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(54) **Title:** DR5-TARGETING MULTABODIES FOR THE TREATMENT OF CANCER

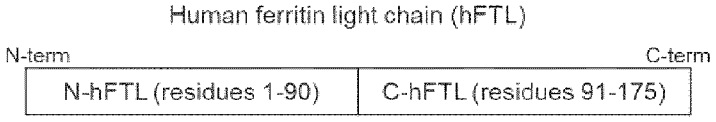

FIG. 1A

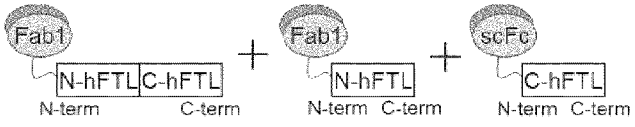

FIG. 1B

(57) **Abstract:** Self-assembled polypeptide complexes comprising 1) fusion polypeptides comprising Fc polypeptides linked to nanocage monomers or subunits thereof and 2) fusion polypeptides comprising an antigen-binding antibody fragment capable of binding to DR5.

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DR5-TARGETING MULTABODIES FOR THE TREATMENT OF CANCER

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims the benefit of and priority to U.S. Provisional Application No. 63/243,372 filed September 13, 2021, the entire content of which is hereby incorporated by reference in its entirety for all purposes.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted electronically in XML format and is hereby incorporated by reference in its entirety. Said XML file, created on September 13, 2022, is named Sequence Listing Sep-2022 3206-5069_R and is 57.6 kilobytes in size.

BACKGROUND

[0003] Death Receptor 5 (DR5) is a member of the TNF-receptor superfamily. DR5 becomes activated by receptor trimerization upon ligand-binding. When activated, DR5 delivers an intracellular apoptosis signal to the cell. DR5 is up-regulated in various types of cancer cells and presents an attractive target for cancer therapy. However, efficacy of a candidate therapeutic based on targeting DR5 may be limited by factors such as, e.g., insufficient ability to cross-link the receptors in the cell membrane.

[0004] A need exists for improved compositions and methods for targeting DR5.

SUMMARY

[0005] The present invention addresses this need with the provision of self-assembled polypeptide complexes comprising (a) fusion polypeptides (1) comprising Fc polypeptides and (2) a nanocage monomer or subunit thereof and (b) fusion polypeptides (1) comprising an antibody fragment capable of binding to DR5 and (2) a nanocage monomer or subunit thereof. In certain embodiments, the Fc polypeptides comprise certain amino acid residues at particular positions, and the self-assembled polypeptide complexes.

[0006] In one aspect, provided are self-assembled polypeptide complexes comprising

(a) a plurality of first fusion polypeptides, each first fusion polypeptide comprising (1) an Fc polypeptide and (2) a nanocage monomer or subunit thereof, and

(b) a plurality of second fusion polypeptides, each second fusion polypeptide comprising (1) an antibody fragment that is capable of binding to DR5 and (2) a nanocage monomer or subunit thereof,

wherein the Fc polypeptide comprises an IgG1 Fc chain, wherein said IgG1 chain comprises (1) an amino acid residue other than glycine at position 237; and (2) a proline residue at position 329, according to EU numbering.

[0007] In some embodiments, the nanocage monomer or subunit thereof in each first fusion polypeptide and in each second fusion polypeptide is a ferritin monomer or subunit thereof.

[0008] In some embodiments, the ferritin monomer or subunit thereof is a ferritin light chain or subunit thereof.

[0009] In some embodiments, the ferritin monomer or subunit thereof is a human ferritin or subunit thereof.

[0010] In some embodiments, the self-assembled polypeptide complex does not comprise any ferritin heavy chains or subunits of ferritin heavy chains.

[0011] In some embodiments, the ferritin monomer or subunit thereof is a ferritin monomer subunit.

[0012] In some embodiments,

a. each first fusion polypeptide comprises a C-half-ferritin, and each second fusion polypeptide comprises an N-half-ferritin; or

b. each first fusion polypeptide comprises an N-half ferritin, and each second fusion polypeptide comprises a C-half-ferritin.

[0013] In some embodiments, each first fusion polypeptide comprises an Fc polypeptide linked to the C-half ferritin's N-terminus via an amino acid linker. In some embodiments, the amino acid linker comprises a $(G_nS)_m$ linker. In some embodiments, the $(G_nS)_m$ linker is a $(GGGGS)_m$ (SEQ ID NO:54) linker.

[0014] In some embodiments, the Fc polypeptide comprises a single chain Fc (scFc) comprising two Fc chains, wherein the two Fc chains are linked via an amino acid linker, e.g., an amino acid linker that comprises a $(G_nS)_m$ linker, e.g., a $(GGGGS)_m$ (SEQ ID NO:54) linker.

[0015] In some embodiments, the Fc polypeptide comprises an IgG1 Fc chain.

[0016] In some embodiments, the IgG1 Fc chain comprises an alanine at position 237 according to EU numbering. In some embodiments, the IgG1 Fc chain comprises: an alanine at position 234, an alanine at position 235, an arginine at position 236, and a leucine at position 330, according to EU numbering.

[0017] In some embodiments, within each second fusion polypeptide, the antigen-binding antibody fragment is linked to the N-terminus of the nanocage monomer or subunit thereof.

[0018] In some embodiments, the antigen-binding antibody fragment of each second fusion polypeptide is a Fab fragment. In some embodiments, each second fusion polypeptide does not comprise any antibody CH2 or CH3 domains.

[0019] In some embodiments, the self-assembled polypeptide complex further comprises a plurality of third fusion polypeptides, each third fusion polypeptide comprising (1) an antigen-binding antibody fragment and (2) a nanocage monomer or a subunit thereof, wherein the third fusion polypeptide is different than the second fusion polypeptide.

[0020] In some embodiments, the antigen-binding antibody fragment of each third fusion polypeptide is a Fab fragment.

[0021] In some embodiments, each third fusion polypeptide does not comprise any antibody CH2 or CH3 domains.

[0022] In some embodiments, the nanocage monomer or subunit thereof of each first fusion polypeptide and each second fusion polypeptide is a ferritin monomer subunit, and

a. each first fusion polypeptide comprises a C-half-ferritin, and each second fusion polypeptide comprises a N-half-ferritin; or

b. each first fusion polypeptide comprises an N-half ferritin, and each second fusion polypeptide comprises a C-half-ferritin.

[0023] In some embodiments, the self-assembled polypeptide complex is characterized by a 1:1 ratio of first fusion polypeptides to second fusion polypeptides.

[0024] In some embodiments, the self-assembled polypeptide complex comprises a total of 24 to 48 fusion polypeptides.

[0025] In some embodiments, the self-assembled polypeptide complex comprises a total of least 24 fusion polypeptides.

[0026] In some embodiments, the self-assembled polypeptide complex comprises a total of at least 32 fusion polypeptides.

[0027] In some embodiments, the self-assembled polypeptide complex has a total of about 32 fusion polypeptides.

[0028] In some embodiments, after administration of a composition comprising the self-assembled polypeptide complex, concentrations of the self-assembled polypeptide complex are substantially similar to those of a reference IgG molecule administered by the same route of administration and in a similar composition during the first 7 days after administration to a subject in need thereof.

[0029] In some embodiments, the self-assembled polypeptide complex exhibits no binding to at least one human Fcγ receptor, as determined in an *in vitro* assay.

[0030] In some embodiments, the self-assembled polypeptide complex exhibits no binding to one or more human Fcγ receptors selected from the group consisting of hFcγRI, hFcγRIIa, hFcγRIIb, hFcγRIIIa, hFcγRIIIb, and combinations thereof, as determined in an *in vitro* assay.

[0031] In some embodiments, the self-assembled polypeptide complex exhibits no binding to hFcγRI, as determined in an *in vitro* assay. In some embodiments, the self-assembled polypeptide complex exhibits no binding to hFcγRIIa, as determined in an *in vitro* assay. In some embodiments, the self-assembled polypeptide complex exhibits no binding to hFcγRIIIa, as determined in an *in vitro* assay. In some embodiments, the self-assembled polypeptide complex exhibits no binding to hFcγRIIb, as determined in an *in vitro* assay. In some embodiments, the self-assembled polypeptide complex exhibits no binding to hFcγRIIIb, as determined in an *in vitro* assay.

[0032] In certain embodiments, provided are self-assembled polypeptide complexes comprising:

(a) a plurality of first fusion polypeptides, each first fusion polypeptide comprising (1) a scFc and (2) a ferritin monomer or subunit thereof, and

(b) a plurality of second fusion polypeptides, each second fusion polypeptide comprising (1) an antibody fragment that is capable of binding to DR5 and (2) a ferritin monomer or subunit thereof, wherein the scFc comprises two IgG1 Fc chains, each IgG1 Fc chain comprising: an alanine at position 234, an alanine at position 235, an arginine at position 236, an alanine at position 237, a proline at position 329, and a leucine at position 330, according to EU numbering.

[0033] In some embodiments,

a. each first fusion polypeptide comprises a C-half-ferritin, and each second fusion polypeptide comprises an N-half-ferritin; or

b. each first fusion polypeptide comprises an N-half ferritin, and each second fusion polypeptide comprises a C-half-ferritin.

[0034] In some embodiments, each first fusion polypeptide comprises a scFc linked to the C-half-ferritin's N-terminus, and each second fusion polypeptide comprises a Fab linked to the N-half-ferritin's N-terminus.

[0035] In some embodiments, each first fusion polypeptide, the scFc is linked via an amino acid linker to the C-half ferritin's N-terminus and/or (2) in each second fusion polypeptide, the Fab is linked via an amino acid linker to N-half ferritin's N-terminus.

[0036] In some embodiments, the self-assembled polypeptide complex is characterized by a 1:1 ratio of first fusion polypeptides to second fusion polypeptides.

[0037] In some embodiments, the self-assembled polypeptide complex further comprises a plurality of third fusion polypeptides, each third fusion polypeptide comprising (1) an antigen-binding antibody fragment and (2) a nanocage monomer or a subunit thereof, wherein the third fusion polypeptide is different than the second fusion polypeptide.

[0038] In some embodiments, the self-assembled polypeptide complex comprises a total of 24 to 48 fusion polypeptides.

[0039] In some embodiments, the self-assembled polypeptide complex comprises a total of least 24 fusion polypeptides.

[0040] In some embodiments, the self-assembled polypeptide complex comprises a total of at least 32 fusion polypeptides.

[0041] In some embodiments, the self-assembled polypeptide complex has total of about 32 fusion polypeptides.

[0042] In some embodiments, the antibody fragment comprises (1) a heavy chain comprising CDR-H1, CDR-H2, and CDR-H3 and (2) a light chain comprising CDR-L1, CDR-L2, and CDR-L3, wherein

(a) (i) the CDR-H1 has a sequence of SEQ ID NO:27 or a sequence differing by one or two amino acids therefrom;

(ii) the CDR-H2 has a sequence of SEQ ID NO:28 or a sequence differing by one or two amino acids therefrom;

(iii) the CDR-H3 has a sequence of SEQ ID NO:29 or a sequence differing by one or two amino acids therefrom;

(iv) the CDR-L1 has a sequence of SEQ ID NO:24 or a sequence differing by one or two amino acids therefrom;

(v) the CDR-L2 has a sequence of SEQ ID NO:25 or a sequence differing by one or two amino acids therefrom; and

(vi) the CDR-L3 has a sequence of SEQ ID NO:26 or a sequence differing by one or two amino acids therefrom;

(b) (i) the CDR-H1 has a sequence of SEQ ID NO:35 or a sequence differing by one or two amino acids therefrom;

(ii) the CDR-H2 has a sequence of SEQ ID NO:36 or a sequence differing by one or two amino acids therefrom;

(iii) the CDR-H3 has a sequence of SEQ ID NO:37 or a sequence differing by one or two amino acids therefrom;

(iv) the CDR-L1 has a sequence of SEQ ID NO:32 or a sequence differing by one or two amino acids therefrom;

(v) the CDR-L2 has a sequence of SEQ ID NO:33 or a sequence differing by one or two amino acids therefrom; and

(vi) the CDR-L3 has a sequence of SEQ ID NO:34 or a sequence differing by one or two amino acids therefrom;

(c) (i) the CDR-H1 has a sequence of SEQ ID NO:43 or a sequence differing by one or two amino acids therefrom;

(ii) the CDR-H2 has a sequence of SEQ ID NO:44 or a sequence differing by one or two amino acids therefrom;

(iii) the CDR-H3 has a sequence of SEQ ID NO:45 or a sequence differing by one or two amino acids therefrom;

(iv) the CDR-L1 has a sequence of SEQ ID NO:40 or a sequence differing by one or two amino acids therefrom;

(v) the CDR-L2 has a sequence of SEQ ID NO:41 or a sequence differing by one or two amino acids therefrom; and

(vi) the CDR-L3 has a sequence of SEQ ID NO:42 or a sequence differing by one or two amino acids therefrom;

or

(d) (i) the CDR-H1 has a sequence of SEQ ID NO:51 or a sequence differing by one or two amino acids therefrom;

(ii) the CDR-H2 has a sequence of SEQ ID NO:52 or a sequence differing by one or two amino acids therefrom;

(iii) the CDR-H3 has a sequence of SEQ ID NO:53 or a sequence differing by one or two amino acids therefrom;

(iv) the CDR-L1 has a sequence of SEQ ID NO:48 or a sequence differing by one or two amino acids therefrom;

(v) the CDR-L2 has a sequence of SEQ ID NO:49 or a sequence differing by one or two amino acids therefrom; and

(vi) the CDR-L3 has a sequence of SEQ ID NO:50 or a sequence differing by one or two amino acids therefrom.

[0043] In some embodiments, the antibody fragment comprises (1) a heavy chain comprising CDR-H1, CDR-H2, and CDR-H3 and (2) a light chain comprising CDR-L1, CDR-L2, and CDR-L3, wherein

(a) (i) the CDR-H1 has a sequence of SEQ ID NO:27,
(ii) the CDR-H2 has a sequence of SEQ ID NO:28,
(iii) the CDR-H3 has a sequence of SEQ ID NO:29,
(iv) the CDR-L1 has a sequence of SEQ ID NO:24,
(v) the CDR-L2 has a sequence of SEQ ID NO:25, and
(vi) the CDR-L3 has a sequence of SEQ ID NO:26;

(b) (i) the CDR-H1 has a sequence of SEQ ID NO:35,
(ii) the CDR-H2 has a sequence of SEQ ID NO:36,
(iii) the CDR-H3 has a sequence of SEQ ID NO:37,
(iv) the CDR-L1 has a sequence of SEQ ID NO:32,
(v) the CDR-L2 has a sequence of SEQ ID NO:33, and
(vi) the CDR-L3 has a sequence of SEQ ID NO:34;

(c) (i) the CDR-H1 has a sequence of SEQ ID NO:43,
(ii) the CDR-H2 has a sequence of SEQ ID NO:44,
(iii) the CDR-H3 has a sequence of SEQ ID NO:45,
(iv) the CDR-L1 has a sequence of SEQ ID NO:40,
(v) the CDR-L2 has a sequence of SEQ ID NO:41, and
(vi) the CDR-L3 has a sequence of SEQ ID NO:42;

or

(d) (i) the CDR-H1 has a sequence of SEQ ID NO:51,
(ii) the CDR-H2 has a sequence of SEQ ID NO:52,
(iii) the CDR-H3 has a sequence of SEQ ID NO:53,
(iv) the CDR-L1 has a sequence of SEQ ID NO:48,
(v) the CDR-L2 has a sequence of SEQ ID NO:49, and
(vi) the CDR-L3 has a sequence of SEQ ID NO:50.

[0044] In some embodiments, the antibody fragment comprises

(1) a heavy chain variable region having at least 85% identical to a reference VH sequence and

(2) a light chain variable region having at least 85% identical to a reference VL sequence, wherein:

(a) the reference VH sequence has a sequence of SEQ ID NO:23 and the reference VL sequence has a sequence of SEQ ID NO:22;

(b) the reference VH sequence has a sequence of SEQ ID NO:31 and the reference VL sequence has a sequence of SEQ ID NO:30;

(c) the reference VH sequence has a sequence of SEQ ID NO:39 and the reference VL sequence has a sequence of SEQ ID NO:38; or

(d) the reference VH sequence has a sequence of SEQ ID NO:47 and the reference VL sequence has a sequence of SEQ ID NO:46.

[0045] In one aspect, provided are methods comprising administering a composition comprising the self-assembled polypeptide complex as disclosed herein to a mammalian subject.

[0046] In some embodiments, the subject is human.

[0047] In some embodiments, the subject is diagnosed as having, or is at risk of developing, a tumor at the time of administering.

[0048] In some embodiments, the step of administering results in slowing or inhibiting progression of the tumor.

[0049] In some embodiments, the step of administering results in regression of the tumor.

[0050] In some embodiments, the step of administering results in complete regression of the tumor.

[0051] In some embodiments, the method comprises administration by a systemic route. e.g., a systemic route that comprises subcutaneous, intravenous, or intramuscular injection, inhalation, or intranasal administration.

[0052] In one aspect, provided are uses of the composition comprising the self-assembled polypeptide complex as described herein for administration to a mammalian subject.

[0053] In some embodiments, the subject is human.

[0054] In some embodiments, the subject is diagnosed as having, or is at risk of developing, a tumor at the time of administration.

[0055] In some embodiments, the use is for slowing or inhibiting progression of the tumor.

[0056] In some embodiments, the use is for causing regression of the tumor.

[0057] In some embodiments, the use is for causing complete regression of the tumor.

[0058] In some embodiments, the us is for systemic administration.

[0059] In some embodiments, systemic administration comprises subcutaneous, intravenous, or intramuscular injection, inhalation, or intranasal administration.

[0060] In one aspect, provided are compositions comprising the self-assembled polypeptide complex as described herein for use in administration to a mammalian subject.

[0061] In some embodiments, the subject is human.

[0062] In some embodiments, the subject is diagnosed as having, or is at risk of developing, a tumor at the time of administration.

[0063] In some embodiments, the compositions are for slowing or inhibiting progression of the tumor.

[0064] In some embodiments, the compositions are for causing regression of the tumor.

[0065] In some embodiments, the compositions are for causing complete regression of the tumor.

[0066] In some embodiments, the compositions are for systemic administration.

[0067] In some embodiments, systemic administration comprises subcutaneous, intravenous, or intramuscular injection, inhalation, or intranasal administration.

BRIEF DESCRIPTION OF THE DRAWINGS

[0068] **FIG. 1A** is a diagrammatic representation of human ferritin light chain (hFTL) and exemplary N-half ferritin (N-hFTL) and C-half ferritin (C-hFTL) molecules.

[0069] **FIG. 1B** is a diagrammatic representation of fusion polypeptides that together form exemplary Multabodies of the disclosure.

[0070] **FIGs. 2A**, **2B**, **2C**, **2D**, and **2E** depict biolayer interferometry (BLI) time-response curves for the binding of a DR5-targeting Multabody (Cona MB IgG1 LLRAL) to human DR5, human DR4, human osteoprotegerin (OPG), human decoy receptor 1 (DcR1), and human DcR2, respectively.

[0071] **FIG. 3** depicts exemplary BLI time-response curves for the binding of DR5-targeting Multabodies containing various Fc chains to human, cynomolgus monkey, and mouse FcRns, measured at pH 6.0 for association and pH 7.4 for dissociation.

[0072] **FIG. 4** illustrates the dose-dependent killing of cancer cells by Cona MB IgG1 wt, Cona MB IgG1 LLRAL, and conatumumab (Cona) in different human tumor cell lines, quantified and represented as the percentage viable cells following Multabody or antibody treatment to vehicle-treated tumor cells.

[0073] **FIG. 5A** is a schematic illustrating the designs of pharmacokinetic studies described

in Example 6.

[0074] **FIG. 5B** depict plots showing the plasma levels of Cona MB IgG1 wt, Cona MB IgG1 LLRAL, or conatumumab (Cona) following a single intraperitoneal (i.p.) or intravenous (i.v.) dose administered to severe combined immunodeficiency (SCID) mice.

[0075] **FIG. 5C** illustrates plasma levels of Cona MB IgG1 LLRAL or conatumumab in SCID mice administered with two i.p. doses, 96 hours apart.

[0076] **FIG. 6A** is a schematic that illustrates the design of an *in vivo* efficacy experiment described in Example 7 and conducted in a COLO 205 xenograft mouse model.

[0077] **FIG. 6B** and **6C** show tumor volumes at various timepoints (**FIG. 6B**) and on Day 88 (**FIG. 6C**) in mice bearing COLO 205 xenograft tumors and treated with vehicle, conatumumab, or Cona MB IgG1 LLRAL.

[0078] **FIGs. 6D-6G** shows tumor growth curves for individual COLO 205 xenograft tumor-bearing mice treated with vehicle (FIG. 6D), conatumumab (FIG. 6E), or Cona MB IgG1 LLRAL (FIGs. 6F and 6G).

[0079] **FIG. 6H** is a time plot that depicts plasma levels of Cona MB IgG1 LLRAL or conatumumab following i.p. administration to COLO 205 xenograft tumor-bearing mice.

[0080] **FIG. 7A** is a plot that depicts tumor volumes of mice in various groups over time since the first dose. "MB" indicates the Cona MB IgG1 LLRAL group. The triangles on the x-axis denote the treatment time points. **FIGs. 7B-7F** show the tumor volumes of individual mice within the vehicle (**FIG. 7B**) and 5 mg/kg, 1 mg/kg, 0.25 mg/kg, and 0.1 mg/kg Cona MB IgG1 LLRAL treatment (**FIG. 7C, FIG. 7D, FIG. 7E,** and **FIG. 7F,** respectively) groups. In FIGs. 7B-7F, "CR" denotes complete regression.

