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(54) Title: ANTI-FOLATE RECEPTOR ALPHA ANTIBODIES AND METHODS OF USE

(57) Abstract: Antibody constructs that bind human folate receptor alpha (FRα or FOLR1) and antibody-drug conjugates (ADCs) comprising an anti-FRα antibody construct conjugated to a drug, such as a cytotoxin or an immune modulator, and their use as therapeutics or diagnostics, for example, in the treatment or diagnosis of cancer.

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ANTI-FOLATE RECEPTOR ALPHA ANTIBODIES AND METHODS OF USE

FIELD

[0001] The present disclosure relates to the field of antibody therapeutics and, in particular, to antibodies targeting human folate receptor alpha (hFRα).

BACKGROUND

[0002] Folate receptor alpha (FRα) is a glycosyl-phosphatidylinositol (GPI)-anchored cell-surface protein encoded by *FOLR1* and is one of a family of high-affinity FRs that also includes FRβ (*FOLR2*), FRγ (*FOLR3*) and FRδ (*FOLR4*). FRα has been identified as a highly relevant cancer therapy target as it is overexpressed in a variety of cancers including ovarian cancer, triple-negative breast cancer (TNBC), endometrial cancer, mesothelioma and lung cancer, with minimal expression in non-malignant tissues.

[0003] Several clinical studies involving FRα-targeted agents in the treatment of cancer are currently ongoing, including the anti-FRα antibody, farletuzumab, and the FRα-targeted antibody-drug conjugates (ADCs), mirvetuximab soravtansine (ImmunoGen, Inc.), MORAb-202 (Eisai Inc.) and STRO-002 (Sutro Biopharma, Inc.).

[0004] This background information is provided for the purpose of making known information believed by the applicant to be of possible relevance to the present disclosure. No admission is necessarily intended, nor should be construed, that any of the preceding information constitutes prior art against the claimed invention.

SUMMARY

[0005] Described herein are anti-FRα antibodies and methods of use. One aspect of the present disclosure relates to an antibody construct comprising an antigen-binding domain that specifically binds to human folate receptor alpha (hFRα), wherein the antibody construct competes for binding

to hFRα with a reference antibody that specifically binds to an epitope within hFRα comprising amino acid residues E120, D121, R123, T124, S125 and Y126 of SEQ ID NO: 15.

[0006] Another aspect of the present disclosure relates to an antibody construct comprising an antigen-binding domain that specifically binds to an epitope within human folate receptor alpha (hFRα) comprising amino acid residues E120, D121, R123, T124, S125 and Y126 of SEQ ID NO: 15.

[0007] Another aspect of the present disclosure relates to an antibody construct comprising an antigen-binding domain that specifically binds to human folate receptor alpha (hFRα), the antigen-binding domain comprising heavy chain CDR amino acid sequences (HCDR1, HCDR2 and HCDR3) comprising the sequences as set forth in SEQ ID NOs: 3, 4 and 5, and light chain CDR amino acid sequences (LCDR1, LCDR2 and LCDR3) comprising the sequences as set forth in SEQ ID NOs: 6, 7 and 8.

[0008] Another aspect of the present disclosure relates to an antibody construct comprising two antigen-binding domains operably linked to an IgG Fc region, wherein each of the antigen-binding domains specifically binds to human folate receptor alpha (hFRα) and comprises:

(a) a VL amino acid sequence as set forth in SEQ ID NO: 39, and a VH amino acid sequence as set forth in SEQ ID NO: 19; or

(b) a VL amino acid sequence as set forth in SEQ ID NO: 124, and a VH amino acid sequence as set forth in SEQ ID NO: 91; or

(c) a VL amino acid sequence as set forth in SEQ ID NO: 64, and

(i) a VH amino acid sequence as set forth in SEQ ID NO: 50, or

(ii) a VH amino acid sequence as set forth in SEQ ID NO: 54, or

(iii) a VH amino acid sequence as set forth in SEQ ID NO: 57, or

(iv) a VH amino acid sequence as set forth in SEQ ID NO: 61, or

(v) a VH amino acid sequence as set forth in SEQ ID NO: 76, or

(vi) a VH amino acid sequence as set forth in SEQ ID NO: 79, or

(vii) a VH amino acid sequence as set forth in SEQ ID NO: 82, or

(viii) a VH amino acid sequence as set forth in SEQ ID NO: 85, or

(ix) a VH amino acid sequence as set forth in SEQ ID NO: 88, or

(x) a VH amino acid sequence as set forth in SEQ ID NO: 106; or

(d) a VL amino acid sequence as set forth in SEQ ID NO: 130, and

(i) a VH amino acid sequence as set forth in SEQ ID NO: 99, or

(ii) a VH amino acid sequence as set forth in SEQ ID NO: 106, or

(iii) a VH amino acid sequence as set forth in SEQ ID NO: 113, or

(iv) a VH amino acid sequence as set forth in SEQ ID NO: 116, or

(v) a VH amino acid sequence as set forth in SEQ ID NO: 133, or

(vi) a VH amino acid sequence as set forth in SEQ ID NO: 136; or

(e) a VL amino acid sequence as set forth in SEQ ID NO: 119, and

(i) a VH amino acid sequence as set forth in SEQ ID NO: 106, or

(ii) a VH amino acid sequence as set forth in SEQ ID NO: 116.

[0009] Another aspect of the present disclosure relates to a polynucleotide or set of polynucleotides encoding the anti-FRα antibody construct as described herein.

[0010] Another aspect of the present disclosure relates to an expression vector or set of expression vectors comprising a polynucleotide or set of polynucleotides encoding the anti-FRα antibody construct as described herein. Another aspect of the present disclosure relates to a host cell comprising the expression vector or set of expression vectors.

[0011] Another aspect of the present disclosure relates to an antibody-drug conjugate comprising the anti-FRα antibody construct as described herein conjugated to one or more drug moieties.

[0012] Another aspect of the present disclosure relates to an antibody-drug conjugate having general Formula I:

$$A\text{-}(L\text{-}(D)_m)_n \quad \textbf{(I)}$$

wherein:

A is an anti-FRα antibody construct as described herein;

L is a linker;

D is a drug moiety;

m is between 1 and about 8, and

n is 1 and about 12.

[0013] Another aspect of the present disclosure relates to a pharmaceutical composition comprising an anti-FRα antibody construct as described herein or an antibody-drug conjugate as described herein, and a pharmaceutically acceptable carrier or diluent.

[0014] Another aspect of the present disclosure relates to an anti-FRα antibody construct as described herein or an antibody-drug conjugate as described herein for use in therapy, for example, in the treatment of cancer.

[0015] Another aspect of the present disclosure relates to a use of an anti-FRα antibody construct as described herein or an antibody-drug conjugate as described herein in the manufacture of a medicament for the treatment of cancer.

[0016] Another aspect of the present disclosure relates to a method of inhibiting the growth of FRα-positive tumor cells comprising contacting the cells with an anti-FRα antibody construct as described herein or an antibody-drug conjugate as described herein.

[0017] Another aspect of the present disclosure relates to a method of treating a subject having a cancer comprising administering to the subject an effective amount of an anti-FRα antibody construct as described herein or an antibody-drug conjugate as described herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0018] **Fig. 1A** shows the sequence of the rabbit heavy chain variable domain CDRs of the chimeric antibody v23924 ported onto a human VH framework (IGHV3-23*01) (SEQ ID NO: 155), and **Fig. 1B** shows the sequence of the rabbit light chain variable domain CDRs of chimeric antibody v23924 ported onto a human VL framework (IGKVI-39*01) (SEQ ID NO: 156). The CDRs were assigned with the AbM definition and are marked in bold italic font.

[0019] **Fig. 2A-D** show the profiles of purified parental chimeric variant v23924 and purified representative humanized variant v30384 as analyzed by electrophoresis and UPLC-SEC. **Fig. 2A & C** show the profiles from electrophoresis under non-reducing (NR) and reducing (R) conditions after preparative SEC purification (post prep-SEC) or after Protein A purification (post-pA) of parental chimeric variant v23924 (**2A**) and purified representative humanized variant v30384 (**2C**), **Fig. 2B & D** show the UPLC-SEC profiles of parental chimeric variant v23924 after preparative SEC purification (**2B**) and purified representative humanized variant v30384 after Protein A purification (**2D**).

[0020] **Fig. 3A & B** depict the bio-layer interferometry (BLI) sensorgrams of parental chimeric variant v23924 (**3A**) and purified representative humanized variant v30384 (**3B**).

[0021] **Fig. 4A-D** depict the intact LC/MS profiles for representative humanized variants v30384 (**4A**, with an expanded view of the main peak in **4B**) and v31422 (**4C**, with an expanded view of the main peak in **4D**).

[0022] **Fig. 5A & B** shows the receptor-mediated internalization capabilities of the chimeric antibody v23924, a representative humanized variant, v30384, and the FRα-targeting antibodies mirvetuximab and farletuzumab at various concentrations in the FRα-expressing cell line IGROV-1 as determined by flow cytometry after a 6-hour incubation (**5A**) and a 24-hour incubation (**5B**). The anti-RSV antibody, palivizumab, was included as a negative control.

[0023] **Fig. 6A & B** show the receptor-mediated internalization capabilities of the chimeric antibody v23924, a representative humanized variant, v30384, and the FRα-targeting antibodies mirvetuximab and farletuzumab at various concentrations in the FRα-expressing cell line OVCAR-3 as determined by flow cytometry after a 6-hour incubation (**6A**) and a 24-hour incubation (**6B**). The anti-RSV antibody, palivizumab, was included as a negative control.

[0024] **Fig. 7** shows the coverage of the hFRα sequence (SEQ ID NO: 15) by peptides generated by pepsin digestion of hFRα. Each bar below the sequence represents a peptide.

[0025] **Fig. 8A & B** show a summary plot (**8A**) and a differential plot (**8B**) of the hydrogen/deuterium exchange mass spectrometry (HDX-MS) kinetics of the peptides generated by pepsin digestion of hFRα: hFOLR1 (hFRα) *vs.* hFOLR1-v23924 complex.

[0026] **Fig. 9A-C** show the amide deuteration level of peptide 119-126 (WEDCRTSY) (SEQ ID NO: 152) after hydrogen/deuterium exchange mass spectrometry (HDX-MS) for 1h: hFOLR1 (**9A**) *vs.* hFOLR1-v23924 complex (**9B**), and the differential plot (**9C**).

[0027] **Fig. 10A & B** show the receptor-mediated internalization capabilities of a parental humanized variant, v30384, and a representative affinity matured variant, v35356, in FRα-expressing cell lines IGROV-1 (**10A**) and JEG-3 (**10B**) as determined by flow cytometry after 5h and 24h incubation periods. Palivizumab was included as a non-targeted control.

[0028] **Fig. 11A-D** show the intracellular payload delivery capabilities of representative ADCs in the cell-lines JEG-3 (**11A**), Caov-3 (**11B**), H2110 (**11C**) and HEC-1-A (**11D**) as assessed by mass spectrometry. ADCs were humanized antibody variant v30384 and affinity matured variant v35356 each conjugated to drug-linker DL1.

[0029] **Fig. 12A-H** show the *in vivo* anti-tumor activities of the chimeric anti-FRα antibody v23924 conjugated to drug-linker DL1 or DL7 assessed in the xenograft models: CTG-0848 PDX (**12A**), OV90 CDX (**12B**), OVCAR-3 CDX (**12C**), LXFA737 PDX (**12D**), JEG3 CDX (**12E**), HCC1954 CDX (**12F**), SKOV3 CDX (**12G**) and KB CDX (**12H**). Control ADCs were v17717 (mirvetuximab Fab with HetFc) conjugated to drug-linker DL1 or DL6.

[0030] **Fig. 13** shows the *in vivo* anti-tumor activities of ADCs comprising the chimeric antibody v23924 or the humanized variants v30384 or v30399 each conjugated to drug-linker DL1, administered at 4 mg/kg or 9 mg/kg, in the mid/high-level FRα expressing OVCAR3 ovarian cancer model.

[0031] **Fig. 14A-E** show the *in vivo* anti-tumor activities of an ADC comprising the humanized variant v30384 conjugated to drug-linker DL1 or DL8, administered at the dosages shown, as assessed in the xenograft models: H2110 CDX (**14A**), SKOV3 CDX (**14B & 14C**), IGROV-1 CDX (**14D**) and LXFA737 PDX (**14E**). Control ADCs were v17717 (mirvetuximab Fab with HetFc) conjugated to drug-linker DL6; v17716 (mirvetuximab Fab with HomoFc) conjugated to drug-linker DL6, and for the H2110 CDX, v31629 (farletuzumab) conjugated to drug-linker DL4.

[0032] **Fig. 15A-D** show the *in vivo* anti-tumor activities of an ADC comprising the humanized variant v30384 conjugated to drug-linker DL5, administered at the dosages shown, in the xenograft models: OV90 (**15A**), H2110 (**15B**) and OVCAR-3 (**15C & 15D**).

[0033] **Fig. 16A-H** show the results of pharmacokinetic analysis indicating concentrations of serum IgG or ADC over time in serum taken from animals treated with ADCs comprising various anti-FRα antibodies; chimeric anti-FRα antibody v23924 conjugated to drug-linker DL1 in OV90 model (**16A**), OVCAR-3 model (**16B**), LXFA737 model (**16C**), JEG3 model (**16D**) or SKOV-3 model (**16E**); humanized variant v30384 conjugated to drug-linker DL1 in H2110 model (**16F**), and humanized variant v30384 conjugated to drug-linker DL5 in OV90 model (**16G**) or H2100 model (**16H**).

[0034] **Fig. 17** presents a table showing the CDR sequences of representative anti-FRα antibodies as defined by IMGT, Chothia, Kabat, Contact and AbM definitions.

[0035] **Fig. 18** presents a table showing the VH and VL sequences of representative anti-FRα antibodies.

[0036] **Fig. 19** shows cell growth inhibition (cytotoxicity) capabilities of an ADC comprising humanized antibody v30384 conjugated to drug-linker DL1 and an ADC comprising the affinity-matured variant v35356 conjugated to drug-linker DL1 in the cell lines: KB-HeLa (**19A**), IGROV-1 (**19B**), JEG-3 (**19C**), SKOV-3 (**19D**) and MDA-MB-468 (**19E**).

[0037] **Fig. 20A-D** shows penetration of the anti-FRα humanized antibody variant v36675 in JEG-3 cell spheroids compared to mirvetuximab and negative control, palivizumab, at 4 hours (**20A**), 24 hours (**20B**), 48 hours (**20C**), and 96 hours (**20D**).

[0038] **Fig. 21A & B** show fixed cell confirmation screen images from a screen for specific off-target binding interactions using Retrogenix Cell Microarray Technology for the anti-FRα humanized antibody variant v36675 at 20 μg/mL (**21A**) and control antibody (rituximab biosimilar) at 1μg/mL (**21B**).

[0039] **Fig. 22** shows competition binding between the chimeric anti-FRα antibody v23294 and the anti-FRα antibodies mirvetuximab and farletuzumab assessed in H2110 cells.

[0040] **Fig. 23** shows the receptor-mediated internalization capabilities of a humanized variant, v30384 compared to the biparatopic anti-FRα antibody B5327A (v36264) and the anti-FRα antibody mirvetuximab (v17716) in the FRα-expressing cell line IGROV-1 as determined by flow cytometry after a 5h incubation period.

[0041] **Fig. 24** shows penetration of the anti-FRα humanized antibody variant v36675 in JEG-3 cell spheroids compared to the biparatopic anti-FRα antibody B5327A (v36264) and the anti-FRα antibody mirvetuximab (v17716) at 96 hours.

DETAILED DESCRIPTION

[0042] The present disclosure relates to antibody constructs that bind human folate receptor alpha (FRα; also referred to herein as FOLR1), but do not show significant binding to folate receptor beta (FOLR2), gamma (FOLR3) or delta (FOLR4). In certain embodiments, the anti-FRα antibody constructs of the present disclosure are also capable of binding to cynomolgus monkey FRα.

