATTG ACCATA CACCTA CCTCAC CTCCAA GTATTG
      A K   [E] K   P R   E E   Q Y   N S   T Y   R V   V S   V L
1141 GCCAAG ACAAAA CCCCGC GAAGAG CAGTAC AATAGC ACATAT AGGGTC GTGAGC GTCCTC
      CGGTTC TGTTTT GGGGCG CTTCTC GTCATG TTATCG TGTATA TCCAG CACTCG CAGGAG
      T V   L H   Q D   W L   N G   K E   Y K   C K   V S   N K
1201 ACTGTC CTCCAC CAGGAC TGGTTG AACGGT AAGGAA TATAAA TGTAAG GTCTCC AACAAAG
      TGACAG GAGGTG GTCCTG ACCAAC TTGCCA TTCCTT ATATTT ACATTT CAGAGG TTGTTC
      A L   P A   P I   E K   T I   S K   A K   G Q   P R   E P
1261 GCTCTG CCCGCG CCAATT GAAAAA ACAATC TCAAAG GCAAAG GGCCAG CCACGG GAACCT
      CGAGAC GGGCGC GGTAA CTTTTT TGTTAG AGTTTC CGTTTC CCGGTC GGTGCC CTTGGA
      Q V   Y T   L P   P S   R E   E M   T K   [N] Q   V S   L T
1321 CAGGTC TACACA CTGCCA CCCAGC CGAGAG GAGATG ACTAAG AATCAG GTCTCT CTGACA
      GTCCAG ATGTGT GACGGT GGGTCG GCTCTC CTCTAC TGATTC TTAGTC CAGAGA GACTGT
      C L   V K   G F   Y P   S D   I A   V E   W E   S N   G Q
1381 TGTCTG GTGAAG GGGTTT TATCCA TCTGAC ATTGCC GTTGAA TGGGAA TCAAAC GGGCAG
      ACAGAC CACTTC CCCAAA ATAGGT AGACTG TAACGG CAACCT ACCCTT AGTTTG CCCGTC
      P E   N N   Y K   T T   P P   V L   D S   D G   S F   F L
1441 CCTGAA AATAAT TACAAG ACTACT CCTCCC GTACTG GACTCC GACGGG TCATTT TTCTC
      GGACTT TTATTA ATGTTC TGATGA GGAGGG CATGAC CTGAGG CTGCCC AGTAAA AAGGAG
      Y S   K L   T V   D K   S R   W Q   Q G   N [V] F S   C S
1501 TACTCT AAACCT ACTGTC GATAAG TCAAGA TGGCAA CAGGGC AACGTC TTCAGT TGCAGC
      ATGAGA TTTGAA TGACAG CTATTC AGTTCT ACCGTT GTCCCG TTGCAG AAGTCA ACGTCG
      V M   H E   A L   H N   H Y   T Q   K S   L S   L S   P G
1561 GTGATG CATGAA GCCCTC CATAAC CATTAT ACGCAG AAATCT CTCAGT CTGTCT CCCGGG
      CACTAC GTACTT CGGGAG GTATTG GTAATA TGC GTC TTTAGA GAGTCA GACAGA GGGCCC
      K *   *
1621 AAGTAA TGA
      TTCATT ACT

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[0283]

Amino acid sequences of the bi-specific binding agents with mutation in the heavy chain constant region of A118C, S119C, S239C, V282C, T289C, N361C and V422C, i.e. bi-specific binding agents hF6-A118C-HN-G7, hF6-S119C-HN-G7, hF6-S239C-HN-G7, hF6-V282C-HN-G7, hF6-T289C-HN-G7, hF6-N361C-HN-G7 and hF6-V422C-HN-G7 are provided as SEQ ID NOs:135-141.

[0284]

Each of the representative bi-specific binding agents were conjugated to the exemplary toxic pyrrolbenzodiazepine (PBD) of formula II (also see Fig. 18). Stochastically conjugated bi-specific binding agents were conjugated in-house using a modified DHAA reduction protocol. Briefly, antibody agents were diluted to 1.1 mg in dPBS and reduced with 2x-20x stabilized TCEP (bond breaker) for 1-3 hours at 25 to 37 °C with occasional swirling. Samples were then buffer exchanged into dPBS with 1mM EDTA to remove TCEP and were oxidized using 2.5X DHAA for 2.5 hours at

room temperature. Antibodies were buffer exchanged into dPBS to remove DHAA and incubated with toxin II at 8X for 1.5 hours at room temperature. Conjugated agents were then buffer exchanged into dPBS and the concentrations were determined by nanodrop. Site specific conjugations were achieved under less stringent reducing conditions. The resulting drug-conjugated bi-specific binding agents that were site-specifically conjugated were designated as hF6-S119C-HN-G7-II, hF6-V282C-HN-G7-II, hF6-T289C-HN-G7-II, hF6-N361C-HN-G7-II and hF6-V422C-HN-G7-II.

[0285]

10 A 5-day cytotoxicity assay was carried out (N=3) using the ‘high CD138’ expressing cell line (KMS11), ‘medium CD138’ cell line (RT112), and negative control cell line (ARH77). The results of the cytotoxicity assay are shown in the table below and in Fig. 20.

[TABLE 30]

|                    | KMS-11    |            | RT112     |            | ARH-77    |            |
|--------------------|-----------|------------|-----------|------------|-----------|------------|
|                    | IC50 (pM) | % max kill | IC50 (pM) | % max kill | IC50 (pM) | % max kill |
| hF6-S119C-HN-G7-II | 27.9      | 99.6       | 35.3      | 99.7       | 3301.7    | 98.1       |
| hF6-T289C-HN-G7-II | 83.8      | 99.6       | 52.6      | 99.7       | 4005.7    | 98.0       |
| hF6-V282C-HN-G7-II | 89.3      | 99.7       | 28.9      | 99.7       | 3552.7    | 98.3       |
| hF6-N361C-HN-G7-II | 28.4      | 98.4       | 75.0      | 99.7       | 5373.7    | 97.1       |
| hF6-V422C-HN-G7-II | 17.0      | 99.5       | 28.1      | 99.7       | 3876.7    | 97.9       |
| hF6-HN-G7-II       | 17.9      | 99         | 7.1       | 99         | 1331.0    | 99         |
| Secukinumab-II     | 38.4      | 99         | 393.9     | 99         | 839.0     | 99         |
| hF6-II             | 114.1     | 99         | 19.6      | 99         | 1126.5    | 98         |

15

[0286]

The representative drug-conjugated bi-specific binding agents designated as hF6-S119C-HN-G7-II, hF6-V282C-HN-G7-II, and hF6-T289C-HN-G7-II, were tested in an in vitro toxicity assay against KMS-11 cells (a high expressing CD138 cell line),

RT-112 cells, AN3CA cells (a CD138/FGFR3 positive cell line), HCC1806 cells (a CD138/FGFR3 positive cell line) (e.g., see Fig. 20) and other cell types. The cell cytotoxicity results are summarized in the table below.

[TABLE 31]

| Cell line      | CD138<br>receptor<br>number on<br>cell line<br>(qFACS) | FGFR3<br>receptor<br>number on<br>cell line<br>(qFACS) | hF6-S119C-HN-G7-II    |                   | hF6-T289C-HN-G7-II    |                   | hF6-V282C-HN-G7-II    |                   |
|----------------|--------------------------------------------------------|--------------------------------------------------------|-----------------------|-------------------|-----------------------|-------------------|-----------------------|-------------------|
|                |                                                        |                                                        | EC <sub>50</sub> , pM | % cell<br>killing | EC <sub>50</sub> , pM | % cell<br>killing | EC <sub>50</sub> , pM | % cell<br>killing |
| <b>KMS-11</b>  | 122,818                                                | 37,455                                                 | 113.2                 | 99.6              | 311.7                 | 99.4              | NT                    | NT                |
| <b>RT-112</b>  | 589,939                                                | 4,264                                                  | 23.41                 | 99.7              | 50.82                 | 99.5              | NT                    | NT                |
| <b>8505C</b>   | 144,618                                                | 1,128                                                  | 559.1                 | 96.1              | 630.8                 | 95.3              | NT                    | NT                |
| <b>OE19</b>    | 418,816                                                | 6,410                                                  | 554.4                 | 70.3              | 696.4                 | 69                | NT                    | NT                |
| <b>MHHES-1</b> | 789                                                    | 11,410                                                 | 1366.5                | 99.3              | 1394.5                | 99.2              | NT                    | NT                |
| <b>An3CA</b>   | 216,058                                                | 1,406                                                  | 936.5                 | 97.3              | 1044.1                | 96.5              | 1054                  | 97.6              |
| <b>HCC1806</b> | 135,321                                                | 1,554                                                  | 3810.3                | 95.9              | 3712.4                | 97.5              | 8630                  | 90                |
| <b>HEC59</b>   | 67,033                                                 | 961                                                    | 3642.5                | 91.7              | 6104                  | 89.8              | NT                    | NT                |

[0287]

The representative drug-conjugated bi-specific binding agents designated as hF6-S119C-HN-G7-II, hF6-V282C-HN-G7-II, and hF6-T289C-HN-G7-II, were tested in an *in vivo* xenograft study at different doses (Fig. 21). Briefly, immunodeficient female mice (strain:Nu/Nu) were inoculated subcutaneously with  $5 \times 10^6$  An3CA or HCC1806 cells. Tumors were measured using digital calipers and the tumor volume was determined according to the formula  $0.5236 * L * W^2$ . Mice were randomized and dosed intravenously with the indicated bi-specific binding agent when tumors reached an average of 175 mm<sup>3</sup> (AN3CA) or 195 mm<sup>3</sup> (HCC1806). The results are summarized in Tables 32 (AN3CA) and 33 (ACC1806) below.

[TABLE 32]

*(AN3CA xenograft summary table)*

| Compound            | Dose      | % T/C | Adjusted p-value |
|---------------------|-----------|-------|------------------|
| hF6-S119C-HN-G7-II  | 1 mg/kg   | 22.5  | 0.0001           |
| hF6-S119C-HN-G7- II | 0.5 mg/kg | 44.6  | 0.0006           |
| hF6-T289C-HN-G7- II | 1 mg/kg   | 23.1  | 0.0001           |
| hF6-T289C-HN-G7- II | 0.5 mg/kg | 32.4  | 0.0001           |

[TABLE 33]

*(HCC1806 xenograft summary table)*

| Compound            | Dose      | % T/C | Adjusted p-value |
|---------------------|-----------|-------|------------------|
| hF6-S119C-HN-G7- II | 1 mg/kg   | 5.1   | 0.0001           |
| hF6-S119C-HN-G7- II | 0.5 mg/kg | 8.3   | 0.0001           |
| hF6-T289C-HN-G7- II | 1 mg/kg   | 3.9   | 0.0001           |
| hF6-T289C-HN-G7- II | 0.5 mg/kg | 10.6  | 0.0001           |

15 [0288]

## Example 12 – Certain Representative Sequences

Human syndecan-1 (syndecan-1) - UniProtKB - P18827

SEQ ID NO:1

MRRAALWLWLCALALSLQPALPQIVATNLPPEDQDGS GDDSDNFSGS  
 5 GAGALQDITLSQQTPSTWKDTQLLTAIPTSPEPTGLEATAASTSTLPAGEGPK  
 EAVVLPEVEPGLTAREQEATPRPRETTQLPTTHLASTTTATTAQEPATSHPHRD  
 MQPGHHETSTPAGPSQADLHTPHTEDGGPSATERAAEDGASSQLPAAEGSGEQ  
 DFTFETSGENTAVVAVEPDRRNQSPVDQGATGASQGLLDRKEVLGGVIAGGLV  
 GLIFAVCLVGFMLYRMKKKDEGSYSLEEPKQANGGAYQKPTKQEEFYA

10 [0289]

Mouse syndecan-1 (syndecan-1) - UniProtKB - P18828

SEQ ID NO:126

MRRAALWLWLCALALRLQPALPQIVAVNVPPEDQDGS GDDSDNFSGS  
 GTGALPDTLSRQTPSTWKDVWLLTATPTAPEPTSSNTETAFTSVLPAGEKPEEG  
 15 EPVLHVEAEPGFTARDKEKEVTTRPRETVQLPITQRASTVRVTTAQA AVTSHPH  
 GGMQPGLHETSAPTAPGQPDHQPPRVEGGGTSVIKEVVEDGTANQLPAGEGSG  
 EQDFTFETSGENTAVAAVEPGLRNQPPVDEGATGASQSLLDRKEVLGGVIAGG  
 LVGLIFAVCLVAFMLYRMKKKDEGSYSLEEPKQANGGAYQKPTKQEEFYA