[0081] **FIG. 7G** is a plot that depicts that amount of Cona MB IgG1 LLRAL detectable in blood samples collected at various timepoints after the first dose was administered. Cona MB IgG1 LLRAL was detectable at all time points tested in the 0.1 mg/kg, 0.25 mg/kg, 1 mg/kg/ and 5 mg/kg Cona MB IgG1 LLRAL treatment groups, with the pharmacokinetics appearing linear in all dose groups.

[0082] **FIG. 8** is a schematic depicting the design of a study of large tumor penetration and apoptosis induction by a DR5-targeting Multabody in an COLO 205 xenograft mouse model. Experiments are described in Example 9.

[0083] **FIGs. 9A-9D** depict representative tumor sections from mice that were untreated (FIGs. 9A and 9C) or treated with a DR5-targeting Multabody ("MB") (FIGs. 9B and 9D). Sections were stained with an antibody against cleaved caspase-3, a marker of apoptosis. Experiments are described in Example 9.

DETAILED DESCRIPTION OF CERTAIN EMBODIMENTS OF THE INVENTION

Definitions

[0084] The terms "**about**" and "**approximately**," when used herein in reference to a value, are used interchangeably and refer to a value that is similar to the referenced value. In general, those skilled in the art, familiar with the context, will appreciate the relevant degree of variation encompassed by "about" or "approximately" in that context. For example, in some embodiments, the terms "about" and "approximately" may encompass a range of values that fall within 25%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less of the referred value.

[0085] As used herein, the terms "**alter**," "**altered**," "**decrease**," "**decreased**," "**increase**," "**increased**," or "**reduction**," "**reduced**," (e.g., in reference to certain outcomes or effects) have meanings relative to a reference level. In some embodiments, in the context of discussing mutations in an Fc chain or Fc polypeptide, the reference level is a level known or as determined with an IgG that does not contain the referenced mutation(s) in the Fc region.

[0086] As used herein, the term "**binding**," unless otherwise specified, refers to a non-covalent association between or among two or more entities. "Direct" binding involves physical contact between entities or moieties; indirect binding involves physical interaction by way of physical contact with one or more intermediate entities. Binding between two or more entities can typically be assessed in any of a variety of contexts--including where interacting entities or moieties are studied in isolation or in the context of more complex systems (e.g., while covalently or otherwise associated with a carrier entity and/or in a biological system or cell). As used herein, the phrases "**non-binding**" or "**no binding,**" or similar phrases, between two entities refers to 1) a lack of detectable binding or 2) binding below a set threshold that corresponds to no binding in an appropriate assay, e.g., an *in vitro* binding assay such as biolayer interferometry. For example, in some embodiments, in an *in vitro* biolayer interferometry assay, a maximal association binding response of less than 0.1 nm after 180 seconds to a biosensor loaded with 0.8 nm of target when the test article is present at a concentration of 20 nM is classified as "non-binding."

[0087] The terms "**ferritin**" and "**apoferritin**" are used interchangeably herein and generally refer to a polypeptide (e.g., a ferritin chain) that is capable of assembling into a ferritin complex which typically comprises 24 protein subunits. In some embodiments, the ferritin is a human ferritin, e.g., a human ferritin light chain, e.g., a human ferritin light chain having at least 85% sequence identity to SEQ ID NO:1 or UniProt P02792. In some

embodiments, the ferritin is a wild-type ferritin. For example, the ferritin may be a wild-type human ferritin.

[0088] The term **"ferritin monomer,"** is used herein to refer to a single chain of a ferritin that, in the presence of other ferritin chains, is capable of self-assembling into a polypeptide complex comprising a plurality of ferritin chains, e.g., 24 or more ferritin chains.

[0089] As used herein, the term "**linker**" is used to refer to an entity that connects two or more elements to form a multi-element agent. For example, those of ordinary skill in the art appreciate that a polypeptide (e.g., fusion polypeptide) whose structure includes two or more functional or organizational domains often includes a stretch of amino acids between such domains that links them to one another. In some embodiments, a polypeptide comprising a linker element has an overall structure of the general form S1-L-S2, wherein S1 and S2 may be the same or different and represent two domains associated with one another by the linker (L). In some embodiments, the linker is an "**amino acid linker**," that is, it comprises amino acid residues, e.g., an amino acid linker may comprise at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100 or more amino acid residues. In some embodiments, a linker is characterized in that it tends not to adopt a rigid three-dimensional structure, but rather provides flexibility to the polypeptide.

[0090] The term "**multispecific**," as used herein, refers to the characteristic of having at least two binding sites at which at least two different binding partners, e.g., an antigen or receptor (e.g., Fc receptor), can bind. For example, a polypeptide complex that comprises at least two Fab fragments, wherein each of the two Fab fragments is capable of binding to a different antigen, is "multispecific." As an additional example, a polypeptide complex that comprises an Fc fragment (which is capable of binding to an Fc receptor) and a Fab fragment (which is capable of binding to an antigen) is "multispecific."

[0091] The term "**multivalent**," as used herein, refers to the characteristic of having at least two binding sites at which a binding partner, e.g., an antigen or receptor (e.g., Fc receptor), can bind. The binding partners that can bind to at least two binding sites may be the same or different.

[0092] The term "**nanocage monomer**," as used herein, refers to a single chain of a polypeptide that is capable of self-assembling with other nanocage monomers to form a self-assembled polypeptide complex comprising a plurality of nanocage monomers. In some embodiments, the nanocage monomer is selected from monomers of ferritin, apoferritin, encapsulin, sulfur oxygenase reductase (SOR), lumazine synthase, pyruvate dehydrogenase,

carboxysome, vault proteins, GroEL, heat shock protein, E2P coat protein, MS2 coat protein, fragments thereof, and variants thereof.

[0093] The term "**polypeptide,**" as used herein, generally has its art-recognized meaning of a polymer of at least three amino acids, e.g., linked to each other by peptide bonds. Those of ordinary skill in the art will appreciate that the term "polypeptide" is intended to be sufficiently general as to encompass not only polypeptides having a complete sequence recited herein, but also to encompass polypeptides that represent functional fragments (i.e., fragments retaining at least one activity) of such complete polypeptides. Moreover, those of ordinary skill in the art understand that protein sequences generally tolerate some substitution without destroying activity. Thus, any polypeptide that retains activity and shares at least about 30-40% overall sequence identity, often greater than about 50%, 60%, 70%, or 80%, and further usually including at least one region of much higher identity, often greater than 90% or even 95%, 96%, 97%, 98%, or 99% in one or more highly conserved regions, usually encompassing at least 3-4 and often up to 20 or more amino acids, with another polypeptide of the same class, is encompassed within the relevant term "polypeptide" as used herein. Polypeptides may contain L-amino acids, D-amino acids, or both and may contain any of a variety of amino acid modifications or analogs known in the art. Useful modifications include, e.g., terminal acetylation, amidation, methylation, glycosylation etc. In some embodiments, proteins may comprise natural amino acids, non-natural amino acids, synthetic amino acids, and combinations thereof

[0094] The term "**self-assembled**," when used in reference to a macromolecular complex (e.g., a polypeptide complex), refers to the spontaneous formation of that complex when sufficient constituents of the complex (e.g., fusion polypeptides) to be formed are present. In some embodiments, complexes self-assemble in physiological conditions, or in a buffer (e.g., a solution) that corresponds to physiological conditions.

[0095] As used herein, the term "**subject**" to an organism, typically a mammal (e.g., a human). In some embodiments, a subject is suffering from or susceptible to a relevant disease, disorder or condition. In some embodiments, a subject displays one or more symptoms or characteristics of a disease, disorder or condition. In some embodiments, a subject is someone with one or more features characteristic of susceptibility to or risk of a disease, disorder, or condition. In some embodiments, a subject is a patient. In some embodiments, a subject is a subject to whom diagnosis and/or therapy is and/or has been administered.

[0096] As used herein, the term **"treatment"** (also "treat" or "treating") refers to any administration of a therapy that partially or completely alleviates, ameliorates, relieves, inhibits, delays onset of, reduces severity of, and/or reduces incidence of one or more symptoms, features, and/or causes of a particular disease, disorder, and/or condition. In some embodiments, such treatment may be of a subject who does not exhibit signs of the relevant disease, disorder and/or condition and/or of a subject who exhibits only early signs of the disease, disorder, and/or condition. Alternatively, or additionally, such treatment may be of a subject who exhibits one or more established signs of the relevant disease, disorder and/or condition. In some embodiments, treatment may be of a subject who has been diagnosed as suffering from the relevant disease, disorder, and/or condition. In some embodiments, treatment may be of a subject known to have one or more susceptibility factors that are statistically correlated with increased risk of development of the relevant disease, disorder, and/or condition.

A. Fusion polypeptides

[0097] In many embodiments, fusion polypeptides compatible with compositions and methods disclosed herein generally comprise a nanocage monomer or subunit thereof linked to either an Fc polypeptide or to an antigen-binding antibody fragment. Within the fusion polypeptide, the Fc polypeptide or the antigen-binding antibody fragment may be linked to the nanocage monomer or subunit thereof at a particular terminus of the nanocage monomer or subunit thereof, e.g., the N-terminus or the C-terminus. In some embodiments, the Fc polypeptide or antigen-binding antibody fragment is linked via an amino acid linker, such as a linker as described herein.

1. Nanocage monomers and subunits thereof

[0098] In some embodiments, the nanocage monomer is a ferritin monomer.

[0099] The term **"ferritin monomer,"** is used herein to refer to a single chain of a ferritin that, in the presence of other ferritin chains, is capable of self-assembling into a polypeptide complex comprising a plurality of ferritin chains, e.g., 24 or more ferritin chains. In some embodiments, the ferritin monomer is a ferritin light chain. In some embodiments, the ferritin monomer does not include a ferritin heavy chain or other ferritin components capable of binding to iron or capable of ferroxidase activity.

[0100] In some embodiments, each fusion polypeptide within the self-assembled polypeptide complex comprises a ferritin light chain or a subunit of a ferritin light chain. In

these embodiments, the self-assembled polypeptide complex does not comprise any ferritin heavy chains or subunits of ferritin heavy chains.

[0101] In some embodiments, the ferritin monomer is a human ferritin chain, e.g., a human ferritin light chain, e.g., a human ferritin light chain having the sequence of at least residues 2-175 of SEQ ID NO:1.

[0102] A "subunit" of a ferritin monomer refers to a portion of a ferritin monomer that is capable of spontaneously associating with another, distinct subunit of a ferritin monomer, so that the subunits together form a ferritin monomer, which ferritin monomer, in turn, is capable of self-assembling with other ferritin monomers to form a polypeptide complex.

[0103] In some embodiments, the ferritin monomer subunit comprises approximately half of a ferritin monomer. As used herein, the term "N-half ferritin" refers to approximately half of a ferritin chain, which half comprises the N-terminus of the ferritin chain. As used herein, the term "C-half ferritin" refers to approximately half a ferritin chain, which half comprises the C-terminus of the ferritin chain. The exact point at which a ferritin chain may be divided to form the N-half ferritin and the C-half ferritin may vary depending on the embodiment. In the context of ferritin monomer subunits based on human ferritin light chain, for example, the halves may be divided at a point that corresponds to a position between about position 75 to about position 100 of SEQ ID NO:1 (or a substantial portion thereof). For example, in some embodiments, an N-half ferritin based on a human ferritin light chain has an amino acid sequence corresponding to residues 1-95 of SEQ ID NO: 1 (or a substantial portion thereof, e.g., residues 2-95 of SEQ ID NO: 1), and a C-half ferritin based on a human ferritin light chain has an amino acid sequence corresponding to residues 96-175 of SEQ ID NO: 1 (or a substantial portion thereof).

[0104] In some embodiments, the halves are divided at a point that corresponds to a position between about position 85 to about position 92 of SEQ ID NO: 1. For example, in some embodiments, an N-half ferritin based on a human ferritin light chain has an amino acid sequence corresponding to residues 1-90 of SEQ ID NO:1 (or a substantial portion thereof, e.g., residues 2-90 of SEQ ID NO: 1), and a C-half ferritin based on a human ferritin light chain has an amino acid sequence corresponding to residues 91-175 of SEQ ID NO:1 (or a substantial portion thereof.

2. Fc polypeptides

[0105] In certain embodiments, fragment crystallizable (Fc) polypeptides comprise Fc chains that each have one or more mutations relative to a reference Fc chain of the same Ig

class. As explained further herein below, the reference Fc chain may be of, e.g., the IgG1 class.

[0106] Unless otherwise noted, numbering of residues within an antibody fragment, e.g., an Fc chain, throughout this disclosure is according to the EU numbering.

[0107] In some embodiments, the Fc polypeptide comprises one or more human IgG1 Fc chains that is, except for mutations noted herein, the Fc polypeptide comprises an Fc chain that is substantially similar to that of the Fc chains within a wild type human IgG1.

[0108] In some embodiments, the Fc polypeptide comprises one or more IgG1 Fc chains (e.g., human IgG1 Fc chains or a human Fc chains), that is, except for having particular residue(s) (which may be different than the residue(s) in the corresponding wild type Fc chains) at certain positions as noted herein, the Fc polypeptide comprises an Fc chain that has an amino acid sequence that is substantially similar to that of the chains within a wild type IgG1 Fc. In some embodiments, the wild type IgG1 Fc is a human IgG1 Fc, in which each Fc chain has an amino acid sequence of SEQ ID NO:4.

For example, an Fc polypeptide may comprise an Fc chain with an amino acid sequence that is at least 85%, at least 87.5%, 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%, or at least 99% identical to that of an Fc chain within a wild-type IgG1 Fc. In some embodiments, an Fc polypeptide comprises an Fc chain that comprises the particular residue(s) at certain position(s) specifically described for that Fc chain, but has an amino acid sequence that is otherwise 100% identical to a corresponding Fc chain within a wild type Fc chain, e.g., wild type IgG1 Fc chain. In some embodiments, the Fc polypeptide comprises an Fc chain that has an amino acid sequence that differs by at least one, at least two, at least three, or at least four amino acid residues from the sequence of SEQ ID NO:4. In some embodiments, the Fc polypeptide comprises an Fc chain that has an amino acid sequence that differs by no more than ten, no more than nine, no more than eight, no more than seven, no more than six, no more than five, or no more than four amino acid residues from the sequence of SEQ ID NO:4.

[0109] In some embodiments, the Fc polypeptide is a single chain Fc (scFc), which comprises two Fc chains linked together by a covalent linker, e.g., via an amino acid linker.

[0110] In certain embodiments, the Fc chain comprises (1) an amino acid residue other than glycine at position 237; and (2) a proline residue at position 329. In some embodiments, the Fc chain comprises an alanine at position 237.

[0111] In some embodiments, the Fc chain is an IgG1 Fc chain and further comprises a mutation or set of mutations at one or more positions selected from position 234, position 235, position 236, position 330, and combinations thereof.

[0112] For example, in some embodiments, the Fc chain is an IgG1 Fc chain that comprises an alanine at position 234, an alanine at position 235, an arginine at position 236, an alanine at position 237, a proline at position 329, and a leucine at position 330.

[0113] In some embodiments, the Fc chain further comprises a mutation at a position associated with glycosylation, e.g., position 297 (e.g., by comprising a glutamine at position 297).

[0114] In some embodiments, the Fc chain comprises a mutation or set of mutations (relative to a corresponding wild type Fc chain) associated with an altered characteristic as further described herein. By "associated with," it is meant that the mutation or set of mutations has been previously characterized, in the context of antibodies such as IgG antibodies, as conferring the altered characteristic (e.g., altered binding to FcRn, altered effector function, etc.) By "altered" it is meant that the characteristic (e.g., binding to an Fc receptor (e.g., an Fcγ receptor or an FcRn)), is different than that observed without the mutation or set of mutations.

[0115] For example, in some embodiments, the altered characteristic comprises altered binding to an Fc receptor.

[0116] In some embodiments, the altered characteristic comprising altered binding to an Fcγ receptor, e.g., a human FcγR. In some embodiments, the FcγR is a human FcγR selected from the group consisting of hFcγRI, hFcγRIIa, hFcγRIIb, hFcγRIIIa, hFcγRIIIb, and combinations thereof.

[0117] In some embodiments, the altered binding comprises no binding, or significantly reduced binding, relative to a corresponding control (e.g., binding levels typically observed under similar circumstances with a corresponding wild type chain), in an assay, e.g., an *in vitro* assay.

3. Antibody fragments capable of binding to DR5

[0118] Antibody fragments are typically capable of binding to an epitope within DR5, exemplary sequences of which are shown in SEQ ID NO:18 to SEQ ID NO:21.

[0119] In some embodiments, the antibody fragment is a Fab. In some embodiments, the antibody fragment is a single-chain Fab (scFab); for example, a fusion polypeptide

comprising both the heavy and light chains of a Fab, optionally linked by a linker (e.g., amino acid linker as disclosed herein) is used.

[0120] In certain embodiments, the antibody fragment comprises a heavy chain variable region (e.g., a V_H). In certain embodiments, the antibody fragment comprises a heavy chain variable domain (e.g., V_H) and a light chain variable domain (e.g., a V_L or V_K). In certain embodiments, the antibody fragment comprises a Fab which comprises a heavy chain variable domain (e.g., V_H) and a light chain variable domain (e.g., a V_L or V_K).

[0121] In certain embodiments, the antibody fragment does not comprise any domains from the Fc region, e.g., does not comprise any CH2 or CH3 domains. In certain embodiments, the antibody fragment capable of binding to DR5 is an antibody fragment of, or derived from, any of a variety of DR5 antibodies, including, e.g., fully human, humanized or chimeric DR5 antibodies. The DR5 antibody from which the antibody fragment is obtained or derived can be of any of a variety of antibody classes, including, e.g., an IgG1 antibody, an IgG2 antibody, an IgG4 antibody. In some embodiments, the antibody fragment is obtained or derived from an agonistic DR5 antibody, e.g., an agonistic humanized DR5 antibody.

[0122] Non-limiting examples of DR5 antibodies include, e.g., conatumumab, tigatuzumab, lexatumumab, and drozitumab.

[0123] In some embodiments, antibody fragments capable of binding to DR5 comprises heavy chain and light chain CDRs having similar sequences (e.g., each CDR being identical, or having one or two amino acid substitutions) to that of the heavy and light chain CDRs of a DR5 antibody.

[0124] In some embodiments, antibody fragments capable of binding to DR5 comprise (1) a heavy chain comprising CDR-H1, CDR-H2, and CDR-H3 and (2) a light chain comprising CDR-L1, CDR-L2, and CDR-L3, wherein

(a) (i) the CDR-H1 has a sequence of SEQ ID NO:27 or a sequence differing by one or two amino acid residues therefrom,

(ii) the CDR-H2 has a sequence of SEQ ID NO:28 or a sequence differing by one or two amino acid residues therefrom,

(iii) the CDR-H3 has a sequence of SEQ ID NO:29 or a sequence differing by one or two amino acid residues therefrom,

(iv) the CDR-L1 has a sequence of SEQ ID NO:24 or a sequence differing by one or two amino acid residues therefrom,

(v) the CDR-L2 has a sequence of SEQ ID NO:25 or a sequence differing by one or two amino acid residues therefrom, and

(vi) the CDR-L3 has a sequence of SEQ ID NO:26 or a sequence differing by one or two amino acid residues therefrom;

(b) (i) the CDR-H1 has a sequence of SEQ ID NO:35 or a sequence differing by one or two amino acid residues therefrom,

(ii) the CDR-H2 has a sequence of SEQ ID NO:36 or a sequence differing by one or two amino acid residues therefrom,

(iii) the CDR-H3 has a sequence of SEQ ID NO:37 or a sequence differing by one or two amino acid residues therefrom,

(iv) the CDR-L1 has a sequence of SEQ ID NO:32 or a sequence differing by one or two amino acid residues therefrom,

(v) the CDR-L2 has a sequence of SEQ ID NO:33 or a sequence differing by one or two amino acid residues therefrom, and

(vi) the CDR-L3 has a sequence of SEQ ID NO:34 or a sequence differing by one or two amino acid residues therefrom,

or

(c) (i) the CDR-H1 has a sequence of SEQ ID NO:43 or a sequence differing by one or two amino acid residues therefrom,

(ii) the CDR-H2 has a sequence of SEQ ID NO:44 or a sequence differing by one or two amino acid residues therefrom,

(iii) the CDR-H3 has a sequence of SEQ ID NO:45 or a sequence differing by one or two amino acid residues therefrom,

(iv) the CDR-L1 has a sequence of SEQ ID NO:40 or a sequence differing by one or two amino acid residues therefrom,

(v) the CDR-L2 has a sequence of SEQ ID NO:41 or a sequence differing by one or two amino acid residues therefrom, and

(vi) the CDR-L3 has a sequence of SEQ ID NO:42 or a sequence differing by one or two amino acid residues therefrom;

or

(d) (i) the CDR-H1 has a sequence of SEQ ID NO:51 or a sequence differing by one or two amino acid residues therefrom,

(ii) the CDR-H2 has a sequence of SEQ ID NO:52 or a sequence differing by one or two amino acid residues therefrom,

(iii) the CDR-H3 has a sequence of SEQ ID NO:53 or a sequence differing by one or two amino acid residues therefrom,

(iv) the CDR-L1 has a sequence of SEQ ID NO:48 or a sequence differing by one or two amino acid residues therefrom,

(v) the CDR-L2 has a sequence of SEQ ID NO:49 or a sequence differing by one or two amino acid residues therefrom, and

(vi) the CDR-L3 has a sequence of SEQ ID NO:50 or a sequence differing by one or two amino acid residues therefrom.