[0043] The present disclosure also relates to antibody-drug conjugates (ADCs) comprising an anti-FRα antibody construct as described herein conjugated to a drug, such as a cytotoxin or an immune modulator. The anti-FRα antibody constructs and ADCs of the present disclosure may find use, for example, as therapeutics or diagnostics. Certain aspects of the present disclosure relate to therapeutic methods and uses of the anti-FRα antibody constructs and ADCs, for example, in the treatment of cancer. Some aspects relate to diagnostic methods and uses of the anti-FRα antibody constructs and ADCs, for example, in the diagnosis or analysis of cancer.

Definitions

[0044] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art.

[0045] As used herein, the term “about” refers to an approximately +/-10% variation from a given value. It is to be understood that such a variation is always included in any given value provided herein, whether or not it is specifically referred to.

[0046] The use of the word "a" or "an" when used herein in conjunction with the term "comprising" may mean "one," but it is also consistent with the meaning of "one or more," "at least one" and "one or more than one."

[0047] As used herein, the terms "comprising," "having," "including" and "containing," and grammatical variations thereof, are inclusive or open-ended and do not exclude additional, unrecited elements and/or method steps. The term "consisting essentially of" when used herein in connection with a composition, use or method, denotes that additional elements and/or method steps may be present, but that these additions do not materially affect the manner in which the recited composition, method or use functions. The term "consisting of" when used herein in connection with a composition, use or method, excludes the presence of additional elements and/or method steps. A composition, use or method described herein as comprising certain elements and/or steps may also, in certain embodiments consist essentially of those elements and/or steps, and in other embodiments consist of those elements and/or steps, whether or not these embodiments are specifically referred to.

[0048] A "complementarity determining region" or "CDR" is an amino acid sequence that contributes to antigen-binding specificity and affinity. "Framework" regions (FR) can aid in maintaining the proper conformation of the CDRs to promote binding between the antigen-binding region and an antigen. From N-terminus to C-terminus, both the light chain variable region (VL) and the heavy chain variable region (VH) of an antibody typically comprise the domains FR1, CDR1, FR2, CDR2, FR3, CDR3, and FR4. The three heavy chain CDRs are referred to herein as HCDR1, HCDR2, and HCDR3, and the three light chain CDRs are referred to as LCDR1, LCDR2, and LCDR3. CDRs provide the majority of contact residues for the binding of the antibody to the antigen or epitope. Often, the three heavy chain CDRs and the three light chain CDRs are required to bind antigen. However, in some instances, even a single variable domain can confer binding specificity to the antigen. Furthermore, as is known in the art, in some cases, antigen-binding may also occur through a combination of a minimum of one or more CDRs selected from the VH and/or VL domains, for example HCDR3.

[0049] A number of different definitions of the CDR sequences are in common use, including those described by Kabat *et al.* (1983, *Sequences of Proteins of Immunological Interest*, NIH

Publication No. 369-847, Bethesda, MD), by Chothia *et al.* (1987, *J Mol Biol*, 196:901-917), as well as the IMGT, AbM (University of Bath) and Contact (MacCallum, *et al.*, 1996, *J Mol Biol*, 262(5):732-745) definitions. By way of example, CDR definitions according to Kabat, Chothia, IMGT, AbM and Contact are provided in Table 1 below. Accordingly, as would be readily apparent to one skilled in the art, the exact numbering and placement of CDRs may differ based on the numbering system employed. However, it is to be understood that the disclosure herein of a VH includes the disclosure of the associated (inherent) heavy chain CDRs (HCDRs) as defined by any of the known numbering systems. Similarly, disclosure herein of a VL includes the disclosure of the associated (inherent) light chain CDRs (LCDRs) as defined by any of the known numbering systems.

Table 1: Common CDR Definitions[1]

Definition	Heavy Chain			Light Chain		
	CDR1[2]	CDR2	CDR3	CDR1	CDR2	CDR3
Kabat	H31-H35B	H50-H65	H95-H102	L24-L34	L50-L56	L89-L97
Chothia	H26-H32, H33 or H34	H52-H56	H95-H102	L24-L34	L50-L56	L89-L97
IMGT	H26-H33, H34, H35, H35A or H35B	H51-H57	H93-H102	L27-L32	L50-L52	L89-L97
AbM	H26-H35B	H50-H58	H95-H102	L24-L34	L50-L56	L89-L97
Contact	H30-H35B	H47-H58	H93-H101	L30-L36	L46-L55	L89-L96

[1] Either the Kabat or Chothia numbering system may be used for HCDR2, HCDR3 and the light chain CDRs for all definitions except Contact, which uses Chothia numbering

[2] Using Kabat numbering. The position in the Kabat numbering scheme that demarcates the end of the Chothia and IMGT CDR-H1 loop varies depending on the length of the loop because Kabat places insertions outside of those CDR definitions at positions 35A and 35B. However, the IMGT and Chothia CDR-H1 loop can be unambiguously defined using Chothia numbering. CDR-H1 definitions using Chothia numbering: Kabat H31-H35, Chothia H26-H32, AbM H26-H35, IMGT H26-H33, Contact H30-H35.

[0050] The term "identical" in the context of two or more polynucleotide or polypeptide sequences, refers to two or more sequences or subsequences that are the same. Sequences are "substantially identical" if they have a percentage of amino acid residues or nucleotides that are the same (for example, about 80%, about 85%, about 90%, about 95%, or about 98% identity, over a specified region) when compared and aligned for maximum correspondence over a comparison window or over a designated region as measured using one of the commonly used sequence comparison algorithms as known to persons of ordinary skill in the art or by manual alignment and visual inspection. For sequence comparison, typically test sequences are compared to a designated reference sequence. When using a sequence comparison algorithm, test and reference sequences are entered into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. Default program parameters can be used, or alternative parameters can be designated. The sequence comparison algorithm then calculates the percent sequence identities for the test sequences relative to the reference sequence, based on the program parameters.

[0051] A "comparison window" refers to a segment of a sequence comprising contiguous amino acid or nucleotide positions which may be, for example, from about 10 to 600 contiguous amino acid or nucleotide positions, or from about 10 to about 200, or from about 10 to about 150 contiguous amino acid or nucleotide positions over which a test sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned. Methods of alignment of sequences for comparison are known to those of ordinary skill in the art. Optimal alignment of sequences for comparison can be conducted, for example, by the local homology algorithm of Smith & Waterman, 1970, *Adv. Appl. Math.*, 2:482c; by the homology alignment algorithm of Needleman & Wunsch, 1970, *J. Mol. Biol.*, 48:443; by the search for similarity method of Pearson & Lipman, 1988, *Proc. Natl. Acad. Sci. USA*, 85:2444, or by computerized implementations of these algorithms (for example, GAP, BESTFIT, FASTA or TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, Madison, WI), or by manual alignment and visual inspection (see, for example, Ausubel *et al.*, Current Protocols in Molecular Biology, (1995 supplement), Cold Spring Harbor Laboratory Press). Examples of available algorithms suitable for determining percent sequence identity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul *et al.*, 1997, *Nuc. Acids Res.*, 25:3389-3402, and Altschul *et al.*, 1990, *J. Mol. Biol.*, 215:403-410, respectively. Software for

performing BLAST analyses is publicly available through the website for the National Center for Biotechnology Information (NCBI).

[0052] The term "subject," as used herein, refers to an animal, in some embodiments a mammal, which is the object of treatment, observation or experiment. The animal may be a human, a non-human primate, a companion animal (for example, dog, cat, or the like), farm animal (for example, cow, sheep, pig, horse, or the like) or a laboratory animal (for example, rat, mouse, guinea pig, non-human primate, or the like). In certain embodiments, the subject is a human.

[0053] It is contemplated that any embodiment discussed herein can be implemented with respect to any method, use or composition disclosed herein, and vice versa.

[0054] Particular features, structures and/or characteristics described in connection with an embodiment disclosed herein may be combined with features, structures and/or characteristics described in connection with another embodiment disclosed herein in any suitable manner to provide one or more further embodiments.

[0055] It is also to be understood that the positive recitation of a feature in one embodiment, serves as a basis for excluding the feature in an alternative embodiment. For example, where a list of options is presented for a given embodiment or claim, it is to be understood that one or more option may be deleted from the list and the shortened list may form an alternative embodiment, whether or not such an alternative embodiment is specifically referred to.

ANTI-FRα ANTIBODY CONSTRUCTS

[0056] The present disclosure relates to antibody constructs that specifically bind to human FRα (hFRα). In this context, the term "antibody construct" refers to a polypeptide or a set of polypeptides that comprises one or more antigen-binding domains, where each of the one or more antigen-binding domains specifically binds to an epitope or antigen. Where the antibody construct comprises two or more antigen-binding domains, each of the antigen-binding domains may bind the same epitope or antigen (*i.e.* the antibody construct is monospecific) or they may bind to different epitopes or antigens (*i.e.* the antibody construct is bispecific or multispecific). The antibody construct may further comprise a scaffold and the one or more antigen-binding domains can be fused or covalently attached to the scaffold, optionally via a linker, as described herein.

[0057] In accordance with the present disclosure, the anti-FRα antibody construct comprises at least one antigen-binding domain that specifically binds to hFRα. By "specifically binds" to hFRα, it is meant that the antibody construct binds to hFRα but does not exhibit significant binding to any of human folate receptor beta (FOLR2), gamma (FOLR3) or delta (FOLR4). In certain embodiments, the anti-FRα antibody constructs of the present disclosure may be capable of binding to an FRα from one or more non-human species. In certain embodiments, the anti-FRα antibody constructs of the present disclosure are capable of binding to cynomolgus monkey FRα.

[0058] Human FRα is also known as "human folate receptor 1" or "FOLR1." The protein sequences of hFRα from various sources are known in the art and readily available from publicly accessible databases, such as GenBank or UniProtKB. Examples of hFRα sequences include for example those provided under NCBI reference numbers P15328, AAX29268.1, AAX37119.1, NP_057937.1 and NP_057936.1. An exemplary hFRα protein sequence is provided in Table 2 as SEQ ID NO: 1 (NCBI Reference Sequence: NP_057936.1). An exemplary cynomolgus monkey FRα protein sequence is also provided in Table 2 (SEQ ID NO: 2; NCBI Reference Sequence: XP_005579002.2).

Table 2: Human and Cynomolgus Monkey FRα Protein Sequences

Organism	**Sequence**	**SEQ ID NO**
Human	MAQRMTTQLLLLLVWVAVVGEAQTRIAWARTELLNVCMNA KHHKEKPGPEDKLHEQCRPWRKNACCSTNTSQEAHKDVSYL YRFNWNHCGEMAPACKRHFIQDTCLYECSPNLGPWIQQVDQ SWRKERVLNVPLCKEDCEQWWEDCRTSYTCKSNWHKGWN WTSGFNKCAVGAACQPFHFYFPTPTVLCNEIWTHSYKVSNYS RGSGRCIQMWFDPAQGNPNEEVARFYAAAMSGAGPWAAWP FLLSLALMLLWLLS	1
Cynomolgus Monkey	MAQRMTTQLLLLLVWVAVVGEAQTRTTRARTELLNVCMNA KHHKEKPGPEDKLHEQCRPWKKNACCSTNTSQEAHKDVSYL YRFNWNHCGEMAPACKRHFIQDTCLYECSPNLGPWIQQVDQ SWRKERVLNVPLCKEDCEQWWEDCRTSYTCKSNWHKGWN WTSGFNKCPVGAACQPFHFYFPTPTVLCNEIWTYSYKVSNYS RGSGRCIQMWFDPAQGNPNEEVARFYAAAMSGAGPWAAWP LLLSLALTLLWLLS	2

[0059] Specific binding of an antigen-binding domain to a target antigen or epitope may be measured, for example, through an enzyme-linked immunosorbent assay (ELISA), a surface plasmon resonance (SPR) technique (employing, for example, a BIAcore instrument) (Liljeblad *et al.*, 2000, *Glyco J,* 17:323-329), flow cytometry or a traditional binding assay (Heeley, 2002, *Endocr Res*, 28:217-229). In certain embodiments, specific binding may be defined as the extent of binding to a non-target protein (such as FOLR2, FOLR3 or FOLR4) being less than about 10% of the binding to hFRα as measured by ELISA or flow cytometry, for example. In certain embodiments, specific binding of an antibody construct for FRα may be defined by a dissociation constant (K_D) of $\leq$1 μM, for example, $\leq$500 nM, $\leq$250 nM, $\leq$100 nM, $\leq$50 nM, or $\leq$10 nM. In certain embodiments, specific binding of an antibody construct for a particular antigen or an epitope may be defined by a dissociation constant (K_D) of 10^{-6} M or less, for example, 10^{-7} M or less, or 10^{-8} M or less. In some embodiments, specific binding of an antibody construct for a particular antigen or an epitope may be defined by a dissociation constant (K_D) between 10^{-6} M and 10^{-9} M, for example, between 10^{-7} M and 10^{-9} M.

[0060] In certain embodiments, the anti-FRα antibody constructs of the present disclosure show higher internalization into FRα-expressing cells than the reference antibodies mirvetuximab (huMovl9 or huFR107) and farletuzumab (MORAb-003).

[0061] Antibody internalization may be measured using art-known methods, for example, by a direct internalization method according to the protocol detailed in Schmidt, M. *et al.*, 2008, *Cancer Immunol. Immunother.*, 57:1879-1890, or using commercially available fluorescent dyes such as the pHAb Dyes (Promega Corporation, Madison, WI), pHrodo iFL and Deep Red Dyes (ThermoFisher Scientific Corporation, Waltham, MA) and Incucyte® Fabfluor-pH Antibody Labeling Reagent (Sartorius AG, Göttingen, Germany), and analysis techniques such as microscopy, FACS, high content imaging or other plate-based assays.

[0062] In certain embodiments, the anti-FRα antibody construct is considered to demonstrate a higher internalization into FRα-expressing cells than a corresponding reference antibody (mirvetuximab or farletuzumab) when the amount of anti-FRα antibody construct internalized into the FRα-expressing cells is at least 1.2 times greater than the amount of reference antibody internalized into the same FRα-expressing cells under the same test conditions. In certain

embodiments, the amount of internalized antibody is determined using an appropriate fluorescent dye and high content imaging. In some embodiments, the amount of internalized antibody is determined in cells that express FRα at a high level. In some embodiments, the amount of internalized antibody is determined in IGROV-1 cells or cells that express FRα at a similar level to IGROV-1 cells. In some embodiments, the amount of internalized antibody is determined after a 6-hour incubation period. In some embodiments, the amount of internalized antibody is determined after a 24-hour incubation period.

[0063] In certain embodiments, the anti-FRα antibody construct is considered to demonstrate a higher internalization into FRα-expressing cells than a corresponding reference antibody (mirvetuximab or farletuzumab) when the amount of anti-FRα antibody construct internalized into the FRα-expressing cells is at least 1.3 times greater, at least 1.4 times greater, at least 1.5 times greater, 1.6 times greater, 1.7 times greater, 1.8 times greater, 1.9 times greater, or 2.0 times greater, than the amount of reference antibody internalized into the same FRα-expressing cells under the same test conditions. In certain embodiments, the amount of internalized antibody is determined using an appropriate fluorescent dye and high content imaging. In some embodiments, the amount of internalized antibody is determined in cells that express FRα at a high level. In some embodiments, the amount of internalized antibody is determined in IGROV-1 cells or cells that express FRα at a similar level to IGROV-1 cells. In some embodiments, the amount of internalized antibody is determined after a 6-hour incubation period. In some embodiments, the amount of internalized antibody is determined after a 24-hour incubation period.