[0290]

20 Rat syndecan-1 (syndecan-1) - UniProtKB - P26260

SEQ ID NO:127

MRRAALWLWLCALALRLQPALPQIVTANVPPEDQDGS GDDSDNFSGS  
 GTGALPDMTSLRQTPSTWKDVWLLTATPTAPEPTSRDTEATLTSILPAGEKPEE  
 GEPVAHVEAEPDFTARDKEKEATTRPRETTQLPVTQQASTAARATTAQASVTS  
 25 HPHGDVQPGLHETLAPTAPGQPDHQPPSVEDGGT SVIKEVVEDETTNQLPAGEG  
 SGEQDFTFETSGENTAVAGVEPDLRNQSPVDEGATGASQGLLDRKEVLGGVIA  
 GGLVGLIFAVCLVAFMLYRMKKKDEGSYSLEEPKQANGGAYQKPTKQEEFYA

[0291]

Macaca mulatta (Rhesus macaque) syndecan-1 - UniProtKB - A0A1D5RIX8

30 SEQ ID NO:128

MGATAYIPNSNSLSALLRGLELPHQTELLRVRALPTLLCPCALCRAPGC  
 VQIVATNLPPEDQDGSGDDSDNFSGSGAGALQDITLSQQTPSTWKDTWLLTATP  
 MSPEPTGLEATAASTSTLPAGEGPKEGEAVVLLEVEPDLTAREQEATPQPTETT  
 QLPTTHQAPTARATTAQEPATSHPHRDMQPGHHETSAPAGPGQADLHTPRTE  
 5 GGPSATERAAEDGASSQLPAAEGSGEQDFTFETSGENTAIVAVEPDHRNQSPVD  
 PGATGASQGLLDRKEVLGGIAGGLVGLIFAVCLVGFMLYRMKKKDEGSYSLE  
 EPKQANGGAYQKPTKQEEFYA

[0292]

Canis lupus familiaris (Dog) (Canis familiaris) syndecan-1 - UniProtKB - E2RT70

10 SEQ ID NO:129

MRRAALWLWLCALALRLQPALPQIVATNVPPEDQDGSGDDSDNFSGS  
 GAGALQDITLSQQTPSTWKDMALLTAMPTAQEPTGADDIDSSTSILLTREGPEG  
 GEAVLVAAEAPGFTDREKETAHPPSETTPHPTTHRSTARATTAQGPATLHPHR  
 DAQPDHHQISVLAEPSQLDPHTPRVEDGGPSATERAAEDGVSTQLPAGEGSGEQ  
 15 DFTFDVSGENTAGTAVEPDQRNQPPVDRGATGASQGLLDRKEVLGGVIAGGLV  
 GLIFAVCLVGFMLYRMKKKDEGSYSLEPKQANGGAYQKPSKQEEFYA

[0293]

Macaca fascicularis (Cynomolgus Monkey) Syndecan-1

SEQ ID NO:130

20 MRRAALWLWLCALALSLQPAMPQIVATNLPPEDQDGSGDDSDNFSGS  
 GAGALQDITLSQQTPSTWKDTWLVRATPMSPEPTGLEATAASTSTIQAGEGPKE  
 GEAVVLLEVEPDLTAREQEATPQPTETTQLPTTHQAPTARATTAQEPATSHPHR  
 DMQPGHHETSAPAGPGQADLHTPRTEGGPSATERAAEDGASSQLPAAEGSGE  
 QDFTFETSGENTAIVAVEPDHRNQSPVDPGATGASQGLLDRKEVLGGIAGGLV  
 25 GLIFAVCLVGFMLYRMKKKDEGSYSLEPKQANGGAYQKPTKQEEFYA

[0294]

Human, FGFR3, isoform b (SEQ ID NO:97)

ISESLGTEQRVVGRAAEVPGPEPGQEQLVFGSGDAVELSCPPPGGGP  
 MGPTVWVKDGTGLVPSERVLVGPQRLQVLNASHEDSGAYSCRQRLTQRVLCH  
 30 FSVRVTDAPSSGDDDEDEDEAEDTGVDTGAPYWTRPERMDKKLLAVPAANTV  
 RFRCPAAGNPTPSISWLKNGREFRGEHRIGGIKLRHQQWSLVMESVVPDRGNY

TCVVENKFGSIRQTYTLDVRLERSPHRPILQAGLPANQTAVLGSDVEFHCKVYSD  
 AQPHIQWLKHVEVNGSKVGPDPYVTVLKSWISESVEADVRLRLANVSERDG  
 GEYLCRATNFIGVAEKAFWLSVHGPRAAEEELVEADEAGSVYAG

[0295]

- 5 Human, FGFR3, isoform c (SEQ ID NO:98)

ISESLGTEQRVVGRAAEVPGPEPGQEQLVFGSGDAVELSCPPPGGGP  
 MGPTVWVKDGTGLVPSEVLVGPQRLQVLNASHEDSGAYSCRQRLTQRVLCH  
 FSVRVTDAPSSGDDDEDGEDEAEDTGVDTGAPYWTRPERMDKKLLAVPAANTV  
 RFRCPAAGNPTPSISWLKNGREFRGEHRIGGIKLRHQWLSVMESVVPDRGNY

- 10 TCVVENKFGSIRQTYTLDVRLERSPHRPILQAGLPANQTAVLGSDVEFHCKVYSD  
 AQPHIQWLKHVEVNGSKVGPDPYVTVLKTAGANTTDKELEVLSLHNVTFE  
 DAGEYTCLAGNSIGFSHSAWLVLPAEEELVEADEAGSVYAG

[0296]

Example 13 – Certain Embodiments

- 15 A1. A bi-specific binding agent comprising (a) an antibody, or antigen binding portion thereof, that binds specifically to syndecan-1 (CD138); and (b) a Fynomer that binds specifically to a fibroblast growth factor receptor 3 (FGFR3).

[0297]

- A1.1. The bi-specific binding agent of embodiment A1, wherein the syndecan-1 is a mammalian syndecan-1.

20

[0298]

A1.2. The bi-specific binding agent of embodiment A1 or A1.1, wherein the syndecan-1 is selected from a human syndecan-1, a mouse syndecan-1 and a monkey syndecan-1 .

25

[0299]

A1.3. The bi-specific binding agent of any one of embodiments A1 to A1.2, wherein the bi-specific agent and/or the antibody, or antigen binding portion thereof, binds specifically to human syndecan-1 and mouse syndecan-1.

[0300]

A1.4. The bi-specific binding agent of any one of embodiments A1 to A1.3, wherein the bi-specific agent and/or the antibody, or antigen binding portion thereof, binds specifically to human syndecan-1 and monkey syndecan-1.

[0301]

- 5 A1.5. The bi-specific binding agent of any one of embodiments A1 to A1.4, wherein the bi-specific agent and/or the antibody, or antigen binding portion thereof, binds specifically to human syndecan-1, mouse syndecan-1 and monkey syndecan-1.

[0302]

- 10 A1.6. The bi-specific binding agent of embodiment A1.2, A1.4 or A1.5, wherein the monkey syndecan-1 comprises a syndecan-1 expressed in, obtained from or isolated from a monkey of the genus *Macaca*.

[0303]

- 15 A1.7. The bi-specific binding agent of embodiment A1.6, wherein the monkey syndecan-1 is a syndecan-1 expressed in, obtained from or isolated from a monkey of the species *Macaca fascicularis* (*Cynomolgus* Monkey).

[0304]

- A1.8. The bi-specific binding agent of embodiment A1.6, wherein the monkey syndecan-1 comprises or consists of the amino acid sequence of SEQ ID NO:128 or 130.

20 [0305]

- A2. The bi-specific binding agent of any one of embodiments A1.2 to A1.8, wherein the bi-specific agent and/or the antibody, or antigen binding portion thereof, binds specifically to the human syndecan-1, the monkey syndecan-1 and/or the mouse syndecan-1 with a KD of 50 nM or lower.

25 [0306]

- A3. The bi-specific binding agent of any one of embodiments A1 to A2, wherein the antibody, or antigen binding portion thereof, is covalently attached to the Fynomer.

[0307]

- 30 A4. The bi-specific binding agent of any one of embodiments A1 to A3, wherein the antibody, or antigen binding portion thereof, binds specifically to an extracellular region of the syndecan-1.

[0308]

A5. The bi-specific binding agent of any one of embodiments A1 to A4, wherein the antibody, or antigen binding portion thereof, binds specifically to a polypeptide comprising the amino acid sequence of AGE GPKEGEAVVLP (SEQ ID NO:94).

5 [0309]

A5.1. The bi-specific binding agent of any one of embodiments A1 to A5, wherein the antibody, or antigen binding portion thereof, competes for binding with another binding agent that binds specifically to a polypeptide comprising or consisting of the amino acid sequence of AGE GPKEGEAVVLP (SEQ ID NO:94).

10 [0310]

A5.2. The bi-specific binding agent of any one of embodiments A1 to A5.1, wherein the antibody, or antigen binding portion thereof, competes for binding to syndecan-1 with a second binding agent comprising or consisting of a CDR-L1 selected from Table 1, a CDR-L2 selected from Table 2, a CDR-L3 selected from Table 3, a CDR-H1 selected from Table 6, a CDR-H2 selected from Table 7, and a CDR-H3 selected from Table 8.

[0311]

A5.3. The bi-specific binding agent of embodiment A5.2, wherein the second binding agent comprises a CDR-L1 having an amino acid sequence at least 85%, at least 90%, at least 95% or 100% identical to the amino acid sequence of SEQ ID NO:3, a CDR-L2 having an amino acid sequence at least 85%, at least 90%, at least 95% or 100% identical to the amino acid sequence of SEQ ID NO:18, a CDR-L3 having an amino acid sequence at least 85%, at least 90%, at least 95% or 100% identical to the amino acid sequence of SEQ ID NO:28, a CDR-H1 having an amino acid sequence at least 85%, at least 90%, at least 95% or 100% identical to the amino acid sequence of SEQ ID NO:47, a CDR-H2 having an amino acid sequence at least 85%, at least 90%, at least 95% or 100% identical to the amino acid sequence of SEQ ID NO:60, and a CDR-H3 having an amino acid sequence at least 85%, at least 90%, at least 95% or 100% identical to the amino acid sequence of SEQ ID NO:73.

30 [0312]

A5.4. The bi-specific binding agent of embodiment A5.2, wherein the second binding agent comprises a CDR-L1 having an amino acid sequence at least 85%, at least 90%, at least 95% or 100% identical to the amino acid sequence of SEQ ID NO:2, a CDR-L2 having an amino acid sequence at least 85%, at least 90%, at least 95% or 100% identical to the amino acid sequence of SEQ ID NO:17, a CDR-L3 having an amino acid sequence at least 85%, at least 90%, at least 95% or 100% identical to the amino acid sequence of SEQ ID NO:27, a CDR-H1 having an amino acid sequence at least 85%, at least 90%, at least 95% or 100% identical to the amino acid sequence of SEQ ID NO:47, a CDR-H2 having an amino acid sequence at least 85%, at least 90%, at least 95% or 100% identical to the amino acid sequence of SEQ ID NO:61, and a CDR-H3 having an amino acid sequence at least 85%, at least 90%, at least 95% or 100% identical to the amino acid sequence of SEQ ID NO:73.

[0313]

A5.5. The bi-specific binding agent of embodiment A5.4, wherein the second binding agent comprises a CDR-L2 having an amino acid sequence at least 85%, at least 90%, at least 95% or 100% identical to the amino acid sequence of SEQ ID NO:16, a CDR-H1 having an amino acid sequence at least 85%, at least 90%, at least 95% or 100% identical to the amino acid sequence of SEQ ID NO:45 or 46, and a CDR-H3 having an amino acid sequence at least 85%, at least 90%, at least 95% or 100% identical to the amino acid sequence of SEQ ID NO:72.

[0314]

A5.6. The bi-specific binding agent of embodiment A5.2, wherein the second binding agent comprises a humanized light chain variable region and a humanized heavy chain variable region.