[0125] In some embodiments, antibody fragments capable of binding to DR5 comprises heavy and light chain CDRs having sequences identical to those of the heavy and light chain CDRs of a DR5 antibody, except for one or two amino acid substitutions total across all six CDRs.

[0126] In some embodiments, antibody fragments capable of binding to DR5 comprises heavy chain and light chain complementarity-determining regions (CDRs) having the same sequences as the CDRs of a DR5 antibody.

[0127] In some embodiments, antibody fragments capable of binding to DR5 comprise (1) a heavy chain comprising CDR-H1, CDR-H2, and CDR-H3 and (2) a light chain comprising CDR-L1, CDR-L2, and CDR-L3, wherein

(a) (i) the CDR-H1 has a sequence of SEQ ID NO:27;
(ii) the CDR-H2 has a sequence of SEQ ID NO:28;
(iii) the CDR-H3 has a sequence of SEQ ID NO:29;
(iv) the CDR-L1 has a sequence of SEQ ID NO:24;
(v) the CDR-L2 has a sequence of SEQ ID NO:25; and
(vi) the CDR-L3 has a sequence of SEQ ID NO:26;

(b) (i) the CDR-H1 has a sequence of SEQ ID NO:35,
(ii) the CDR-H2 has a sequence of SEQ ID NO:36,
(iii) the CDR-H3 has a sequence of SEQ ID NO:37,
(iv) the CDR-L1 has a sequence of SEQ ID NO:32,
(v) the CDR-L2 has a sequence of SEQ ID NO:33, and
(vi) the CDR-L3 has a sequence of SEQ ID NO:34;

(c) (i) the CDR-H1 has a sequence of SEQ ID NO:43,
(ii) the CDR-H2 has a sequence of SEQ ID NO:44,
(iii) the CDR-H3 has a sequence of SEQ ID NO:45,
(iv) the CDR-L1 has a sequence of SEQ ID NO:40,

(v) the CDR-L2 has a sequence of SEQ ID NO:41, and

(vi) the CDR-L3 has a sequence of SEQ ID NO:42;

or

(d) (i) the CDR-H1 has a sequence of SEQ ID NO:51,

(ii) the CDR-H2 has a sequence of SEQ ID NO:52,

(iii) the CDR-H3 has a sequence of SEQ ID NO:53,

(iv) the CDR-L1 has a sequence of SEQ ID NO:48,

(v) the CDR-L2 has a sequence of SEQ ID NO:49, and

(vi) the CDR-L3 has a sequence of SEQ ID NO:50.

[0128] In some embodiments, the antibody fragment capable of binding to DR5 comprises

(1) a heavy chain variable region having at least 85%, at least 87.5%, 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 a heavy chain variable region from a DR5 antibody and

(2) a light chain variable region having at least 85%, at least 87.5%, 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 a light chain variable region from a DR5 antibody.

[0129] In some embodiments, the antibody fragment capable of binding to DR5 comprises

(1) a heavy chain variable region having at least 85%, at least 87.5%, 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 a reference V_H sequence and

(2) a light chain variable region having at least 85%, at least 87.5%, 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 a reference V_L sequence, wherein:

(a) the reference V_H sequence has a sequence of SEQ ID NO:23 and the reference V_L sequence has a sequence of SEQ ID NO:22,

(b) the reference V_H sequence has a sequence of SEQ ID NO:31 and the reference V_L sequence has a sequence of SEQ ID NO:30,

(c) the reference V_H sequence has a sequence of SEQ ID NO:39 and the reference V_L sequence has a sequence of SEQ ID NO:38, or

(d) the reference V_H sequence has a sequence of SEQ ID NO:47 and the reference V_L sequence has a sequence of SEQ ID NO:46.

[0130] In embodiments where multiple types of fusion polypeptides having antibody fragments are used, the antibody fragments in the various types of fusion polypeptides may be capable of binding to the same epitope on DR5, capable of binding to epitopes that are distinct and non-overlapping on DR5, or capable of binding to epitopes that are distinct but overlapping on DR5.

4. Linkers

[0131] In certain embodiments, linkers are used within fusion polypeptides and/or within single-chain molecules such as scFcs. In some embodiments, the linker is an amino acid linker. For example, a linker as employed herein may comprise from about 1 to about 100 amino acid residues, e.g., about 1 to about 70, about 2 to about 70, about 1 to about 30, or about 2 to about 30 amino acid residues. In some embodiments, the linker comprises at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, or at least 10 amino acid residues.

[0132] In certain embodiments, the linker comprises a glycine-serine sequence, e.g., a $(G_nS)_m$ sequence (e.g., GGS, GGGS (SEQ ID NO:55), or GGGGS (SEQ ID NO:54) sequence) that is present in at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, or at least 14 copies within the linker.

B. Self-assembled polypeptide complexes

[0133] In one aspect, provided are self-assembled polypeptide complexes comprising a plurality of fusion polypeptides as disclosed herein. Generally, provided self-assembled polypeptide complexes comprise (a) a plurality of first fusion polypeptides, each first fusion polypeptide comprising (1) an Fc polypeptide linked to (2) a nanocage monomer or subunit thereof, wherein the Fc polypeptide comprises an Fc chain having one or more mutations relative to a reference Fc chain of the same Ig class, and (b) a plurality of second fusion polypeptides, each second fusion polypeptide comprising (1) an antigen-binding antibody fragment linked to (2) a nanocage monomer or subunit thereof.

[0134] In some embodiments, the nanocage monomer is a ferritin monomer, and each fusion polypeptide within the self-assembled polypeptide complex comprises a ferritin light chain or a subunit of a ferritin light chain. In these embodiments, the self-assembled polypeptide complex does not comprise any ferritin heavy chains, subunits of ferritin heavy

chains, or other ferritin components capable of binding to iron or capable of ferroxidase activity.

[0135] In some embodiments, the nanocage monomer or subunit thereof is a ferritin monomer subunit, and (a) each first fusion polypeptide comprises a ferritin monomer subunit which is C-half-ferritin and each second fusion polypeptide comprises a ferritin monomer subunit which is N-half-ferritin; or (b) each first fusion polypeptide comprises a ferritin monomer subunit which is N-half ferritin and each second fusion polypeptide comprises a ferritin monomer subunit which is C-half-ferritin.

[0136] In some embodiments, the self-assembled polypeptide complex comprises between 24 and 48 fusion polypeptides in total. In some embodiments, the self-assembled polypeptide complex comprises 24 fusion polypeptides in total. In some embodiments, the self-assembled polypeptide complex comprises more than 24 fusion polypeptides, e.g., at least 26, at least 28, at least 30, at least 32 fusion polypeptides, at least 34 fusion polypeptides, at least 36 fusion polypeptides, at least 38 fusion polypeptides, at least 40 fusion polypeptides, at least 42 fusion polypeptides, at least 44 fusion polypeptides, at least 46 fusion polypeptides, or at least 48 fusion polypeptides in total. In some embodiments, the self-assembled polypeptide complex comprises about 32 fusion polypeptides.

[0137] In some embodiments, the self-assembled polypeptide complex comprises at least 4, at least 5, least 6, at least 7, or at least 8 first fusion polypeptides.

[0138] In some embodiments, the self-assembled polypeptide complex comprises at least 4, at least 5, least 6, at least 7, or at least 8 second fusion polypeptides.

[0139] In some embodiments, the self-assembled polypeptide complex further comprises at least 4, at least 5, least 6, at least 7, at least 8, at least 9, at least 10, least 11, at least 12, at least 13, at least 14, at least 15, or at least 16 third fusion polypeptides.

[0140] In some embodiments, the self-assembled polypeptide complex comprises a ratio of approximately 1:1, 11:13, 3:5, 1:2, 7:17, 1:3, 2:7, 5:19, 1:4, 1:5, 1:6, 1:7, 1:8, 1:12, 1:24 of first fusion polypeptides to all other fusion polypeptides.

Pharmacokinetic characteristics

[0141] In certain embodiments, when administered to a subject in need thereof, a provided self-assembled polypeptide complex, has one or more pharmacokinetic features similar to that of a reference IgG molecule (e.g., an IgG molecule whose class matches the class of an Fc chain within an Fc polypeptide of a first fusion polypeptide within the self-assembled polypeptide complex). In some embodiments, the ranges for the pharmacokinetic

characteristics discussed herein (e.g., half-life, AUC, and/or C_{max}) are obtained when the self-assembled polypeptide complex is administered to a human subject. In some embodiments, the ranges for the pharmacokinetic characteristics discussed herein are obtained when the self-assembled polypeptide complex is administered via a systemic route, e.g., via intravenous or subcutaneous administration.

[0142] In some embodiments, a self-assembled polypeptide complex as disclosed herein has a similar half-life to that of reference IgG molecule. The reference IgG molecule may be, e.g., an antibody from which the antigen-binding antibody fragment within the second and/or third fusion polypeptide within the self-assembled polypeptide complex is derived. For example, if the antigen-binding fragment within the second and/or third fusion polypeptide comprises variable regions from "Antibody A," then the reference IgG molecule may, in some embodiments, be "Antibody A."

[0143] In some embodiments, after administration to a subject in need thereof, the self-assembled polypeptide complex has a half-life of between about 3 to 35 days, about 3 to about 28 days, about 3 to about 21 days, about 3 to about 14 days, about 3 to about 10 days, about 3 to about 7 days, about 3 to about 5 days, about 5 to about 35 days, about 5 to about 28 days, about 5 to about 21 days, about 5 to about 14 days, about 5 to about 10 days, about 5 to about 7 days, about 7 to about 35 days, about 7 to about 28 days, about 7 to about 21 days, about 7 to about 14 days, about 7 to about 10 days, about 10 to about 35 days, about 10 to about 28 days, about 10 to about 21 days, about 10 to about 14 days, about 14 to about 35 days, about 14 to about 28 days, about 14 to about 21 days, about 21 to about 35 days, or about 21 to about 28 days. In some embodiments, after administration to a subject in need thereof, the self-assembled polypeptide complex has a half-life of at least 3 days, at least 5 days, at least 7 days, at least 10 days, at least 14 days, at least 21 days, or at least 28 days. In some embodiments, after administration to a subject in need thereof, the self-assembled polypeptide complex is detectable in serum after at least 3 days, at least 5 days, at least 7 days, at least 10 days, at least 14 days, at least 21 days, or at least 28 days.

[0144] In some embodiments, a self-assembled polypeptide complex as disclosed herein has a similar bioavailability to that of reference IgG molecule, e.g., an antibody from which the Fab fragment comprised in the self-assembled polypeptide complex is derived. For example, in some embodiments, after administration to a subject in need thereof, the self-assembled polypeptide complex has an area-under-the-curve (AUC) of between about 10 to about 8000 day·μg/mL, about 10 to about 7000 day·μg/mL, about 10 to about 6000 day·μg/mL, about 10 to about 5000 day·μg/mL, about 10 to about 4000 day·μg/mL, about 10

to about 3000 day·μg/mL, about 10 to about 2500 day·μg/mL, about 10 to about 1000 day·μg/mL, about 10 to about 1500 day·μg/mL, about 10 to about 1000 day·μg/mL, about 10 to about 750 day·μg/mL, about 10 to about 500 day·μg/mL, about 10 to about 400 day·μg/mL, about 10 to about 300 day·μg/mL, about 10 to about 200 day·μg/mL, about 10 to about 100 day·μg/mL, about 10 to about 50 day·μg/mL, about 10 to about 25 day·μg/mL, about 25 to about 8000 day·μg/mL, about 25 to about 7000 day·μg/mL, about 25 to about 6000 day·μg/mL, about 25 to about 5000 day·μg/mL, about 25 to about 4000 day·μg/mL, about 25 to about 3000 day·μg/mL, about 25 to about 2500 day·μg/mL, about 25 to about 1000 day·μg/mL, about 25 to about 1500 day·μg/mL, about 25 to about 1000 day·μg/mL, about 25 to about 750 day·μg/mL, about 25 to about 500 day·μg/mL, about 25 to about 400 day·μg/mL, about 25 to about 300 day·μg/mL, about 25 to about 200 day·μg/mL, about 25 to about 100 day·μg/mL, about 25 to about 50 day·μg/mL, about 50 to about 8000 day·μg/mL, about 50 to about 7000 day·μg/mL, about 50 to about 6000 day·μg/mL, about 50 to about 5000 day·μg/mL, about 50 to about 4000 day·μg/mL, about 50 to about 3000 day·μg/mL, about 50 to about 2500 day·μg/mL, about 50 to about 2000 day·μg/mL, about 50 to about 1500 day·μg/mL, about 50 to about 1000 day·μg/mL, about 50 to about 750 day·μg/mL, about 50 to about 500 day·μg/mL, about 50 to about 400 day·μg/mL, about 50 to about 300 day·μg/mL, about 50 to about 200 day·μg/mL, about 50 to about 100 day·μg/mL, about 100 to about 8000 day·μg/mL, about 100 to about 7000 day·μg/mL, about 100 to about 6000 day·μg/mL, about 100 to about 5000 day·μg/mL, about 100 to about 4000 day·μg/mL, about 100 to about 3000 day·μg/mL, about 100 to about 2500 day·μg/mL, about 100 to about 1000 day·μg/mL, about 100 to about 1500 day·μg/mL, about 100 to about 1000 day·μg/mL, about 100 to about 750 day·μg/mL, about 100 to about 500 day·μg/mL, about 100 to about 400 day·μg/mL, about 100 to about 300 day·μg/mL, about 100 to about 200 day·μg/mL, about 200 to about 8000 day·μg/mL, about 200 to about 7000 day·μg/mL, about 200 to about 6000 day·μg/mL, about 200 to about 5000 day·μg/mL, about 200 to about 4000 day·μg/mL, about 200 to about 3000 day·μg/mL, about 200 to about 2000 day·μg/mL, about 200 to about 1000 day·μg/mL, about 200 to about 1500 day·μg/mL, about 200 to about 1000 day·μg/mL, about 200 to about 750 day·μg/mL, about 200 to about 500 day·μg/mL, about 200 to about 400 day·μg/mL, about 200 to about 300 day·μg/mL, about 300 to about 8000 day·μg/mL, about 300 to about 7000 day·μg/mL, about 300 to about 6000 day·μg/mL, about 300 to about 5000 day·μg/mL, about 300 to about 4000 day·μg/mL, about 300 to about 3000 day·μg/mL, about 300 to about 2500 day·μg/mL, about 300 to about 2000 day·μg/mL, about 300 to about 1500 day·μg/mL, about 300 to about 1000 day·μg/mL, about 300 to about 750 day·μg/mL, about

300 to about 500 day·μg/mL, about 300 to about 400 day·μg/mL, about 400 to about 8000 day·μg/mL, about 400 to about 7000 day·μg/mL, about 400 to about 6000 day·μg/mL, about 400 to about 5000 day·μg/mL, about 400 to about 4000 day·μg/mL, about 400 to about 3000 day·μg/mL, about 400 to about 2500 day·μg/mL, about 400 to about 2000 day·μg/mL, about 400 to about 1500 day·μg/mL, about 400 to about 1000 day·μg/mL, about 400 to about 750 day·μg/mL, about 400 to about 500 day·μg/mL, about 500 to about 8000 day·μg/mL, about 500 to about 7000 day·μg/mL, about 500 to about 6000 day·μg/mL, about 500 to about 5000 day·μg/mL, about 500 to about 4000 day·μg/mL, about 500 to about 3000 day·μg/mL, about 500 to about 2500 day·μg/mL, about 500 to about 2000 day·μg/mL, about 500 to about 1500 day·μg/mL, about 500 to about 1000 day·μg/mL, about 500 to about 750 day·μg/mL, about 750 to about 8000 day·μg/mL, about 750 to about 7000 day·μg/mL, about 750 to about 6000 day·μg/mL, about 750 to about 5000 day·μg/mL, about 750 to about 4000 day·μg/mL, about 750 to about 3000 day·μg/mL, about 750 to about 2500 day·μg/mL, about 750 to about 2000 day·μg/mL, about 750 to about 1500 day·μg/mL, about 750 to about 1000 day·μg/mL, about 1000 to about 8000 day·μg/mL, about 1000 to about 7000 day·μg/mL, about 1000 to about 6000 day·μg/mL, about 1000 to about 5000 day·μg/mL, about 1000 to about 4000 day·μg/mL, about 1000 to about 3000 day·μg/mL, about 1000 to about 2500 day·μg/mL, about 1000 to about 2000 day·μg/mL, about 1000 to about 1500 day·μg/mL, about 1500 to about 8000 day·μg/mL, about 1500 to about 7000 day·μg/mL, about 1500 to about 6000 day·μg/mL, about 1500 to about 5000 day·μg/mL, about 1500 to about 4000 day·μg/mL, about 1500 to about 3000 day·μg/mL, about 1500 to about 2500 day·μg/mL, about 1500 to about 2000 day·μg/mL, about 2000 to about 8000 day·μg/mL, about 2000 to about 7000 day·μg/mL, about 2000 to about 6000 day·μg/mL, about 2000 to about 5000 day·μg/mL, about 2000 to about 4000 day·μg/mL, about 2000 to about 3000 day·μg/mL, about 2000 to about 2500 day·μg/mL, about 2500 to about 8000 day·μg/mL, about 2500 to about 7000 day·μg/mL, about 2500 to about 6000 day·μg/mL, about 2500 to about 5000 day·μg/mL, about 2500 to about 4000 day·μg/mL, about 2500 to about 3000 day·μg/mL, about 3000 to about 8000 day·μg/mL, about 3000 to about 7000 day·μg/mL, about 3000 to about 6000 day·μg/mL, about 3000 to about 5000 day·μg/mL, about 3000 to about 4000 day·μg/mL, about 4000 to about 8000 day·μg/mL, about 4000 to about 7000 day·μg/mL, about 4000 to about 6000 day·μg/mL, about 4000 to about 5000 day·μg/mL, about 5000 to about 8000 day·μg/mL, about 5000 to about 7000 day·μg/mL, about 5000 to about 6000 day·μg/mL, about 6000 to about 8000 day·μg/mL, about 6000 to about 7000 day·μg/mL, or about 7000 to about 8000 day·μg/mL. In some embodiments, after administration to a subject in need

thereof, the self-assembled polypeptide complex has an AUC of at least 10 day·μg/mL, at least 25 day·μg/mL, at least 50 day·μg/mL, at least 100 day·μg/mL, at least 200 day·μg/mL, at least 300 day·μg/mL, at least 400 day·μg/mL, at least 500 day·μg/mL, at least 750 day·μg/mL, at least 1000 day·μg/mL, at least 1500 day·μg/mL, at least 2000 day·μg/mL, at least 2500 day·μg/mL, at least 3000 day·μg/mL, at least 4000 day·μg/mL, at least 5000 day·μg/mL, at least 6000 day·μg/mL, at least 7000 day·μg/mL, or at least 8000 day·μg/mL.