Antigen-Binding Domains

[0064] The anti-FRα antibody constructs of the present disclosure comprise at least one antigen-binding domain that is capable of binding to hFRα. The at least one antigen-binding domain capable of binding to hFRα typically is an immunoglobulin-based binding domain, such as an antigen-binding antibody fragment. Examples of an antigen-binding antibody fragment include, but are not limited to, a Fab fragment, a Fab' fragment, a single chain Fab (scFab), a single chain Fv (scFv) and a single domain antibody (sdAb).

[0065] A "Fab fragment" contains the constant domain of the light chain (CL) and the first constant domain of the heavy chain (CH1) along with the variable domains of the light and heavy

chains (VL and VH, respectively). Fab′ fragments differ from Fab fragments by the addition of a few amino acid residues at the C-terminus of the heavy chain CH1 domain, including one or more cysteines from the antibody hinge region. A Fab fragment may also be a single-chain Fab molecule, *i.e.* a Fab molecule in which the Fab light chain and the Fab heavy chain are connected by a peptide linker to form a single peptide chain. For example, the C-terminus of the Fab light chain may be connected to the N-terminus of the Fab heavy chain in the single-chain Fab molecule.

[0066] An "scFv" includes a heavy chain variable domain (VH) and a light chain variable domain (VL) of an antibody in a single polypeptide chain. The scFv may optionally further comprise a polypeptide linker between the VH and VL domains which enables the scFv to form a desired structure for antigen binding. For example, an scFv may include a VL connected from its C-terminus to the N-terminus of a VH by a polypeptide linker. Alternately, an scFv may comprise a VH connected through its C-terminus to the N-terminus of a VL by a polypeptide linker (see review in Pluckthun in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenburg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994)).

[0067] An "sdAb" format refers to a single immunoglobulin domain. The sdAb may be, for example, of camelid origin. Camelid antibodies lack light chains and their antigen-binding sites consist of a single domain, termed a "VHH." An sdAb comprises three CDR/hypervariable loops that form the antigen-binding site: CDR1, CDR2 and CDR3. sdAbs are fairly stable and easy to express, for example, as a fusion with the Fc chain of an antibody (see, for example, Harmsen & De Haard, 2007, *Appl. Microbiol Biotechnol.,* 77(1):13-22).

[0068] In those embodiments in which the anti-FRα antibody constructs comprise two or more antigen-binding domains, each additional antigen-binding domain may independently be an immunoglobulin-based domain, such as an antigen-binding antibody fragment, or a non-immunoglobulin-based domain, such as a non-immunoglobulin-based antibody mimetic, or other polypeptide or small molecule capable of specifically binding to its target, for example, a natural or engineered ligand. Non-immunoglobulin-based antibody mimetic formats include, for example, anticalins, fynomers, affimers, alphabodies, DARPins and avimers.

[0069] The present disclosure describes herein the identification of an antibody that specifically binds hFRα (variant v23924), as well as representative humanized versions of this antibody

(variants v30384, v30389, v30394, v30399, v31422, v31423, v31424, v31425 and v31426) and representative affinity-matured versions of this antibody (variants v35305, v35342, v35347, v35348, v35350, v35354, v35356, v35358, v36167, v36168 and v36675) (see Examples and Sequence Tables). Epitope mapping using the hFRα sequence shown in Fig. 7 (SEQ ID NO: 15) determined that the epitope within the hFRα protein bound by variant v23924 comprises the amino acid residues E120, D121, R123, T124, S125 and Y126 of SEQ ID NO: 15 (see Example 13).

[0070] In certain embodiments, the at least one antigen-binding domain that binds hFRα comprised by the anti-FRα antibody constructs of the present disclosure binds an epitope within the hFRα protein that comprises the amino acid residues E120, D121, R123, T124, S125 and Y126 of SEQ ID NO: 15. In some embodiments, the hFRα epitope bound by the anti-FRα antibody constructs is a non-linear (or discontinuous) epitope comprising the amino acid residues E120, D121, R123, T124, S125 and Y126 of SEQ ID NO: 15. In certain embodiments, the anti-FRα antibody constructs of the present disclosure comprise an antigen-binding domain that competes for binding to hFRα with an antibody that binds to an epitope within the hFRα protein comprising the amino acid residues E120, D121, R123, T124, S125 and Y126. In certain embodiments, the anti-FRα antibody constructs of the present disclosure comprise an antigen-binding domain that competes for binding to hFRα with antibody v23924 described herein.

[0071] One can determine whether an antibody construct competes for binding to hFRα with an antibody that binds to an epitope within the hFRα protein comprising the amino acid residues E120, D121, R123, T124, S125 and Y126 or with antibody v23924 using competition assays known in the art. For example, the antibody that binds to an epitope within the hFRα protein comprising the amino acid residues E120, D121, R123, T124, S125 and Y126 or antibody v23924 (the reference antibody) is first allowed to bind to hFRα under saturating conditions and then the ability of the test antibody construct to bind to hFRα is measured. If the test antibody construct is able to bind to hFRα at the same time as the reference antibody, then the test antibody construct is considered to bind to a different epitope than the reference antibody. Conversely, if the test antibody construct is not able to bind to hFRα at the same time as the reference antibody, then the test antibody construct is considered to bind to the same epitope, to an overlapping epitope, or to an epitope that is in close proximity to the epitope bound by the reference antibody. Competition assays may also be run in which the binding order of the reference and test antibodies is reversed,

that is, the test antibody is first allowed to bind to hFRα under saturating conditions and then the ability of the reference antibody construct to bind to hFRα is measured.

[0072] Such competition assays can be performed using techniques such as ELISA, radioimmunoassay, surface plasmon resonance (SPR), bio-layer interferometry, flow cytometry and the like. An "antibody that competes with" a reference antibody refers to an antibody that blocks binding of the reference antibody to its epitope in a competition assay by 50% or more.

[0073] In certain embodiments, the anti-FRα antibody constructs of the present disclosure comprise at least one antigen-binding domain that specifically binds to hFRα, where the antigen-binding domain comprises a set of CDRs based on the CDRs of antibody variant v23924 described herein. The CDR sequences of the antibody v23924 and representative humanized or affinity-matured versions of this antibody are shown in Fig. 17. Analysis of the CDR sequences from the parental and affinity-matured anti-FRα antibodies identified a minimal amino acid sequence present in each CDR as defined by any one of the IMGT, Chothia, Kabat, Contact or AbM numbering systems. These amino acid sequences are represented by the minimal consensus CDR sequences provided in Table 3. Extended versions of these CDR consensus sequences based on CDR sequences defined by the AbM numbering system are shown in Table 4.

Table 3: Minimal CDR Consensus Sequences of Anti-FRα Antibodies

CDR	Sequence	Variable (X)	SEQ ID NO
Heavy Chain			
HCDR1	X^1YGVS	X^1 is S or L	3
HCDR2	NSGGS		4
HCDR3	SGSGYPMDYX^2X^3I	X^2 is L or H X^3 is A or P	5
Light Chain			
LCDR1	QSIX^4X^5W	X^4 is G or W X^5 is D or Y	6
LCDR2	EAS		7
LCDR3	QQGYGR$X^6X^7X^8X^9$N	X^6 is S or W X^7 is N or H X^8 is V or I	8

CDR	Sequence	Variable (X)	SEQ ID NO
		X^9 is D or L	

Table 4: CDR Consensus Sequences of Anti-FRα Antibodies based on AbM Numbering System

CDR	Sequence	Variable	SEQ ID NO
Heavy Chain			
HCDR1	GFSLSX^{10}YGVS	X^{10} is S or L	9
HCDR2	X^{11}X^{12}NSGGSAY	X^{11} is S or A X^{12} is V or L	10
HCDR3	SGSGYPMDYX^{13}X^{14}I	X^{13} is L or H X^{14} is A or P	11
Light Chain			
LCDR1	X^{15}ASQSIX^{16}X^{17}WLA	X^{15} is R or Q X^{16} is G or W X^{17} is D or Y	12
LCDR2	EASTLAS		13
LCDR3	QQGYGRX^{18}X^{19}X^{20}X^{21}NI	X^{18} is S or W X^{19} is N or H X^{20} is V or I X^{21} is D or L	14

[0074] In certain embodiments, the anti-FRα antibody constructs of the present disclosure comprise an antigen-binding domain having heavy chain CDR amino acid sequences (HCDR1, HCDR2 and HCDR3) comprising the sequences as set forth in SEQ ID NOs: 3, 4 and 5, and light chain CDR amino acid sequences (LCDR1, LCDR2 and LCDR3) comprising the sequences as set forth in SEQ ID NOs: 6, 7 and 8.

[0075] In certain embodiments, the anti-FRα antibody constructs of the present disclosure comprise an antigen-binding domain having:

(i) an HCDR1 amino acid sequence as set forth in SEQ ID NO: 3; an HCDR2 amino acid sequence as set forth in SEQ ID NO: 4, and an HCDR3 amino acid sequence as set forth in SEQ ID NO: 5, where X^2 is L and X^3 is A, or X^2 is H and X^3 is P, and

(ii) an LCDR1 amino acid sequence as set forth in SEQ ID NO: 6, where X^4 is G and X^5 is D, or X^4 is W and X^5 is Y; an LCDR2 amino acid sequence as set forth in SEQ ID NO: 7, and an LCDR3 amino acid sequence as set forth in SEQ ID NO: 8, where X^6 is S, X^7 is N, X^8 is V and X^9 is D, or X^6 is W, X^7 is H, X^8 is I and X^9 is L.

[0076] In certain embodiments, the anti-FRα antibody constructs of the present disclosure comprise an antigen-binding domain having heavy chain CDR amino acid sequences (HCDR1, HCDR2 and HCDR3) comprising the sequences as set forth in SEQ ID NOs: 9, 10 and 11, and light chain CDR amino acid sequences (LCDR1, LCDR2 and LCDR3) comprising the sequences as set forth in SEQ ID NOs: 12, 13 and 14.

[0077] In certain embodiments, the anti-FRα antibody constructs of the present disclosure comprise an antigen-binding domain having:

(i) an HCDR1 amino acid sequence as set forth in SEQ ID NO: 9; an HCDR2 amino acid sequence as set forth in SEQ ID NO: 10, where X^{11} is S or A and X^{12} is V, or X^{11} is S and X^{12} is L, and an HCDR3 amino acid sequence as set forth in SEQ ID NO: 11, where X^{13} is L and X^{14} is A, or X^{13} is H and X^{14} is P, and

(ii) an LCDR1 amino acid sequence as set forth in SEQ ID NO: 12, where X^{15} is R or Q, X^{16} is G and X^{17} is D, or X^{15} is R, X^{16} is W and X^{17} is Y; an LCDR2 amino acid sequence as set forth in SEQ ID NO: 13, and an LCDR3 amino acid sequence as set forth in SEQ ID NO: 14, where X^{18} is S, X^{19} is N, X^{20} is V and X^{21} is D, or X^{18} is W, X^{19} is H, X^{20} is I and X^{21} is L.

[0078] In certain embodiments, the anti-FRα antibody constructs of the present disclosure comprise an antigen-binding domain having:

(i) an HCDR1 amino acid sequence selected from the HCDR1 amino acid sequences of any one of variants v23924, v30618, v30384, v30389, v30394, v30399, v31422, v31423, v31424, v31425, v31426, v35305, v35342, v35347, v35348, v35350, v35354, v35356, v35358, v36167, v36168 or v36675; an HCDR2 amino acid sequence selected from the HCDR2 amino acid sequences of any one of variants v23924, v30618, v30384, v30389, v30394, v30399, v31422, v31423, v31424, v31425, v31426, v35305, v35342, v35347, v35348, v35350, v35354, v35356,

v35358, v36167, v36168 or v36675, and an HCDR3 amino acid sequence selected from the HCDR3 amino acid sequences of any one of variants v23924, v30618, v30384, v30389, v30394, v30399, v31422, v31423, v31424, v31425, v31426, v35305, v35342, v35347, v35348, v35350, v35354, v35356, v35358, v36167, v36168 or v36675, and

(ii) a LCDR1 amino acid sequence selected from the LCDR1 amino acid sequences of any one of variants v23924, v30618, v30384, v30389, v30394, v30399, v31422, v31423, v31424, v31425, v31426, v35305, v35342, v35347, v35348, v35350, v35354, v35356, v35358, v36167, v36168 or v36675; a LCDR2 amino acid sequence selected from the LCDR2 amino acid sequences of any one of variants v23924, v30618, v30384, v30389, v30394, v30399, v31422, v31423, v31424, v31425, v31426, v35305, v35342, v35347, v35348, v35350, v35354, v35356, v35358, v36167, v36168 or v36675, and a LCDR3 amino acid sequence selected from the LCDR3 amino acid sequences of any one of variants v23924, v30618, v30384, v30389, v30394, v30399, v31422, v31423, v31424, v31425, v31426, v35305, v35342, v35347, v35348, v35350, v35354, v35356, v35358, v36167, v36168 or v36675,

where the CDR amino acid sequences are as defined by any one of the IMGT, Chothia, Kabat, Contact or AbM numbering systems (see Fig. 17).

[0079] In certain embodiments, the anti-FRα antibody constructs of the present disclosure comprise an antigen-binding domain having heavy chain CDR amino acid sequences (HCDR1, HCDR2 and HCDR3) selected from the heavy chain CDR amino acid sequences (HCDR1, HCDR2 and HCDR3) of any one of variants v23924, v30618, v30384, v30389, v30394, v30399, v31422, v31423, v31424, v31425, v31426, v35305, v35342, v35347, v35348, v35350, v35354, v35356, v35358, v36167, v36168 or v36675, as defined by any one of the IMGT, Chothia, Kabat, Contact or AbM numbering systems, and light chain CDR amino acid sequences (LCDR1, LCDR2 and LCDR3) selected from the light chain CDR amino acid sequences (LCDR1, LCDR2 and LCDR3) of any one of variants v23924, v30618, v30384, v30389, v30394, v30399, v31422, v31423, v31424, v31425, v31426, v35305, v35342, v35347, v35348, v35350, v35354, v35356, v35358, v36167, v36168 or v36675, as defined by any one of the IMGT, Chothia, Kabat, Contact or AbM numbering systems.

[0080] In certain embodiments, the anti-FRα antibody constructs of the present disclosure comprise an antigen-binding domain comprising heavy chain CDR amino acid sequences (HCDR1, HCDR2 and HCDR3) and light chain CDR amino acid sequences (LCDR1, LCDR2 and LCDR3) of any one of variants v23924, v30618, v30384, v30389, v30394, v30399, v31422, v31423, v31424, v31425, v31426, v35305, v35342, v35347, v35348, v35350, v35354, v35356, v35358, v36167, v36168 or v36675, as defined by any one of the IMGT, Chothia, Kabat, Contact or AbM numbering systems.

[0081] In certain embodiments, the anti-FRα antibody constructs of the present disclosure comprise an antigen-binding domain comprising the CDR sequences of the VH domain of any one of variants v23924, v30618, v30384, v30389, v30394, v30399, v31422, v31423, v31424, v31425, v31426, v35305, v35342, v35347, v35348, v35350, v35354, v35356, v35358, v36167, v36168 or v36675. In certain embodiments, the anti-FRα antibody constructs of the present disclosure comprise an antigen-binding domain comprising the CDR sequences of the VL domain of any one of variants v23924, v30618, v30384, v30389, v30394, v30399, v31422, v31423, v31424, v31425, v31426, v35305, v35342, v35347, v35348, v35350, v35354, v35356, v35358, v36167, v36168 or v36675. The VH and VL sequences of v23924, v30618, v30384, v30389, v30394, v30399, v31422, v31423, v31424, v31425, v31426, v35305, v35342, v35347, v35348, v35350, v35354, v35356, v35358, v36167, v36168 and v36675 are provided in Fig. 18.