[0315]

A5.7. The bi-specific binding agent of embodiment A5.6, wherein the second binding agent comprises a humanized light chain variable region having an amino acid sequence at least 85%, at least 90%, at least 95% or 100% identical to the amino acid sequence of SEQ ID NO:41 and a humanized heavy chain variable region having an amino acid sequence at least 85%, at least 90%, at least 95% or 100% identical to the amino acid sequence of SEQ ID NO:90.

[0316]

A5.8. The bi-specific binding agent of embodiment A5.6, wherein the second binding agent comprises a humanized light chain having an amino acid sequence at least 85%, at least 90%, at least 95% or 100% identical to the amino acid sequence of SEQ ID NO:44  
5 and a humanized heavy chain having an amino acid sequence at least 85%, at least 90%, at least 95% or 100% identical to the amino acid sequence of SEQ ID NO:93.

[0317]

A6. The bi-specific binding agent of any one of embodiments A1 to A5.1, wherein the antibody, or antigen binding portion thereof, comprises the following light chain  
10 complementarity determining regions (CDR): (i) a CDR-L1 (light chain CDR1) comprising an amino acid sequence having 90% identity to an amino acid sequence selected from SEQ ID NOs:2-15; (ii) a CDR-L2 (light chain CDR2) comprising an amino acid sequence having at least 85%, or at least 90% identity to an amino acid sequence selected from SEQ ID NOs:16-26, and (iii) a CDR-L3 (light chain CDR3)  
15 comprising an amino acid sequence having at least 85%, or at least 90% identity to an amino acid sequence selected from SEQ ID NOs:27-33.

[0318]

A6.1. The bi-specific binding agent of any one of embodiments A1 to A5.1, wherein the antibody, or antigen binding portion thereof, comprises the following light chain  
20 complementarity determining regions (CDR): (i) a CDR-L1 (light chain CDR1) comprising or consisting of an amino acid sequence selected from SEQ ID NOs:2-15; (ii) a CDR-L2 (light chain CDR2) comprising or consisting of an amino acid sequence selected from SEQ ID NOs:16-26, and (iii) a CDR-L3 (light chain CDR3) comprising or consisting of an amino acid sequence selected from SEQ ID NOs:27-33.

25 [0319]

A7. The bi-specific binding agent of any one of embodiments A1 to A5.1, and A6 to A6.1, wherein the antibody, or antigen binding portion thereof, comprises the following heavy chain complementarity determining regions (CDR): (i) a CDR-H1 (heavy chain CDR1) comprising or consisting of an amino acid sequence having at least  
30 85%, or at least 90% identity to an amino acid sequence selected from SEQ ID NOs:45-59; (ii) a CDR-H2 (heavy chain CDR2) comprising or consisting of an amino

acid sequence having at least 85%, or at least 90% identity to an amino acid sequence selected from SEQ ID NOs:60-71, (iii) a CDR-H3 (heavy chain CDR3) comprising or consisting of an amino acid sequence having at least 85%, or at least 90% identity to an amino acid sequence selected from SEQ ID NOs:72-81.

5 [0320]

A7.1. The bi-specific binding agent of any one of embodiments A1 to A5.1, and A6 to A6.1, wherein the antibody, or antigen binding portion thereof, comprises the following heavy chain complementarity determining regions (CDR): (i) a CDR-H1 (heavy chain CDR1) comprising or consisting of an amino acid sequence selected from  
10 SEQ ID NOs:45-59; (ii) a CDR-H2 (heavy chain CDR2) comprising or consisting of an amino acid sequence selected from SEQ ID NOs:60-71, (iii) a CDR-H3 (heavy chain CDR3) comprising or consisting of an amino acid sequence selected from SEQ ID NOs:72-81.

[0321]

15 A8. The bi-specific binding agent of any one of embodiments A6 to A7.1, wherein the CDR-L3 is selected from SEQ ID NO:27 or SEQ ID NO:29; the CDR-L2 is selected from SEQ ID NO:17 or SEQ ID NO:20; and the CDR-L1 is selected from SEQ ID NO:2 or SEQ ID NO:4.

[0322]

20 A9. The bi-specific binding agent of any one of embodiments A6 to A8, wherein the CDR-H3 is selected from SEQ ID NO:73 or SEQ ID NO:75; the CDR-H2 is selected from SEQ ID NO:61 or SEQ ID NO:63; and the CDR-H1 is selected from SEQ ID NO:47 or SEQ ID NO:50.

[0323]

25 A10. The bi-specific binding agent of any one of embodiments A1 to A9, wherein the CDR-L1 comprises or consists of the amino acid sequence of SEQ ID NO:2, the CDR-L2 comprises or consists of the amino acid sequence of SEQ ID NO:17, the CDR-L3 comprises or consists of the amino acid sequence of SEQ ID NO:27, the CDR-H1 comprises the amino acid sequence of SEQ ID NO:47, the CDR-H2 comprises  
30 or consists of the amino acid sequence of SEQ ID NO:61, and the CDR-H3 comprises or consists of the amino acid sequence of SEQ ID NO:73.

[0324]

A10.1. The bi-specific binding agent of embodiment A10, wherein the antibody, or antigen binding portion thereof, comprises a light chain variable region comprising or consisting of an amino acid sequence at least 85%, at least 90%, at least 5 95% or 100% identical to the amino acid sequence of SEQ ID NO:41 and a heavy chain variable region comprising or consisting of an amino acid sequence at least 85%, at least 90%, at least 95% or 100% identical to the amino acid sequence of SEQ ID NO:90.

[0325]

10 A10.2. The bi-specific binding agent of embodiment A10.1, wherein the antibody, or antigen binding portion thereof, comprises a light chain comprising or consisting of an amino acid sequence at least 85%, at least 90%, at least 95% or 100% identical to the amino acid sequence of SEQ ID NO:44 and a heavy chain comprising or consisting of an amino acid sequence at least 85%, at least 90%, at least 95% or 100% 15 identical to the amino acid sequence of SEQ ID NO:93.

[0326]

A11. The bi-specific binding agent of any one of embodiments A1 to A9, wherein the CDR-L1 comprises or consists of the amino acid sequence of SEQ ID NO:4, the CDR-L2 comprises or consists of the amino acid sequence of SEQ ID NO:20, the 20 CDR-L3 comprises or consists of the amino acid sequence of SEQ ID NO:29, the CDR-H1 comprises or consists of the amino acid sequence of SEQ ID NO:50, the CDR-H2 comprises or consists of the amino acid sequence of SEQ ID NO:63, and the CDR-H3 comprises or consists of the amino acid sequence of SEQ ID NO:75.

[0327]

25 A12. The bi-specific binding agent of any one of embodiments A1 to A11, wherein the antibody, comprises a constant region of an IgG, IgD, IgE, IgA or IgM.

[0328]

A13. The bi-specific binding agent of any one of embodiments A1 to A12, wherein the antibody, or antigen binding portion thereof, is a chimeric antibody and/or 30 humanized antibody.

[0329]

A13.1. The bi-specific binding agent of embodiments A13, wherein the chimeric antibody comprises a human constant domain of an IgG1.

[0330]

5 A13.2. The bi-specific binding agent of embodiments A13 or A13.1, wherein the humanized antibody comprises one or more human framework regions, or 1, 2, 3, 4, 5 or more framework regions comprising or consisting an amino acid sequence having at least 85%, at least 90% identity, at least 95% identity, or at least 100% identity to a corresponding human framework region.

[0331]

10 A14. The bi-specific binding agent of any one of embodiments A1 to A13.2, wherein the antibody, or antigen binding portion thereof binds specifically to an extracellular domain of a human syndecan-1, an extracellular domain of a monkey syndecan-1 and/or an extracellular domain of a mouse syndecan-1.

[0332]

15 A15. The bi-specific binding agent of any one of embodiments A1 to A13, wherein the antibody, or antigen binding portion thereof binds specifically to the extracellular domain of the human syndecan-1, the monkey syndecan-1 and/or the mouse syndecan-1 with a binding affinity (KD) of 50 nM or less.

[0333]

20 A16. The bi-specific binding agent of any one of embodiments A1 to A15, wherein the FGFR3 is a human fibroblast growth factor receptor 3 (FGFR3).

[0334]

A17. The bi-specific binding agent of any one of embodiments A1 to A16, wherein the human FGFR3 comprises an FGFR3 isoform 3b or FGFR3 isoform 3c.

25 [0335]

A18. The bi-specific binding agent of embodiment A16 or A17, wherein the Fynomer binds specifically to a human FGFR3 isoform 3b and an FGFR3 isoform 3c.

[0336]

30 A19. The bi-specific binding agent of any one of embodiments A1 to A18, wherein the Fynomer comprises an RT-loop comprising or consisting of the amino acid

sequence EVMSTTA (SEQ ID NO:114) and a SRC loop comprising or consisting of the amino acid sequence SQSPH (SEQ ID NO:115).

[0337]

A20. The bi-specific binding agent of any one of embodiments A1 to A19, wherein  
5 the Fynomer comprises or consists of a polypeptide having an amino acid sequence at least 85%, at least 90%, or at least 95% identical to the amino acid sequence GVTLFVALYDYEVMSTTALS FHKGEKFQILSQSPHGQYWEARSLTTGETG(X<sub>7</sub>)I PSNYVAPVDSIQ (SEQ ID NO:113), wherein the amino acid at position (X<sub>7</sub>) is any amino acid.

10 [0338]

A20.1. The bi-specific binding agent of any one of embodiments A1 to A19, wherein the Fynomer comprises or consists of a polypeptide having the amino acid sequence of  
GVTLFVALYDYEVMSTTALS FHKGEKFQILSQSPHGQYWEARSLTTGETG(X<sub>7</sub>)I  
15 PSNYVAPVDSIQ (SEQ ID NO:113), wherein the amino acid at position (X<sub>7</sub>) is any amino acid.

[0339]

A21. The bi-specific binding agent of embodiment A20 or A20.1, wherein (X<sub>7</sub>) is selected from N, R, W and K.

20 [0340]

A22. The bi-specific binding agent of any one of embodiments A1 to A18, wherein the Fynomer comprises an RT-loop comprising or consisting of the amino acid sequence EVYGPTPM (SEQ ID NO:100).

[0341]

25 A23. The bi-specific binding agent of any one of embodiments A1 to A18, or A22, wherein the Fynomer comprises or consists of a polypeptide having an amino acid sequence at least 85%, at least 90%, at least 95% or 100% identical to the amino acid sequence

GVTLFVALYDYE VYGPTPMLS FHKGEKFQIL(X<sub>1</sub>)(X<sub>2</sub>)(X<sub>3</sub>)(X<sub>4</sub>)GPYWEARSL(X<sub>5</sub>)  
30 TGETG(X<sub>6</sub>)IPSNYVAPVDSIQ (SEQ ID NO:99), wherein the amino acids (X<sub>1</sub>), (X<sub>2</sub>), (X<sub>3</sub>), (X<sub>4</sub>), (X<sub>5</sub>) and (X<sub>6</sub>) are selected from any amino acid.

[0342]

A23.1. The bi-specific binding agent of A23, wherein the Fynomer comprises or consists of the amino acid sequence  
 GVTLFVALYDYEYVGPTPMLSFHKGEKFQIL(X<sub>1</sub>)(X<sub>2</sub>)(X<sub>3</sub>)(X<sub>4</sub>)GPYWEARSL(X<sub>5</sub>)  
 5 TGETG(X<sub>6</sub>)IPSNYVAPVDSIQ (SEQ ID NO:99), wherein the amino acids (X<sub>1</sub>), (X<sub>2</sub>), (X<sub>3</sub>), (X<sub>4</sub>), (X<sub>5</sub>) and (X<sub>6</sub>) are selected from any amino acid.

[0343]

A24. The bi-specific binding agent of embodiment A23 or A23.1, wherein

(X<sub>1</sub>) is N, R, or K;

10 (X<sub>2</sub>) is S, G, K or R;

(X<sub>3</sub>) is S or G;

(X<sub>4</sub>) is E, Q, D, S or K;

(X<sub>5</sub>) is T or A; and

(X<sub>6</sub>) is Y, W or L.