[0145] In some embodiments, a self-assembled polypeptide complex as disclosed herein has a similar bioavailability to that of reference IgG molecule. For example, in some embodiments, after administration to a subject in need thereof, the self-assembled polypeptide complex has a maximum concentration (C_{max}) of between about 10 μg/mL to about 750 mg/mL, about 25 μg/mL to about 750 mg/mL, about 50 μg/mL to about 750 mg/mL, about 75 μg/mL to about 750 mg/mL, about 100 μg/mL to about 750 mg/mL, about 250 μg/mL to about 750 mg/mL, about 500 μg/mL to about 750 mg/mL, about 750 μg/mL to about 750 mg/mL, about 1 mg/mL to about 750 mg/mL, about 10 mg/mL to about 750 mg/mL, about 25 mg/mL to about 750 mg/mL, about 50 mg/mL to about 750 mg/mL, about 75 mg/mL to about 750 mg/mL, about 100 mg/mL to about 750 mg/mL, about 250 mg/mL to about 750 mg/mL, about 500 mg/mL to about 750 mg/mL, about 10 μg/mL to about 500 mg/mL, about 25 μg/mL to about 500 mg/mL, about 50 μg/mL to about 500 mg/mL, about 75 μg/mL to about 500 mg/mL, about 100 μg/mL to about 500 mg/mL, about 250 μg/mL to about 500 mg/mL, about 500 μg/mL to about 500 mg/mL, about 750 μg/mL to about 500 mg/mL, about 1 mg/mL to about 500 mg/mL, about 10 mg/mL to about 500 mg/mL, about 25 mg/mL to about 500 mg/mL, about 50 mg/mL to about 500 mg/mL, about 75 mg/mL to about 500 mg/mL, about 100 mg/mL to about 500 mg/mL, about 250 mg/mL to about 500 mg/mL, about 10 μg/mL to about 250 mg/mL, about 25 μg/mL to about 250 mg/mL, about 50 μg/mL to about 250 mg/mL, about 75 μg/mL to about 250 mg/mL, about 100 μg/mL to about 250 mg/mL, about 250 μg/mL to about 250 mg/mL, about 500 μg/mL to about 250 mg/mL, about 750 μg/mL to about 250 mg/mL, about 1 mg/mL to about 250 mg/mL, about 10 mg/mL to about 250 mg/mL, about 25 mg/mL to about 250 mg/mL, about 50 mg/mL to about 250 mg/mL, about 75 mg/mL to about 250 mg/mL, about 100 mg/mL to about 250 mg/mL, about 10 μg/mL to about 100 mg/mL, about 25 μg/mL to about 100 mg/mL, about 50 μg/mL to about 100 mg/mL, about 75 μg/mL to about 100 mg/mL, about 100 μg/mL to about 100 mg/mL, about 250 μg/mL to about 100 mg/mL, about 500 μg/mL to about 100 mg/mL, about 750 μg/mL to about 100 mg/mL, about 1 mg/mL to about 100 mg/mL, about 10 mg/mL to about 100 mg/mL, about 25 mg/mL to about 100 mg/mL, about 50 mg/mL to

about 100 mg/mL, about 75 mg/mL to about 100 mg/mL, about 10 μg/mL to about 75 mg/mL, about 25 μg/mL to about 75 mg/mL, about 50 μg/mL to about 75 mg/mL, about 75 μg/mL to about 75 mg/mL, about 100 μg/mL to about 75 mg/mL, about 250 μg/mL to about 75 mg/mL, about 500 μg/mL to about 75 mg/mL, about 750 μg/mL to about 75 mg/mL, about 1 mg/mL to about 75 mg/mL, about 10 mg/mL to about 75 mg/mL, about 25 mg/mL to about 75 mg/mL, about 50 mg/mL to about 75 mg/mL, about 10 μg/mL to about 50 mg/mL, about 25 μg/mL to about 50 mg/mL, about 50 μg/mL to about 50 mg/mL, about 75 μg/mL to about 50 mg/mL, about 100 μg/mL to about 50 mg/mL, about 250 μg/mL to about 50 mg/mL, about 500 μg/mL to about 50 mg/mL, about 750 μg/mL to about 50 mg/mL, about 1 mg/mL to about 50 mg/mL, about 10 mg/mL to about 50 mg/mL, about 25 mg/mL to about 50 mg/mL, about 10 μg/mL to about 25 mg/mL, about 25 μg/mL to about 25 mg/mL, about 50 μg/mL to about 25 mg/mL, about 75 μg/mL to about 25 mg/mL, about 100 μg/mL to about 25 mg/mL, about 250 μg/mL to about 25 mg/mL, about 500 μg/mL to about 25 mg/mL, about 750 μg/mL to about 25 mg/mL, about 1 mg/mL to about 25 mg/mL, about 10 mg/mL to about 25 mg/mL, about 10 μg/mL to about 10 mg/mL, about 25 μg/mL to about 10 mg/mL, about 50 μg/mL to about 10 mg/mL, about 75 μg/mL to about 10 mg/mL, about 100 μg/mL to about 10 mg/mL, about 250 μg/mL to about 10 mg/mL, about 500 μg/mL to about 10 mg/mL, about 750 μg/mL to about 10 mg/mL, about 1 mg/mL to about 10 mg/mL, about 10 μg/mL to about 1 mg/mL, about 25 μg/mL to about 1 mg/mL, about 50 μg/mL to about 1 mg/mL, about 75 μg/mL to about 1 mg/mL, about 100 μg/mL to about 1 mg/mL, about 250 μg/mL to about 1 mg/mL, about 500 μg/mL to about 1 mg/mL, about 750 μg/mL to about 1 mg/mL, about 10 μg/mL to about 750 μg/mL, about 25 μg/mL to about 750 μg/mL, about 50 μg/mL to about 750 μg/mL, about 75 μg/mL to about 750 μg/mL, about 100 μg/mL to about 750 μg/mL, about 250 μg/mL to about 750 μg/mL, about 500 μg/mL to about 750 μg/mL, about 10 μg/mL to about 500 μg/mL, about 25 μg/mL to about 500 μg/mL, about 50 μg/mL to about 500 μg/mL, about 75 μg/mL to about 500 μg/mL, about 100 μg/mL to about 500 μg/mL, about 250 μg/mL to about 500 μg/mL, about 10 μg/mL to about 250 μg/mL, about 25 μg/mL to about 250 μg/mL, about 50 μg/mL to about 250 μg/mL, about 75 μg/mL to about 250 μg/mL, about 100 μg/mL to about 250 μg/mL, about 10 μg/mL to about 100 μg/mL, about 25 μg/mL to about 100 μg/mL, about 50 μg/mL to about 100 μg/mL, about 75 μg/mL to about 100 μg/mL, about 10 μg/mL to about 75 μg/mL, about 25 μg/mL to about 75 μg/mL, about 50 μg/mL to about 75 μg/mL, about 10 μg/mL to about 50 μg/mL, about 25 μg/mL to about 50 μg/mL, or about 10 μg/mL to about 25 μg/mL. In some embodiments, after administration to a subject in need thereof , the self-assembled polypeptide complex has

a maximum concentration (C_{max}) of at least 10 μg/mL, at least 25 μg/mL, at least 50 μg/mL, at least 100 μg/mL, at least 250 μg/mL, at least 500 μg/mL, at least 750 μg/mL, at least 1 mg/mL, at least 10 mg/mL, at least 25 mg/mL, at least 50 mg/mL, at least 75 mg/mL, at least 100 mg/mL, at least 250 mg/mL, at least 500 mg/mL, or at least 750 mg/mL when administered to a subject in need thereof.

Effects

[0146] In certain embodiments, a provided self-assembled polypeptide complex is capable of inducing multimerization (e.g., trimerization) of DR5 receptors on a target cell (e.g., a cancer cell).

[0147] In some embodiments, administration of a self-assembled polypeptide complex as disclosed herein to a subject results in an improvement in a clinical outcome or metric in the subject. For example, in a subject with a tumor, administration of the self-assembled polypeptide complex may inhibit or slow progression of the tumor, e.g., cause regression of the tumor. In some embodiments administration of the self-assembled polypeptide complex results in complete regression of the tumor.

C. Methods of treatment

[0148] In one aspect, provided are methods that may be useful for treating, ameliorating, or preventing a disease or a condition (e.g., an infectious disease, cancer, or an autoimmune disease), generally comprising a step of administering a composition comprising a self-assembled polypeptide complex of the present disclosure to a subject.

[0149] In some embodiments, the subject is a mammal, e.g., a human.

[0150] Compositions for administration to subjects generally comprise a self-assembled polypeptide complex as disclosed herein. In some embodiments, such compositions further comprise a pharmaceutically acceptable excipient.

[0151] Compositions may be formulated for administration for any of a variety of routes of administration, including systemic routes (e.g., oral, inhalation, intranasal, intravenous, intraperitoneal, subcutaneous, or intramuscular administration).

[0152] In some embodiments, the step of administering results in improvement in one or more clinical outcomes or metrics in the subject.

[0153] In some embodiments, the step of administering results in slowing or inhibiting progression of the tumor, e.g., regression of the tumor. In some embodiments, the step of administering results in complete regression of the tumor.

EXAMPLES

Example 1. Construction and expression of representative DR5-targeting Multabodies

[0154] This Example describes the generation of DR5-targeting Multabodies (MBs) comprising a combination of fusion proteins comprising a (1) human ferritin light chain or subunit thereof and (2) a single-chain Fab (scFab) or a single chain Fc-dimer (scFc), fused via a linker, such as a $(Gly_n\text{-}Ser)_m$ amino acid linker as described herein.

[0155] Genes encoding fusion proteins (1) scFab of an anti-DR5 antibody (in this case, conatumumab) fused to the N-terminus of hFTL (aDR5-hFTL, SEQ ID NO:9), (2) scFab of an anti-DR5 antibody fused to the N-terminus of an N-half ferritin (aDR5-N_hFTL, SEQ ID NO:11), and (3) scFc fused to the N-terminus of a C-half ferritin (various scFc-C_hFTL constructs, as described further below) were prepared, mixed at a molar ratio of 2:1:1 and transiently transfected into ExpiCHO-S cells for the production and formation of the DR5-targeting MBs. See **FIG. 1B**.

[0156] The scFc-ferritin fusion proteins comprised wild-type (WT) or engineered IgG1 Fc chains. The engineered IgG1 Fc chains contained various combinations of the L234A, L235A, G236R, G237A, P329G, and A330L mutations, as shown in Table 1 below. Numbering in Table 1 is according to the EU numbering scheme.

Table 1. Residues at certain positions within IgG1 Fc chains used in Multabodies and corresponding SEQ ID NOs of scFc-C_hFTL constructs

[0157] In rows corresponding to sets of engineered Fc chains, residues are only shown at positions that differ from the wild type residue.

Amino acid position →	**234**	**235**	**236**	**237**	**329**	**330**	**SEQ ID NO: of corresponding scFc-C_hFTL construct**
WT (wild type)	**L**	**L**	**G**	**G**	**P**	**A**	SEQ ID NO:13
LALA	A	A					SEQ ID NO:14
LALAP	A	A			G		SEQ ID NO:15
LLRAL	A	A	R	A		L	SEQ ID NO:16
LLGRAL	A	A	R	A	G	L	SEQ ID NO:17

Example 2. Target binding of Multabodies determined by biolayer interferometry

[0158] The binding kinetics and affinity of exemplary MBs generated as described in Example 1 to recombinant human DR5 (hDR5), cynomolgus DR5 (cDR5), mouse DR5 (mDR5), and rat DR5 (rDR5) were determined by biolayer interferometry (BLI) using an Octet RED96 instrument. Binding characteristics were determined for (1) conatumumab, a fully human monoclonal IgG1 antibody that binds to DR5, and (2) Cona MB IgG1 LLRAL, a Multabody that includes fusion polypeptides that include a scFc with the "LLRAL" mutations described in Table 1, and which also includes fusion polypeptides that comprise a scFab derived from conatumumab.

[0159] Briefly, Ni-NTA biosensors were coated with hDR5-His, cDR5-His, mDR5-His, or rDR5-His (extracellular domain of hDR5, cDR5, mDR5, or rDR5 with a C-terminal polyhistidine tag) to reach a signal response of 0.8 nm. The coated biosensors were dipped into wells containing serial dilutions of the test MBs (20-10-5-2.5-1.25-0.63 nM) in PBS-0.02%T-0.01%BSA (PBS supplemented with 0.02% (v/v) Tween 20 and 0.01% (w/v) BSA) for 180 s (association phase) and then into PBS-0.02%T-0.01%BSA for 180 s (dissociation phase). All measurements were performed at 30 °C in PBS-0.02%T-0.01%BSA, pH 7.4, with shaking speed 1000 rpm and monitored in real time. Biosensors were regenerated between experiments by applying 10 mM glycine, pH 1.7, for 5 s for four times, followed by recharging with 10 mM $NiSO_4$ for 1 min.

[0160] Target binding was evaluated based on the maximal association binding response at the end of the association phase, the dissociation rate (k_{off}), and/or the equilibrium dissociation constant (K_D) calculated using a 1:1 fitting model. A maximal association binding response of less than 0.1 nm when test MB at 20 nM was classified as "non-binding."

[0161] The values determined for k_{on}, k_{off}, and the resulting K_D for the test molecules are summarized in Table 2. Cona MB IgG1 LLRAL bound to human and cynomolgus DR5 with approximately picomolar affinity or less and showed no binding to mouse or rat DR5. (The instrument has detection limit of 1 picomolor.) These observations are consistent with conatumumab's ("Cona") binding profile.

Table 2. Kinetic constants and affinities to target binding of Multabodies determined by BLI

Test article	hDR5			cDR5			mDR5	rDR5
	k_{on} [M^{-1} x s^{-1}]	k_{off} [s^{-1}]	K_D [M]	k_{on} [M^{-1} x s^{-1}]	k_{off} [s^{-1}]	K_D [M]		
Cona MB IgG1 LLRAL	1.4×10^{6}	$<1.0 \times 10^{-7}$	$<1.0 \times 10^{-12}$	1.5×10^{6}	$<1.0 \times 10^{-7}$	$<1.0 \times 10^{-12}$	Non-binding	Non-binding
Cona	2.4×10^{5}	2.2×10^{-5}	8.9×10^{-11}	ND	ND	ND		

Example 3. Binding specificity of Multabodies determined by biolayer interferometry

[0162] BLI was employed to assess the non-specific binding of exemplary DR-targeting MBs to related tumor necrosis factor receptor superfamily (TNFRSF) members.

[0163] The experiments were performed similarly as described in Example 2, except that His-tagged extracellular domains of human DR4, human osteoprotegerin (OPG), human decoy receptor 1 (DcR1), or human DcR2—hDR4-His, hOPG-His, hDcR1-His, or hDcR2-His were coated onto Ni-NTA biosensors and titrated with test MBs at various concentrations.

[0164] FIGs. 2A, 2B, 2C, 2D, and 2E show representative examples of relevant segments of the resulting sensorgrams. Cona MB IgG1 LLRAL bound DR5 with high specificity and showed no binding to other tested TNFRSF members.

Example 4. Binding of Multabodies to Fc receptors determined by biolayer interferometry

[0165] The binding kinetics and affinities of various DR5-targeting MBs (each comprising different sets of Fc mutations and generated as described in Example 1) to various Fc receptors were determined by BLI.

[0166] All MBs tested in this Example contained polypeptides comprising scFabs derived from conatumumab, which binds to DR5. These DR5-targeting MBs ("Cona MB") also included polypeptides having scFcs with the either wild type Fc chains (IgG1 wt) or Fc chains with a certain combination of mutations (IgG1 LLRAL). (See Table 1)

[0167] Binding to the following human, cynomolgus monkey, and mouse Fc receptors were determined: human Fc gamma receptor type I (hFcγRI), hFcγRIIa, hFcγRIIb, hFcγRIIIa, hFcγRIIIb, human neonatal Fc receptor (hFcRn), cynomolgus monkey FcγRI (cFcγRI), cFcγRIIa, cFcγRIIb, cFcγRIII, cFcRn, mouse FcγRI (mFcγRI), mFcγRIIb, mFcγRIII, mFcγRIV, and mFcRn.

[0168] Experiments were performed similarly as described in Example 2, except that His-tagged Fc receptors were coated onto Ni-NTA biosensors and titrated with test MBs at various concentrations. To assess the potential of the MBs to undergo endosomal recycling, binding to FcRn was measured at pH 6.0 for association and pH 7.4 for dissociation.

[0169] Table 3 summarizes the k_{on}, k_{off}, and K_D values determined for the MBs binding to various FcγRs. Cona MB with wild type IgG1 Fc (IgG1 WT) bound to human, cynomolgus monkey, and mouse FcγRs. Cona MB with IgG1 LLRAL showed diminished binding to human, cynomolgus monkey, and mouse FcγRs.

[0170] **FIG. 3** shows representative examples of relevant segments of resulting sensorgrams from the FcRn binding studies. All tested MBs bound to human, cynomolgus monkey, and mouse FcRns at pH 6.0 and dissociated from the receptors at pH 7.4. Cona MB IgG1 LLRAL dissociated from human or cynomolgus monkey FcRn at pH 7.4 at a comparable rate to Cona MB IgG1 WT.

Table 3. Kinetic constants and affinities to FcγRs of Multabodies determined by BLI

		Cona MB IgG1 WT	Cona MB IgG1 LLRAL
hFcγRI	k_{on} [M^{-1} x s^{-1}]	5.67×10^{5}	Non-binding
	k_{off} [s^{-1}]	$<1.0 \times 10^{-7}$	
	K_D [M]	$<1.0 \times 10^{-12}$	
hFcγRIIa	k_{on} [M^{-1} x s^{-1}]	1.34×10^{6}	Non-binding
	k_{off} [s^{-1}]	$<1.0 \times 10^{-7}$	
	K_D [M]	$<1.0 \times 10^{-12}$	
hFcγRIIb	k_{on} [M^{-1} x s^{-1}]	1.47×10^{6}	Non-binding
	k_{off} [s^{-1}]	$<1.0 \times 10^{-7}$	
	K_D [M]	4.81×10^{-12}	
hFcγRIIIa	k_{on} [M^{-1} x s^{-1}]	1.32×10^{6}	Non-binding
	k_{off} [s^{-1}]	$<1.0 \times 10^{-7}$	
	K_D [M]	$<1.0 \times 10^{-12}$	
hFcγRIIIb	k_{on} [M^{-1} x s^{-1}]	1.57×10^{6}	Non-binding
	k_{off} [s^{-1}]	8.44×10^{-5}	
	K_D [M]	5.37×10^{-11}	
cFcγRI	k_{on} [M^{-1} x s^{-1}]	4.72×10^{5}	Non-binding
	k_{off} [s^{-1}]	$<1.0 \times 10^{-7}$	
	K_D [M]	$<1.0 \times 10^{-12}$	
cFcγRIIa	k_{on} [M^{-1} x s^{-1}]	1.01×10^{6}	Non-binding
	k_{off} [s^{-1}]	2.01×10^{-4}	
	K_D [M]	1.99×10^{-10}	
cFcγRIIb	k_{on} [M^{-1} x s^{-1}]	1.07×10^{6}	Non-binding

	k_{off} [s^{-1}]	1.79×10^{-4}	
	K_D [M]	1.67×10^{-10}	
cFcγRIII	k_{on} [$M^{-1} \times s^{-1}$]	3.22×10^{5}	Non-binding
	k_{off} [s^{-1}]	$<1.0 \times 10^{-7}$	
	K_D [M]	$<1.0 \times 10^{-12}$	
mFcγRI	k_{on} [$M^{-1} \times s^{-1}$]	7.46×10^{5}	Non-binding
	k_{off} [s^{-1}]	$<1.0 \times 10^{-7}$	
	K_D [M]	$<1.0 \times 10^{-12}$	
mFcγRIIb	k_{on} [$M^{-1} \times s^{-1}$]	8.89×10^{5}	Non-binding
	k_{off} [s^{-1}]	2.98×10^{-4}	
	K_D [M]	3.35×10^{-10}	
mFcγRIII	k_{on} [$M^{-1} \times s^{-1}$]	7.04×10^{5}	Non-binding
	k_{off} [s^{-1}]	4.90×10^{-4}	
	K_D [M]	6.96×10^{-10}	
mFcγRIV	k_{on} [$M^{-1} \times s^{-1}$]	3.56×10^{5}	Non-binding
	k_{off} [s^{-1}]	$<1.0 \times 10^{-7}$	
	K_D [M]	$<1.0 \times 10^{-12}$	

Example 5. Assessment of *in vitro* tumor cell cytotoxicity

[0171] The cytotoxicity of exemplary DR5-targeting MBs (generated as described in Example 1) was evaluated using different tumor cell lines. Cona MB IgG1 wt and Cona MB IgG1 LLRAL were tested on COLO 205 (human colon carcinoma), HCT-15 (human colon carcinoma), NCI-H2122 (human lung carcinoma), SNU-5 (human gastric carcinoma), Capan-1 (human pancreatic carcinoma), MDA-MB-231 (invasive ductal carcinoma), BxPC-3 (human pancreatic carcinoma), and NCI-H2228 (human lung carcinoma) cell lines.

[0172] Tumor cell lines were seeded at 5000 cells per well in a 96-well plate and incubated overnight to facilitate attachment. The next day, cells were treated with serial dilutions of DR5-targeting MBs or anti-DR5 (conatumumab) and incubated for 24 h at 37 °C. Cell viability was measured using the CellTiter-Glo Luminescence Cell Viability Assay (Promega), according to the manufacturer's instruction. Briefly, assay reagent (100 µL) was added to 100 µL cells at room temperature, mixed using a plate shaker at 500 rpm for 2 min, and incubated at room temperature for 10 min. Luminescent signals were read using the Synergy Neo2 Multi-Mode Assay Microplate Reader (BioTek Instruments).

[0173] **FIG. 4** shows results from these experiments. In FIG. 4, the percentage of viable cells (compared to vehicle-treated tumor cells) was plotted on the y-axis versus the test molecule concentration on the x-axis. The data were fitted using Prism 9.1.2 software (GraphPad) with nonlinear regression (log inhibitor vs. response, variable slope, 4

parameters). Table 4 provides the resulting IC_{50} values. Both of the tested DR5-targeting MBs (Cona MB IgG1 wt and Cona MB IgG1 LLRAL) were capable of inducing cytotoxicity of human cancer cells lines. On the other hand, conatumumab—an IgG1 antibody—did not induce, or very poorly induced, tumor cell cytotoxicity.

Table 4. Assessment of tumor cell cytotoxicity

		Cona	Cona MB IgG1 wt	Cona MB IgG1 LLRAL
IC_{50} [pM]	**COLO 205**	> 33000	4.06	2.38
	HCT-15	> 33000	13.58	9.53
	NCI-2122	> 33000	4.99	5.86
	SNU-5	> 33000	11.14	9.51
	Capan-1	> 33000	13.73	15.7
	MDA-MB-231	> 33000	34.17	23.96
	BxPC-3	> 33000	54.64	50.2
	NCI-H2228	> 33000	79.67	77.15

Example 6. Pharmacokinetics of Multabodies in mice

[0174] The pharmacokinetics (PK) of exemplary DR5-targeting MBs generated as described in Example 1 (Cona MB IgG1 LLRAL and conatumumab) were analyzed in 8-week old female BALB/c SCID mice (Jackson Labs).

[0175] For single-dose PK studies, mice received a single bolus injection of 200 µL test molecule at 5 mg/kg intraperitoneally (i.p.) or intravenously (i.v.) (N=8 mice per group, N=4 mice per timepoint). 50-100 µL blood samples were collected at the timepoints of 3 h, 24 h, 48 h, 72 h, 5 days, 7 days, 15 days, and 21 days.

[0176] For multi-dose PK studies, mice were injected with 200 µL test molecule at 5 mg/kg on day 9, followed by a second injection of 200 µL test molecule at 5 mg/kg 96 h after. 50-100 µL blood samples were collected 3 h, 24 h, 48 h, 72 h, and 96 h after the first dose and 3 h, 24 h, 48 h, 72 h, 5 days, 7 days, and 14 days after the second dose.

[0177] Blood samples were collected from the saphenous vein into heparin-coated tubes and finger-vortexed to ensure mixing. Subsequently, the samples were centrifuged at 1500 x g at 4 °C for 15 min, and the plasma samples were collected and immediately stored at -80 °C until use in ELISA assay.