[0082] In certain embodiments, the anti-FRα antibody constructs of the present disclosure comprise an antigen-binding domain comprising a VH amino acid sequence selected from the VH amino acid sequences of any one of variants v23924, v30618, v30384, v30389, v30394, v30399, v31422, v31423, v31424, v31425, v31426, v35305, v35342, v35347, v35348, v35350, v35354, v35356, v35358, v36167, v36168 or v36675. In certain embodiments, the anti-FRα antibody constructs of the present disclosure comprise an antigen-binding domain comprising a VL amino acid sequence selected from the VL amino acid sequences of any one of variants v23924, v30618, v30384, v30389, v30394, v30399, v31422, v31423, v31424, v31425, v31426, v35305, v35342, v35347, v35348, v35350, v35354, v35356, v35358, v36167, v36168 or v36675.

[0083] In certain embodiments, the anti-FRα antibody constructs of the present disclosure comprise an antigen-binding domain comprising a VH amino acid sequence and a VL amino acid

sequence selected from the VH and VL amino acid sequences of any one of variants v23924, v30618, v30384, v30389, v30394, v30399, v31422, v31423, v31424, v31425, v31426, v35305, v35342, v35347, v35348, v35350, v35354, v35356, v35358, v36167, v36168 or v36675.

[0084] One skilled in the art will appreciate that a limited number of amino acid substitutions may be introduced into the CDR sequences or into the VH or VL sequences of known antibodies without the antibody losing its ability to bind its target. Candidate amino acid substitutions may be identified by computer modeling or by art-known techniques such as alanine scanning, with the resulting variants being tested for binding activity by standard techniques. Accordingly, in certain embodiments, the anti-FRα antibody constructs of the present disclosure comprise an antigen-binding domain that comprises a set of CDRs (*i.e.* heavy chain HCDR1, HCDR2 and HCDR3, and light chain LCDR1, LCDR2 and LCDR3) that have 90% or greater, 95% or greater, 98% or greater, 99% or greater, or 100% sequence identity to a set of CDRs of any one of variants v23924, v30618, v30384, v30389, v30394, v30399, v31422, v31423, v31424, v31425, v31426, v35305, v35342, v35347, v35348, v35350, v35354, v35356, v35358, v36167, v36168 or v36675, where the % sequence identity is calculated across all six CDRs and where the antigen-binding domain retains the ability to bind hFRα.

[0085] In certain embodiments, the anti-FRα antibody constructs of the present disclosure comprise an antigen-binding domain that comprises a variant of the set of CDR sequences of any one of variants v23924, v30618, v30384, v30389, v30394, v30399, v31422, v31423, v31424, v31425, v31426, v35305, v35342, v35347, v35348, v35350, v35354, v35356, v35358, v36167, v36168 or v36675, where the variant comprises between 1 and 10 amino acid substitutions across the set of CDRs (*i.e.* the CDRs may be modified by up to 10 amino acid substitutions with any combination of the six CDRs being modified), and where the antigen-binding domain retains the ability to bind hFRα. In some embodiments, the anti-FRα antibody constructs of the present disclosure comprise an antigen-binding domain that comprises a variant of the set of CDR sequences of any one of variants v23924, v30618, v30384, v30389, v30394, v30399, v31422, v31423, v31424, v31425, v31426, v35305, v35342, v35347, v35348, v35350, v35354, v35356, v35358, v36167, v36168 or v36675, where the variant comprises between 1 and 7 amino acid substitutions, between 1 and 5 amino acid substitutions, between 1 and 4 amino acid substitutions, between 1 and 3 amino acid substitutions, between 1 and 2 amino acid substitutions, or 1 amino

acid substitution, across the set of CDRs, and where the antigen-binding domain retains the ability to bind hFRα.

[0086] In certain embodiments, the anti-FRα antibody constructs of the present disclosure comprise an antigen-binding domain that comprises a VH sequence that is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the VH sequence of any one of variants v23924, v30618, v30384, v30389, v30394, v30399, v31422, v31423, v31424, v31425, v31426, v35305, v35342, v35347, v35348, v35350, v35354, v35356, v35358, v36167, v36168 or v36675, where the antigen-binding domain retains the ability to bind hFRα. In certain embodiments, the anti-FRα antibody constructs of the present disclosure comprise an antigen-binding domain that comprises a VL sequence that is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the VL sequence of any one of variants v23924, v30618, v30384, v30389, v30394, v30399, v31422, v31423, v31424, v31425, v31426, v35305, v35342, v35347, v35348, v35350, v35354, v35356, v35358, v36167, v36168 or v36675, where the antigen-binding domain retains the ability to bind hFRα.

[0087] In certain embodiments, the anti-FRα antibody constructs of the present disclosure comprise an antigen-binding domain having:

(i) an HCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 20, 23, 26, 28, 31, 92, 93, 94, 95 or 96; an HCDR2 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 21, 24, 27, 29, 32, 51, 58, 100, 101, 102, 103, 109, 137, 138 or 139, and an HCDR3 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 22, 25, 30, 107, 108 or 110, and

(ii) a LCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 40, 43, 45, 65, 125, 126 or 127; a LCDR2 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 41, 44 or 46, and a LCDR3 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 42, 47, 120 or 121.

[0088] In certain embodiments, the anti-FRα antibody constructs of the present disclosure comprise an antigen-binding domain comprising the CDR sequences of the VH domain having a sequence as set forth in any one of SEQ ID NOs: 19, 50, 54, 57, 61, 76, 79, 82, 85, 88, 91, 99, 106, 113, 116, 133 or 136. In certain embodiments, the anti-FRα antibody constructs of the present disclosure comprise an antigen-binding domain comprising the CDR sequences of the VL domain having a sequence as set forth in any one of SEQ ID NOs: 39, 64, 119, 124 or 130.

[0089] In certain embodiments, the anti-FRα antibody constructs of the present disclosure comprise an antigen-binding domain having:

(a) a LCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 40, 43 or 45; a LCDR2 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 41, 44 or 46, and a LCDR3 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 42 or 47, and an HCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 20, 23, 26, 28 or 31; an HCDR2 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 21, 24, 27, 29 or 32; an HCDR3 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 22, 25 or 30;

(b) a LCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 125, 126 or 127; a LCDR2 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 41, 44 or 46, and a LCDR3 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 42 or 47, and an HCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 92, 93, 94, 95 or 96; an HCDR2 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 21, 24, 29, 32 or 51; an HCDR3 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 22, 25 or 30;

(c) a LCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 40, 45 or 65; a LCDR2 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 41, 44 or 46, and a LCDR3 amino acid

sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 42 or 47, and

(i) an HCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 20, 23, 26, 28 or 31; an HCDR2 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 21, 24, 29, 32 or 51; an HCDR3 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 22, 25 or 30, or

(ii) an HCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 20, 23, 26, 28 or 31; an HCDR2 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 21, 24, 29, 32 or 58; an HCDR3 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 22, 25 or 30, or

(iii) an HCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 92, 93, 94, 95 or 96; an HCDR2 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 24, 100, 101, 102 or 103; an HCDR3 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 22, 25 or 30; or

(d) a LCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 125, 126 or 127; a LCDR2 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 41, 44 or 46, and a LCDR3 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 120 or 121, and

(i) an HCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 92, 93, 94, 95 or 96; an HCDR2 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 24, 100, 101, 102 or 103; an HCDR3 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 22, 25 or 30, or

(ii) an HCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 92, 93, 94, 95 or 96; an HCDR2 amino acid sequence

selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 21, 24, 29, 32 or 109; an HCDR3 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 107, 108 or 110, or

(iii) an HCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 92, 93, 94, 95 or 96; an HCDR2 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 21, 24, 29, 32 or 109; an HCDR3 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 22, 25 or 30, or

(iv) an HCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 20, 23, 26, 28 or 31; an HCDR2 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 21, 24, 29, 32 or 109; an HCDR3 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 107, 108 or 110, or

(v) an HCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 20, 23, 26, 28 or 31; an HCDR2 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 21, 24, 29, 32 or 109; an HCDR3 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 22, 25 or 30, or

(vi) an HCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 92, 93, 94, 95 or 96; an HCDR2 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 21, 24, 137, 138 or 139; an HCDR3 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 22, 25 or 30; or

(e) a LCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 40, 45 or 65; a LCDR2 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 41, 44 or 46, and a LCDR3 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 120 or 121, and

(i) an HCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 92, 93, 94, 95 or 96; an HCDR2 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 21, 24, 29, 32 or 109; an HCDR3 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 107, 108 or 110, or

(ii) an HCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 20, 23, 26, 28 or 31; an HCDR2 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 21, 24, 29, 32 or 109; an HCDR3 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 107, 108 or 110.

[0090] In certain embodiments, the anti-FRα antibody constructs of the present disclosure comprise an antigen-binding domain having:

(a) an HCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 20, 23, 26, 28 or 31; an HCDR2 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 21, 24, 27, 29 or 32; an HCDR3 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 22, 25 or 30; a LCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 40, 43 or 45; a LCDR2 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 41, 44 or 46, and a LCDR3 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 42 or 47, or

(b) an HCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 20, 23, 26, 28 or 31; an HCDR2 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 21, 24, 29, 32 or 51; an HCDR3 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 22, 25 or 30; a LCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 40, 45 or 65; a LCDR2 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 41, 44 or 46, and a LCDR3 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 42 or 47, or

(c) an HCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 20, 23, 26, 28 or 31; an HCDR2 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 21, 24, 29, 32 or 58; an HCDR3 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 22, 25 or 30; a LCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 40, 45 or 65; a LCDR2 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 41, 44 or 46, and a LCDR3 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 42 or 47, or

(d) an HCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 92, 93, 94, 95 or 96; an HCDR2 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 21, 24, 29, 32 or 51; an HCDR3 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 22, 25 or 30; a LCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 125, 126 or 127; a LCDR2 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 41, 44 or 46, and a LCDR3 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 42 or 47, or

(e) an HCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 92, 93, 94, 95 or 96; an HCDR2 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 24, 100, 101, 102 or 103; an HCDR3 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 22, 25 or 30; a LCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 125, 126 or 127; a LCDR2 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 41, 44 or 46, and a LCDR3 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 120 or 121, or

(f) an HCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 92, 93, 94, 95 or 96; an HCDR2 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 21, 24, 29, 32 or 109; an HCDR3

amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 107, 108 or 110; a LCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 40, 45 or 65; a LCDR2 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 41, 44 or 46, and a LCDR3 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 42 or 47, or

(g) an HCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 92, 93, 94, 95 or 96; an HCDR2 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 21, 24, 29, 32 or 109; an HCDR3 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 107, 108 or 110; a LCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 40, 45 or 65; a LCDR2 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 41, 44 or 46, and a LCDR3 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 120 or 121, or

(h) an HCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 92, 93, 94, 95 or 96; an HCDR2 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 21, 24, 29, 32 or 109; an HCDR3 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 107, 108 or 110; a LCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 125, 126 or 127; a LCDR2 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 41, 44 or 46, and a LCDR3 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 120 or 121, or

(i) an HCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 92, 93, 94, 95 or 96; an HCDR2 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 21, 24, 29, 32 or 109; an HCDR3 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 22, 25 or 30; a LCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 125, 126 or 127; a LCDR2 amino acid sequence selected from

the amino acid sequences as set forth in any one of SEQ ID NOs: 41, 44 or 46, and a LCDR3 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 120 or 121, or

(j) an HCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 20, 23, 26, 28 or 31; an HCDR2 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 21, 24, 29, 32 or 109; an HCDR3 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 107, 108 or 110; a LCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 40, 45 or 65; a LCDR2 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 41, 44 or 46, and a LCDR3 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 120 or 121, or

(k) an HCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 20, 23, 26, 28 or 31; an HCDR2 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 21, 24, 29, 32 or 109; an HCDR3 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 107, 108 or 110; a LCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 125, 126 or 127; a LCDR2 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 41, 44 or 46, and a LCDR3 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 120 or 121, or

(l) an HCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 20, 23, 26, 28 or 31; an HCDR2 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 21, 24, 29, 32 or 109; an HCDR3 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 22, 25 or 30; a LCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 125, 126 or 127; a LCDR2 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 41, 44 or 46, and a LCDR3 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 120 or 121, or

(m) an HCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 92, 93, 94, 95 or 96; an HCDR2 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 21, 24, 137, 138 or 139; an HCDR3 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 22, 25 or 30; a LCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 125, 126 or 127; a LCDR2 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 41, 44 or 46, and a LCDR3 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 120 or 121.

[0091] In certain embodiments, the anti-FRα antibody constructs of the present disclosure comprise an antigen-binding domain comprising a VH amino acid sequence selected from the VH amino acid sequences as set forth in any one of SEQ ID NOs: 19, 50, 54, 57, 61, 76, 79, 82, 85, 88, 91, 99, 106, 113, 116, 133 or 136. In certain embodiments, the anti-FRα antibody constructs of the present disclosure comprise an antigen-binding domain comprising a VL amino acid sequence selected from the VL amino acid sequences as set forth in any one of SEQ ID NOs: 39, 64, 119, 124 or 130.

[0092] In certain embodiments, the anti-FRα antibody constructs of the present disclosure comprise an antigen-binding domain comprising a VH amino acid sequence selected from the VH amino acid sequences as set forth in any one of SEQ ID NOs: 19, 50, 54, 57, 61, 76, 79, 82, 85, 88, 91, 99, 106, 113, 116, 133 or 136, and a VL amino acid sequence selected from the VL amino acid sequences as set forth in any one of SEQ ID NOs: 39, 64, 119, 124 or 130.

[0093] In certain embodiments, the anti-FRα antibody constructs of the present disclosure comprise an antigen-binding domain comprising:

(a) a VL amino acid sequence as set forth in SEQ ID NO: 39, and a VH amino acid sequence as set forth in SEQ ID NO: 19; or

(b) a VL amino acid sequence as set forth in SEQ ID NO: 124, and a VH amino acid sequence as set forth in SEQ ID NO: 91; or

(c) a VL amino acid sequence as set forth in SEQ ID NO: 64, and

(i) a VH amino acid sequence as set forth in SEQ ID NO: 50, or

(ii) a VH amino acid sequence as set forth in SEQ ID NO: 54, or

(iii) a VH amino acid sequence as set forth in SEQ ID NO: 57, or

(iv) a VH amino acid sequence as set forth in SEQ ID NO: 61, or

(v) a VH amino acid sequence as set forth in SEQ ID NO: 76, or

(vi) a VH amino acid sequence as set forth in SEQ ID NO: 79, or

(vii) a VH amino acid sequence as set forth in SEQ ID NO: 82, or

(viii) a VH amino acid sequence as set forth in SEQ ID NO: 85, or

(ix) a VH amino acid sequence as set forth in SEQ ID NO: 88, or

(x) a VH amino acid sequence as set forth in SEQ ID NO: 106; or

(d) a VL amino acid sequence as set forth in SEQ ID NO: 130, and

(i) a VH amino acid sequence as set forth in SEQ ID NO: 99, or

(ii) a VH amino acid sequence as set forth in SEQ ID NO: 106, or

(iii) a VH amino acid sequence as set forth in SEQ ID NO: 113, or

(iv) a VH amino acid sequence as set forth in SEQ ID NO: 116, or

(v) a VH amino acid sequence as set forth in SEQ ID NO: 133, or

(vi) a VH amino acid sequence as set forth in SEQ ID NO: 136; or

(e) a VL amino acid sequence as set forth in SEQ ID NO: 119, and

(i) a VH amino acid sequence as set forth in SEQ ID NO: 106, or

(ii) a VH amino acid sequence as set forth in SEQ ID NO: 116.