15 [0344]

A25. The bi-specific binding agent of any one of embodiments A1 to A18, wherein the Fynomer comprises a polypeptide comprising an amino acid sequence that is at least 85% identical, at least 90% identical, at least 95% identical or 100% identical to an amino acid sequence selected from:

20 (SEQ ID NO:101; FF2L4C3)  
 GVTLFVALYDYEYVGPTPMLSFHKGEKFQILNSSEGPYWEARSLTTGETGLIPS  
 NYVAPVDSIQ;

(SEQ ID NO:103; FF44L65G12)  
 GVTLFVALYDYEYVGPTPMLSFHKGEKFQILRGGQGPYWEARSLTTGETGLIPS  
 25 NYVAPVDSIQ;

(SEQ ID NO:105; FF44L65G7)  
 GVTLFVALYDYEYVGPTPMLSFHKGEKFQILRGGDGPYWEARSLTTGETGLIPS  
 NYVAPVDSIQ;

(SEQ ID NO:107; FF48L66G7; "G7")  
 30 GVTLFVALYDYEYVGPTPMLSFHKGEKFQILKGGSGPYWEARSLTTGETGLIPS  
 NYVAPVDSIQ;

(SEQ ID NO:109; FF43L65D5)  
 GVTLFVALYDYEEVYGPTPMLSFHKGEKFQILRKGKGPYWEARSLATGETGLIPS  
 NYVAPVDSIQ;

(SEQ ID NO:111; FF44L65B7)  
 5 GVTLFVALYDYEEVYGPTPMLSFHKGEKFQILRRGSGPYWEARSLTTGETGLIPS  
 NYVAPVDSIQ; and

(SEQ ID NO:116; FF40L54A5)  
 GVTLFVALYDYEVMSTTALSSFHKGEKFQILSQSPHGQYWEARSLTTGETGWIPS  
 NYVAPVDSIQ.

10 [0345]

A25.1. The bi-specific binding agent of any one of embodiments A1 to A18, wherein the Fynomer comprises a polypeptide comprising an amino acid sequence that is at least 85% identical, at least 90% identical, at least 95% identical or 100% identical to the amino acid sequence of SEQ ID NO:107, where the amino acid sequence EVYGPTPM  
 15 (SEQ ID NO:100) at amino acid positions 12 to 19 of SEQ ID NO:107 and amino acids P and Y at amino acid positions 37 and 38 of SEQ ID NO:107 are conserved, and the Fynomer binds specifically to an FGFR3.

[0346]

A25.2. The bi-specific binding agent of any one of embodiments A1 to A18, wherein  
 20 the Fynomer comprises a polypeptide comprising an amino acid sequence that is at least 85% identical, at least 90% identical, at least 95% identical or 100% identical to the amino acid sequence of SEQ ID NO:107, where the amino acid sequence EVYGPTPM (SEQ ID NO:100) at amino acid positions 12 to 19 of SEQ ID NO:107 and amino acids at amino acid positions 32 to 38 of SEQ ID NO:107 are conserved, and the Fynomer  
 25 binds specifically to an FGFR3.

[0347]

A25.3. The bi-specific binding agent of any one of embodiments A1 to A18, wherein the Fynomer comprises or consists of a polypeptide comprising or consisting of the amino acid sequence of SEQ ID NO:107.

30 [0348]

A25.4. The bi-specific binding agent of any one of embodiments A1 to A25.3, wherein the Fynomer specifically binds to both human FGFR3 isoform b and FGFR3 isoform c.

[0349]

- 5 A26. The bi-specific binding agent of any one of embodiments A1 to A25.4, wherein the Fynomer binds specifically to an extracellular region of the FGFR3, FGFR3b or FGFR3c.

[0350]

- 10 A27. The bi-specific binding agent of any one of embodiments A1 to A26, wherein the Fynomer binds specifically to the FGFR3, FGFR3b, FGFR3c, or a portion thereof, with a binding affinity (KD) of from about  $10^{-5}$  M to about  $10^{-15}$  M.

[0351]

- 15 A28. The bi-specific binding agent of any one of embodiments A1 to A27, wherein the Fynomer binds specifically to the FGFR3, FGFR3b, FGFR3c, or a portion thereof, with a binding affinity (KD) of  $10^{-8}$  M or less.

[0352]

- A29. The bi-specific binding agent of any one of embodiments A1 to A28, wherein the Fynomer is glycosylated.

[0353]

- 20 A30. The bi-specific binding agent of any one of embodiments A1 to A29, wherein the Fynomer is covalently attached to the antibody, or antigen binding portion thereof, by a linker.

[0354]

- 25 A30.1 The bi-specific binding agent of embodiment A30, wherein the linker comprises or consists of a peptide bond.

[0355]

- 30 A31. The bi-specific binding agent of embodiment A30 or A30.1, wherein the linker comprises or consists of a peptide comprising or consisting of one or more amino acids, 5 to 100 amino acids, 5 to 50 amino acids, 5 to 25 amino acids, 5 to 20 amino acids, or 5 to 10 amino acids.

[0356]

- A32. The bi-specific binding agent of embodiment A30, A30.1, or A31, wherein the linker comprises or consists of an optionally substituted C1-C50 alkyl, an optionally substituted C2-C50 alkenyl, an optionally substituted C2-C50 alkynyl, acyl, acyloxy, alkyloxycarbonyloxy, aryloxycarbonyloxy, cycloalkyl, cycloalkenyl, alkoxy, cycloalkoxy, aryl, heteroaryl, arylalkoxy carbonyl, alkoxy carbonylacyl, aminocarbonyl, aminocarboxyloxy, azido, phenyl, cycloalkylacyl, alkylthio, arylthio, oxysulfonyl, carboxy, thio, sulfoxide, sulfone, sulfonate esters, thiocyno, amide, amino, ester, halogenated alkyl, or a combination thereof.  
[0357]
- 10 A33. The bi-specific binding agent of any one of embodiments A1 to A29, wherein the antibody, or antigen binding portion thereof is non-covalently attached to the Fynomer by means of a binding pair.  
[0358]
- 15 A34. The bi-specific binding agent of any one of embodiments A1 to A33, wherein the Fynomer is attached to an N-terminal end of a heavy chain or light chain of the antibody, or antigen binding portion thereof.  
[0359]
- 20 A35.1. The bi-specific binding agent of any one of embodiments A1 to A33, wherein the Fynomer is covalently attached to an N-terminal end of a heavy chain of the antibody by a peptide bond.  
[0360]
- A35.2. The bi-specific binding agent of embodiments A35.1, wherein a C-terminal amino acid of the Fynomer is covalently attached to an N-terminal amino acid of a heavy chain of the antibody by a peptide bond.  
25 [0361]
- A35.3. The bi-specific binding agent of embodiments A35.1, wherein a C-terminal amino acid of the Fynomer is covalently attached to an N-terminal amino acid of a light chain of the antibody by a peptide bond.  
[0362]

A35.4. The bi-specific binding agent of any one of embodiments A1 to A33, wherein the Fynomer is attached to a carboxy-terminal end of a heavy chain or light chain of the antibody, or antigen binding portion thereof.

[0363]

- 5 A35.5. The bi-specific binding agent of embodiments A35.4, wherein an N-terminal amino acid of the Fynomer is covalently attached to a carboxy-terminal amino acid of a heavy chain of the antibody by a peptide bond.

[0364]

- 10 A35.6. The bi-specific binding agent of embodiments A35.4, wherein an N-terminal amino acid of the Fynomer is covalently attached to a carboxy-terminal amino acid of a light chain of the antibody by a peptide bond.

[0365]

- 15 A35.7. The bi-specific binding agent of any one of embodiments A1 to A29, comprising (i) the Fynomer and heavy chain amino acid sequence of SEQ ID NO:125 and (ii) the light chain amino acid sequence of SEQ ID NO:44.

[0366]

- 20 A35.8. The bi-specific binding agent of any one of embodiments A1 to A29, comprising or consisting of (i) the Fynomer and heavy chain amino acid sequence of SEQ ID NO:125, wherein the serine at amino acid position 213 of SEQ ID NO:125 is mutated to a cysteine, (ii) the light chain amino acid sequence of SEQ ID NO:44, and (iii) an anti-neoplastic agent covalently attached to the cysteine.

[0367]

- 25 A36. The bi-specific binding agent of any one of embodiments A1 to A35.7, further comprising an anti-neoplastic agent.

[0368]

- 30 A37. The bi-specific binding agent of embodiment A35.8 or A36, wherein the anti-neoplastic agent is selected from the group consisting of an auristatin, a dolastatin, a maytansine, a tubulysin, a calicheamicin, a pyrrolobenzodiazepine (PBD), a duocarmycin, a doxorubicin, a pseudomonas exotoxin-A (PE38), an irinotecan and a derivative of any one of the foregoing.

[0369]

A38. The bi-specific binding agent of embodiment A35.8, A36 or A37, wherein the anti-neoplastic agent is covalently or non-covalently attached to the bi-specific binding agent.

[0370]

- 5 A39. The bi-specific binding agent of embodiment A38, wherein the anti-neoplastic agent is attached to the antibody, or antigen binding portion thereof.

[0371]

A40. The bi-specific binding agent of embodiment A38, wherein the anti-neoplastic agent is attached to the Fynomer.

- 10 [0372]

A41. The bi-specific binding agent of any one of embodiments A35.8 to A40, wherein the anti-neoplastic agent is non-covalently attached to the bi-specific binding agent by means of a binding pair.

[0373]

- 15 A42. The bi-specific binding agent of any one of embodiments A35.8 to A40, wherein the anti-neoplastic agent is covalently attached to the bi-specific binding agent by a linker.

[0374]

- A43. The bi-specific binding agent of any one of embodiments A35.8 to A42,  
20 wherein the anti-neoplastic agent comprises monomethyl auristatin E (MMAE) or monomethyl auristatin F (MMAF).

[0375]

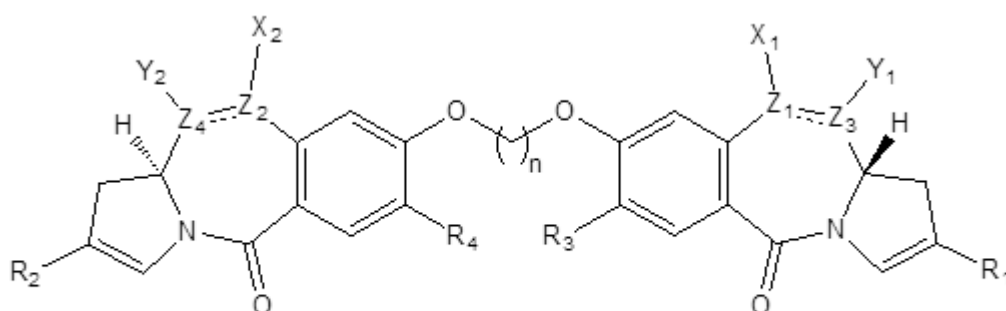
- A44. The bi-specific binding agent of any one of embodiments A35.8 to A40, and  
A42, wherein the anti-neoplastic agent comprises or consists of a  
25 pyrrolbenzodiazepine toxin and a linking group;

wherein the pyrrolbenzodiazepine toxin is covalently linked to the linking group and the linking group is covalently linked to the bi-specific binding agent.

[0376]

A45. The bi-specific binding agent of embodiment A44, wherein the pyrrolobenzodiazepine toxin comprises or consists of the structure of chemical formula I:

[Chem. 36.]



( I )

wherein

Z<sub>1</sub> and Z<sub>2</sub> are both N;

Z<sub>3</sub> and Z<sub>4</sub> are both C;

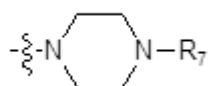
[Chem. 37.]

the double-dash lines  $\equiv$  represent a single bond or a double bond;

n is 1 to 10; each of R<sub>3</sub> and R<sub>4</sub> are independently H, or a C<sub>1-4</sub> alkoxy; and each of R<sub>1</sub> and R<sub>2</sub> are independently selected from the group consisting of H, C<sub>1-5</sub> alkyl, C<sub>3-6</sub> cycloalkyl, C<sub>2-5</sub> alkenyl, and a phenyl optionally substituted with R<sub>5</sub>,

wherein R<sub>5</sub> is selected from the group consisting of -NH<sub>2</sub>, -NHR<sub>6</sub>, and a piperazinyl substituted with R<sub>7</sub> having the structure

[Chem. 38.]



wherein R<sub>6</sub> comprises the linking group, and R<sub>7</sub> is H, or a C<sub>1-5</sub> alkyl;

X<sub>1</sub> is null, a protecting group, or comprises the linking group; X<sub>2</sub> is null, a protecting group, or comprises the linking group; only one of X<sub>1</sub>, X<sub>2</sub>, R<sub>1</sub>, and R<sub>2</sub> comprises the linking group; and each of Y<sub>1</sub> and Y<sub>2</sub> are independently either null, OH, or SO<sub>3</sub>H;

provided that:

[Chem. 39.]