[0178] To measure plasma drug level with ELISA, recombinant DR5 at 2 µg/mL in PBS was coated onto Maxisorp plates (Fisher Scientific) overnight at 4 °C. After washing twice

with PBS-0.05%T (PBS supplemented with 0.05% (v/v) Tween-20), the plate was blocked with 3% (w/v) BSA in PBS for 1 h at room temperature and subsequently washed twice with PBS-0.05%T. Plasma samples were diluted in PBS-0.05%T-0.5%BSA (PBS supplemented with 0.05% (v/v) Tween-20 and 0.5% (w/v) BSA), added to wells, and incubated for 1 h at room temperature and a shaking frequency of 500 rpm, followed by another wash step with PBS-0.05%T. Bound molecules were detected by incubation with 1:10000 diluted goat polyclonal anti-human Fc-HRP secondary antibody (Jackson Immunoresearch). After a further wash step with PBS-0.05%T, OptEIA™ TMB Substrate Reagent Set (BD Biosciences) was used for detection following the manufacturer's instruction, and the absorbances at 450 nm were read using the Synergy Neo2 Multi-Mode Assay Microplate Reader (BioTek Instruments). A calibration curve was prepared using the dilutions of test molecule in PBS-0.05%T-0.5%BSA.

[0179] **FIG. 5A** is a schematic illustrating the design of these pharmacokinetic studies.

[0180] **FIG. 5B** shows plots of the plasma concentration over time in a single-dose PK study for Cona MB IgG1 wt, Cona MB IgG1 LLRAL, and conatumumab ("Cona"). Table 5 presents a summary of calculated half-lives. Cona MB IgG1 LLRAL showed significantly enhanced PK properties compared to Cona MB IgG1 wt. For the first 7 days, the plasma concentrations of Cona MB IgG1 LLRAL remained comparable to that of conatumumab. The Cona MB IgG1 LLRAL plasma concentration on day 15 was 125x the IC_{50} value (~ 0.04 µg/mL) determined in an *in vitro* cytotoxicity assay (see Example 5) and on day 21, 40x the IC_{50} value.

[0181] **FIG. 5C** shows plots of plasma concentration over time in a multi-dose PK study for Cona MB IgG1 LLRAL and conatumumab.

Table 5. Half-lives in mice after a single dose

Half-life [days]	**Cona i.v.**	**Cona MB IgG1 LLRAL i.v.**	**Cona MB IgG1 wt i.v.**
3 h – 24 h	3.5	1.0	0.9
24 h - 5 days	5.7	5.2	1.5
5 days - 15 days	14.8	3.1	2.2

Example 7. Therapeutic effect of DR5-targeting Multabodies in a xenograft mouse model

[0182] The therapeutic effect of exemplary Multabodies was evaluated in a colon cancer xenograft model. **FIG. 6A** shows a schematic illustrating the study design of the *in vivo* efficacy study.

[0183] 5 x 10^6 COLO 205 cells were injected subcutaneously in the flank of BALB/c SCID mice (n = 12 per group). Tumors were allowed to grow to an average size of 200 mm^3. Mice were sorted into treatment or control groups such that the mean tumor volumes were equal across groups (**FIG. 6B**). Mice received treatment (5 mg/kg per dose) or control via intraperitoneal (i.p.) injection once or twice weekly for two weeks (**FIG. 6A**). Tumor volume was measured twice weekly using calipers. Blood samples were collected 24 h, 7 days, 14 days and 21 days following the initial dose, and the drug levels in plasma were measured as described in Example 6.

[0184] **FIGs. 6B-6G** are plots that depict tumor volumes of mice in various groups. The plots in **FIGs. 6B and 6D-6G** show tumor volumes over time, and the plot in **FIG. 6C** shows the tumor volume at day 88 after treatment initiation. Treatment with Cona MB IgG1 LLRAL once or twice weekly significantly inhibited the growth of large established tumors, suggesting that Cona MB IgG1 LLRAL was able to penetrate tumors even of large size. Cona MB IgG1 LLRAL inhibited tumor growth more strongly than conatumumab did, resulting in 9/12 and 11/12 complete regressions in the once weekly-treated and twice weekly-treated group, respectfully. This compares to only 1/12 complete remissions in the conatumumab-treated group.

[0185] **FIG. 6H** is a plot showing the concentrations of test molecules in plasma (y-axis) as a function of the time after the first dose (x-axis). The pharmacokinetic profile of Cona MB IgG1 LLRAL as observed in the COLO205 colon cancer mouse model is similar to that observed in the multi-dose PK study described in Example 6. (See **FIG. 5B**). These results suggest that the presence of tumor does not impact the clearance of the Multabody.

[0186] These experiments were repeated in a second study with the same study design, using vehicle, conatumumab, or Cona MB IgG1 LLRAL once weekly for two weeks, at 5 mg/kg per dose. Results from this second study were similar with those from the first study: no tumor regression was observed in the vehicle group, none of the ten mice in the conatumumab group exhibited complete tumor regression, and all ten mice in the Cona MB IgG1 LLRAL group showed complete tumor regression (data not shown). Results from this

second study indicate that the therapeutic effect of Cona MB IgG1 LLRAL is highly reproducible with once weekly dosing.

Example 8. Dose range efficacy of DR5-targeting Multabodies in a xenograft mouse model

[0187] The dose range efficacy of Cona IgG1 MB LLRAL was also explored in the same colon cancer xenograft model used in Example 6.

[0188] 5 x 10^6 COLO 205 cells were injected subcutaneously in the flank of Balb/c SCID mice. Tumors were allowed to grow to an average size of 200 mm^3. Mice were sorted into one of five groups (vehicle or Cona MB IgG1 LLRAL at 0.1 mg/kg, 0.25 mg/kg, 1 mg/kg, or 5 mg/kg per dose) such that the mean tumor volumes were equal across the groups. Mice received treatment or vehicle via intraperitoneal (i.p.) injection once weekly for three weeks. Tumor volume was measured twice weekly using calipers. Blood samples were obtained throughout the experiment.

[0189] **FIG. 7A** is a plot that depicts tumor volumes of mice in various groups over time following the first dose. "MB" indicates the Cona MB IgG1 LLRAL group. **FIGs. 7B-7F** show the tumor volumes of individual mice within the vehicle (**FIG. 7B**) and 5 mg/kg, 1 mg/kg, 0.25 mg/kg, and 0.1 mg/kg Cona MB IgG1 LLRAL treatment (**FIG. 7C, FIG. 7D, FIG. 7E,** and **FIG. 7F,** respectively) groups. As shown in **FIG. 7A**, mice treated with Cona MB IgG1 LLRAL at 0.25 mg/kg, 1 mg/kg/ and 5 mg/kg doses showed significant improvement over the vehicle group. Cona MB IgG1 LLRAL appeared to have similar efficacy at doses of 1 mg/kg and 5 mg/kg initially. However, the 1 mg/kg showed fewer complete responses and more regrowth than did the 5 mg/kg treatment group, in which complete regression was seen in twelve out of twelve mice.

[0190] **FIG. 7G** is a plot that depicts the amount of Cona MB IgG1 LLRAL detectable in blood samples collected at various timepoints after the first dose was administered. Cona MB IgG1 LLRAL was detectable in the plasma at all time points tested in the 0.1 mg/kg, 0.25 mg/kg, 1 mg/kg/ and 5 mg/kg Cona MB IgG1 LLRAL treatment groups, with the pharmacokinetics appearing linear in all dose groups.

[0191] These results demonstrate that Cona MB IgG1 LLRAL exhibited therapeutic efficacy at multiple doses tested, including comparable efficacy at a dose level that was five-fold lower than that tested in Example 6. However, whereas complete tumor regression was

observed at the 5 mg/kg dose level, some tumor regrowth was observed when the dose level was lowered to 1 mg/kg.

Example 9. Penetration of large tumors and induction of apoptosis by a DR5-targeting Multabody

[0192] Exemplary Multabodies generated as described in Example 1 were evaluated for their ability to penetrate large tumors and induce apoptosis in a colon xenograft model.

[0193] 5 x 10^6 COLO 205 cells were injected subcutaneously in the flank of Balb/c SCID mice. Tumors were allowed to grow to an average size of 500 mm^3. Tumor volume was measured twice weekly using calipers. Mice were randomly assigned to a vehicle or treatment groups such that the average tumor size was the same across the two groups, about 500 mm^3 in each group. Mice were then administered one dose of vehicle or 5 mg/kg Cona MB IgG1 LLRAL by intraperitoneal (i.p.) injection. Blood samples were collected 24 hours after injection of vehicle or Cona MB IgG1 LLRAL, and tumors were harvested for subsequent histological analyses. **FIG. 8** depicts a schematic and timeline for these experiments.

[0194] Tumors were fixed in formalin, embedded in paraffin, and sectioned. Tumor sections were stained immunohistochemically for cleaved caspase-3, a marker of apoptosis. Representative images of stained tissue sections are shown in **FIGs. 9A and 9C** (vehicle-treated mice) and **FIGs. 9B and 9D** (Cona MB IgG1 LLRAL-treated mice). As shown in these figures, few apoptotic cells were detected in tumor sections from vehicle-treated mice. In contrast, tissue sections from Cona MB IgG1 LLRAL-treated mice contained a large proportion of apoptotic cells throughout the tumor including deep within the core of the tumor, away from the tumor margin (see stained cells in FIGs. 9B and 9D).

[0195] These results confirm that Cona MB IgG1 LLRAL induced apoptosis of tumor cells throughout the tumor. Moreover, these results confirm that Multabodies are able to penetrate into the core of large established tumors.

SEQUENCE LISTING

Underlining within fusion sequences indicate linker sequences.

Bolding within fusion sequences indicate ferritin or ferritin subunit sequences.

Within variable region sequences, underlining and bolding together indicate complementary determining regions sequences.
Boxed and bolded residues indicate residues that are mutated relative to a reference molecule, e.g. relative to an IgG1 Fc.

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SEQ ID NO:16 scFc-C_hFTL IgG1 LLRAL

DKTHTCPPCPAPEAARAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV
EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPLPIEKTISKAKGQPR
EPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL
YSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGGGGSGGGGSGGGGSG
GGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSDKTHTCPPCPAPE
AARAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ
YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPLPIEKTISKAKGQPREPQVYTLPPSREE

MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ
GNVFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGGGGSGGGGSGGGGSGGGGSGG**GKTPDAM**
KAAMALEKKLNQALLDLHALGSARTDPHLCDFLETHFLDEEVKLIKKMGDHLTNLHRLGGPE
AGLGEYLFERLTLRHD

SEQ ID NO:17 scFc-C_hFTL IgG1 LLGRAL

DKTHTCPPCPAPE**AARA**PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV
EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL**GL**PIEKTISKAKGQPR
EPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL
YSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGGGGSGGGGSGGGGSG
GGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSDKTHTCPPCPAPE
AARAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ
YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL**GL**PIEKTISKAKGQPREPQVYTLPPSREE
MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ
GNVFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGGGGSGGGGSGGGGSGGGGSGG**GKTPDAM**
KAAMALEKKLNQALLDLHALGSARTDPHLCDFLETHFLDEEVKLIKKMGDHLTNLHRLGGPE
AGLGEYLFERLTLRHD

SEQ ID NO:18 human DR5 (GenBank accession no. AAB67109.1)

MEQRGQNAPA ASGARKRHGP GPREARGARP GPRVPKTLVL VVAAVLLLVS
AESALITQQD LAPQQRAAPQ QKRSSPSEGL CPPGHHISED GRDCISCKYG
QDYSTHWNDL LFCLRCTRCD SGEVELSPCT TTRNTVCQCE EGTFREEDSP
EMCRKCRTGC PRGMVKVGDC TPWSDIECVH KESGIIIGVT VAAVVLIVAV
FVCKSLLWKK VLPYLKGICS GGGGDPERVD RSSQRPGAED NVLNEIVSIL
QPTQVPEQEM EVQEPAEPTG VNMLSPGESE HLLEPAEAER SQRRRLLVPA
NEGDPTETLR QCFDDFADLV PFDSWEPLMR KLGLMDNEIK VAKAEAAGHR
DTLYTMLIKW VNKTGRDASV HTLLDALETL GERLAKQKIE DHLLSSGKFM
YLEGNADSAM S

SEQ ID NO:19 human DR5 (GenBank accession no. AAB71412.1)

MEQRGQNAPA ASGARKRHGP GPREARGARP GLRVPKTLVL VVAAVLLLVS
AESALITQQD LAPQQRVAPQ QKRSSPSEGL CPPGHHISED GRDCISCKYG
QDYSTHWNDL LFCLRCTRCD SGEVELSPCT TTRNTVCQCE EGTFREEDSP
EMCRKCRTGC PRGMVKVGDC TPWSDIECVH KESGIIIGVT VAAVVLIVAV
FVCKSLLWKK VLPYLKGICS GGGGDPERVD RSSQRPGAED NVLNEIVSIL
QPTQVPEQEM EVQEPAEPTG VNMLSPGESE HLLEPAEAER SQRRRLLVPA

NEGDPTETLR QCFDDFADLV PFDSWEPLMR KLGLMDNEIK VAKAEAAGHR
DTLYTMLIKW VNKTGRDASV HTLLDALETL GERLAKQKIE DHLLSSGKFM
YLEGNADSAM S

SEQ ID NO:20 human DR5 (GenBank accession no. AAC01565.1)

MEQRGQNAPA ASGARKRHGP GPREARGARP GLRVPKTLVL VVAAVLLLVS
AESALITQQD LAPQQRVAPQ QKRSSPSEGL CPPGHHISED GRDCISCKYG
QDYSTHWNDL LFCLRCTRCD SGEVELSPCT TTRNTVCQCE EGTFREEDSP
EMCRKCRTGC PRGMVKVGDC TPWSDIECVH KESGIIIGVT VAAVVLIVAV
FVCKSLLWKK VLPYLKGICS GGGGDPERVD RSSQRPGAED NVLNEIVSIL
QPTQVPEQEM EVQEPAEPTG VNMLSPGESE HLLEPAEAER SQRRRLLVPA
NEGDPTETLR QCFDDFADLV PFDSWEPLMR KLGLMDNEIK VAKAEAAGHR
DTLYTMLIKW VNKTGRDASV HTLLDALETL GERLAKQKIE DHLLSSGKFM
YLEGNADSAM S

SEQ ID NO:21 human DR5 (GenBank accession no. AAB67103.1)

MEQRGQNAPA ASGARKRHGP GPREARGARP GLRVPKTLVL VVAAVLLLVS
AESALITQQD LAPQQRAAPQ QKRSSPSEGL CPPGHHISED GRDCISCKYG
QDYSTHWNDL LFCLRCTRCD SGEVELSPCT TTRNTVCQCE EGTFREEDSP
EMCRKCRTGC PRGMVKVGDC TPWSDIECVH KESGIIIGVT VAAVVLIVAV
FVCKSLLWKK VLPYLKGICS GGGGDPERVD RSSQRPGAED NVLNEIVSIL
QPTQVPEQEM EVQEPAEPTG VNMLSPGESE HLLEPAEAER SQRRRLLVPA
NEGDPTETLR QCFDDFADLV PFDSWEPLMR KLGLMDNEIK VAKAEAAGHR
DTLYTMLIKW VNKTGRDASV HTLLDALETL GERLAKQKIE DHLLSSGKFM
YLEGNADSAL S

SEQ ID NO:22 V_L from Conatumumab

EIVLTQSPGTLSLSPGERATLSC**RASQGISRSYLA**WYQQKPGQAPSLLIY**GASSRAT**GIPDR
FSGSGSGTDFTLTISRLEPEDFAVYYC**QQFGSSPWT**FGQGTKVEIK

SEQ ID NO:23 V_H from Conatumumab

QVQLQESGPGLVKPSQTLSLTCTVS**GGSISSGDYFWS**WIRQLPGKGLEWIG**HIHNSGTTYYN**
PSLKSRVTISVDTSKKQFSLRLSSVTAADTAVYYCAR**DRGGDYYYGMDV**WGQGTTVTVSS

SEQ ID NO:24 CDR-L1 from Conatumumab

RASQGISRSYLA

SEQ ID NO:25 CDR-L2 from Conatumumab

GASSRAT

SEQ ID NO:26 CDR-L3 from Conatumumab

QQFGSSPWT

SEQ ID NO:27 CDR-H1 from Conatumumab

GGSISSGDYFWS

SEQ ID NO:28 CDR-H2 from Conatumumab

HIHNSGTTYYNPSLKS

SEQ ID NO:29 CDR-H3 from Conatumumab

DRGGDYYYGMDV

SEQ ID NO:30 V_L from Tigatuzumab

DIQMTQSPSSLSASVGDRVTITC**KASQDVGTAVA**WYQQKPGKAPKLLIY**WASTRHT**GVPSRF
SGSGSGTDFTLTISSLQPEDFATYYC**QQYSSYRT**FGQGTKVEIK

SEQ ID NO:31 V_H from Tigatuzumab

VQLVESGGGLVQPGGSLRLSCAAS**GFTFSSYVMS**WVRQAPGKGLEWVA**TISSGGSYTYYPDS**
VKGRFTISRDNAKNTLYLQMNSLRAEDTAVYYCAR**RGDSMITTDY**WGQGTLVTVSS

SEQ ID NO:32 CDR-L1 from Tigatuzumab

KASQDVGTAVA

SEQ ID NO:33 CDR-L2 from Tigatuzumab

WASTRHT

SEQ ID NO:34 CDR-L3 from Tigatuzumab

QQYSSYRT

SEQ ID NO:35 CDR-H1 from Tigatuzumab

GFTFSSYVMS

SEQ ID NO:36 CDR-H2 from Tigatuzumab

TISSGGSYTYYPDSVKG

SEQ ID NO:37 CDR-H3 from Tigatuzumab

RGDSMITTDY

SEQ ID NO:38 V_L from Drozitumab

SELTQDPAVSVALGQTVRITC**SGDSLRSYYAS**WYQQKPGQAPVLVIY**GANNRPS**GIPDRFSG
SSSGNTASLTITGAQAEDEADYYC**NSADSSGNHVV**FGGGTKLTVL

SEQ ID NO:39 V_H from Drozitumab

EVQLVQSGGGVERPGGSLRLSCAAS**GFTFDDYAMS**WVRQAPGKGLEWVSG**INWQGGSTGYAD**
SVKGRVTISRDNAKNSLYLQMNSLRAEDTAVYYCAK**ILGAGRGWYFDY**WGKGTTVTVSS

SEQ ID NO:40 CDR-L1 from Drozitumab

SGDSLRSYYAS

SEQ ID NO:41 CDR-L2 from Drozitumab

GANNRPS

SEQ ID NO:42 CDR-L3 from Drozitumab

NSADSSGNHVV

SEQ ID NO:43 CDR-H1 from Drozitumab

GFTFDDYAMS

SEQ ID NO:44 CDR-H2 from Drozitumab

INWQGGSTGYADSVKG

SEQ ID NO:45 CDR-H3 from Drozitumab

ILGAGRGWYFDY

SEQ ID NO:46 V_L from Lexatumumab

SELTQDPAVSVALGQTVRITC**QGDSLRSYYAS**WYQQKPGQAPVLVIY**GKNNRPS**GIPDRFSG
SSSGNTASLTITGAQAEDEADYYC**NSRDSSGNHVV**FGGGTKLTVL

SEQ ID NO:47 V_H from Lexatumumab

EVQLVQSGGGVERPGGSLRLSCAAS**GFTFDDYGMS**WVRQAPGKGLEWVSG**INWNGGSTGYAD**
SVKGRVTISRDNAKNSLYLQMNSLRAEDTAVYYCAK**ILGAGRGWYFDL**WGKGTTVTVSS

SEQ ID NO:48 CDR-L1 from Lexatumumab

QGDSLRSYYAS

SEQ ID NO:49 CDR-L2 from Lexatumumab

GKNNRPS

SEQ ID NO:50 CDR-L3 from Lexatumumab

NSRDSSGNHVV

SEQ ID NO:51 CDR-H1 from Lexatumumab

GFTFDDYGMS

SEQ ID NO:52 CDR-H2 from Lexatumumab

INWNGGSTGYADSVKG

SEQ ID NO:53 CDR-H3 from Lexatumumab

ILGAGRGWYFDL

SEQ ID NO:54 GS linker sequence

GGGGS

SEQ ID NO:55 GS linker sequence

GGGS

EQUIVALENTS / OTHER EMBODIMENTS

[0196] While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure that come within known or customary practice within the art to which the invention pertains and may be applied to the essential features herein before set forth.

CLAIMS

1. A self-assembled polypeptide complex comprising

(a) a plurality of first fusion polypeptides, each first fusion polypeptide comprising (1) an Fc polypeptide and (2) a nanocage monomer or subunit thereof, and

(b) a plurality of second fusion polypeptides, each second fusion polypeptide comprising (1) an antibody fragment that is capable of binding to DR5 and (2) a nanocage monomer or subunit thereof,

wherein the Fc polypeptide comprises an IgG1 Fc chain, wherein said IgG1 chain comprises (1) an amino acid residue other than glycine at position 237; and (2) a proline residue at position 329, according to EU numbering.

2. The self-assembled polypeptide complex of claim 1, wherein the nanocage monomer or subunit thereof in each first fusion polypeptide and in each second fusion polypeptide is a ferritin monomer or subunit thereof.

3. The self-assembled polypeptide complex of claim 2, wherein the ferritin monomer or subunit thereof is a ferritin light chain or subunit thereof.