[0094] In certain embodiments, the anti-FRα antibody constructs of the present disclosure comprise an antigen-binding domain comprising:

(i) a VH amino acid sequence as set forth in SEQ ID NO: 19, and a VL amino acid sequence as set forth in SEQ ID NO: 39, or

(ii) a VH amino acid sequence as set forth in SEQ ID NO: 50, and a VL amino acid sequence as set forth in SEQ ID NO: 64, or

(iii) a VH amino acid sequence as set forth in SEQ ID NO: 54, and a VL amino acid sequence as set forth in SEQ ID NO: 64, or

(iv) a VH amino acid sequence as set forth in SEQ ID NO: 57, and a VL amino acid sequence as set forth in SEQ ID NO: 64, or

(v) a VH amino acid sequence as set forth in SEQ ID NO: 61, and a VL amino acid sequence as set forth in SEQ ID NO: 64, or

(vi) a VH amino acid sequence as set forth in SEQ ID NO: 76, and a VL amino acid sequence as set forth in SEQ ID NO: 64, or

(vii) a VH amino acid sequence as set forth in SEQ ID NO: 79, and a VL amino acid sequence as set forth in SEQ ID NO: 64, or

(viii) a VH amino acid sequence as set forth in SEQ ID NO: 82, and a VL amino acid sequence as set forth in SEQ ID NO: 64, or

(ix) a VH amino acid sequence as set forth in SEQ ID NO: 85, and a VL amino acid sequence as set forth in SEQ ID NO: 64, or

(x) a VH amino acid sequence as set forth in SEQ ID NO: 88, and a VL amino acid sequence as set forth in SEQ ID NO: 64, or

(xi) a VH amino acid sequence as set forth in SEQ ID NO: 91, and a VL amino acid sequence as set forth in SEQ ID NO: 124, or

(xii) a VH amino acid sequence as set forth in SEQ ID NO: 99, and a VL amino acid sequence as set forth in SEQ ID NO: 130, or

(xiii) a VH amino acid sequence as set forth in SEQ ID NO: 106, and a VL amino acid sequence as set forth in SEQ ID NO: 64, or

(xiv) a VH amino acid sequence as set forth in SEQ ID NO: 106, and a VL amino acid sequence as set forth in SEQ ID NO: 119, or

(xv) a VH amino acid sequence as set forth in SEQ ID NO: 106, and a VL amino acid sequence as set forth in SEQ ID NO: 130, or

(xvi) a VH amino acid sequence as set forth in SEQ ID NO: 113, and a VL amino acid sequence as set forth in SEQ ID NO: 130, or

(xvii) a VH amino acid sequence as set forth in SEQ ID NO: 116, and a VL amino acid sequence as set forth in SEQ ID NO: 119 or

(xviii) a VH amino acid sequence as set forth in SEQ ID NO: 116, and a VL amino acid sequence as set forth in SEQ ID NO: 130, or

(xix) a VH amino acid sequence as set forth in SEQ ID NO: 133, and a VL amino acid sequence as set forth in SEQ ID NO: 130, or

(xx) a VH amino acid sequence as set forth in SEQ ID NO: 136, and a VL amino acid sequence as set forth in SEQ ID NO: 130.

Formats

[0095] The anti-FRα antibody constructs of the present disclosure may have various formats. The minimal component of the anti-FRα antibody construct is an antigen-binding domain that binds to hFRα. The anti-FRα antibody constructs may further optionally comprise one or more additional antigen-binding domains and/or a scaffold. In those embodiments in which the anti-FRα antibody construct comprises two or more antigen-binding domains, each additional antigen-binding domain may bind to the same epitope within hFRα, may bind to a different epitope within hFRα, or may bind to a different antigen. Thus, the anti-FRα antibody construct may be, for example, monospecific, biparatopic, bispecific or multispecific.

[0096] In certain embodiments, the anti-FRα antibody construct comprises at least one antigen-binding domain that binds to hFRα and a scaffold, where the antigen-binding domain is operably linked to the scaffold. The term “operably linked,” as used herein, means that the components described are in a relationship permitting them to function in their intended manner. Examples of suitable scaffolds are described below.

[0097] In certain embodiments, the anti-FRα antibody construct comprises two antigen-binding domains optionally operably linked to a scaffold. In some embodiments, the anti-FRα antibody

construct may comprise three or four antigen-binding domains and optionally a scaffold. In these formats, when comprising a scaffold, at least a first antigen-binding domain is operably linked to the scaffold and the remaining antigen-binding domain(s) may each independently be operably linked to the scaffold or to the first antigen-binding domain or, when more than two antigen-binding domains are present, to another antigen-binding domain.

[0098] Anti-FRα antibody constructs that lack a scaffold may comprise a single antigen-binding domain in an appropriate format, such as an sdAb, or they may comprise two or more antigen-binding domains optionally operably linked by one or more linkers. In such anti-FRα antibody constructs, the antigen-binding domains may be in the form of scFvs, Fabs, sdAbs, or a combination thereof. For example, using scFvs as the antigen-binding domains, formats such as a tandem scFv ($(scFv)_2$ or taFv) may be constructed, in which the scFvs are connected together by a flexible linker. scFvs may also be used to construct diabody formats, which comprise two scFvs connected by a short linker (usually about 5 amino acids in length). The restricted length of the linker results in dimerization of the scFvs in a head-to-tail manner. In any of the preceding formats, the scFvs may be further stabilized by inclusion of an interdomain disulfide bond. For example, a disulfide bond may be introduced between VL and VH through introduction of an additional cysteine residue in each chain (for example, at position 44 in VH and position 100 in VL) (see, for example, Fitzgerald *et al.*, 1997, *Protein Engineering*, 10:1221-1225), or a disulfide bond may be introduced between two VHs to provide a construct having a DART format (see, for example, Johnson *et al.*, 2010, *J Mol. Biol.*, 399:436-449).

[0099] Similarly, formats comprising two sdAbs, such as VHs or VHHs, connected together through a suitable linker may be employed in some embodiments. Other examples of anti-FRα antibody construct formats that lack a scaffold include those based on Fab fragments, for example, Fab_2 and $F(ab')_2$ formats, in which the Fab fragments are connected through a linker or an IgG hinge region.

[00100] Combinations of antigen-binding domains in different forms may also be employed to generate alternative scaffold-less formats. For example, an scFv or a sdAb may be fused to the C-terminus of either or both of the light and heavy chain of a Fab fragment resulting in a bivalent (Fab-scFv/sdAb) construct.

[00101] In certain embodiments, the anti-FRα antibody construct may be in an antibody format that is based on an immunoglobulin (Ig). In certain embodiments, the anti-FRα antibody construct may be based on an IgG class immunoglobulin, for example, an IgGl, IgG2, IgG3 or IgG4 immunoglobulin. In some embodiments, the anti-FRα antibody construct may be based on an IgG1 immunoglobulin. In the context of the present disclosure, when an anti-FRα antibody construct is based on a specified immunoglobulin isotype, it is meant that the anti-FRα antibody construct comprises all or a portion of the constant region of the specified immunoglobulin isotype. For example, an anti-FRα antibody construct based on a given Ig isotype may comprise at least one antigen-binding domain operably linked to an Ig scaffold, where the scaffold comprises an Fc region from the given isotype and optionally an Ig hinge region from the same or a different isotype. It is to be understood that the anti-FRα antibody constructs may also comprise hybrids of isotypes and/or subclasses in some embodiments. It is also to be understood that the Fc region and/or hinge region may optionally be modified to impart one or more desirable functional properties as is known in the art.

[00102] In some embodiments, the anti-FRα antibody constructs may be derived from two or more immunoglobulins that are from different species, for example, the anti-FRα antibody construct may be a chimeric antibody or a humanized antibody. The terms "chimeric antibody" and "humanized antibody" both refer generally to antibodies that combine immunoglobulin regions or domains from more than one species.

[00103] A "chimeric antibody" typically comprises at least one variable domain from a non-human antibody, such as a rabbit or rodent (for example, murine) antibody, and at least one constant domain from a human antibody. The human constant domain of a chimeric antibody need not be of the same isotype as the non-human constant domain it replaces. Chimeric antibodies are discussed, for example, in Morrison *et al.*, 1984, *Proc. Natl. Acad. Sci. USA*, 81:6851-55, and U.S. Patent No. 4,816,567.

[00104] A "humanized antibody" is a type of chimeric antibody that contains minimal sequence derived from a non-human antibody. Generally, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a hypervariable region (CDR) of the recipient are replaced by residues from a hypervariable region (CDR) of a non-human species

(donor antibody), such as mouse, rat, rabbit or non-human primate, having the desired specificity and affinity for a target antigen. This technique for creating humanized antibodies is often referred to as "CDR grafting."

[00105] In some instances, additional modifications may be made to a humanized antibody to further refine antibody performance. For example, framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues, or the humanized antibodies may comprise residues that are not found in either the recipient antibody or the donor antibody. In general, a variable domain in a humanized antibody will comprise all or substantially all of the hypervariable regions from a non-human immunoglobulin and all or substantially all of the FRs from a human immunoglobulin sequence. Humanized antibodies are described in more detail in Jones, *et al.*, 1986, *Nature,* 321:522-525; Riechmann, *et al.,* 1988, *Nature,* 332:323-329, and Presta, 1992, *Curr. Op. Struct. Biol.,* 2:593-596, for example.

[00106] A number of approaches are known in the art for selecting the most appropriate human frameworks in which to graft the non-human CDRs. Early approaches used a limited subset of well-characterised human antibodies, irrespective of the sequence identity to the non-human antibody providing the CDRs (the "fixed frameworks" approach). More recent approaches have employed variable regions with high amino acid sequence identity to the variable regions of the non-human antibody providing the CDRs ("homology matching" or "best-fit" approach). An alternative approach is to select fragments of the framework sequences within each light or heavy chain variable region from several different human antibodies. CDR-grafting may in some cases result in a partial or complete loss of affinity of the grafted molecule for its target antigen. In such cases, affinity can be restored by back-mutating some of the residues of human origin to the corresponding non-human ones. Methods for preparing humanized antibodies by these approaches are well-known in the art (see, for example, Tsurushita & Vasquez, 2004, *Humanization of Monoclonal Antibodies*, Molecular Biology of B Cells, 533-545, Elsevier Science (USA); Jones *et al.*, 1986, *Nature*, 321:522-525; Riechmann *et al.*, 1988, *Nature*, 332:323-329; Presta *et al.,* 1997, *Cancer Res,* 57(20):4593-4599).

[00107] Alternatively, or in addition to, these traditional approaches, more recent technologies may be employed to further reduce the immunogenicity of a CDR-grafted humanized antibody.

For example, frameworks based on human germline sequences or consensus sequences may be employed as acceptor human frameworks rather than human frameworks with somatic mutation(s). Another technique that aims to reduce the potential immunogenicity of non-human CDRs is to graft only specificity-determining residues (SDRs). In this approach, only the minimum CDR residues required for antigen-binding activity (the "SDRs") are grafted into a human germline framework. This method improves the "humanness" (*i.e.* the similarity to human germline sequence) of the humanized antibody and thus may help reduce the risk of immunogenicity of the variable region. These techniques have been described in various publications (see, for example, Almagro & Fransson, 2008, *Front Biosci,* 13:1619-1633; Tan, *et al.*, 2002, *J Immunol,* 169:1119-1125; Hwang, *et al.*, 2005, *Methods,* 36:35-42; Pelat, *et al.*, 2008, *J Mol Biol,* 384:1400-1407; Tamura, *et al.*, 2000, *J Immunol*, 164:1432-1441; Gonzales, *et al.*, 2004, *Mol Immunol,* 1:863-872, and Kashmiri, *et al.*, 2005, *Methods*, 36:25-34).

[00108] In certain embodiments, the anti-FRα antibody construct of the present disclosure comprises humanized antibody sequences, for example, one or more humanized variable domains. In some embodiments, the anti-FRα antibody construct can be a humanized antibody. Non-limiting examples of humanized antibodies based on the anti-FRα antibody v23924 are described herein (v30384, v30389, v30394, v30399, v31422, v31423, v31424, v31425 and v31426; see Examples and Sequence Tables).

Scaffolds

[00109] In certain embodiments, the anti-FRα antibody constructs of the present disclosure comprise one or more antigen-binding domains operably linked to a scaffold. The antigen-binding domain(s) may be in one or a combination of the forms described above (for example, scFvs, Fabs and/or sdAbs). Examples of suitable scaffolds are described in more detail below and include, but are not limited to, immunoglobulin Fc regions, albumin, albumin analogues and derivatives, heterodimerizing peptides (such as leucine zippers, heterodimer-forming "zipper" peptides derived from Jun and Fos, IgG CH1 and CL domains or barnase-barstar toxins), cytokines, chemokines or growth factors. Other examples include antibodies based on the DOCK-AND-LOCK™ (DNL™) technology developed by IBC Pharmaceuticals, Inc. and Immunomedics, Inc. (see, for example, Chang, *et al.*, 2007, *Clin. Cancer Res.*, 13:5586s-5591s).

[00110] A scaffold may be a peptide, polypeptide, polymer, nanoparticle or other chemical entity. Where the scaffold is a polypeptide, each antigen-binding domain of the anti-FRα antibody construct may be linked to either the N- or C-terminus of the polypeptide scaffold. Anti-FRα antibody constructs comprising a polypeptide scaffold in which one or more of the antigen-binding domains are linked to a region other than the N- or C-terminus, for example, via the side chain of an amino acid with or without a linker, are also contemplated in certain embodiments.

[00111] In embodiments where the anti-FRα antibody construct comprises a scaffold that is a peptide or polypeptide, the antigen-binding domain(s) may be linked to the scaffold by genetic fusion or chemical conjugation. Typically, when the scaffold is a peptide or polypeptide, the antigen-binding domain(s) are linked to the scaffold by genetic fusion. In some embodiments, where the scaffold is a polymer or nanoparticle, the antigen-binding domain(s) may be linked to the scaffold by chemical conjugation.

[00112] A number of protein domains are known in the art that comprise selective pairs of two different polypeptides and may be used to form a scaffold. An example is leucine zipper domains such as Fos and Jun that selectively pair together (Kostelny, *et al.,* J Immunol, 148:1547-53 (1992); Wranik, *et al.,* J. Biol. Chem., 287: 43331-43339 (2012)). Other selectively pairing molecular pairs include, for example, the barnase-barstar pair (Deyev, *et al.,* Nat Biotechnol, 21:1486-1492 (2003)), DNA strand pairs (Chaudri, *et al.,* FEBS Letters, 450(1–2):23-26 (1999)) and split fluorescent protein pairs (International Patent Application Publication No. WO 2011/135040).

[00113] Other examples of protein scaffolds include immunoglobulin Fc regions, albumin, albumin analogues and derivatives, toxins, cytokines, chemokines and growth factors. The use of protein scaffolds in combination with antigen-binding moieties has been described (see, for example, Müller *et al.,* 2007, *J. Biol. Chem.,* 282:12650-12660; McDonaugh *et al.,* 2012, *Mol. Cancer Ther.,* 11:582-593; Vallera *et al.,* 2005, *Clin. Cancer Res.,* 11:3879-3888; Song *et al.,* 2006, *Biotech. Appl. Biochem.*, 45:147-154, and U.S. Patent Application Publication No. 2009/0285816).

[00114] For example, fusing antigen-binding moieties such as scFvs, diabodies or single chain diabodies to albumin has been shown to improve the serum half-life of the antigen-binding

moieties (Müller *et al., ibid.*). Antigen-binding moieties may be fused at the N- and/or C-termini of albumin, optionally via a linker.

[00115] Derivatives of albumin in the form of heteromultimers that comprise two transporter polypeptides obtained by segmentation of an albumin protein such that the transporter polypeptides self-assemble to form quasi-native albumin have been described (see International Patent Application Publication Nos. WO 2012/116453 and WO 2014/012082). As a result of the segmentation of albumin, the heteromultimer includes four termini and thus can be fused to up to four different antigen-binding moieties, optionally via linkers.

[00116] In certain embodiments, the anti-FRα antibody construct may comprise a protein scaffold. In some embodiments, the anti-FRα antibody construct may comprise a protein scaffold that is based on an immunoglobulin Fc region, an albumin or an albumin analogue or derivative. In some embodiments, the anti-FRα antibody construct may comprise a protein scaffold that is based on an immunoglobulin Fc region, for example, an IgG Fc region.