- (i) when  $X_1$  comprises or consists of the linking group,  $Z_1 \text{-----} Z_3$  is N-C,
- (ii) when  $X_2$  comprises or consists of the linking group,  $Z_2 \text{-----} Z_4$  is N-C,
- (iii) when  $X_1$  is the protecting group,  $Z_1 \text{-----} Z_3$  is N-C, and
- (iv) when  $X_2$  is the protecting group,  $Z_2 \text{-----} Z_4$  is N-C,

wherein null means that an indicated moiety is absent from the structure of chemical formula I or indicates the presence of one or more hydrogens, or where not explicitly  
 5 specified, the one or more hydrogens may be present to complete a required valence.

[0377]

A46. The bi-specific binding agent of embodiment A45, wherein n is 3, 4 or 5.

[0378]

10 A47. The bi-specific binding agent of embodiment A45 or A46, wherein  $R_3$  and  $R_4$  are both  $-\text{O}-\text{CH}_3$ .

[0379]

A48. The bi-specific binding agent of any one of embodiments A45 to A47, wherein  $R_1$  and  $R_2$  are both methyl.

15 [0380]

A49. The bi-specific binding agent of any one of embodiments A45 to A47, wherein  $R_1$  and  $R_2$  are both  $-\text{CH}=\text{CH}-\text{CH}_3$ .

[0381]

A50. The bi-specific binding agent of any one of embodiments A45 to A47, wherein  
 20  $R_2$  is a cyclopropyl.

[0382]

A51. The bi-specific binding agent of any one of embodiments A45 to A47, wherein  $R_2$  is phenyl substituted with 4-methylpiperazin-1-yl.

[0383]

25 A52. The bi-specific binding agent of embodiment A50 or A51, wherein  $R_1$  is a phenyl optionally substituted with  $R_5$ ,  $R_5$  is  $-\text{NHR}_6$  and  $R_6$  comprises or consists of the linking group.

[0384]

A53. The bi-specific binding agent of any one of embodiments A45 to A47 and A50 to A52, wherein  $X_1$  is null,  $Y_1$  is null,

[Chem. 40.]

- 5  $Z_1 \text{---} Z_3$  is  $N=C$ ,  $X_2$  is null,  $Y_2$  is null and  $Z_2 \text{---} Z_4$  is  $N=C$ .

[0385]

A54. The bi-specific binding agent of any one of embodiments A45 to A51, wherein  $X_1$  comprises or consists of the linking group,  $Y_1$  is OH,

[Chem. 41.]

- 10  $Z_2 \text{---} Z_4$  is  $N=C$ ,  $X_2$  is null, and  $Y_2$  is null.

[0386]

A55. The bi-specific binding agent of any one of embodiments A45 to A51, wherein  $X_1$  comprises or consists of the linking group,  $Y_1$  is OH,

[Chem. 42.]

- 15  $Z_2 \text{---} Z_4$  is  $N-C$ ,  $X_2$  is a protecting group, and  $Y_2$  is OH.

[0387]

A56. The bi-specific binding agent of any one of embodiments A44 to A55, wherein the linking group is attached to the pyrrolobenzodiazepine toxin by a carbamate group.

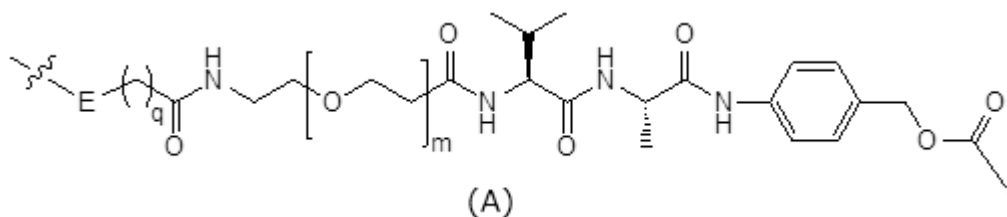
[0388]

- 20 A57. The bi-specific binding agent of any one of embodiments A44 to A55, wherein the linking group is attached to the pyrrolobenzodiazepine toxin by an amide group.

[0389]

A58. The bi-specific binding agent of any one of embodiments A44 to A57, wherein the linking group comprises or consists of the structure of chemical formula A:

- 25 [Chem. 43.]



wherein the asterisk indicates the point of attachment to the pyrrolobenzodiazepine toxin;

the wavy line indicates the point of attachment to the binding agent;

m is 1 to 20;

5 q is 0 to 10; and

E is a connecting group.

[0390]

A59. The bi-specific binding agent of embodiment A58, wherein m is 4 or 8.

[0391]

10 A60. The bi-specific binding agent of embodiment A58 or A59, wherein q is 0, 1 or 2.

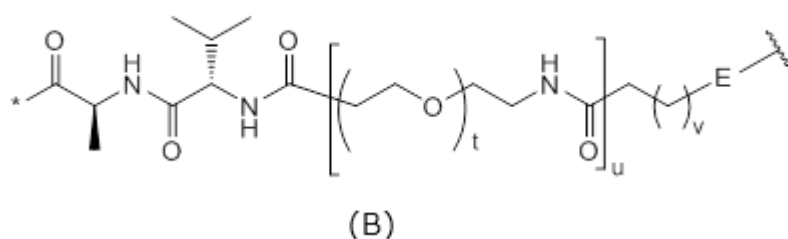
[0392]

A61. The bi-specific binding agent of embodiment A58, wherein m is 8 and q is 2.

[0393]

15 A62. The bi-specific binding agent of any one of embodiments A44 to A57, wherein the linking group comprises or consists of the structure of chemical formula B:

[Chem. 44.]



wherein

20 the asterisk indicates the point of attachment to the pyrrolobenzodiazepine toxin;

the wavy line indicates the point of attachment to the binding agent;

E is a connecting group;

v is 0 to 10; and

u is 0 or 1; wherein when u is 1, t is 1 to 10.

25 [0394]

A63. The bi-specific binding agent of embodiment A62, wherein v is 1.

[0395]

A64. The bi-specific binding agent of embodiment A62 or A63, wherein u is 1, and t is 8.

5 [0396]

A65. The bi-specific binding agent of embodiment A62, wherein u is 0, and v is 4.

[0397]

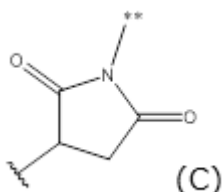
A66. The bi-specific binding agent of any one of embodiments A58 to A65, wherein the binding agent is connected to E by a thioether bond formed between a cysteine thiol residue of the binding agent and E.

10

[0398]

A67. The bi-specific binding agent of any one of embodiments A58 to A66, wherein E comprises or consists of the structure of chemical formula C:

[Chem. 45.]



15

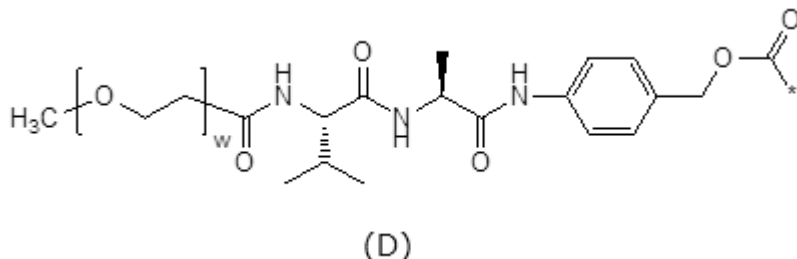
wherein the wavy line indicates the point of attachment to the binding agent and the double asterisk indicates the point of attachment to the linking group.

[0399]

A68. The bi-specific binding agent of any one of embodiments A45 to A67, wherein the protecting group has the following structure (D):

20

[Chem. 46.]



wherein the asterisk indicates the point of attachment to the pyrrolobenzodiazepine toxin; and  
w is 1 to 5.

5 [0400]

A69. The bi-specific binding agent of embodiment A68, wherein w is 2.

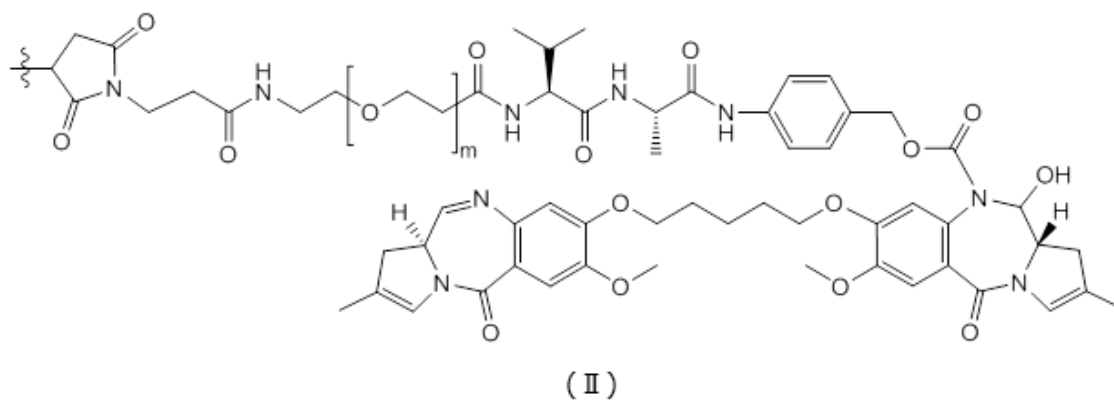
[0401]

A70. The bi-specific binding agent of any one of embodiments A45 to A69, wherein  
10 the protecting group is a cleavable protecting group.

[0402]

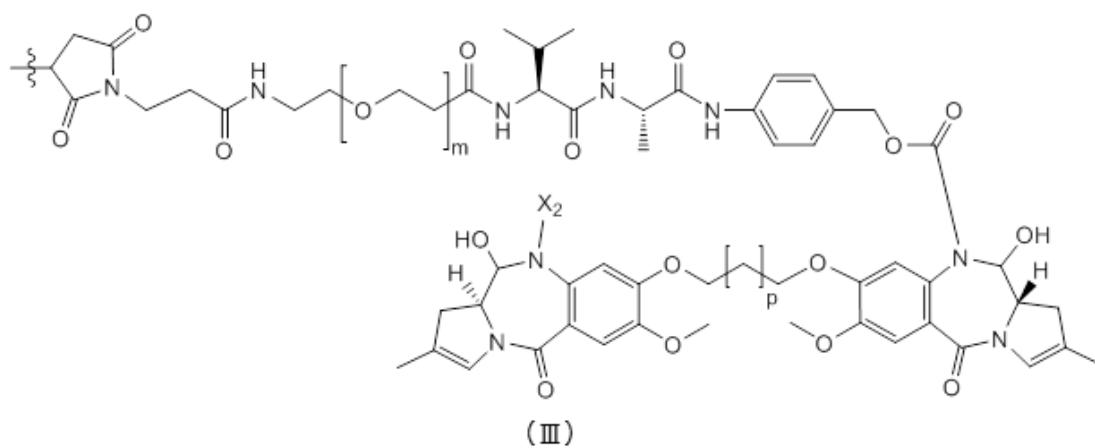
A71. The bi-specific binding agent of embodiment A44, wherein the anti-neoplastic agent comprises or consists of a structure selected from the group consisting of

15 [Chem. 47.]



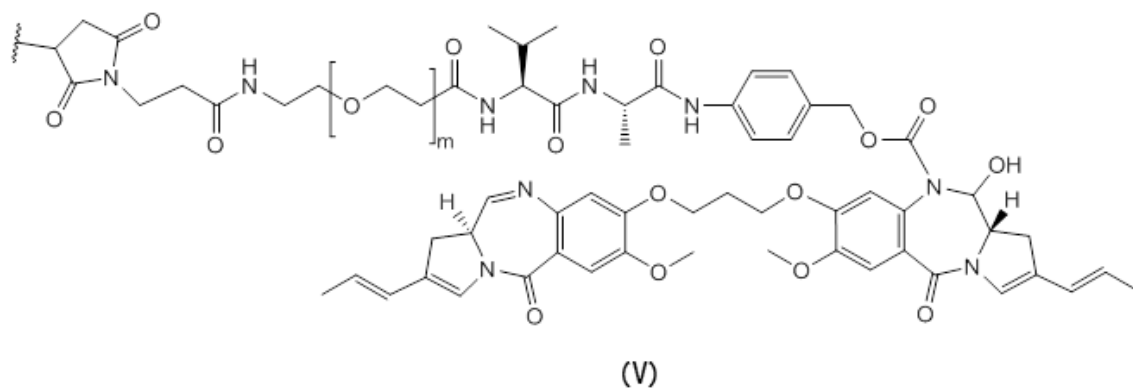
wherein m is 8;

[Chem. 48.]