4. The self-assembled polypeptide complex of claim 2 or 3, wherein the ferritin monomer or subunit thereof is a human ferritin or subunit thereof.

5. The self-assembled polypeptide complex of any one of claims 2-4, which does not comprise any ferritin heavy chains or subunits of ferritin heavy chains.

6. The self-assembled polypeptide complex of any one of claims 2-5, wherein the ferritin monomer or subunit thereof is a ferritin monomer subunit.

7. The self-assembled polypeptide complex of claim 6, wherein

a. each first fusion polypeptide comprises a C-half-ferritin, and each second fusion polypeptide comprises an N-half-ferritin; or

b. each first fusion polypeptide comprises an N-half ferritin, and each second fusion polypeptide comprises a C-half-ferritin.

8. The self-assembled polypeptide complex of claim 7, wherein each first fusion polypeptide comprises an Fc polypeptide linked to the C-half ferritin's N-terminus via an amino acid linker.

9. The self-assembled polypeptide complex of claim 8, wherein the amino acid linker comprises a $(G_nS)_m$ linker.

10. The self-assembled polypeptide complex of claim 9, wherein the $(G_nS)_m$ linker is a $(GGGGS)_m$ (SEQ ID NO:54) linker.

11. The self-assembled polypeptide complex of any one of claims 1-10, wherein the Fc polypeptide comprises a single chain Fc (scFc) comprising two Fc chains, wherein the two Fc chains are linked via an amino acid linker.

12. The self-assembled polypeptide complex of claim 11, wherein the amino acid linker that links the two Fc chains comprises a $(G_nS)_m$ linker.

13. The self-assembled polypeptide complex of claim 12, wherein the $(G_nS)_m$ linker is a $(GGGGS)_m$ (SEQ ID NO:54) linker.

14. The self-assembled polypeptide complex of any one of claims 1-13, wherein the Fc polypeptide comprises an IgG1 Fc chain.

15. The self-assembled polypeptide complex of claim 14, wherein the IgG1 Fc chain comprises an alanine at position 237 according to EU numbering.

16. The self-assembled polypeptide complex of claim 14 or 15, wherein the IgG1 Fc chain comprises: an alanine at position 234, an alanine at position 235, an arginine at position 236, and a leucine at position 330, according to EU numbering.

17. The self-assembled polypeptide complex of any one of claims 1-16, wherein, within each second fusion polypeptide, the antigen-binding antibody fragment is linked to the N-terminus of the nanocage monomer or subunit thereof.

18. The self-assembled polypeptide complex of any one of claims 1-17, wherein the antigen-binding antibody fragment of each second fusion polypeptide is a Fab fragment.

19. The self-assembled polypeptide complex of any one of claims 1-18, wherein each second fusion polypeptide does not comprise any antibody CH2 or CH3 domains.

20. The self-assembled polypeptide complex of any one of claims 1-19, further comprising a plurality of third fusion polypeptides, each third fusion polypeptide comprising (1) an antigen-binding antibody fragment and (2) a nanocage monomer or a subunit thereof, wherein the third fusion polypeptide is different than the second fusion polypeptide.

21. The self-assembled polypeptide complex of claim 20, wherein the antigen-binding antibody fragment of each third fusion polypeptide is a Fab fragment.

22. The self-assembled polypeptide complex of claim 21, wherein each third fusion polypeptide does not comprise any antibody CH2 or CH3 domains.

23. The self-assembled polypeptide complex of any one of claims 1-22, wherein the nanocage monomer or subunit thereof of each first fusion polypeptide and each second fusion polypeptide is a ferritin monomer subunit, and

a. each first fusion polypeptide comprises a C-half-ferritin, and each second fusion polypeptide comprises a N-half-ferritin; or
b. each first fusion polypeptide comprises an N-half ferritin, and each second fusion polypeptide comprises a C-half-ferritin.

24. The self-assembled polypeptide complex of any one of claims 1-23, wherein the self-assembled polypeptide complex is characterized by a 1:1 ratio of first fusion polypeptides to second fusion polypeptides.

25. The self-assembled polypeptide complex of any one of claims 1-24, comprising a total of 24 to 48 fusion polypeptides.

26. The self-assembled polypeptide complex of any one of claims 1-25, comprising a total of least 24 fusion polypeptides.

27. The self-assembled polypeptide complex of claim 26, comprising a total of at least 32 fusion polypeptides.

28. The self-assembled polypeptide complex of claim 27, having a total of about 32 fusion polypeptides.

29. The self-assembled polypeptide complex of any one of claims 1-28, characterized in that, after administration of a composition comprising the self-assembled polypeptide complex, concentrations of the self-assembled polypeptide complex are substantially similar to those of a reference IgG molecule administered by the same route of administration and in a similar composition during the first 7 days after administration to a subject in need thereof.

30. The self-assembled polypeptide complex of any one of claims 1-29, which exhibits no binding to at least one human Fcγ receptor, as determined in an *in vitro* assay.

31. The self-assembled polypeptide complex of claim 30, which exhibits no binding to one or more human Fcγ receptors selected from the group consisting of hFcγRI, hFcγRIIa, hFcγRIIb, hFcγRIIIa, hFcγRIIIb, and combinations thereof, as determined in an *in vitro* assay.

32. The self-assembled polypeptide complex of claim 31, which exhibits no binding to hFcγRI, as determined in an *in vitro* assay.

33. The self-assembled polypeptide complex of claim 31 or 32, which exhibits no binding to hFcγRIIa, as determined in an *in vitro* assay.

34. The self-assembled polypeptide complex of claim 31, 32, or 33, which exhibits no binding to hFcγRIIIa, as determined in an *in vitro* assay.

35. The self-assembled polypeptide complex of any one of claims 31-34, which exhibits no binding to hFcγRIIb, as determined in an *in vitro* assay.

36. The self-assembled polypeptide complex of any one of claims 31-36 which exhibits no binding to hFcγRIIIb, as determined in an *in vitro* assay.

37. A self-assembled polypeptide complex comprising:

(a) a plurality of first fusion polypeptides, each first fusion polypeptide comprising (1) a scFc and (2) a ferritin monomer or subunit thereof, and

(b) a plurality of second fusion polypeptides, each second fusion polypeptide comprising (1) an antibody fragment that is capable of binding to DR5 and (2) a ferritin monomer or subunit thereof, wherein the scFc comprises two IgG1 Fc chains, each IgG1 Fc chain comprising: an alanine at position 234, an alanine at position 235, an arginine at position 236, an alanine at position 237, a proline at position 329, and a leucine at position 330, according to EU numbering.

38. The self-assembled polypeptide complex of any 37, wherein:

a. each first fusion polypeptide comprises a C-half-ferritin, and each second fusion polypeptide comprises an N-half-ferritin; or
b. each first fusion polypeptide comprises an N-half ferritin, and each second fusion polypeptide comprises a C-half-ferritin.

39. The self-assembled polypeptide complex of claim 38, wherein each first fusion polypeptide comprises a scFc linked to the C-half-ferritin's N-terminus, and each second fusion polypeptide comprises a Fab linked to the N-half-ferritin's N-terminus.

40. The self-assembled polypeptide complex of claim 39, wherein, (1) in each first fusion polypeptide, the scFc is linked via an amino acid linker to the C-half ferritin's N-terminus and/or (2) in each second fusion polypeptide, the Fab is linked via an amino acid linker to N-half ferritin's N-terminus.

41. The self-assembled polypeptide complex of any one of claims 38-40, wherein the self- assembled polypeptide complex is characterized by a 1:1 ratio of first fusion polypeptides to second fusion polypeptides.

42. The self-assembled polypeptide complex of any one of claims 37-41, further comprising a plurality of third fusion polypeptides, each third fusion polypeptide comprising (1) an antigen-binding antibody fragment and (2) a nanocage monomer or a subunit thereof, wherein the third fusion polypeptide is different than the second fusion polypeptide.

43. The self-assembled polypeptide complex of any one of claims 37-42, comprising a total of 24 to 48 fusion polypeptides.

44. The self-assembled polypeptide complex of any one of claims 37-43, comprising a total of least 24 fusion polypeptides.

45. The self-assembled polypeptide complex of claim 44, comprising a total of at least 32 fusion polypeptides.

46. The self-assembled polypeptide complex of claim 45, having a total of about 32 fusion polypeptides.

47. The self-assembled polypeptide complex of any one of claims 1-46, wherein the antibody fragment comprises (1) a heavy chain comprising CDR-H1, CDR-H2, and CDR-H3 and (2) a light chain comprising CDR-L1, CDR-L2, and CDR-L3, wherein

(a) (i) the CDR-H1 has a sequence of SEQ ID NO:27 or a sequence differing by one or two amino acids therefrom;

(ii) the CDR-H2 has a sequence of SEQ ID NO:28 or a sequence differing by one or two amino acids therefrom;

(iii) the CDR-H3 has a sequence of SEQ ID NO:29 or a sequence differing by one or two amino acids therefrom;

(iv) the CDR-L1 has a sequence of SEQ ID NO:24 or a sequence differing by one or two amino acids therefrom;

(v) the CDR-L2 has a sequence of SEQ ID NO:25 or a sequence differing by one or two amino acids therefrom; and

(vi) the CDR-L3 has a sequence of SEQ ID NO:26 or a sequence differing by one or two amino acids therefrom;

(b) (i) the CDR-H1 has a sequence of SEQ ID NO:35 or a sequence differing by one or two amino acids therefrom;

(ii) the CDR-H2 has a sequence of SEQ ID NO:36 or a sequence differing by one or two amino acids therefrom;

(iii) the CDR-H3 has a sequence of SEQ ID NO:37 or a sequence differing by one or two amino acids therefrom;

(iv) the CDR-L1 has a sequence of SEQ ID NO:32 or a sequence differing by one or two amino acids therefrom;

(v) the CDR-L2 has a sequence of SEQ ID NO:33 or a sequence differing by one or two amino acids therefrom; and

(vi) the CDR-L3 has a sequence of SEQ ID NO:34 or a sequence differing by one or two amino acids therefrom;

(c) (i) the CDR-H1 has a sequence of SEQ ID NO:43 or a sequence differing by one or two amino acids therefrom;

(ii) the CDR-H2 has a sequence of SEQ ID NO:44 or a sequence differing by one or two amino acids therefrom;

(iii) the CDR-H3 has a sequence of SEQ ID NO:45 or a sequence differing by one or two amino acids therefrom;

(iv) the CDR-L1 has a sequence of SEQ ID NO:40 or a sequence differing by one or two amino acids therefrom;

(v) the CDR-L2 has a sequence of SEQ ID NO:41 or a sequence differing by one or two amino acids therefrom; and

(vi) the CDR-L3 has a sequence of SEQ ID NO:42 or a sequence differing by one or two amino acids therefrom;

or

(d) (i) the CDR-H1 has a sequence of SEQ ID NO:51 or a sequence differing by one or two amino acids therefrom;

(ii) the CDR-H2 has a sequence of SEQ ID NO:52 or a sequence differing by one or two amino acids therefrom;

(iii) the CDR-H3 has a sequence of SEQ ID NO:53 or a sequence differing by one or two amino acids therefrom;

(iv) the CDR-L1 has a sequence of SEQ ID NO:48 or a sequence differing by one or two amino acids therefrom;

(v) the CDR-L2 has a sequence of SEQ ID NO:49 or a sequence differing by one or two amino acids therefrom; and

(vi) the CDR-L3 has a sequence of SEQ ID NO:50 or a sequence differing by one or two amino acids therefrom.

48. The self-assembled polypeptide complex of claim 47, wherein the antibody fragment comprises (1) a heavy chain comprising CDR-H1, CDR-H2, and CDR-H3 and (2) a light chain comprising CDR-L1, CDR-L2, and CDR-L3, wherein

(a) (i) the CDR-H1 has a sequence of SEQ ID NO:27,
(ii) the CDR-H2 has a sequence of SEQ ID NO:28,
(iii) the CDR-H3 has a sequence of SEQ ID NO:29,
(iv) the CDR-L1 has a sequence of SEQ ID NO:24,
(v) the CDR-L2 has a sequence of SEQ ID NO:25, and
(vi) the CDR-L3 has a sequence of SEQ ID NO:26;

(b) (i) the CDR-H1 has a sequence of SEQ ID NO:35,
(ii) the CDR-H2 has a sequence of SEQ ID NO:36,
(iii) the CDR-H3 has a sequence of SEQ ID NO:37,
(iv) the CDR-L1 has a sequence of SEQ ID NO:32,
(v) the CDR-L2 has a sequence of SEQ ID NO:33, and
(vi) the CDR-L3 has a sequence of SEQ ID NO:34;

(c) (i) the CDR-H1 has a sequence of SEQ ID NO:43,
(ii) the CDR-H2 has a sequence of SEQ ID NO:44,
(iii) the CDR-H3 has a sequence of SEQ ID NO:45,
(iv) the CDR-L1 has a sequence of SEQ ID NO:40,
(v) the CDR-L2 has a sequence of SEQ ID NO:41, and
(vi) the CDR-L3 has a sequence of SEQ ID NO:42;

or

(d) (i) the CDR-H1 has a sequence of SEQ ID NO:51,
(ii) the CDR-H2 has a sequence of SEQ ID NO:52,
(iii) the CDR-H3 has a sequence of SEQ ID NO:53,
(iv) the CDR-L1 has a sequence of SEQ ID NO:48,
(v) the CDR-L2 has a sequence of SEQ ID NO:49, and
(vi) the CDR-L3 has a sequence of SEQ ID NO:50.

49. The self-assembled polypeptide complex of any one of claims 1-48, wherein the antibody fragment comprises

(1) a heavy chain variable region having at least 85% identical to a reference V_H sequence and

(2) a light chain variable region having at least 85% identical to a reference V_L sequence, wherein:

(a) the reference V_H sequence has a sequence of SEQ ID NO:23 and the reference V_L sequence has a sequence of SEQ ID NO:22;

(b) the reference V_H sequence has a sequence of SEQ ID NO:31 and the reference V_L sequence has a sequence of SEQ ID NO:30;

(c) the reference V_H sequence has a sequence of SEQ ID NO:39 and the reference V_L sequence has a sequence of SEQ ID NO:38; or

(d) the reference V_H sequence has a sequence of SEQ ID NO:47 and the reference V_L sequence has a sequence of SEQ ID NO:46.

50. A method comprising administering a composition comprising the self-assembled polypeptide complex of any one of claims 1-49 to a mammalian subject.

51. The method of claim 50, wherein the subject is human.

52. The method of claim 50 or 51, wherein the subject is diagnosed as having, or is at risk of developing, a tumor at the time of administering.

53. The method of claim 52, wherein said step of administering results in slowing or inhibiting progression of the tumor.

54. The method of claim 53, wherein said step of administering results in regression of the tumor.

55. The method of claim 54, wherein said step of administering results in complete regression of the tumor.

56. The method of any one of claims 50 to 55, comprising administration by a systemic route.

57. The method of claim 56, wherein the systemic route comprises subcutaneous, intravenous, or intramuscular injection, inhalation, or intranasal administration.

58. Use of the composition comprising the self-assembled polypeptide complex of any one of claims 1-49 for administration to a mammalian subject.

59. The use of claim 58, wherein the subject is human.

60. The use of claim 58 or 59, wherein the subject is diagnosed as having, or is at risk of developing, a tumor at the time of administration.

61. The use of claim 60, for slowing or inhibiting progression of the tumor.

62. The use of claim 60 or 61, for causing regression of the tumor.

63. The use of claim 62, for causing complete regression of the tumor.

64. The use of any one of claims 58 to 63, for systemic administration.

65. The use of claim 64, wherein systemic administration comprises subcutaneous, intravenous, or intramuscular injection, inhalation, or intranasal administration.

66. The composition comprising the self-assembled polypeptide complex of any one of claims 1-49 for use in administration to a mammalian subject.

67. The composition for use of claim 66, wherein the subject is human.

68. The composition for use of claim 66 or 67, wherein the subject is diagnosed as having, or is at risk of developing, a tumor at the time of administration.

69. The composition for use of claim 68, for slowing or inhibiting progression of the tumor.

70. The composition for use of claim 68 or 69, for causing regression of the tumor.

71. The composition for use of claim 70, for causing complete regression of the tumor.

72. The composition for use of any one of claims 66 to 71, for systemic administration.

73. The composition for use of claim 72, wherein systemic administration comprises subcutaneous, intravenous, or intramuscular injection, inhalation, or intranasal administration.

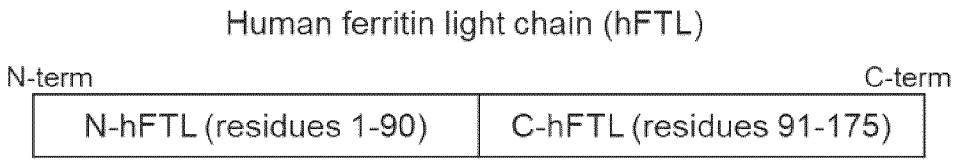

Human ferritin light chain (hFTL)
N-term
C-term
N-hFTL (residues 1-90)
C-hFTL (residues 91-175)

FIG. 1A

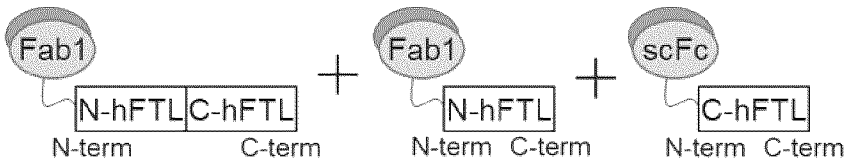

Fab1
N-hFTL
C-hFTL
N-term
C-term
Fab1
N-hFTL
N-term
C-term
scFc
C-hFTL
N-term
C-term