Fc Regions

[00117] The terms "Fc region," "Fc" or "Fc domain" as used herein refer to a C-terminal region of an immunoglobulin heavy chain that contains at least a portion of the constant region. The term includes native sequence Fc regions and variant Fc regions. Unless otherwise specified herein, numbering of amino acid residues in the Fc region or constant region is according to the EU numbering system, also called the EU index, as described in Kabat, *et al.,* Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD (1991).

[00118] In certain embodiments, the anti-FRα antibody constructs of the present disclosure may comprise a scaffold that is based on an immunoglobulin Fc region. The Fc region may be dimeric and composed of two Fc polypeptides or alternatively, the Fc region may be composed of a single polypeptide.

[00119] An "Fc polypeptide" in the context of a dimeric Fc refers to one of the two polypeptides forming the dimeric Fc domain, *i.e.* a polypeptide comprising one or more C-terminal constant regions of an immunoglobulin heavy chain that is capable of stable self-association. When

referring to the polypeptides forming a dimeric Fc region, the terms "first Fc polypeptide" and "second Fc polypeptide" may be used interchangeably provided that the Fc region comprises one first Fc polypeptide and one second Fc polypeptide.

[00120] An Fc region may comprise a CH3 domain or it may comprise both a CH3 and a CH2 domain. For example, in certain embodiments, an Fc polypeptide of a dimeric IgG Fc region may comprise an IgG CH2 domain sequence and an IgG CH3 domain sequence. In such embodiments, the CH3 domain comprises two CH3 sequences, one from each of the two Fc polypeptides of the dimeric Fc region, and the CH2 domain comprises two CH2 sequences, one from each of the two Fc polypeptides of the dimeric Fc region.

[00121] In some embodiments, the anti-FRα antibody construct may comprise a scaffold that is based on an IgG Fc region. In some embodiments, the anti-FRα antibody construct may comprise a scaffold that is based on a human IgG Fc region. In some embodiments, the anti-FRα antibody construct may comprise a scaffold based on an IgG1 Fc region. In some embodiments, the anti-FRα antibody construct may comprise a scaffold based on a human IgG1 Fc region.

[00122] In certain embodiments, the anti-FRα antibody construct may comprise a scaffold based on an IgG Fc region, which is a homodimeric Fc region, comprising a first Fc polypeptide and a second Fc polypeptide, each comprising a CH3 sequence, and optionally a CH2 sequence and in which the amino acid sequences of the first and second Fc polypeptides are the same.

[00123] In certain embodiments, the anti-FRα antibody construct may comprise a scaffold based on an IgG Fc region, which is a heterodimeric Fc region, comprising a first Fc polypeptide and a second Fc polypeptide, each comprising a CH3 sequence, and optionally a CH2 sequence and in which the amino acid sequences of the first and second Fc polypeptides are different. In some embodiments, the anti-FRα antibody construct may comprise a scaffold based on an Fc region which comprises two CH3 sequences, at least one of which comprises one or more amino acid modifications. In some embodiments, the anti-FRα antibody construct may comprise a scaffold based on an Fc region which comprises two CH3 sequences and two CH2 sequences, at least one of the CH2 sequences comprising one or more amino acid modifications.

[00124] In some embodiments, the anti-FRα antibody construct may comprise a heterodimeric Fc region comprising a modified CH3 domain, where the modified CH3 domain is an asymmetrically modified CH3 domain comprising one or more asymmetric amino acid modifications. As used herein, an "asymmetric amino acid modification" refers to a modification, such as a substitution or an insertion, in which an amino acid at a specific position on a first CH3 or CH2 sequence is different to the amino acid on a second CH3 or CH2 sequence at the same position. These asymmetric amino acid modifications can be a result of modification of only one of the two amino acids at the same respective amino acid position on each sequence, or different modifications of both amino acids at the same respective position on each of the first and second CH3 or CH2 sequences. Each of the first and second CH3 or CH2 sequences of a heterodimeric Fc may comprise one or more than one asymmetric amino acid modification.

[00125] In some embodiments, the anti-FRα antibody construct may comprise a heterodimeric Fc comprising a modified CH3 domain, where the modified CH3 domain comprises one or more amino acid modifications that promote formation of the heterodimeric Fc over formation of a homodimeric Fc. In some embodiments, one or more of the amino acid modifications are asymmetric amino acid modifications.

[00126] Amino acid modifications that may be made to the CH3 domain of an Fc in order to promote formation of a heterodimeric Fc are known in the art and include, for example, those described in International Publication No. WO 96/027011 ("knobs into holes"), Gunasekaran *et al.*, 2010, *J Biol Chem*, 285, 19637-46 ("electrostatic steering"), Davis *et al.*, 2010, *Prot Eng Des Sel*, 23(4):195-202 (strand exchange engineered domain (SEED) technology) and Labrijn *et al.*, 2013, *Proc Natl Acad Sci USA*, 110(13):5145-50 (Fab-arm exchange). Other examples include approaches combining positive and negative design strategies to produce stable asymmetrically modified Fc regions as described in International Publication Nos. WO 2012/058768 and WO 2013/063702. In certain embodiments, the anti-FRα antibody construct may comprise a scaffold based on a modified Fc region as described in International Publication No. WO 2012/058768 or WO 2013/063702.

[00127] Table 5 provides the amino acid sequence of the human IgG1 Fc sequence (SEQ ID NO:16), corresponding to amino acids 231 to 447 of the full-length human IgG1 heavy chain. The

CH3 sequence comprises amino acids 341-447 of the full-length human IgG1 heavy chain. Also shown in Table 5 are CH3 domain amino acid modifications that promote formation of a heterodimeric Fc as described in in International Patent Application Publication Nos. WO 2012/058768 and WO 2013/063702.

[00128] In certain embodiments, the anti-FRα antibody construct may comprise a heterodimeric Fc scaffold having a modified CH3 domain comprising the modifications of any one of Variant 1, Variant 2, Variant 3, Variant 4 or Variant 5, as shown in Table 5.

Table 5: Human IgG1 Fc Sequence[1] and CH3 Domain Amino Acid Modifications Promoting Heterodimer Formation

APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 16)		
Variant No	**Chain**	**Mutations**
1	A	L351Y_F405A_Y407V
	B	T366L_K392M_T394W
2	A	L351Y_F405A_Y407V
	B	T366L_K392L_T394W
3	A	T350V_L351Y_F405A_Y407V
	B	T350V_T366L_K392L_T394W
4	A	T350V_L351Y_F405A_Y407V
	B	T350V_T366L_K392M_T394W
5	A	T350V_L351Y_S400E_F405A_Y407V
	B	T350V_T366L_N390R_K392M_T394W

[1] Sequence from positions 231-447 (EU numbering)

[00129] In some embodiments, the anti-FRα antibody construct may comprise a scaffold based on an Fc region comprising two CH3 sequences and two CH2 sequences, at least one of the CH2 sequences comprising one or more amino acid modifications. Modifications in the CH2 domain

can affect the binding of Fc receptors (FcRs) to the Fc, such as receptors of the FcγRI, FcγRII and FcγRIII subclasses.

[00130] In some embodiments, the anti-FRα antibody construct comprises a scaffold based on an IgG Fc having a modified CH2 domain, wherein the modification of the CH2 domain results in altered binding to one or more of the FcγRI, FcγRII and FcγRIII receptors.

[00131] A number of amino acid modifications to the CH2 domain that selectively alter the affinity of the Fc for different Fcγ receptors are known in the art. Amino acid modifications that result in increased binding and amino acid modifications that result in decreased binding can each be useful in certain indications. For example, increasing binding affinity of an Fc for FcγRIIIa (an activating receptor) may result in increased antibody dependent cell-mediated cytotoxicity (ADCC), which in turn results in increased lysis of the target cell. Decreased binding to FcγRIIb (an inhibitory receptor) likewise may be beneficial in some circumstances. In certain indications, a decrease in, or elimination of, ADCC and complement-mediated cytotoxicity (CDC) may be desirable. In such cases, modified CH2 domains comprising amino acid modifications that result in increased binding to FcγRIIb or amino acid modifications that decrease or eliminate binding of the Fc region to all of the Fcγ receptors ("knock-out" variants) may be useful.

[00132] Examples of amino acid modifications to the CH2 domain that alter binding of the Fc by Fcγ receptors include, but are not limited to, the following: S298A/E333A/K334A and S298A/E333A/K334A/K326A (increased affinity for FcγRIIIa) (Lu, *et al.*, 2011, *J Immunol Methods*, 365(1-2):132-41); F243L/R292P/Y300L/V305I/P396L (increased affinity for FcγRIIIa) (Stavenhagen, *et al.* 2007, *Cancer Res* 67(18):8882-90); F243L/R292P/Y300L/L235V/P396L (increased affinity for FcγRIIIa) (Nordstrom JL, *et al.*, 2011, *Breast Cancer Res*, 13(6):R123); F243L (increased affinity for FcγRIIIa) (Stewart, *et al.*, 2011, *Protein Eng Des Sel.*, 24(9):671-8); S298A/E333A/K334A (increased affinity for FcγRIIIa) (Shields, *et al.*, 2001, *J Biol Chem,* 276(9):6591-604); S239D/I332E/A330L and S239D/I332E (increased affinity for FcγRIIIa) (Lazar, *et al.*, 2006, *Proc Natl Acad Sci USA*, 103(11):4005-10), and S239D/S267E and S267E/L328F (increased affinity for FcγRIIb) (Chu, *et al.*, 2008, *Mol Immunol,* 45(15):3926-33). Various amino acid modifications to the CH2 domain that alter binding of the Fc by FcγRIIb are described in International Publication No. WO 2021/232162. Additional

modifications that affect Fc binding to Fcγ receptors are described in *Therapeutic Antibody Engineering* (Strohl & Strohl, Woodhead Publishing series in Biomedicine No 11, ISBN 1 907568 37 9, Oct 2012, page 283).

[00133] In certain embodiments, the anti-FRα antibody construct comprises a scaffold based on an IgG Fc having a modified CH2 domain, in which the modified CH2 domain comprises one or more amino acid modifications that result in decreased or eliminated binding of the Fc region to all of the Fcγ receptors (*i.e.* a "knock-out" variant).

[00134] Various publications describe strategies that have been used to engineer antibodies to produce "knock-out" variants (see, for example, Strohl, 2009, *Curr Opin Biotech* 20:685-691, and Strohl & Strohl, "*Antibody Fc engineering for optimal antibody performance*" In Therapeutic Antibody Engineering, Cambridge: Woodhead Publishing, 2012, pp 225-249). These strategies include reduction of effector function through modification of glycosylation, use of IgG2/IgG4 scaffolds, or the introduction of mutations in the hinge or CH2 domain of the Fc (see also, U.S. Patent Publication No. 2011/0212087, International Publication No. WO 2006/105338, U.S. Patent Publication No. 2012/0225058, U.S. Patent Publication No. 2012/0251531 and Strop *et al.*, 2012, *J. Mol. Biol.*, 420: 204-219).

[00135] Examples of mutations that may be introduced into the hinge or CH2 domain to produce a "knock-out" variant include the amino acid modifications L234A/L235A, and L234A/L235A/D265S.

[00136] In certain embodiments, the anti-FRα antibody constructs described herein may comprise a scaffold based on an IgG Fc in which native glycosylation has been modified. As is known in the art, glycosylation of an Fc may be modified to increase or decrease effector function. For example, mutation of the conserved asparagine residue at position 297 to alanine, glutamine, lysine or histidine (*i.e.* N297A, Q, K or H) results in an aglycoslated Fc that lacks all effector function (Bolt *et al.*, 1993, *Eur. J. Immunol.*, 23:403-411; Tao & Morrison, 1989, *J. Immunol.*, 143:2595-2601).

[00137] Conversely, removal of fucose from heavy chain N297-linked oligosaccharides has been shown to enhance ADCC, based on improved binding to FcγRIIIa (see, for example, Shields *et al.*,

2002, *J Biol Chem.*, 277:26733-26740, and Niwa *et al.*, 2005, *J. Immunol. Methods,* 306:151-160). Such low fucose antibodies may be produced, for example in knockout Chinese hamster ovary (CHO) cells lacking fucosyltransferase (FUT8) (Yamane-Ohnuki *et al.*, 2004, *Biotechnol. Bioeng.*, 87:614-622); in the variant CHO cell line, Lec 13, that has a reduced ability to attach fucose to N297-linked carbohydrates (International Publication No. WO 03/035835), or in other cells that generate afucosylated antibodies (see, for example, Li *et al.*, 2006, *Nat Biotechnol*, 24:210-215; Shields *et al.*, 2002, *ibid*, and Shinkawa *et al.*, 2003, *J. Biol. Chem.*, 278:3466-3473). In addition, International Publication No. WO 2009/135181 describes the addition of fucose analogues to culture medium during antibody production to inhibit incorporation of fucose into the carbohydrate on the antibody.

[00138] Other methods of producing antibodies with little or no fucose on the Fc glycosylation site (N297) are well known in the art. For example, the GlymaX® technology (ProBioGen AG) (see von Horsten *et al.*, 2010, *Glycobiology*, 20(12):1607-1618 and U.S. Patent No. 8,409,572).

[00139] Other glycosylation variants include those with bisected oligosaccharides, for example, variants in which a biantennary oligosaccharide attached to the Fc region of the antibody is bisected by N-acetylglucosamine (GlcNAc). Such glycosylation variants may have reduced fucosylation and/or improved ADCC function (see, for example, International Publication No. WO 2003/011878, U.S. Patent No. 6,602,684 and US Patent Application Publication No. US 2005/0123546). Useful glycosylation variants also include those having at least one galactose residue in the oligosaccharide attached to the Fc region, which may have improved CDC function (see, for example, International Publication Nos. WO 1997/030087, WO 1998/58964 and WO 1999/22764).

[00140] In certain embodiments, the anti-FRα antibody constructs have the format of a full-size antibody (FSA). In some embodiments, the anti-FRα antibody constructs have the format of an IgG FSA, for example, an IgG1 FSA. In some embodiments, the anti-FRα antibody construct is a FSA comprising a first heavy chain sequence (H1), a second heavy chain sequence (H2), a first light chain sequence (L1) and a second light chain sequence (L2). In some embodiments, the anti-FRα antibody construct is a monospecific FSA with a homodimeric Fc and comprises H1, H2, L1 and L2 sequence, where H1 and H2 have the same amino acid sequence, and L1 and L2 have the

same amino acid sequence. In some embodiments, the anti-FRα antibody construct is a monospecific FSA with a heterodimeric Fc and comprises H1, H2, L1 and L2 sequences, where H1 and H2 have different amino acid sequences, and L1 and L2 have the same amino acid sequence. In some embodiments, the anti-FRα antibody construct is a bispecific or biparatopic FSA with a heterodimeric Fc and comprises H1, H2, L1 and L2 sequences, where H1 and H2 have different amino acid sequences, and L1 and L2 have different amino acid sequences.

[00141] In certain embodiments, the anti-FRα antibody construct is a FSA having a set of H1, H2, L1 and L2 sequences comprising the H1, H2, L1 and L2 amino acid sequences as set forth in Tables A & B for any one of variants v23924, v30618, v30384, v30389, v30394, v30399, v31422, v31423, v31424, v31425, v31426, v35305, v35342, v35347, v35348, v35350, v35354, v35356, v35358, v36167, v36168 or v36675. As is known in the art, expression of antibody heavy chain sequences in certain cell lines or from certain expression vector may result in the inclusion of a C-terminal lysine residue on one or both of the heavy chains. Accordingly, certain embodiments of the present disclosure relate to anti-FRα antibody constructs that are FSAs having a set of H1, H2, L1 and L2 sequences comprising the H1, H2, L1 and L2 amino acid sequences as set forth in Tables A & B for any one of variants v23924, v30618, v30384, v30389, v30394, v30399, v31422, v31423, v31424, v31425, v31426, v35305, v35342, v35347, v35348, v35350, v35354, v35356, v35358, v36167, v36168 or v36675, in which one or both of the H1 and H2 sequences comprise a C-terminal lysine (see, for example, SEQ ID NO: 157).