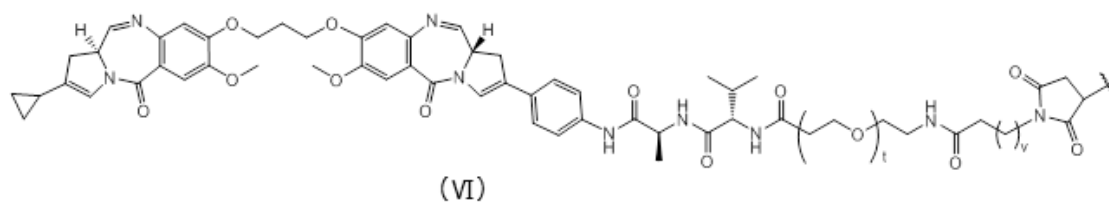
wherein  $m$  is 8,  $p$  is 3, and  $X_2$  is a protecting group;

[Chem. 49.]



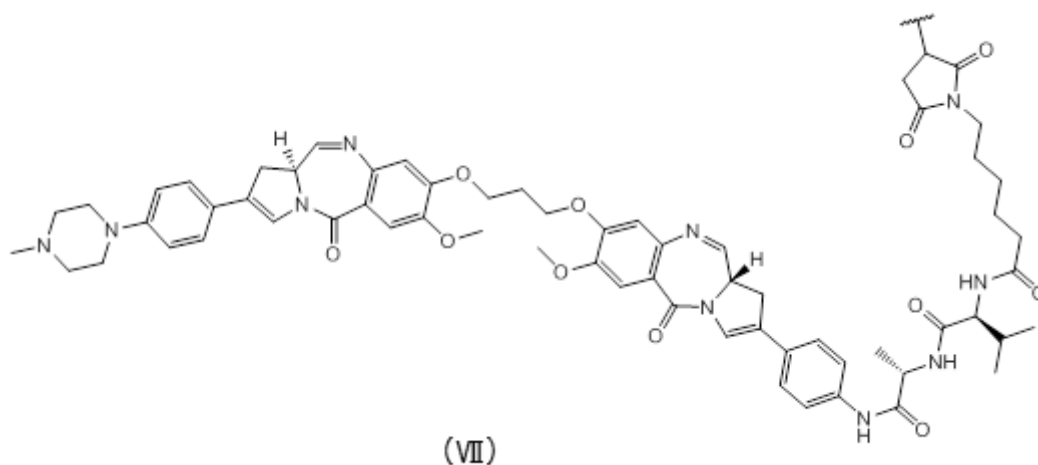
5 wherein  $m$  is 8;

[Chem. 50.]



wherein  $t$  is 8, and  $v$  is 1; and

[Chem. 51.]

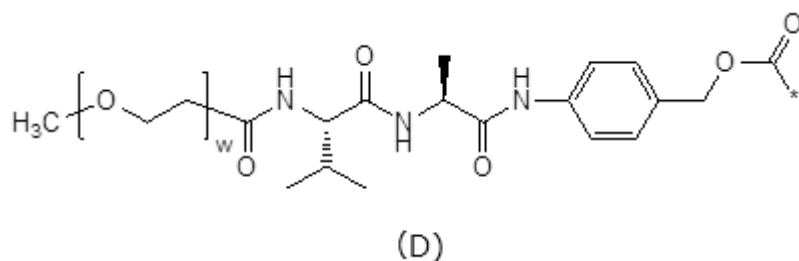


wherein the wavy line indicates the point of attachment to the binding agent.

[0403]

- 5 A72. The bi-specific binding agent of embodiment A71, wherein the protecting group of X<sub>2</sub> has the following structure (D):

[Chem. 52.]



- wherein the asterisk indicates the point of attachment to the anti-neoplastic agent or  
10 PBD toxin; and w is 1 to 5.

[0404]

- A73. The bi-specific binding agent of any one of embodiments A44 to A72, wherein at least one amino acid of the binding agent is mutated to a cysteine, and the cysteine is linked covalently to the linking group by a thiol ether bond.

- 15 [0405]

- A73.1. The bi-specific binding agent of embodiment A73, wherein the antibody comprises a human heavy chain constant region of an IgG<sub>1</sub> or IgG<sub>2</sub>, and the serine at

position 119 of the heavy chain constant region of the antibody is mutated to a cysteine, and the cysteine is linked covalently to the linking group by a thiol ether bond.

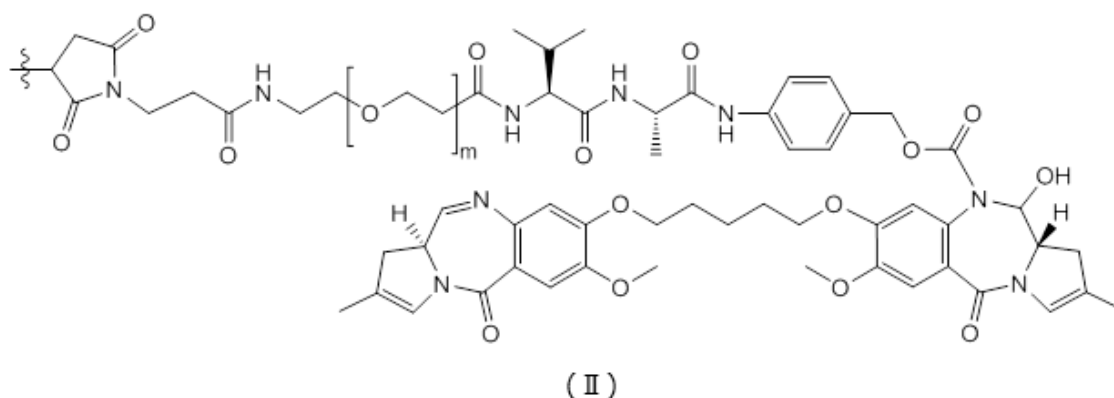
[0406]

A73.2. The bi-specific binding agent of any one of embodiments A44 to A72, wherein at least one amino acid of the binding agent is mutated to a lysine, and a free amino group of the lysine is linked covalently to the linking group.

[0407]

A74. The bi-specific binding agent of embodiment A44 or A73.2, wherein the antineoplastic agent comprises or consists of the structure of chemical formula (II):

10 [Chem. 53.]



wherein  $m$  is 8, and the wavy line indicates the point of attachment to a thiol group of the binding agent.

[0408]

15 A75. A pharmaceutical composition comprising the bi-specific binding agent of any one of embodiments A1 to A74 and a pharmaceutically acceptable excipient, diluent, additive or carrier.

[0409]

A76. The pharmaceutical composition of embodiment A75, wherein the pharmaceutical composition is formulated as a sterile, lyophilized powder.

20

[0410]

A77. The pharmaceutical composition of embodiment A75 or A76, wherein the pharmaceutical composition is formulated for intravenous administration to a mammal.

[0411]

A77.1. The pharmaceutical composition of any one of embodiments A75 to A77 for use in the treatment of a neoplasm.

[0412]

- 5 A77.2. The pharmaceutical composition of embodiment A77.1, wherein the neoplasm comprises a neoplastic cell or cancer cell that expresses syndecan-1.

[0413]

- A77.3. The pharmaceutical composition of any one of embodiments A77.1 to A77.2, wherein the neoplasm is selected from the group consisting of a carcinoma, sarcoma,  
10 nervous system neoplasia, lymphoma, myeloma, leukemia, melanoma, mesothelioma, solid or soft tissue tumors, and a secondary cancer.

[0414]

- A77.4. The pharmaceutical composition of any one of embodiments A77.1 to A77.3, wherein the neoplasm is selected from the group consisting of a bladder cancer, breast  
15 cancer, colorectal cancer, gastric cancer, pancreatic cancer, esophageal cancer, liver cancer, hepatocellular cancer, hypopharynx cancer, lung cancer, adenocarcinoma, ovarian cancer and renal cancer.

[0415]

- A77.5. The pharmaceutical composition of any one of embodiments A77.1 to A77.3,  
20 wherein the neoplasm is selected from the group consisting of a pancreatic adenocarcinoma, pancreatic neuroendocrine cancer, colorectal adenocarcinoma, small intestinal malignancy, cholangiocarcinoma, non-small cell lung cancer (NSCLC), thyroid carcinoma, esophageal or esophagogastric junction (EGJ) cancer, gastric adenocarcinoma, liver hepatocellular carcinoma, head and neck squamous carcinoma,  
25 female genital tract malignancy, breast carcinoma, triple negative breast cancer, lung small cell carcinoma, ovarian surface epithelial carcinoma, retroperitoneal or peritoneal sarcoma, prostatic adenocarcinoma, neuroendocrine tumor, gastrointestinal stromal tumor, glioblastoma and non-epithelial ovarian cancer.

[0416]

- 30 A77.6. The pharmaceutical composition of any one of embodiments A77.1 to A77.3, wherein the neoplasm is selected from the group consisting of multiple myeloma,

ovarian carcinoma, cervical cancer, endometrial cancer, thyroid cancer, testicular cancer, kidney carcinoma, gall bladder carcinoma, transitional cell bladder carcinoma, gastric cancer, prostate cancer, prostate adenocarcinoma, breast cancer, lung cancer, colon carcinoma, Hodgkin's and non-Hodgkin's lymphoma and multiple myeloma,  
 5 chronic lymphocytic leukemia (CLL), acute lymphoblastic leukemia (ALL), T-cell acute lymphoblastic leukemia (T-ALL), B-cell malignancies, B-cell acute lymphoblastic leukemia (B-ALL), acute myeloblastic leukemia (AML), a solid tissue sarcoma, colon carcinoma, non-small cell lung carcinoma, squamous cell lung carcinoma, colorectal carcinoma, hepato-carcinoma, pancreatic cancer, brain cancer (e.g., neuroblastoma or  
 10 meningioma), skin cancer (e.g., melanoma, basal cell carcinoma, or squamous cell carcinoma), and head and neck carcinoma.

[0417]

A78. A method of treating a subject having, or suspected of having, a neoplasm comprising:

- 15 a) providing a subject having, or suspected of having, a neoplasm; and  
 b) administering to the subject a therapeutically effective amount of the bi-specific binding agent of any one of embodiments A1 to A74, or the pharmaceutical composition of any one of embodiments A75 to A77.

[0418]

- 20 A79. The method of embodiment A78, wherein after the administering, the bi-specific binding agent blocks, inhibits, ameliorates, abrogates, or suppresses growth, viability or metastasis of the cancer.

[0419]

- A80. The method of any embodiment A78 to A79, wherein after the administering,  
 25 the bi-specific binding agent induces death, necrosis or apoptosis of some or all of the cancer.

[0420]

- A81. The method of any one of embodiments A78 to A80, wherein the neoplasm comprises or consists of a carcinoma, sarcoma, nervous system neoplasia, lymphoma,  
 30 myeloma, leukemia, melanoma, mesothelioma, solid or soft tissue tumors, or secondary cancers.

[0421]

A82. The method of embodiment A81, wherein the neoplasm comprises or consists of a bladder cancer, breast cancer, colorectal cancer, gastric cancer, pancreatic cancer, esophageal cancer, liver cancer, hepatocellular cancer, hypopharynx cancer, lung cancer, adenocarcinoma, ovarian cancer or renal cancer.

[0422]

A83. The method of embodiment A81 or A82, wherein the neoplasm comprises or consists of a pancreatic adenocarcinoma, pancreatic neuroendocrine cancer, colorectal adenocarcinoma, small intestinal malignancy, cholangiocarcinoma, non-small cell lung cancer (NSCLC), thyroid carcinoma, esophageal or esophagogastric junction (EGJ) cancer, gastric adenocarcinoma, liver hepatocellular carcinoma, head and neck squamous carcinoma, female genital tract malignancy, breast carcinoma, triple negative breast cancer, lung small cell carcinoma, ovarian surface epithelial carcinoma, retroperitoneal or peritoneal sarcoma, prostatic adenocarcinoma, neuroendocrine tumor, gastrointestinal stromal tumor, glioblastoma or non-epithelial ovarian cancer.