FIG. 1B

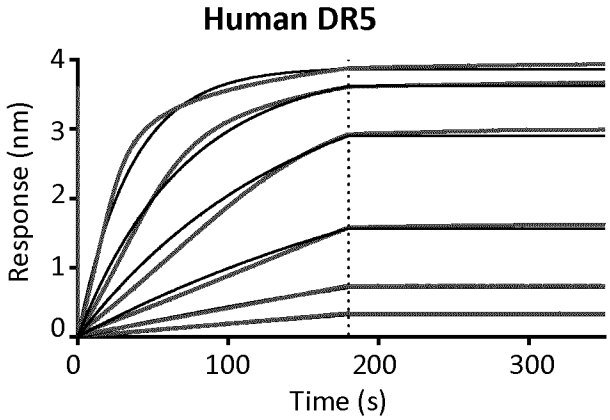


FIG. 2A

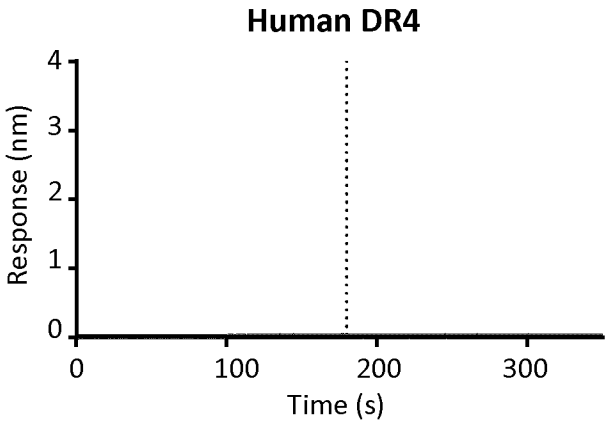


FIG. 2B

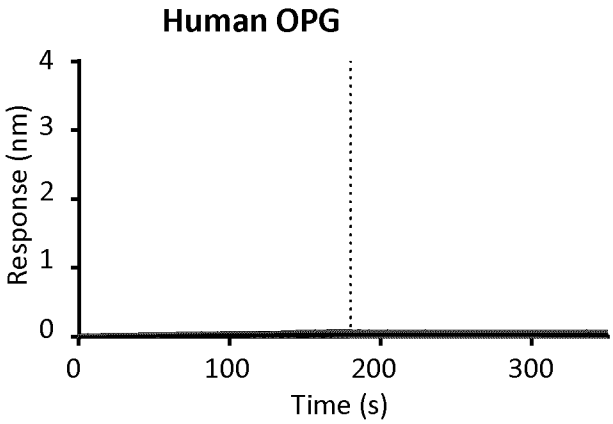


FIG. 2C

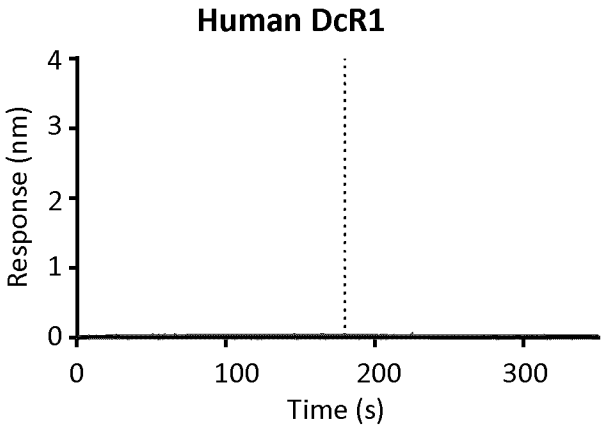


FIG. 2D

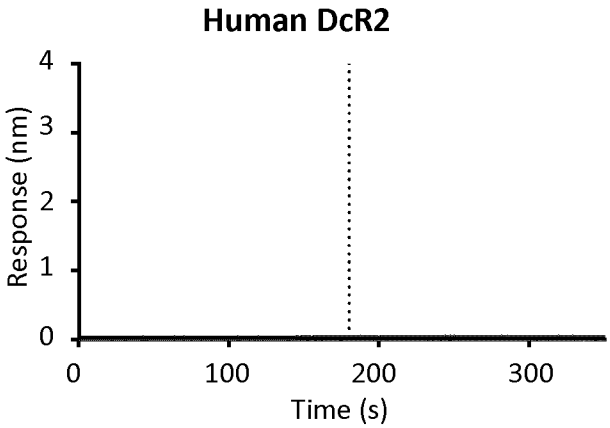


FIG. 2E

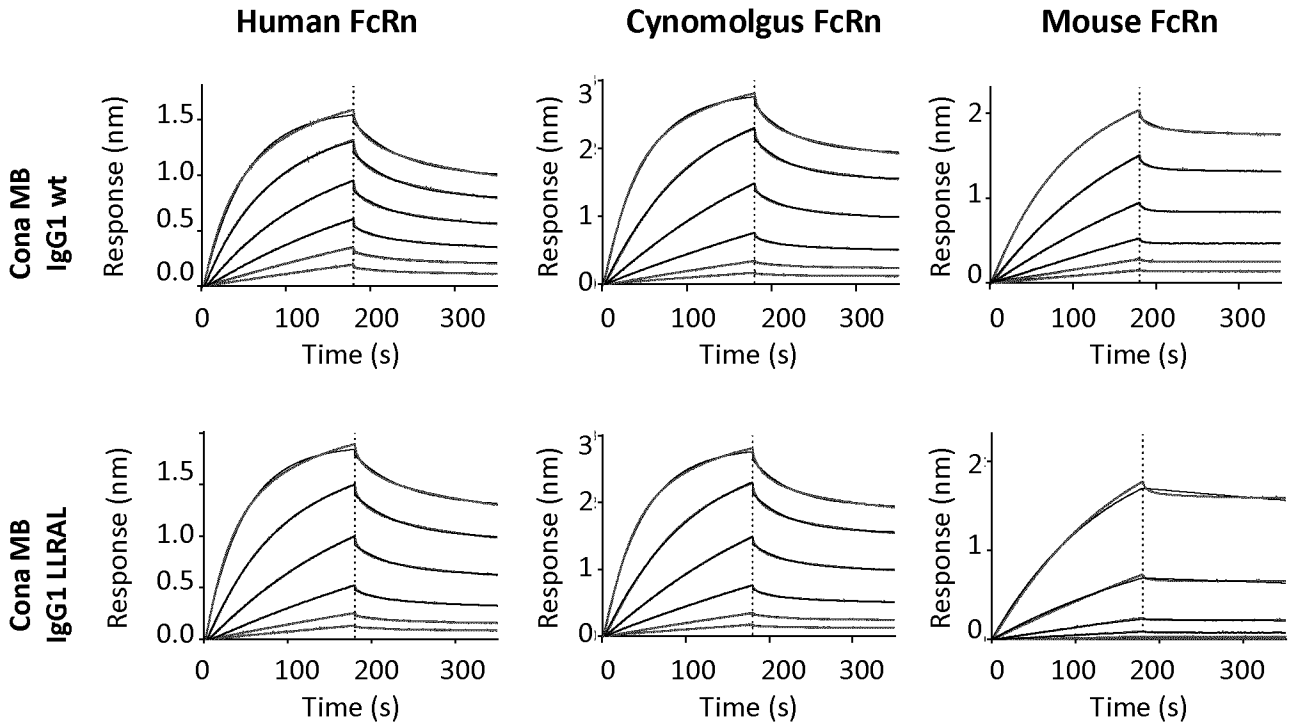

Human FcRn
Cynomolgus FcRn
Mouse FcRn
Cona MB IgG1 wt
Cona MB IgG1 LLRAL
Response (nm)
Time (s)
0
100
200
300
0.0
0.5
1.0
1.5
1
2
3

FIG. 3

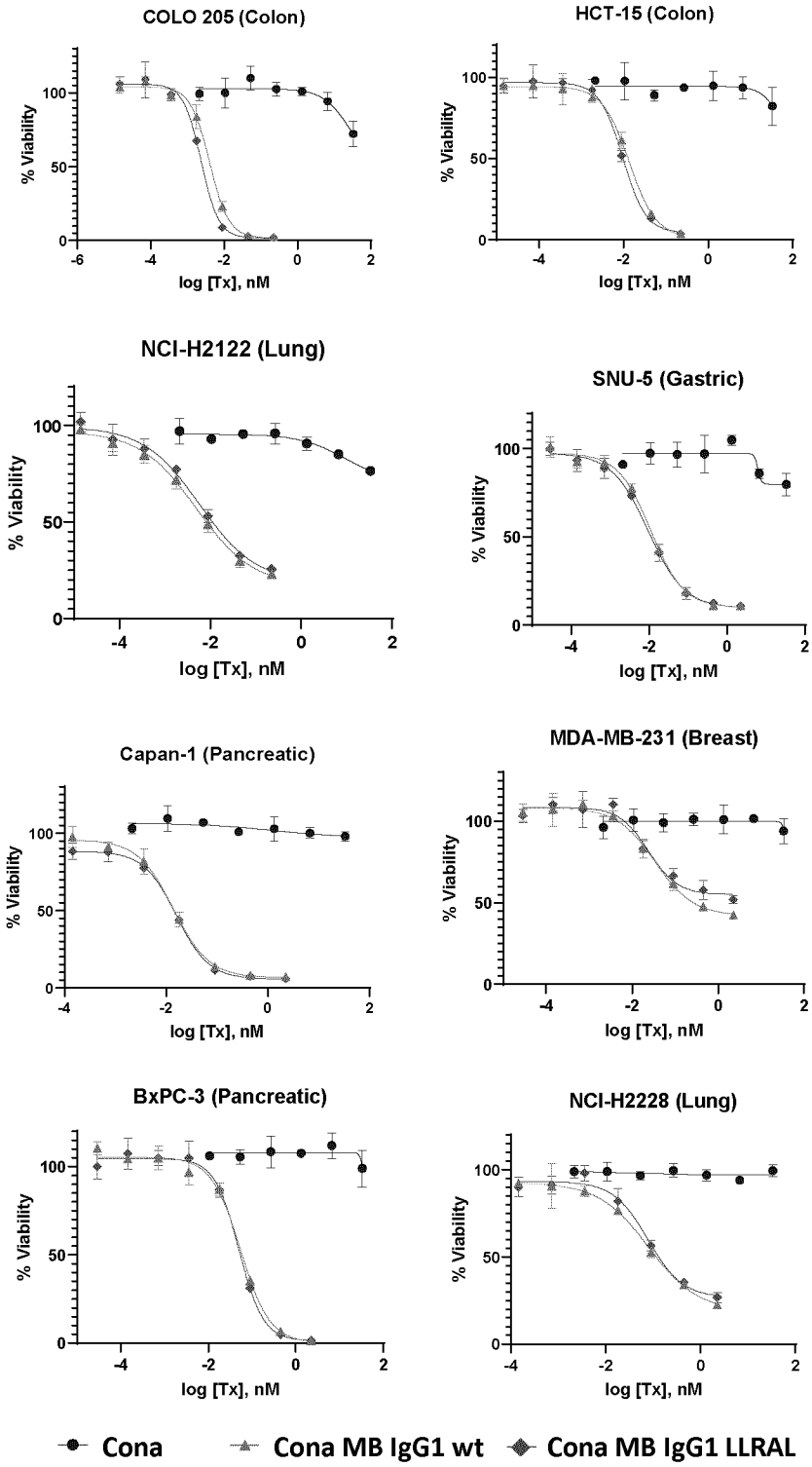
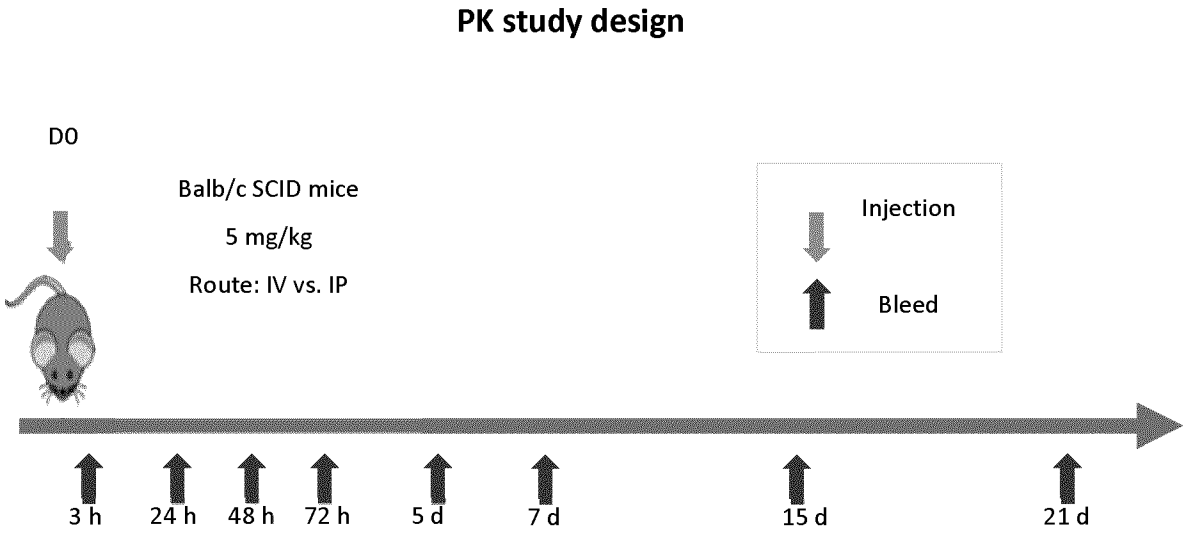

COLO 205 (Colon)
HCT-15 (Colon)
NCI-H2122 (Lung)
SNU-5 (Gastric)
Capan-1 (Pancreatic)
MDA-MB-231 (Breast)
BxPC-3 (Pancreatic)
NCI-H2228 (Lung)
% Viability
log [Tx], nM
Cona
Cona MB IgG1 wt
Cona MB IgG1 LLRAL

FIG. 4


PK study design
D0
Balb/c SCID mice
5 mg/kg
Route: IV vs. IP
Injection
Bleed
3 h
24 h
48 h
72 h
5 d
7 d
15 d
21 d

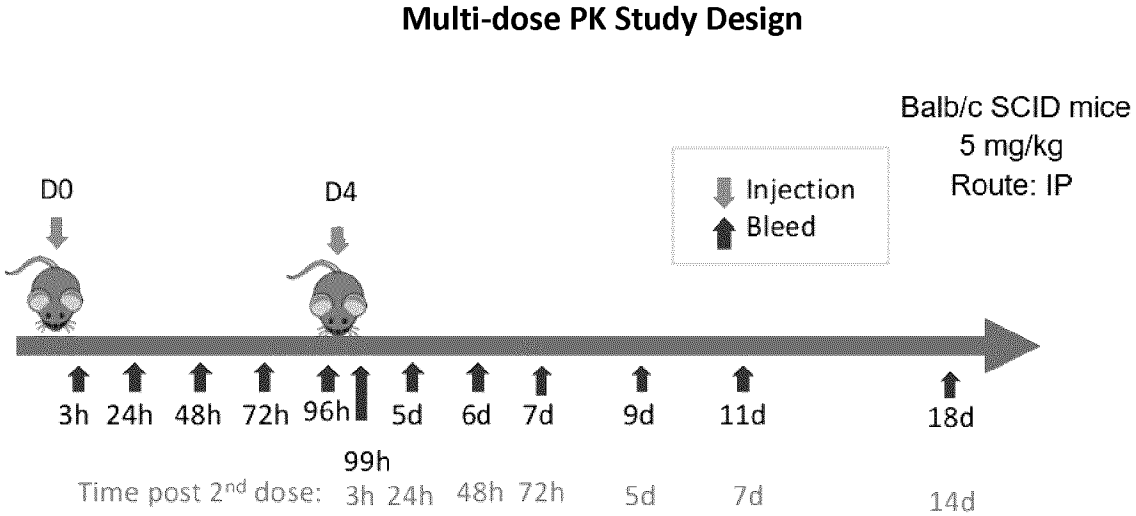
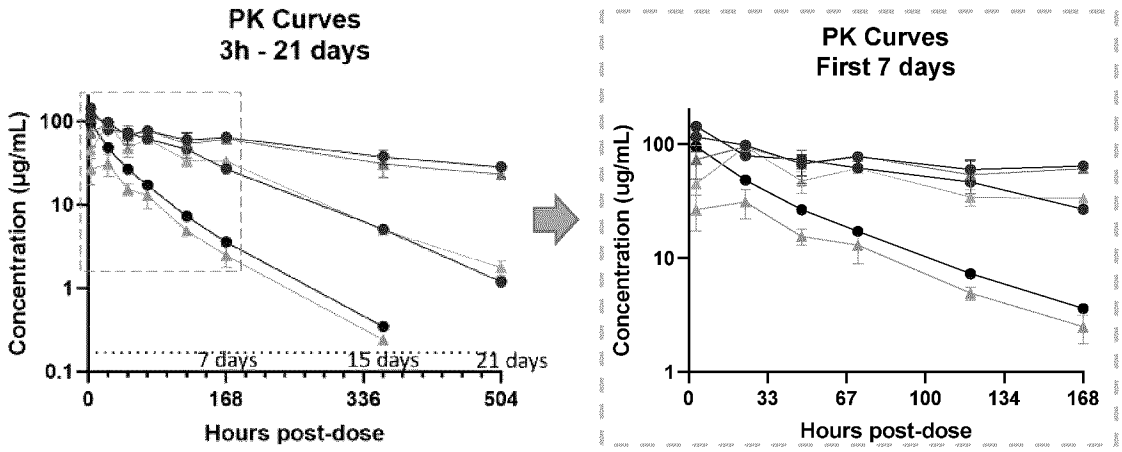

Multi-dose PK Study Design
Balb/c SCID mice
5 mg/kg
Route: IP
D0
D4
Injection
Bleed
3h
24h
48h
72h
96h
99h
5d
6d
7d
9d
11d
18d
Time post 2nd dose:
3h
24h
48h
72h
5d
7d
14d

FIG. 5A


PK Curves
3h - 21 days
Concentration (µg/mL)
100
10
1
0.1
7 days
15 days
21 days
0
168
336
504
Hours post-dose
PK Curves
First 7 days
Concentration (ug/mL)
100
10
1
0
33
67
100
134
168
Hours post-dose

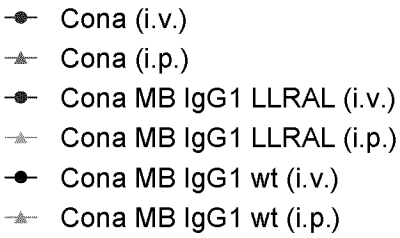

Cona (i.v.)
Cona (i.p.)
Cona MB IgG1 LLRAL (i.v.)
Cona MB IgG1 LLRAL (i.p.)
Cona MB IgG1 wt (i.v.)
Cona MB IgG1 wt (i.p.)

FIG. 5B

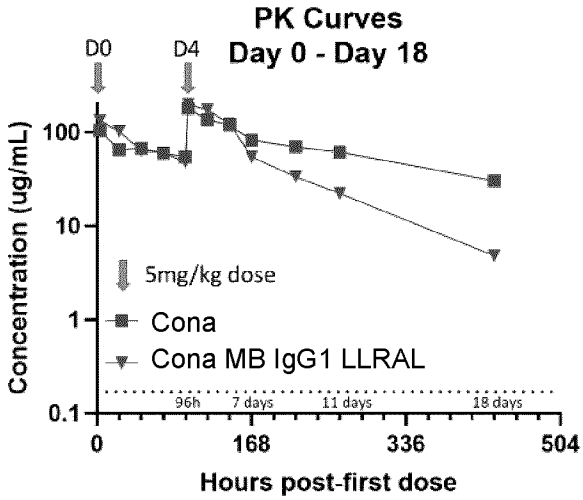

PK Curves
Day 0 - Day 18
D0
D4
Concentration (ug/mL)
100
10
1
0.1
5mg/kg dose
Cona
Cona MB IgG1 LLRAL
96h
7 days
11 days
18 days
0
168
336
504
Hours post-first dose

FIG. 5C

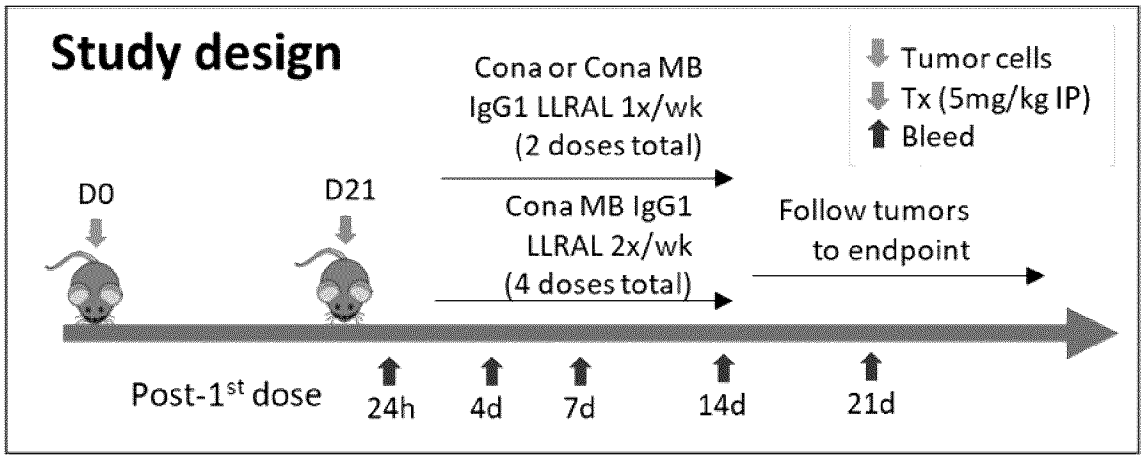

Study design
Cona or Cona MB
IgG1 LLRAL 1x/wk
(2 doses total)
Cona MB IgG1
LLRAL 2x/wk
(4 doses total)
Follow tumors
to endpoint
Tumor cells
Tx (5mg/kg IP)
Bleed
D0
D21
Post-1st dose
24h
4d
7d
14d
21d

FIG. 6A

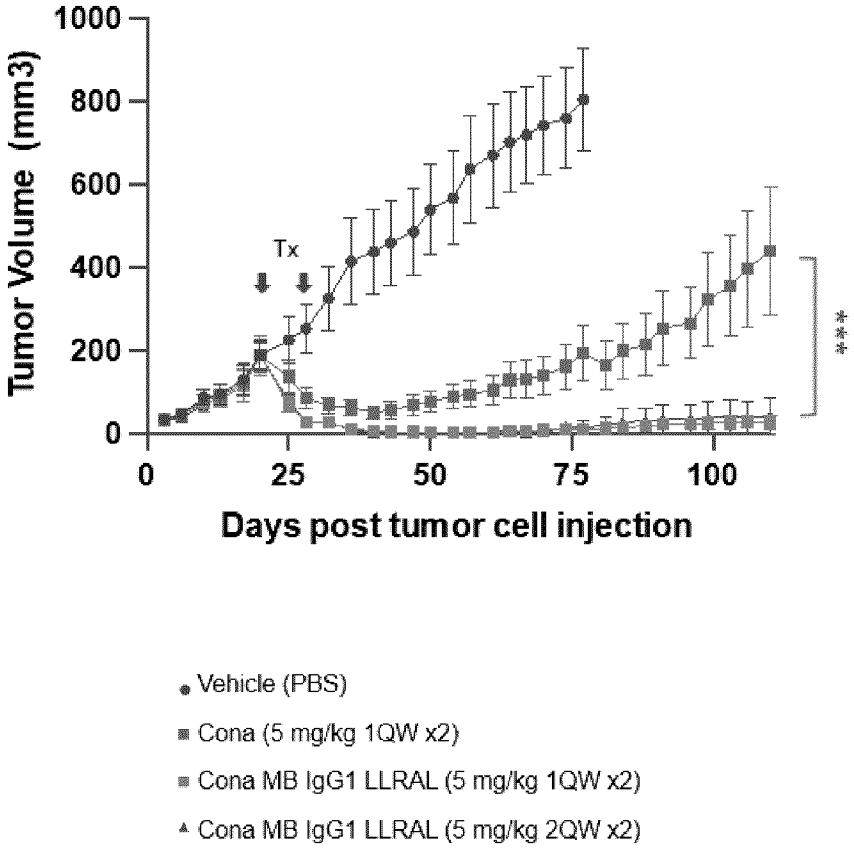

Tumor Volume (mm3)
1000
800
600
400
200
0
Tx
***
0
25
50
75
100
Days post tumor cell injection
Vehicle (PBS)
Cona (5 mg/kg 1QW x2)
Cona MB IgG1 LLRAL (5 mg/kg 1QW x2)
Cona MB IgG1 LLRAL (5 mg/kg 2QW x2)