PREPARATION OF ANTI-FRα ANTIBODY CONSTRUCTS

[00142] The anti-FRα antibody constructs described herein may be produced using standard recombinant methods known in the art (see, for example, U.S. Patent No. 4,816,567 and "*Antibodies: A Laboratory Manual*," 2nd Edition, Ed. Greenfield, Cold Spring Harbor Laboratory Press, New York, 2014).

[00143] Typically, for recombinant production of an antibody construct, a polynucleotide or set of polynucleotides encoding the anti-FRα antibody construct is generated and inserted into one or more vectors for further cloning and/or expression in a host cell. Polynucleotide(s) encoding the anti-FRα antibody construct may be produced by standard methods known in the art (see, for example, Ausubel *et al., Current Protocols in Molecular Biology*, John Wiley & Sons, New York,

1994 & update, and "*Antibodies: A Laboratory Manual*," 2nd Edition, Ed. Greenfield, Cold Spring Harbor Laboratory Press, New York, 2014). As would be appreciated by one of skill in the art, the number of polynucleotides required for expression of the anti-FRα antibody construct will be dependent on the format of the construct, including whether or not the antibody construct comprises a scaffold. For example, when an anti-FRα antibody construct is in a mAb format with a homodimeric Fc, two polynucleotides each encoding one polypeptide chain will be required, whereas when an anti-FRα antibody construct is in a mAb format with a heterodimeric Fc, three polynucleotides each encoding one polypeptide chain will be required. When multiple polynucleotides are required, they may be incorporated into one vector or into more than one vector.

[00144] Generally, for expression, the polynucleotide or set of polynucleotides is incorporated into an expression vector or vectors together with one or more regulatory elements, such as transcriptional elements, which are required for efficient transcription of the polynucleotide. Examples of such regulatory elements include, but are not limited to, promoters, enhancers, terminators, and polyadenylation signals. One skilled in the art will appreciate that the choice of regulatory elements is dependent on the host cell selected for expression of the antibody construct and that such regulatory elements may be derived from a variety of sources, including bacterial, fungal, viral, mammalian or insect genes. The expression vector may optionally further contain heterologous nucleic acid sequences that facilitate expression or purification of the expressed protein. Examples include, but are not limited to, signal peptides and affinity tags such as metal-affinity tags, histidine tags, avidin/streptavidin encoding sequences, glutathione-S-transferase (GST) encoding sequences and biotin encoding sequences. The expression vector may be an extrachromosomal vector or an integrating vector.

[00145] Suitable host cells for cloning or expression of the anti-FRα antibody constructs include various prokaryotic or eukaryotic cells as known in the art. Eukaryotic host cells include, for example, mammalian cells, plant cells, insect cells and yeast cells (such as *Saccharomyces* or *Pichia* cells). Prokaryotic host cells include, for example, *E. coli, A. salmonicida* or *B. subtilis* cells.

[00146] In certain embodiments, the anti-FRα antibody construct may be produced in bacteria, in particular when glycosylation and Fc effector function are not needed, as described for example in U.S. Patent Nos. 5,648,237; 5,789,199, and 5,840,523, and in Charlton, *Methods in Molecular Biology,* Vol. 248, pp. 245-254, B.K.C. Lo, ed., Humana Press, Totowa, N.J., 2003.

[00147] Eukaryotic microbes such as filamentous fungi or yeast may be suitable expression host cells in certain embodiments, in particular fungi and yeast strains whose glycosylation pathways have been "humanized" resulting in the production of an antibody construct with a partially or fully human glycosylation pattern (see, for example, Gerngross, 2004, *Nat. Biotech.* 22:1409-1414, and Li *et al.*, 2006, *Nat. Biotech.* 24:210-215).

[00148] Suitable host cells for the expression of glycosylated anti-FRα antibody constructs are usually eukaryotic cells. For example, U.S. Patent Nos. 5,959,177, 6,040,498, 6,420,548, 7,125,978 and 6,417,429 describe PLANTIBODIES™ technology for producing antigen-binding constructs in transgenic plants. Mammalian cell lines adapted to grow in suspension may be particularly useful for expression of antibody constructs. Examples include, but are not limited to, monkey kidney CV1 line transformed by SV40 (COS-7), human embryonic kidney (HEK) line 293 or 293 cells (see, for example, Graham *et al.,* 1977, *J. Gen Virol.,* 36:59), baby hamster kidney cells (BHK), mouse sertoli TM4 cells (see, for example, Mather, 1980, *Biol Reprod,* 23:243-251), monkey kidney cells (CV1), African green monkey kidney cells (VERO-76), human cervical carcinoma (HeLa) cells, canine kidney cells (MDCK), buffalo rat liver cells (BRL 3A), human lung cells (W138), human liver cells (Hep G2), mouse mammary tumour (MMT 060562), TRI cells (see, for example, Mather *et al.,* 1982, *Annals N.Y. Acad Sci,* 383:44-68), MRC 5 cells, FS4 cells, Chinese hamster ovary (CHO) cells (including $DHFR^-$ CHO cells, see Urlaub *et al.,* 1980, *Proc Natl Acad Sci USA*, 77:4216), and myeloma cell lines (such as Y0, NS0 and Sp2/0). Exemplary mammalian host cell lines suitable for production of antibody constructs are reviewed in Yazaki & Wu, *Methods in Molecular Biology*, Vol. 248, pp. 255-268 (B.K.C. Lo, ed., Humana Press, Totowa, N.J., 2003).

[00149] In certain embodiments, the host cell may be a transient or stable higher eukaryotic cell line, such as a mammalian cell line. In some embodiments, the host cell may be a mammalian HEK293T, CHO, HeLa, NS0 or COS cell line, or a cell line derived from any one of these cell

lines. In some embodiments, the host cell may be a stable cell line that allows for mature glycosylation of the antibody construct.

[00150] The host cells comprising the expression vector(s) encoding the anti-FRα antibody construct may be cultured using routine methods to produce the anti-FRα antibody construct. Alternatively, in some embodiments, host cells comprising the expression vector(s) encoding the anti-FRα antibody construct may be used therapeutically or prophylactically to deliver the anti-FRα antibody construct to a subject, or polynucleotides or expression vectors may be administered to a cell from a subject *ex vivo* and the cell then returned to the body of the subject.

[00151] Typically, the anti-FRα antibody constructs are purified after expression. Proteins may be isolated or purified in a variety of ways known to those skilled in the art (see, for example, *Protein Purification: Principles and Practice*, 3rd Ed., Scopes, Springer-Verlag, NY, 1994). Standard purification methods include chromatographic techniques, including ion exchange, hydrophobic interaction, affinity, sizing or gel filtration, and reverse-phase, carried out at atmospheric pressure or at high pressure using systems such as FPLC and HPLC. Additional purification methods include electrophoretic, immunological, precipitation, dialysis and chromatofocusing techniques. Ultrafiltration and diafiltration techniques, in conjunction with protein concentration, are also useful. As is well known in the art, a variety of natural proteins bind Fc and antibodies, and these proteins may be used for purification of certain antibody constructs. For example, the bacterial proteins A and G bind to the Fc region. Likewise, the bacterial protein L binds to the Fab region of some antibodies. Purification may also be enabled by a particular fusion partner. For example, antibodies may be purified using glutathione resin if a GST fusion is employed, Ni^{+2} affinity chromatography if a His-tag is employed or immobilized anti-flag antibody if a flag-tag is used. The degree of purification necessary will vary depending on the use of the anti-FRα antibody constructs. In some instances, no purification may be necessary.

[00152] In certain embodiments, the anti-FRα antibody constructs are substantially pure. The term "substantially pure" (or "substantially purified") when used in reference to an anti-FRα antibody construct described herein, means that the antibody construct is substantially or essentially free of components that normally accompany or interact with the protein as found in its naturally occurring environment, such as a native cell, or a host cell in the case of recombinantly produced

construct. In certain embodiments, an anti-FRα antibody construct that is substantially pure is a protein preparation having less than about 30%, less than about 25%, less than about 20%, less than about 15%, less than about 10%, or less than about 5% (by dry weight) of contaminating protein.

[00153] Certain embodiments of the present disclosure relate to a method of making an anti-FRα antibody construct comprising culturing a host cell into which one or more polynucleotides encoding the anti-FRα antibody construct, or one or more expression vectors encoding the anti-FRα antibody construct, have been introduced, under conditions suitable for expression of the anti-FRα antibody construct, and optionally recovering the anti-FRα antibody construct from the host cell (or from host cell culture medium).

Post-Translational Modifications

[00154] In certain embodiments, the anti-FRα antibody constructs described herein may comprise one or more post-translational modifications. Such post-translational modifications may occur *in vivo*, or they be conducted *in vitro* after isolation of the anti-FRα antibody construct from the host cell.

[00155] Post-translational modifications include various modifications as are known in the art (see, for example, *Proteins - Structure and Molecular Properties*, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York, 1993; *Post-Translational Covalent Modification of Proteins*, B. C. Johnson, Ed., Academic Press, New York, pgs. 1-12, 1983; Seifter *et al.,* 1990, *Meth. Enzymol.*, 182:626-646, and Rattan *et al.,* 1992, *Ann. N.Y. Acad. Sci.*, 663:48-62). In those embodiments in which the anti-FRα antibody construct comprises one or more post-translational modifications, the construct may comprise the same type of modification at one or several sites, or it may comprise different modifications at different sites.

[00156] Examples of post-translational modifications include glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, formylation, oxidation, reduction, proteolytic cleavage or specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease or $NaBH_4$.

[00157] Other examples of post-translational modifications include, for example, addition or removal of N-linked or O-linked carbohydrate chains, chemical modifications of N-linked or O-linked carbohydrate chains, processing of N-terminal or C-terminal ends, attachment of chemical moieties to the amino acid backbone, and addition or deletion of an N-terminal methionine residue resulting from prokaryotic host cell expression. Post-translational modifications may also include modification with a detectable label, such as an enzymatic, fluorescent, luminescent, isotopic or affinity label to allow for detection and isolation of the protein. Examples of suitable enzyme labels include, but are not limited to, horseradish peroxidase, alkaline phosphatase, beta-galactosidase and acetylcholinesterase. Examples of suitable prosthetic group complexes include, but are not limited to, streptavidin/biotin and avidin/biotin. Examples of suitable fluorescent materials include, but are not limited to, umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride and phycoerythrin. Examples of luminescent materials include luminol, and bioluminescent materials such as luciferase, luciferin and aequorin. Examples of suitable radioactive materials include iodine, carbon, sulfur, tritium, indium, technetium, thallium, gallium, palladium, molybdenum, xenon and fluorine.

[00158] Additional examples of post-translational modifications include acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphotidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, gamma-carboxylation, GPI anchor formation, hydroxylation, iodination, methylation, myristylation, pegylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination.

POLYNUCLEOTIDES, VECTORS AND HOST CELLS

[00159] Certain embodiments of the present disclosure relate to an isolated polynucleotide or a set of polynucleotides encoding an anti-FRα antibody construct described herein. A polynucleotide in this context may encode all or part of an anti-FRα antibody construct.

[00160] The terms "nucleic acid," "nucleic acid molecule" and "polynucleotide" are used interchangeably herein and refer to a polymeric form of nucleotides of any length, either deoxyribonucleotides or ribonucleotides, or analogues thereof. Non-limiting examples of polynucleotides include a gene, a gene fragment, messenger RNA (mRNA), cDNA, recombinant polynucleotides, plasmids, vectors, isolated DNA of any sequence, isolated RNA of any sequence, nucleic acid probes, and primers.

[00161] A polynucleotide that "encodes" a given polypeptide is a polynucleotide that is transcribed (in the case of DNA) and translated (in the case of mRNA) into a polypeptide *in vivo* when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxy) terminus. A transcription termination sequence may be located 3' to the coding sequence.

[00162] Certain embodiments of the present disclosure relate to vectors (such as expression vectors) comprising one or more polynucleotides encoding an anti-FRα antibody construct described herein. The polynucleotide(s) may be comprised by a single vector or by more than one vector. In some embodiments, the polynucleotides are comprised by a multicistronic vector.

[00163] Certain embodiments of the present disclosure relate to host cells comprising polynucleotide(s) encoding an anti-FRα antibody construct described herein or one or more vectors comprising the polynucleotide(s). In some embodiments, the host cell is eukaryotic, for example, a Chinese Hamster Ovary (CHO) cell, a human embryonic kidney (HEK) cell or a lymphoid cell (e.g. Y0, NS0, Sp20 cell).

ANTIBODY-DRUG CONJUGATES

[00164] Certain embodiments of the present disclosure relate to antibody-drug conjugates (ADCs) comprising an anti-FRα antibody construct as described herein conjugated to one or more drug moieties, such as cytotoxins or immune modulators.

[00165] Typically, in an ADC, the anti-FRα antibody construct is conjugated to a drug moiety via a linker, which may be a cleavable or non-cleavable linker. The anti-FRα antibody construct may be conjugated to a single drug molecule, or it may be conjugated to multiple drug molecules. The

number of drug molecules conjugated to a single anti-FRα antibody construct is defined by the drug-to-antibody ratio (DAR). In certain embodiments, in the ADCs of the present disclosure, the DAR is in the range of from about 1 to about 12, or from about 2 to about 12, or from about 2 to about 8.

[00166] In certain embodiments, the ADCs comprising an anti-FRα antibody construct have the general Formula I:

$$A\text{-}(L\text{-}(D)_m)_n \quad \textbf{(I)}$$

[00167] where A is an anti-FRα antibody construct as described herein; L is a linker; D is a drug moiety; m is between 1 and about 8, and n is between 1 and about 12.

[00168] In certain embodiments in Formula I, m is between 1 and 6. In some embodiments, m is 1 or 2. In some embodiments, n is between about 1 and about 8, for example, between about 2 and about 8.

[00169] Various compounds known to be useful as cytotoxic or immunomodulatory ADC payloads may be employed as the drug moiety in the ADCs comprising the anti-FRα antibody constructs. Examples include, but are not limited to, maytansinoids and maytansinoid analogues, benzodiazepines and pyrrolobenzodiazepines, duocarmycins such as CC-1065 and analogues thereof, calicheamicins and calicheamicin analogues, auristatins and auristatin analogues, hemiasterlins and hemiasterlin analogues, tubulysins and tubulysin analogues, amatoxins and amatoxin analogues, camptothecins and camptothecin analogues, eribulin, TLR agonists (such as agonists of TLR7 and/or TLR8) and STING agonists.

[00170] In certain embodiments, the drug moiety comprised by the ADCs of the present disclosure is an auristatin or auristatin analogue, a hemiasterlin or a hemiasterlin analogue, a camptothecin or camptothecin analogue, or eribulin.

[00171] Typically, in the ADCs of the present disclosure, the drug moiety is linked to the anti-FRα antibody construct by a linker. Linkers are bifunctional or multifunctional moieties capable of linking one or more drug molecules to the antibody construct. In some embodiments, the linker may be bifunctional (or monovalent) such that it links a single drug molecule to a single site on

the antibody construct. In some embodiments, the linker may be multifunctional (or polyvalent) such that it links more than one drug molecule to a single site on the antibody construct. Multifunctional linkers may also be used to link one drug molecule to more than one site on the antibody construct in some embodiments.