[0423]

A84. The method of embodiment A81, wherein the neoplasm is selected from the group consisting of multiple myeloma, ovarian carcinoma, cervical cancer, endometrial cancer, thyroid cancer, testicular cancer, kidney carcinoma, gall bladder carcinoma, transitional cell bladder carcinoma, gastric cancer, prostate cancer, prostate adenocarcinoma, breast cancer, lung cancer, colon carcinoma, Hodgkin's and non-Hodgkin's lymphoma and multiple myeloma, chronic lymphocytic leukemia (CLL), acute lymphoblastic leukemia (ALL), T-cell acute lymphoblastic leukemia (T-ALL), B-cell malignancies, B-cell acute lymphoblastic leukemia (B-ALL), acute myeloblastic leukemia (AML), a solid tissue sarcoma, colon carcinoma, non-small cell lung carcinoma, squamous cell lung carcinoma, colorectal carcinoma, hepato-carcinoma, pancreatic cancer, brain cancer (e.g., neuroblastoma or meningioma), skin cancer (e.g., melanoma, basal cell carcinoma, or squamous cell carcinoma), and head and neck carcinoma.

[0424]

A85. The method of any one of embodiments A78 to A84, further comprising administering a chemotherapeutic agent to the subject.

[0425]

5 A86. The method of any one of embodiments A78 to A85, wherein the subject is a human.

[0426]

A87. The bi-specific binding agent of any one of embodiments A1 to A74, or the pharmaceutical composition of any one of embodiments A75 to A77, for use in treating a subject having or suspected of having a neoplasm.

10 [0427]

A88. The method or use of any one of embodiments A78 to A87, wherein the neoplasm comprises a neoplastic cell or cancer cell that expresses syndecan-1 or an FGFR3.

[0428]

15 A89. The method or use of any one of embodiments A78 to A87, wherein the FGFR3 is a human FGFR3.

[0429]

20 A90. The bi-specific binding agent of any one of embodiments A1 to A89, wherein the bi-specific agent is internalized into a cell upon binding to the syndecan-1 that is expressed on the surface of the cell and/or upon binding to an FGFR3 expressed on the surface of the cell.

[0430]

25 A91. The bi-specific binding agent of any one of embodiments A1 to A90, comprising (i) the Fynomer and heavy chain amino acid sequence of SEQ ID NO:125 and (ii) the light chain amino acid sequence of SEQ ID NO:44.

[0431]

30 A92. The bi-specific binding agent of any one of embodiments A1 to A90, comprising (i) the Fynomer and heavy chain amino acid sequence of SEQ ID NO:125, wherein the serine at amino acid position 213 of SEQ ID NO:125 is mutated to a cysteine and (ii) the light chain amino acid sequence of SEQ ID NO:44, wherein the pyrrolobenzodiazepine toxin is covalently attached to the thiol group of the cysteine.

[0432]

B0. A binding agent comprising (a) an antibody, or antigen binding portion thereof, that binds specifically to syndecan-1 (CD138); and (b) an anti-neoplastic agent.

[0433]

5 B1. The binding agent of embodiment B0, wherein the antibody, or antigen binding portion thereof, is covalently or non-covalently attached to the anti-neoplastic agent.

[0434]

B2. The binding agent of embodiment B0 or B1, wherein the antibody, or antigen binding portion thereof, binds specifically to an extracellular region of the syndecan-1.

10 [0435]

B3. The binding agent of any one of embodiments B0 to B2, wherein the syndecan-1 is a mammalian syndecan-1.

[0436]

15 B4. The binding agent of any one of embodiments B0 to B3, wherein the antibody, or antigen binding portion thereof, binds specifically to a polypeptide comprising or consisting of the amino acid sequence of AGEKPKEGEAVVLP (SEQ ID NO:94).

[0437]

20 B5. The binding agent of any one of embodiments B0 to B4, wherein the antibody, or antigen binding portion thereof, comprises the following light chain complementarity determining regions (CDR): (i) a CDR-L1 (light chain CDR1) comprising or consisting of an amino acid sequence having at least 85% identity or at least 90% identity to an amino acid sequence selected from SEQ ID NOs:2-15; (ii) a CDR-L2 (light chain CDR2) comprising or consisting of an amino acid sequence having at least 85% identity or at least 90% identity to an amino acid sequence selected from SEQ ID NOs:16-26, and (iii) a CDR-L3 (light chain CDR3) comprising or consisting of an amino acid sequence having at least 85% identity or at least 90% identity to an amino acid sequence selected from SEQ ID NOs:27-33.

[0438]

30 B6. The binding agent of embodiment B5, wherein the CDR-L1 is selected from SEQ ID NO:2 and SEQ ID NO:4; the CDR-L2 is selected from SEQ ID NO:17 and SEQ ID NO:20; and the CDR-L3 is selected from SEQ ID NO:27 and SEQ ID NO:29.

[0439]

B7. The binding agent of any one of embodiments B0 to B6, wherein the antibody, or antigen binding portion thereof, comprises the following heavy chain complementarity determining regions (CDR): (i) a CDR-H1 (heavy chain CDR1) comprising or consisting of an amino acid sequence having at least 85% identity or at least 90% identity to an amino acid sequence selected from SEQ ID NOs:45-59; (ii) a CDR-H2 (heavy chain CDR2) comprising or consisting of an amino acid sequence having at least 85% identity or at least 90% identity to an amino acid sequence selected from SEQ ID NOs:60-71, and (iii) a CDR-H3 (heavy chain CDR3) comprising or consisting of an amino acid sequence having at least 85% identity or at least 90% identity to an amino acid sequence selected from SEQ ID NOs:72-81.

[0440]

B8. The binding agent of any one of embodiments B0 to B7, wherein the CDR-H3 is selected from SEQ ID NO:73 and SEQ ID NO:75; the CDR-H2 is selected from SEQ ID NO:61 and SEQ ID NO:63; and the CDR-H1 is selected from SEQ ID NO:47 and SEQ ID NO:50.

[0441]

B9. The binding agent of any one of embodiments B0 to B8, wherein the CDR-L1 comprises or consists of the amino acid sequence of SEQ ID NO:2, the CDR-L2 comprises or consists of the amino acid sequence of SEQ ID NO:17, the CDR-L3 comprises or consists of the amino acid sequence of SEQ ID NO:27, the CDR-H1 comprises or consists of the amino acid sequence of SEQ ID NO:47, the CDR-H2 comprises or consists of the amino acid sequence of SEQ ID NO:61, and the CDR-H3 comprises or consists of the amino acid sequence of SEQ ID NO:73.

[0442]

B10. The binding agent of any one of embodiments B0 to B8, wherein the CDR-L1 comprises or consists of the amino acid sequence of SEQ ID NO:4, the CDR-L2 comprises or consists of the amino acid sequence of SEQ ID NO:20, the CDR-L3 comprises or consists of the amino acid sequence of SEQ ID NO:29, the CDR-H1 comprises or consists of the amino acid sequence of SEQ ID NO:50, the CDR-H2

comprises or consists of the amino acid sequence of SEQ ID NO:63, and the CDR-H3 comprises or consists of the amino acid sequence of SEQ ID NO:75.

[0443]

5 B11. The binding agent of any one of embodiments B0 to B10, wherein the antibody, comprises a constant region of an IgG, IgD, IgE, IgA or IgM.

[0444]

B12. The binding agent of any one of embodiments B0 to B11, wherein the antibody, or antigen binding portion thereof, is humanized.

[0445]

10 B13. The binding agent of any one of embodiments B0 to B12, wherein the antibody, or antigen binding portion thereof binds specifically to a human syndecan-1 with a binding affinity (KD) of 50 nM or less.

[0446]

15 B14. The binding agent of any one of embodiments B0 to B13, wherein the anti-neoplastic agent is covalently attached to the antibody, or antigen binding portion thereof, by a linker.

[0447]

20 B15. The binding agent of embodiment B14, wherein the linker comprises or consists of a peptide comprising one or more amino acids, 5 to 100 amino acids, 5 to 50 amino acids, 5 to 25 amino acids, 5 to 20 amino acids, or 5 to 10 amino acids.

[0448]

25 B16. The binding agent of embodiment B14, wherein the linker comprises an optionally substituted C1-C50 alkyl, an optionally substituted C2-C50 alkenyl, an optionally substituted C2-C50 alkynyl, acyl, acyloxy, alkyloxycarbonyloxy, aryloxycarbonyloxy, cycloalkyl, cycloalkenyl, alkoxy, cycloalkoxy, aryl, heteroaryl, arylalkoxy carbonyl, alkoxy carbonylacyl, aminocarbonyl, aminocarboxyloxy, azido, phenyl, cycloalkylacyl, alkylthio, arylthio, oxysulfonyl, carboxy, thio, sulfoxide, sulfone, sulfonate esters, thiocyno, amide, amino, ester, halogenated alkyl, or a combination thereof.

30 [0449]

B17. The binding agent of any one of embodiments B0 to B16, wherein the antibody, or antigen binding portion thereof, is non-covalently attached to the anti-neoplastic agent by means of a binding pair.

[0450]

- 5 B18. The binding agent of any one of embodiments B0 to B17, wherein the anti-neoplastic agent is attached to an amino-terminal end of a heavy chain or light chain of the antibody, or antigen binding portion thereof.

[0451]

- 10 B19. The binding agent of any one of embodiments B0 to B18, wherein the anti-neoplastic agent is attached to a carboxy-terminal end of a heavy chain or light chain of the antibody, or antigen binding portion thereof.

[0452]

- 15 B20. The binding agent of any one of embodiments B0 to B19, wherein the anti-neoplastic agent is selected from the group consisting of an auristatin, a dolastatin, a maytansine, a tubulysin, a calicheamicin, a duocarmycin, a doxorubicin, a pseudomonas exotoxin-A (PE38), an irinotecan and a derivative of any one of the foregoing.

[0453]

- 20 B21. The binding agent of any one of embodiments B0 to B20, wherein the anti-neoplastic agent comprises a monomethyl auristatin E (MMAE) or a monomethyl auristatin F (MMAF).

[0454]

- 25 The entirety of each patent, patent application, publication or any other reference or document cited herein hereby is incorporated by reference. In case of conflict, the specification, including definitions, will control.

[0455]

Citation of any patent, patent application, publication or any other document is not an admission that any of the foregoing is pertinent prior art, nor does it constitute any admission as to the contents or date of these publications or documents.

- 30 [0456]

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable  
5 methods and materials are described herein.

[0457]

All of the features disclosed herein may be combined in any combination. Each feature disclosed in the specification may be replaced by an alternative feature serving a same, equivalent, or similar purpose. Thus, unless expressly stated otherwise, disclosed  
10 features (e.g., antibodies) are an example of a genus of equivalent or similar features.

[0458]

As used herein, all numerical values or numerical ranges include integers within such ranges and fractions of the values or the integers within ranges unless the context clearly indicates otherwise. Further, when a listing of values is described herein (e.g., about  
15 50%, 60%, 70%, 80%, 85% or 86%) the listing includes all intermediate and fractional values thereof (e.g., 54%, 85.4%). Thus, to illustrate, reference to 80% or more identity, includes 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94% etc., as well as 81.1%, 81.2%, 81.3%, 81.4%, 81.5%, etc., 82.1%, 82.2%, 82.3%, 82.4%, 82.5%, etc., and so forth.

20 [0459]

Reference to an integer with more (greater) or less than includes any number greater or less than the reference number, respectively. Thus, for example, a reference to less than 100, includes 99, 98, 97, etc. all the way down to the number one (1); and less than 10, includes 9, 8, 7, etc. all the way down to the number one (1).

25 [0460]

As used herein, all numerical values or ranges include fractions of the values and integers within such ranges and fractions of the integers within such ranges unless the context clearly indicates otherwise. Thus, to illustrate, reference to a numerical range, such as 1-10 includes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, as well as 1.1, 1.2, 1.3, 1.4, 1.5, etc.,  
30 and so forth. Reference to a range of 1-50 therefore includes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10,

11, 12, 13, 14, 15, 16, 17, 18, 19, 20, etc., up to and including 50, as well as 1.1, 1.2, 1.3, 1.4, 1.5, etc., 2.1, 2.2, 2.3, 2.4, 2.5, etc., and so forth.