FIG. 6B

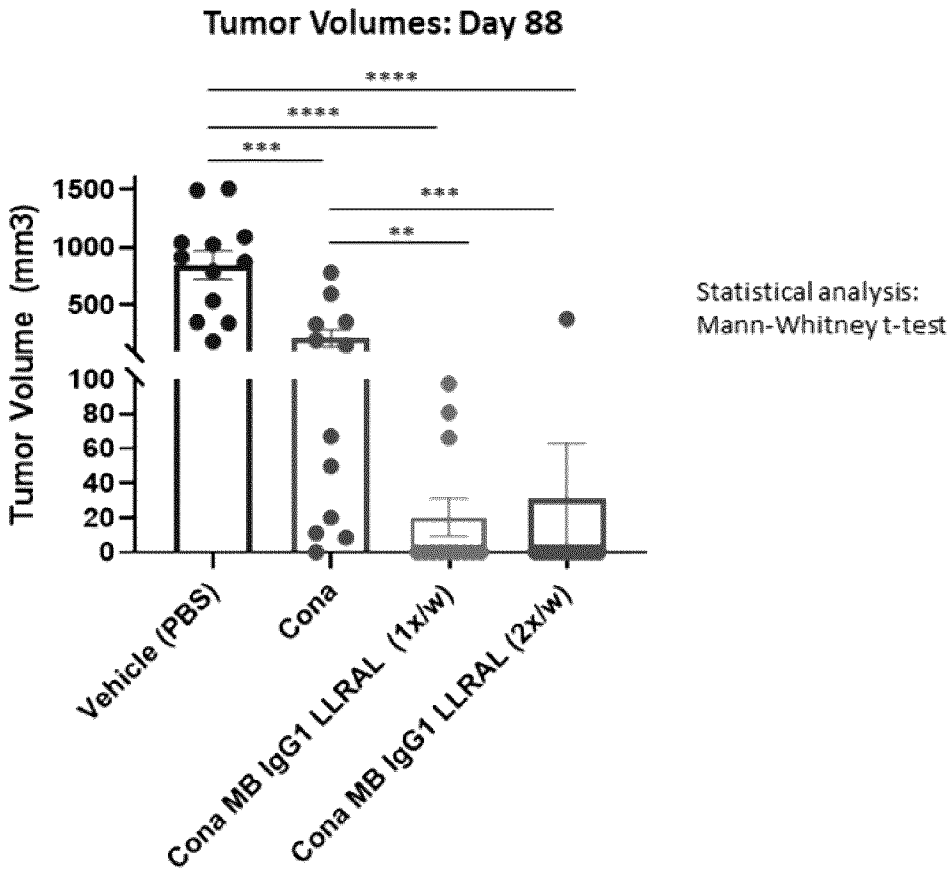

Tumor Volumes: Day 88
****
****
***
***
**
Tumor Volume (mm3)
1500
1000
500
100
80
60
40
20
0
Vehicle (PBS)
Cona
Cona MB IgG1 LLRAL (1x/w)
Cona MB IgG1 LLRAL (2x/w)
Statistical analysis:
Mann-Whitney t-test

FIG. 6C

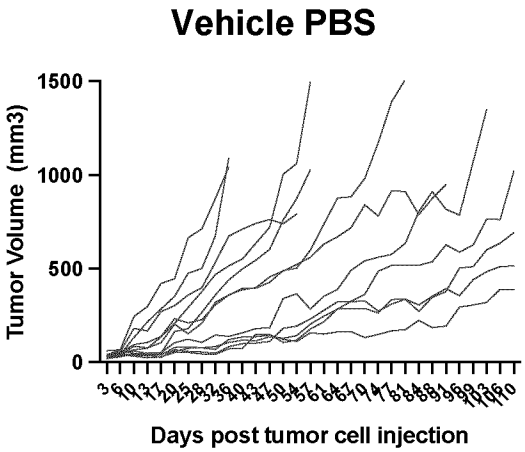


FIG. 6D

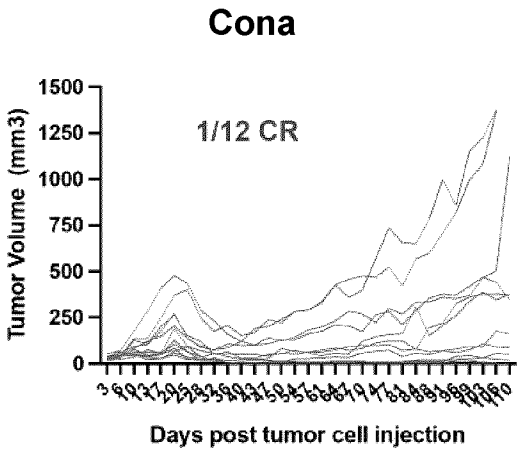


FIG. 6E

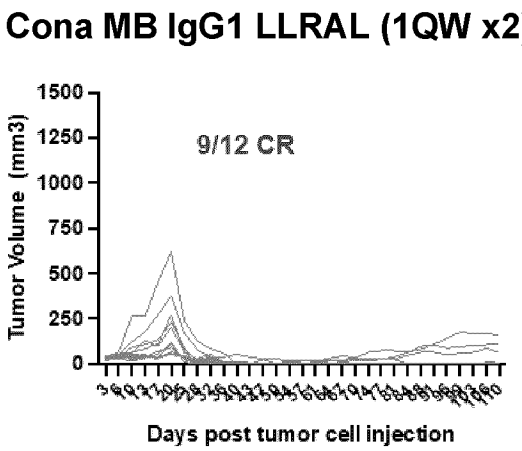


FIG. 6F

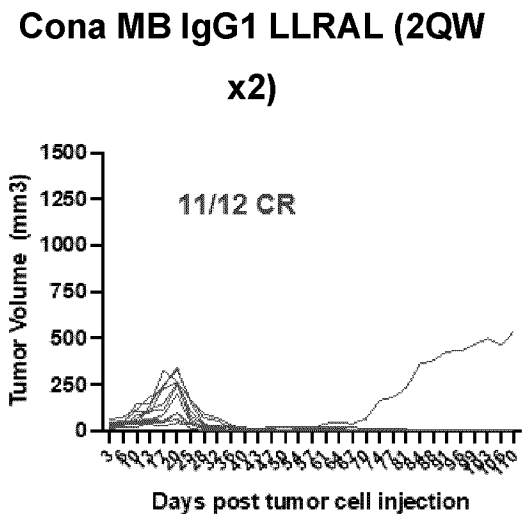


FIG. 6G

CR = complete response

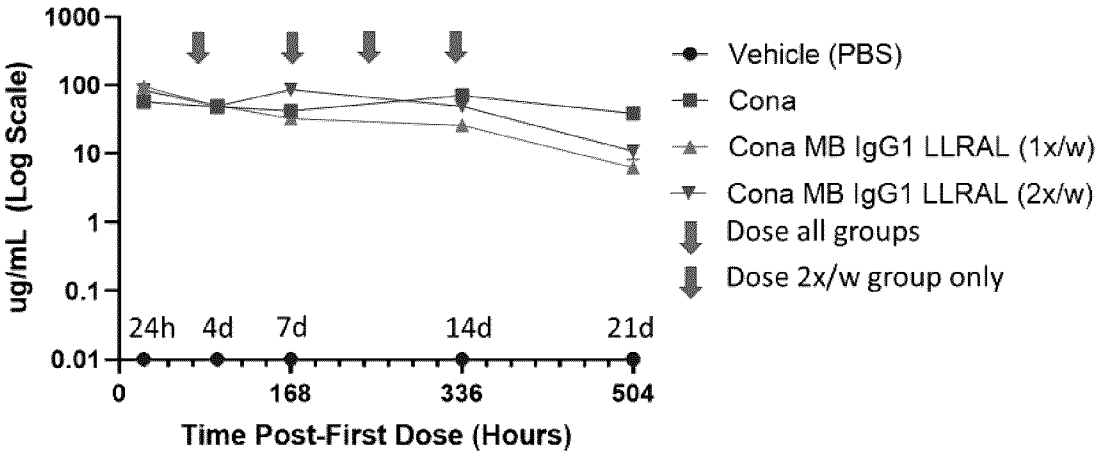

1000
100
10
1
0.1
0.01
ug/mL (Log Scale)
24h
4d
7d
14d
21d
0
168
336
504
Time Post-First Dose (Hours)
Vehicle (PBS)
Cona
Cona MB IgG1 LLRAL (1x/w)
Cona MB IgG1 LLRAL (2x/w)
Dose all groups
Dose 2x/w group only

FIG. 6H

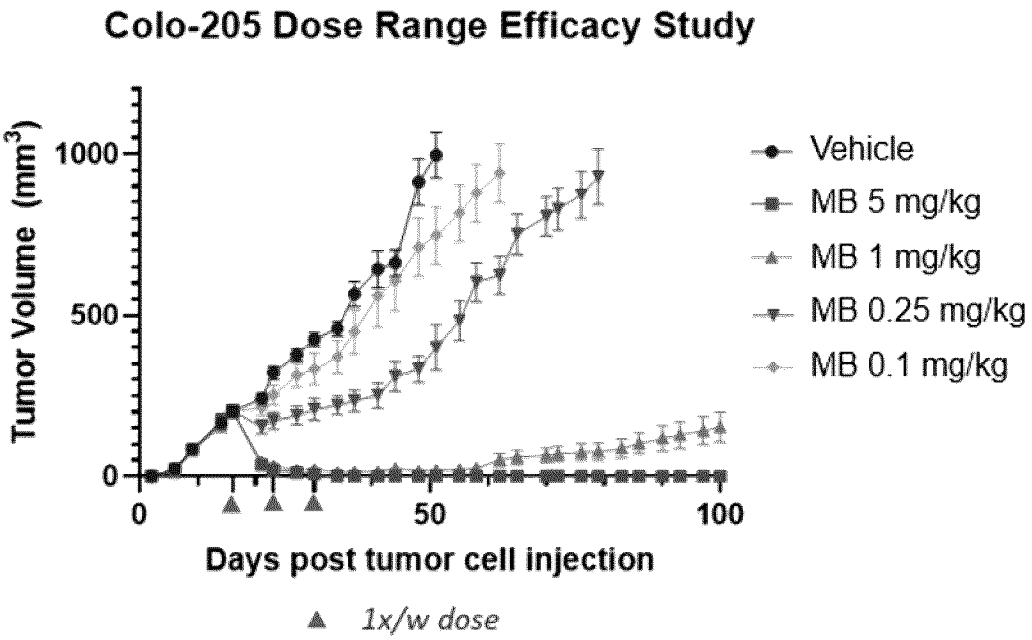

Colo-205 Dose Range Efficacy Study
Tumor Volume (mm3)
1000
500
0
0
50
100
Days post tumor cell injection
1x/w dose
Vehicle
MB 5 mg/kg
MB 1 mg/kg
MB 0.25 mg/kg
MB 0.1 mg/kg

FIG. 7A

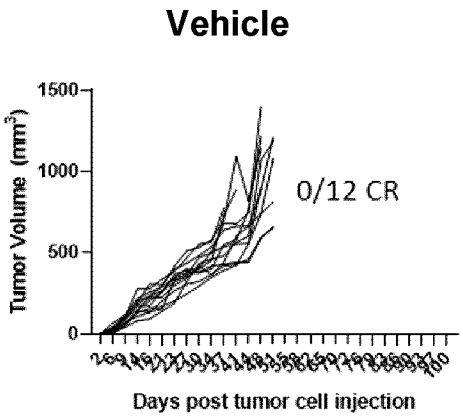


FIG. 7B

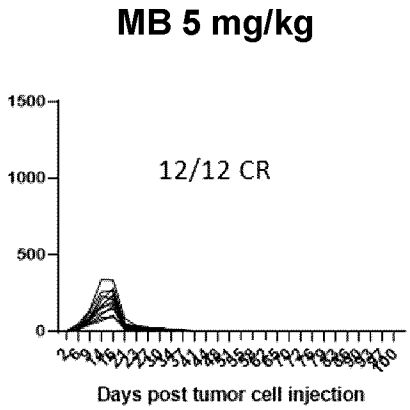


FIG. 7C

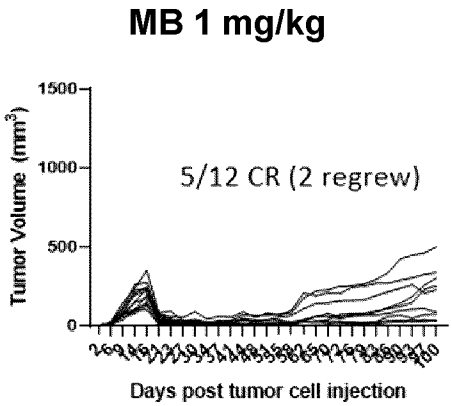


FIG. 7D

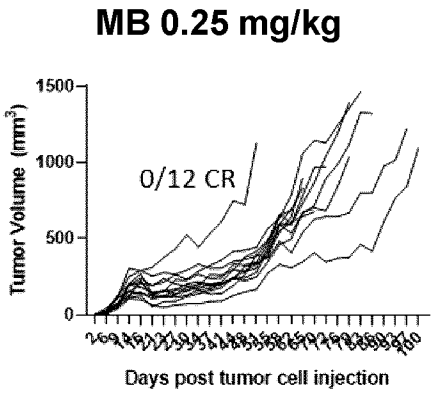


FIG. 7E

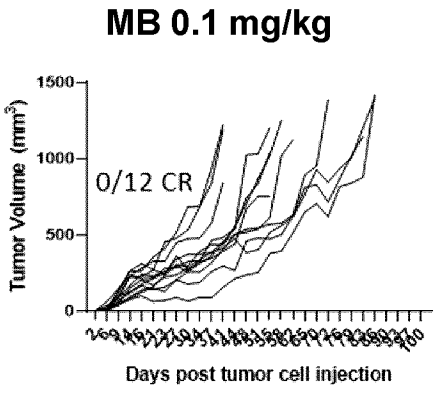


FIG. 7F

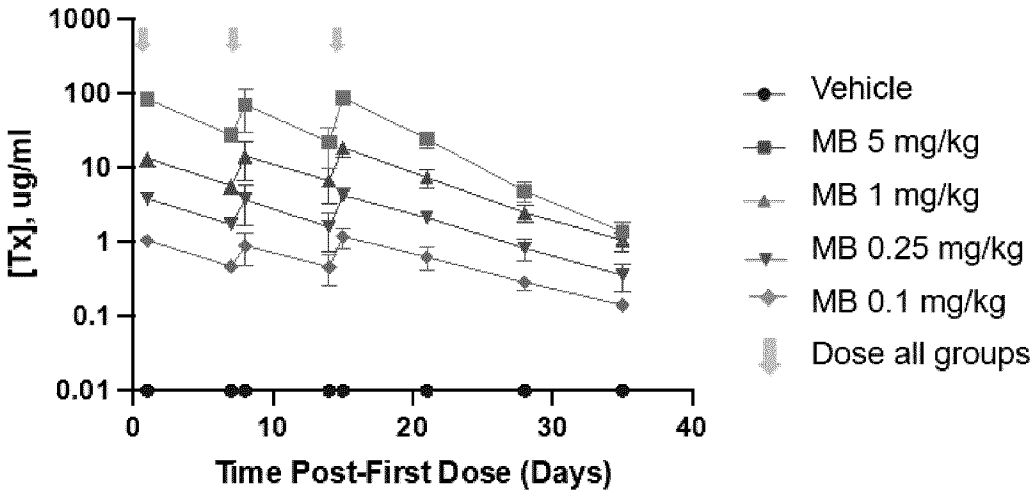

1000
100
10
1
0.1
0.01
[Tx], ug/ml
0
10
20
30
40
Time Post-First Dose (Days)
Vehicle
MB 5 mg/kg
MB 1 mg/kg
MB 0.25 mg/kg
MB 0.1 mg/kg
Dose all groups

FIG. 7G

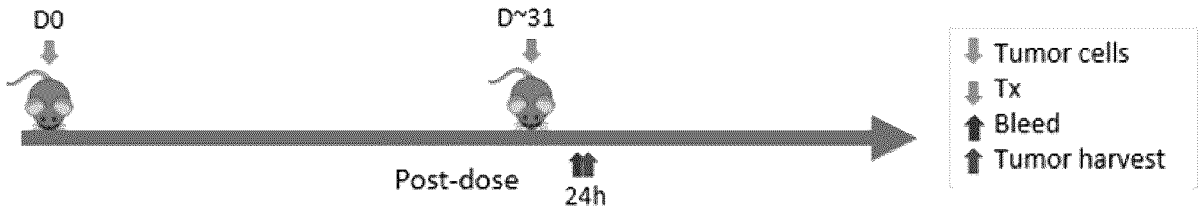


FIG. 8

Vehicle

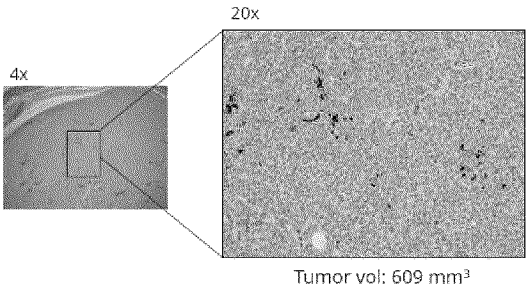


FIG. 9A

MB

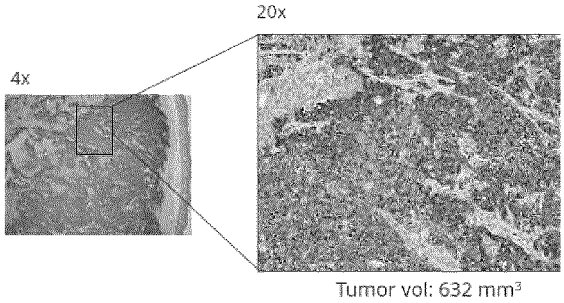


FIG. 9B

Vehicle

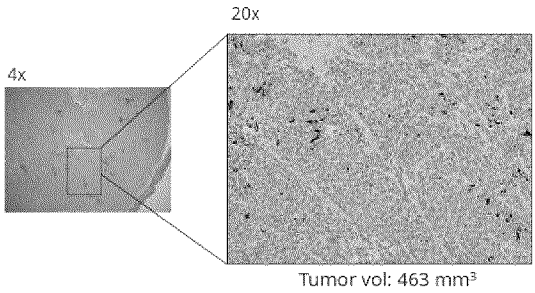


FIG. 9C

MB

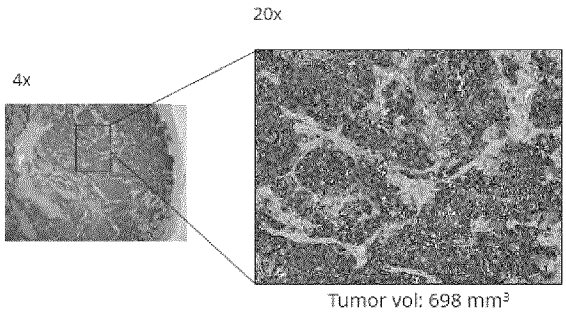


FIG. 9D

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2022/051361

A. CLASSIFICATION OF SUBJECT MATTER

IPC: ***A61K 47/64*** (2017.01) , ***A61P 35/00*** (2006.01) , ***C07K 14/47*** (2006.01) , ***C07K 16/00*** (2006.01) , ***C07K 16/28*** (2006.01) , ***C07K 19/00*** (2006.01)

CPC: **A61K 47/64** (2020.01) , A61P 35/00 (2020.01) , C07K 14/47 (2020.01) , C07K 16/00 (2020.01) , C07K 16/28 (2020.01) , C07K 2319/30 (2020.01) (more CPCs on the last page)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC: *A61K 47/64* (2017.01) , *A61P 35/00* (2006.01) , *C07K 14/47* (2006.01) , *C07K 16/00* (2006.01) , *C07K 16/28* (2006.01) , *C07K 19/00* (2006.01)
Keywords searched across the whole IPC and CPC

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)

Databases: Questel Orbit, SCOPUS, GenomeQuest, Google

Keyword: DR5, death receptor 5, nanocage, nanoparticle, multabody, Fc, ferritin, tigatuzumab, lexatumumab, drozitumab, conatumumab
SEQ ID Nos searched: SEQ ID Nos 22-53

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2021/016724 A1 (JULIEN *et al.*) 04 February 2021 (04-02-2021) *see whole document	1-73
Y	DIVINE *et al.* Designed proteins assemble antibodies into modular nanocages. Science. 2 April 2021 (02-04-2021), Vol. 372, No. 6537, pp.1-22 *see whole document, particularly pages 6, 7 and Fig. 4	1-73

☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

*	Special categories of cited documents:
"A"	document defining the general state of the art which is not considered to be of particular relevance
"D"	document cited by the applicant in the international application
"E"	earlier application or patent but published on or after the international filing date
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O"	document referring to an oral disclosure, use, exhibition or other means
"P"	document published prior to the international filing date but later than the priority date claimed
"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&"	document member of the same patent family

Date of the actual completion of the international search 08 November 2022 (08-11-2022	Date of mailing of the international search report 15 November 2022 (15-11-2022)
Name and mailing address of the ISA/CA Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 819-953-2476	Authorized officer Ryan Killoran (819) 360-4142

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	LIU *et al*. Fc-Engineering for Modulated Effector Functions-Improving Antibodies for Cancer Treatment. Antibodies. 17 November 2020 (17-11-2020), Vol. 9, No. 64, pp. 1-34 *see whole document	1-73
P, X	WO 2022/109743 A1 (JULIEN *et al*.) 02 June 2022 (02-06-2022) *see whole document	1-73

Box No. I **Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)**

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☒ forming part of the international application as filed.

   b. ☐ furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)),

      ☐ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CA2022/051361

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
WO2021016724A1	04 February 2021 (04-02-2021)	WO2021016724A1	04 February 2021 (04-02-2021)
		AU2020320459A1	03 March 2022 (03-03-2022)
		BR112022001800A2	12 April 2022 (12-04-2022)
		CA3149320A1	04 February 2021 (04-02-2021)
		CN114867754A	05 August 2022 (05-08-2022)
		EP4007779A1	08 June 2022 (08-06-2022)
		JP2022543070A	07 October 2022 (07-10-2022)
		KR20220107151A	02 August 2022 (02-08-2022)
WO2022109743A1	02 June 2022 (02-06-2022)	None	

IPC:

CPC:
C07K 2319/31 (2020.01)