[0461]

Reference to a series of ranges includes ranges which combine the values of the  
5 boundaries of different ranges within the series. Thus, to illustrate reference to a series  
of ranges, for example, of 1-10, 10-20, 20-30, 30-40, 40-50, 50-60, 60-75, 75-100,  
100-150, 150-200, 200-250, 250-300, 300-400, 400-500, 500-750, 750-1,000,  
1,000-1,500, 1,500-2,000, 2,000-2,500, 2,500-3,000, 3,000-3,500, 3,500-4,000,  
4,000-4,500, 4,500-5,000, 5,500-6,000, 6,000-7,000, 7,000-8,000, or 8,000-9,000,  
10 includes ranges of 10-50, 50-100, 100-1,000, 1,000-3,000, 2,000-4,000, etc.

[0462]

Modifications can be made to the foregoing without departing from the basic aspects of  
the technology. Although the technology has been described in substantial detail with  
reference to one or more specific embodiments, those of ordinary skill in the art will  
15 recognize that changes can be made to the embodiments specifically disclosed in this  
application, yet these modifications and improvements are within the scope and spirit of  
the technology.

[0463]

The invention is generally disclosed herein using affirmative language to describe the  
20 numerous embodiments and aspects. The invention also specifically includes  
embodiments in which particular subject matter is excluded, in full or in part, such as  
substances or materials, method steps and conditions, protocols, or procedures. For  
example, in certain embodiments or aspects of the invention, materials and/or method  
steps are excluded. Thus, even though the invention is generally not expressed herein  
25 in terms of what the invention does not include aspects that are not expressly excluded  
in the invention are nevertheless disclosed herein.

[0464]

The technology illustratively described herein suitably can be practiced in the absence  
of any element(s) not specifically disclosed herein. Thus, for example, in each  
30 instance herein any of the terms “comprising,” “consisting essentially of,” and  
“consisting of” can be replaced with either of the other two terms. The terms and

expressions which have been employed are used as terms of description and not of limitation, and use of such terms and expressions do not exclude any equivalents of the features shown and described or segments thereof, and various modifications are possible within the scope of the technology claimed. The term “a” or “an” can refer to one of or a plurality of the elements it modifies (e.g., “a reagent” can mean one or more reagents) unless it is contextually clear either one of the elements or more than one of the elements is described. The term “about” as used herein refers to a value within 10% of the underlying parameter (i.e., plus or minus 10%), and use of the term “about” at the beginning of a string of values modifies each of the values (i.e., “about 1, 2 and 3” refers to about 1, about 2 and about 3). For example, a weight of “about 100 grams” can include weights between 90 grams and 110 grams. The term, “substantially” as used herein refers to a value modifier meaning “at least 95%”, “at least 96%”, “at least 97%”, “at least 98%”, or “at least 99%” and may include 100%. For example, a composition that is substantially free of X, may include less than 5%, less than 4%, less than 3%, less than 2%, or less than 1% of X, and/or X may be absent or undetectable in the composition.

[0465]

Thus, it should be understood that although the present technology has been specifically disclosed by representative embodiments and optional features, modification and variation of the concepts herein disclosed can be resorted to by those skilled in the art, and such modifications and variations are considered within the scope of this technology.

## [CLAIMS]

## [Claim 1]

A bi-specific binding agent comprising

5 (a) an antibody, or antigen binding portion thereof, that binds specifically to syndecan-1 (CD138); and

(b) a Fynomer that binds specifically to a fibroblast growth factor receptor 3 (FGFR3), wherein the Fynomer comprises a polypeptide having an amino acid sequence of

10 (i) GVTLFVALYDYEVYGPTPMLSFHKGEKFQIL(X<sub>1</sub>)(X<sub>2</sub>)(X<sub>3</sub>)(X<sub>4</sub>)GPYWEARSL(X<sub>5</sub>)TGETG(X<sub>6</sub>)IPSNYVAPVDSIQ (SEQ ID NO:99) or

(ii) GVTLFVALYDYEVMSSTALSFKGEKFQILSQSPHGQYWEARSLTTGETG(X<sub>7</sub>)IPSNYVAPVDSIQ (SEQ ID NO:113), wherein the amino acids (X<sub>1</sub>), (X<sub>2</sub>), (X<sub>3</sub>), (X<sub>4</sub>), (X<sub>5</sub>), (X<sub>6</sub>) and (X<sub>7</sub>) are selected from any amino acid.

15

## [Claim 2]

The bi-specific binding agent of claim 1, wherein the antibody, or antigen binding portion thereof, comprises the following light chain and heavy chain complementarity determining regions (CDR):

20 (i) a CDR-L1 (light chain CDR1) comprising an amino acid sequence selected from SEQ ID NOs:2-15;

(ii) a CDR-L2 (light chain CDR2) comprising an amino acid sequence selected from SEQ ID NOs:16-26,

25 (iii) a CDR-L3 (light chain CDR3) comprising an amino acid sequence selected from SEQ ID NOs:27-33,

(iv) a CDR-H1 (heavy chain CDR1) comprising an amino acid sequence selected from SEQ ID NOs:45-59;

(v) a CDR-H2 (heavy chain CDR2) comprising an amino acid sequence selected from SEQ ID NOs:60-71, and

30 (vi) a CDR-H3 (heavy chain CDR3) comprising an amino acid sequence selected from SEQ ID NOs:72-81.

## [Claim 3]

The bi-specific binding agent of claim 2, wherein

- (i) the CDR-L1 comprises an amino acid sequence of SEQ ID NO:3;
- 5 (ii) the CDR-L2 comprises an amino acid sequence of SEQ ID NO:18;
- (iii) the CDR-L3 comprises an amino acid sequence of SEQ ID NO:28;
- (iv) the CDR-H1 comprises an amino acid sequence of SEQ ID NO:46;
- (v) the CDR-H2 comprises an amino acid sequence of SEQ ID NO:60; and
- (vi) the CDR-H3 comprises an amino acid sequence of SEQ ID NO:72.

10

## [Claim 4]

The bi-specific binding agent of claim 2, wherein

- (i) the CDR-L1 comprises an amino acid sequence of SEQ ID NO:2;
- (ii) the CDR-L2 comprises an amino acid sequence of SEQ ID NO:17;
- 15 (iii) the CDR-L3 comprises an amino acid sequence of SEQ ID NO:27;
- (iv) the CDR-H1 comprises an amino acid sequence of SEQ ID NO:47;
- (v) the CDR-H2 comprises an amino acid sequence of SEQ ID NO:61; and
- (vi) the CDR-H3 comprises an amino acid sequence SEQ ID NO:73.

## 20 [Claim 5]

The bi-specific binding agent of claim 3 or 4, wherein the antibody, or antigen binding portion thereof comprises a humanized light chain variable region comprising an amino acid sequence having at least 85% identity to an amino acid sequence selected from selected from the group consisting of SEQ ID NOs:41, 42, and 43, and a humanized  
25 heavy chain variable region comprising an amino acid sequence having at least 85% identity to an amino acid sequence selected from selected from the group consisting of SEQ ID NOs:89, 90, 91 and 92 .

## [Claim 6]

30 The bi-specific binding agent of claim 5, wherein the antibody, or antigen binding portion thereof comprises a humanized light chain variable region comprising an amino

acid sequence of SEQ ID NO:41 and a humanized heavy chain variable region comprising an amino acid sequence of SEQ ID NO:90.

[Claim 7]

- 5 The bi-specific binding agent of claim 6, wherein the antibody, or antigen binding portion thereof comprises a humanized light chain comprising an amino acid sequence of SEQ ID NO:44 and/or a humanized heavy chain comprising an amino acid sequence of SEQ ID NO:93.

10 [Claim 8]

The bi-specific binding agent of claim 2, wherein

- (i) the CDR-L1 comprises an amino acid sequence of SEQ ID NO:4;
- (ii) the CDR-L2 comprises an amino acid sequence of SEQ ID NO:20;
- (iii) the CDR-L3 comprises an amino acid sequence of SEQ ID NO:29;
- 15 (iv) the CDR-H1 comprises an amino acid sequence of SEQ ID NO:50;
- (v) the CDR-H2 comprises an amino acid sequence of SEQ ID NO:63; and
- (vi) the CDR-H3 comprises an amino acid sequence of SEQ ID NO:75.

[Claim 9]

- 20 The bi-specific binding agent of any one of claims 1-8, wherein the Fynomer binds specifically to the human FGFR3 isoform 3b and the FGFR3 isoform 3c.

[Claim 10]

The bi-specific binding agent of any one of claims 1-9, wherein

- 25 (X<sub>1</sub>) is N, R, or K;
- (X<sub>2</sub>) is S, G, K or R;
- (X<sub>3</sub>) is S or G;
- (X<sub>4</sub>) is E, Q, D, S or K;
- (X<sub>5</sub>) is T or A; and
- 30 (X<sub>6</sub>) is Y, W or L.

[Claim 11]

The bi-specific binding agent of claim 10, wherein

(X<sub>1</sub>) is R, or K;

(X<sub>2</sub>) is G, K or R;

5 (X<sub>3</sub>) is S;

(X<sub>4</sub>) is Q, D, S or K;

(X<sub>5</sub>) is T or A; and

(X<sub>6</sub>) is W or L.

10 [Claim 12]

The bi-specific binding agent of any one of claims 1-11, wherein the Fynomer comprises a polypeptide comprising an amino acid sequence selected from:

(SEQ ID NO:101; FF2L4C3)

GVTLFVALYDYEEVYGPTPMLS FHKGEKFQILNSSEGPYWEARSLTTGETGLIPS

15 NYVAPVDSIQ;

(SEQ ID NO:103; FF44L65G12)

GVTLFVALYDYEEVYGPTPMLS FHKGEKFQILRGGQGPYWEARSLTTGETGLIPS

NYVAPVDSIQ;

(SEQ ID NO:105; FF44L65G7)

20 GVTLFVALYDYEEVYGPTPMLS FHKGEKFQILRGGDGPYWEARSLTTGETGLIPS

NYVAPVDSIQ;

(SEQ ID NO:107; FF48L66G7; "G7")

GVTLFVALYDYEEVYGPTPMLS FHKGEKFQILKGGSGPYWEARSLTTGETGLIPS

NYVAPVDSIQ;

25 (SEQ ID NO:109; FF43L65D5)

GVTLFVALYDYEEVYGPTPMLS FHKGEKFQILRKGKGPYWEARSLATGETGLIPS

NYVAPVDSIQ;

(SEQ ID NO:111; FF44L65B7)

GVTLFVALYDYEEVYGPTPMLS FHKGEKFQILRRGSGPYWEARSLTTGETGLIPS

30 NYVAPVDSIQ; and

(SEQ ID NO:116; FF40L54A5)

GVTLFVALYDYEVMSTTALSFKGGEKFQILSQSPHGQYWEARSLTTGETGWIPS  
 NYVAPVDSIQ.

[Claim 13]

- 5 The bi-specific binding agent of any one of claims 1-12, wherein the Fynomer is covalently attached to the antibody, or antigen binding portion thereof, by a linker.

[Claim 14]

- 10 The bi-specific binding agent of any one of claims 1-13, further comprising an anti-neoplastic agent.

[Claim 15]

- 15 The bi-specific binding agent of claim 14, wherein the anti-neoplastic agent comprises a pyrrolobenzodiazepine toxin.

[Claim 16]

- 20 The bi-specific binding agent of any one of claims 1-15, wherein the antibody, or antigen binding portion thereof, competes for binding to syndecan-1 with a second binding agent comprises a CDR-L1 having an amino acid sequence selected from SEQ ID NOs:2-15, a CDR-L2 having an amino acid sequence selected from SEQ ID NOs:16-26, a CDR-L3 having an amino acid sequence selected from SEQ ID NOs:27-33, a CDR-H1 having an amino acid sequence selected from SEQ ID NOs:45-59, a CDR-H2 having an amino acid sequence selected from SEQ ID NOs:60-71, and a CDR-H3 having an amino acid sequence selected from SEQ ID  
 25 NOs:72-81.

[Claim 17]

- 30 A pharmaceutical composition comprising the bi-specific binding agent of any one of claims 1-16 and a pharmaceutically acceptable excipient, diluent, additive or carrier.

[Claim 18]

The pharmaceutical composition of claim 17 for use in the treatment of a neoplasm.

[Claim 19]

5 The pharmaceutical composition of claim 17 or 18, wherein the neoplasm is selected from a carcinoma, sarcoma, nervous system neoplasia, lymphoma, myeloma, leukemia, melanoma, mesothelioma, solid or soft tissue tumors, and a secondary cancer.