[00172] Attachment of a linker to an anti-FRα antibody construct can be accomplished in a variety of ways, such as through surface lysines, reductive-coupling to oxidized carbohydrates, or through cysteine residues liberated by reducing interchain disulfide linkages. Alternatively, attachment of a linker to an anti-FRα antibody construct may be achieved by modification of the antibody construct to include additional cysteine residues (see, for example, U.S. Patent Nos. 7,521,541; 8,455,622 and 9,000,130) or non-natural amino acids that provide reactive handles, such as selenomethionine, *p*-acetylphenylalanine, formylglycine or *p*-azidomethyl-L-phenylalanine to allow for site-specific conjugation (see, for example, Hofer *et al.,* 2009, *Biochemistry,* 48:12047-12057; Axup *et al.,* 2012, *PNAS,* 109:16101-16106; Wu *et al.,* 2009, *PNAS,* 106:3000-3005; Zimmerman *et al.,* 2014, *Bioconj. Chem.,* 25:351-361). A further option is the use of GlycoConnect™ technology (Synaffix BV, Nijmegen, Netherlands), which involves enzymatic remodelling of the antibody glycans to allow for attachment of a linker by metal-free click chemistry (see, for example, European Patent No. EP 2 911 699).

[00173] Linkers typically include a functional group capable of reacting with the target group or groups on the antigen binding construct and one or more functional groups capable of reacting with a target group on the drug moiety. Suitable functional groups are known in the art and include those described, for example, in *Bioconjugate Techniques* (G.T. Hermanson, 2013, Academic Press). Non-limiting examples of functional groups for reacting with free cysteines or thiols include maleimide, haloacetamide, haloacetyl, activated esters such as succinimide esters, 4-nitrophenyl esters, pentafluorophenyl esters, tetrafluorophenyl esters, anhydrides, acid chlorides, sulfonyl chlorides, isocyanates and isothiocyanates. Also useful in this context are "self-stabilizing" maleimides such as those described in Lyon *et al.,* 2014, *Nat. Biotechnol.,* 32:1059-1062. Non-limiting examples of functional groups for reacting with surface lysines and amines include activated esters (such as N-hydroxysuccinamide (NHS) esters and sulfo-NHS esters), imido esters (such as Traut's reagent), isothiocyanates, aldehydes and acid anhydrides (such as diethylenetriaminepentaacetic anhydride (DTPA)). Other examples include the use of

succinimido-1,1,3,3-tetra-methyluronium tetrafluoroborate (TSTU) or benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) to convert a carboxylic acid to an activated ester, which may then be reacted with an amine. Non-limiting examples of functional groups capable of reacting with an electrophilic group on the antibody construct or drug moiety (such as an aldehyde or ketone carbonyl group) include hydrazide, oxime, amino, hydrazine, thiosemicarbazone, hydrazine carboxylate and arylhydrazide.

[00174] In certain embodiments, a linker that includes a functional group that allows for bridging of two interchain cysteines on the antibody binding construct may be used, such as a ThioBridge™ linker (Badescu *et al.*, 2014, *Bioconjug. Chem.*, 25:1124–1136), a dithiomaleimide (DTM) linker (Behrens *et al.*, 2015, *Mol. Pharm.*, 12:3986–3998), a dithioaryl(TCEP)pyridazinedione-based linker (Lee *et al.*, 2016, *Chem. Sci.*, 7:799-802) or a dibromopyridazinedione-based linker (Maruani *et al.*, 2015, *Nat. Commun.*, 6:6645).

[00175] A variety of linkers for linking drugs to antibodies are known in the art, including hydrazone-, disulfide- and peptide-based linkers. Linkers may be cleavable or non-cleavable. A cleavable linker is typically susceptible to cleavage under intracellular conditions, for example, through lysosomal processes. Examples include linkers that are protease-sensitive, acid-sensitive or reduction-sensitive. Non-cleavable linkers by contrast, rely on the degradation of the antibody in the cell, which typically results in the release of an amino acid-linker-drug moiety.

[00176] An example of a cleavable linker that may be useful in certain embodiments is a peptide-containing linker cleavable by an intracellular protease, such as lysosomal protease or an endosomal protease. Examples include dipeptide-containing linkers, such as those comprising the dipeptides Val-Cit, Phe-Lys, Val-Lys, Ala-Lys, Phe-Lys, Val-Cit, Phe-Cit, Leu-Cit, Ile-Cit, Trp-Cit, Phe-Arg, Ala-Phe, Val-Ala, Met-Lys, Asn-Lys, Ile-Pro, Ile-Val, Asp-Val, His-Val, Met-(D)Lys, Asn-(D)Lys, Val-(D)Asp, NorVal-(D)Asp, Ala-(D)Asp, Me_3Lys-Pro, PhenylGly-(D)Lys, Met-(D)Lys, Asn-(D)Lys, Pro-(D)Lys or Met-(D)Lys; tripeptide-containing linkers such as those comprising the tripeptides Met-Cit-Val, Gly-Cit-Val, (D)Phe-Phe-Lys or (D)Ala-Phe-Lys, and tetrapeptide-containing linkers such as those comprising the tetrapeptides Gly-Phe-Leu-Gly, Gly-Gly-Phe-Gly or Ala-Leu-Ala-Leu.

[00177] Additional useful cleavable linkers include disulfide-containing linkers and linkers hydrolyzable at a specific pH or within a pH range, such as hydrazone linkers. Examples of disulfide-containing linkers include, but are not limited to, N-succinimydyl-4-(2-pyridyldithio) butanoate (SPDB) and N-succinimydyl-4-(2-pyridyldithio)-2-sulfo butanoate (sulfo-SPDB). Disulfide-containing linkers may optionally include additional groups to provide steric hindrance adjacent to the disulfide bond in order to improve the extracellular stability of the linker, for example, inclusion of a geminal dimethyl group. Linkers comprising combinations of these functionalities may also be useful, for example, linkers comprising both a hydrazone and a disulfide are known in the art.

[00178] A further example of a cleavable linker is a linker comprising a β-glucuronide, which is cleavable by β-glucuronidase, an enzyme present in lysosomes and tumor interstitium (see, for example, De Graaf *et al.*, 2002, *Curr. Pharm. Des.*, 8:1391–1403).

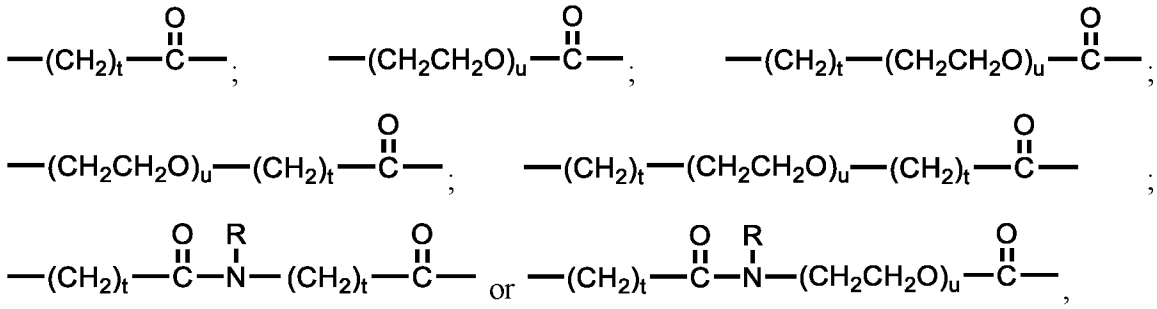
[00179] Cleavable linkers may optionally further comprise one or more additional functionalities such as self-immolative/self-elimination groups, stretchers or hydrophilic moieties.

[00180] Self-immolative and self-elimination groups that find use in linkers include, for example, *p*-aminobenzyl (PAB) and *p*-aminobenzyloxycarbonyl (PABC) groups, methylated ethylene diamine (MED) and hemi-aminal groups. Other examples of self-immolative groups include, but are not limited to, aromatic compounds that are electronically similar to the PABC or PABE group such as heterocyclic derivatives, for example 2-aminoimidazol-5-methanol derivatives as described in U.S. Patent No. 7,375,078. Other examples include groups that undergo cyclization upon amide bond hydrolysis, such as substituted and unsubstituted 4-aminobutyric acid amides (Rodrigues *et al.*, 1995, *Chemistry Biology,* 2:223-227) and 2-aminophenylpropionic acid amides (Amsberry, *et al.*, 1990, *J. Org. Chem.*, 55:5867-5877). Self-immolative/self-elimination groups, alone or in combination, are often included in peptide-based linkers but may also be included in other types of linkers. In some embodiments, the linker may include one or more self-immolative/self-elimination groups, for example, a PABC group, a PABE group, or a combination of a PABC or PABE group and an MED.

[00181] Stretchers that find use in linkers for ADCs include, for example, alkylene groups and stretchers based on aliphatic acids, diacids, amines or diamines, such as diglycolate, malonate,

caproate and caproamide. Other stretchers include, for example, glycine-based stretchers and polyethylene glycol (PEG) or monomethoxy polyethylene glycol (mPEG) stretchers. PEG and mPEG stretchers also function as hydrophilic moieties and may be particularly useful with hydrophobic drugs, although their use in linkers with other drugs is also contemplated in some embodiments.

[00182] In certain embodiments, a stretcher may have one of the following structures:



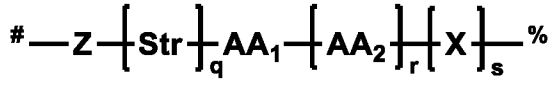
wherein:

R is H or C_1-C_6 alkyl;

t is an integer between 2 and 10, and

u is an integer between 1 and 10.

[00183] In some embodiments, in ADCs of Formula I, linker, L, is a cleavable linker having Formula II:



(II)

wherein:

Z is a linking group that joins the linker to a target group on the anti-FRα antibody construct, A;

Str is a stretcher;

AA_1 and AA_2 are each independently an amino acid, wherein AA_1-$[AA_2]_r$ forms a protease cleavage site;

X is a self-immolative group;

q is 0 or 1;

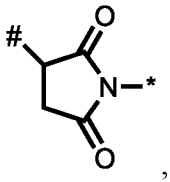
r is 1, 2 or 3;

s is 0, 1 or 2;

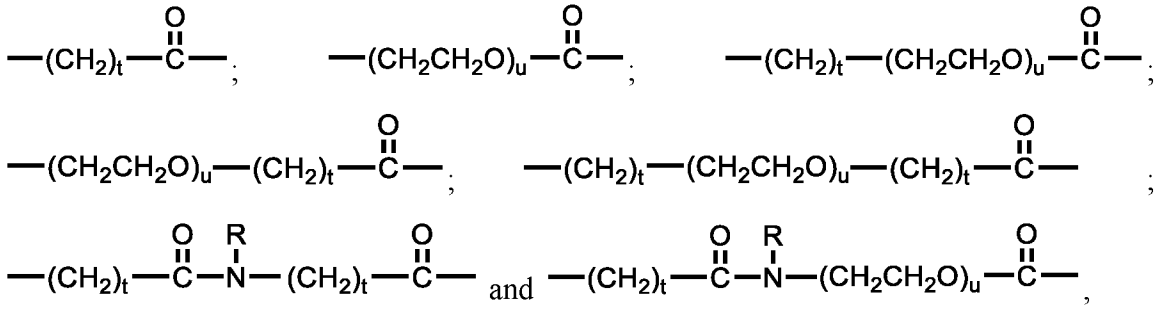
is the point of attachment to the anti-FRα antibody construct, A, and

% is the point of attachment to the drug moiety, D.

[00184] In some embodiments, in linkers of Formula (II):

Z is  , where # is the point of attachment to A, and * is the point of attachment to the remainder of the linker.

[00185] In some embodiments, in linkers of Formula (II), Str is selected from:



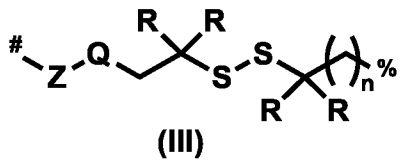
wherein:

R is H or C_1-C_6 alkyl;

t is an integer between 2 and 10, and

u is an integer between 1 and 10.

[00186] In some embodiments, ADCs of Formula I may comprise a disulfide-containing linker. In some embodiments, in ADCs of Formula I, linker, L, is a cleavable linker having Formula III:



(III)

wherein:

Z is a linking group that joins the linker to a target group on the anti-FRα antibody construct, A;

Q is $–(CH_2)_p-$ or $–(CH_2CH_2O)_q-$, wherein p and q are each independently an integer between 1 and 10;

each R is independently H or C_1-C_6 alkyl;

n is 1, 2 or 3;

is the point of attachment to the anti-FRα antibody construct, A, and

% is the point of attachment to the drug moiety, D.

[00187] In some embodiments, ADCs of Formula I may comprise a β-glucuronide-containing linker.

[00188] Various non-cleavable linkers are known in the art for linking drugs to antibodies and may be useful in the ADCs of the present disclosure in certain embodiments. Examples of non-cleavable linkers include linkers having an N-succinimidyl ester or N-sulfosuccinimidyl ester moiety for reaction with the antibody, as well as a maleimido- or haloacetyl-based moiety for reaction with the drug, or *vice versa*. An example of such a non-cleavable linker is based on sulfosuccinimidyl-4-[N-maleimidomethyl]cyclohexane-1-carboxylate (sulfo-SMCC). Sulfo-SMCC conjugation typically occurs via a maleimide group which reacts with sulfhydryls (thiols, —SH), while the sulfo-NHS ester is reactive toward primary amines. Other non-limiting examples of such linkers include those based on N-succinimidyl 4-(maleimidomethyl)cyclohexanecarboxylate (SMCC), N-succinimidyl-4-(N-maleimidomethyl)-cyclohexane-1-carboxy-(6-amidocaproate) (“long chain” SMCC or LC-SMCC), κ-maleimidoundecanoic acid N-succinimidyl ester (KMUA), γ-maleimidobutyric acid N-succinimidyl ester (GMBS), ε–maleimidocaproic acid N-hydroxysuccinimide ester (EMCS), m-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS), N-(α–maleimidoacetoxy)-succinimide ester (AMAS), succinimidyl-6-(β–maleimidopropionamido)hexanoate (SMPH), N-succinimidyl 4-(p-maleimidophenyl)-butyrate (SMPB) and N-(p-maleimidophenyl)isocyanate (PMPI). Other examples include those comprising a haloacetyl-based functional group such as N-succinimidyl-4-(iodoacetyl)-aminobenzoate (SIAB), N-succinimidyl iodoacetate (SIA), N-succinimidyl bromoacetate (SBA) and N-succinimidyl 3-(bromoacetamido)propionate (SBAP).

[00189] ADCs comprising an anti-FRα antibody construct as described herein may be prepared by one of several routes known in the art, employing standard organic chemistry reactions, conditions, and reagents (see, for example, *Bioconjugate Techniques* (G.T. Hermanson, 2013, Academic Press). For example, conjugation may be achieved by (1) reaction of a functional group of an antibody construct with a bivalent linker reagent, to form antibody-linker intermediate A-L, via a covalent bond, followed by reaction with an activated drug moiety D; or (2) reaction of a functional group of a drug moiety with a linker reagent, to form drug-linker intermediate D-L, via a covalent bond, followed by reaction with a functional group of an antibody construct. Conjugation methods (1) and (2) may be employed with a variety of antibody constructs, drug moieties, and linkers to prepare the ADCs described here.

[00190] Various prepared linkers, linker components and drugs are commercially available or may be prepared using standard synthetic organic chemistry techniques (see, for example, March's Advanced Organic Chemistry (Smith & March, 2006, Sixth Ed., Wiley); Toki *et al.,* 2002, *J. Org. Chem.,* 67:1866-1872; Frisch *et al.,* 1997, *Bioconj. Chem.,* 7:180-186; *Bioconjugate Techniques* (G.T. Hermanson, 2013, Academic Press), and *Antibody-Drug Conjugates: Methods in Molecular Biology* (Ducry (Ed.), 2013, Springer)). In addition, a number of pre-formed drug-linkers suitable for reaction with a selected antibody construct are also available commercially, for example, drug-linkers comprising DM1, DM4, MMAE, MMAF or Duocarmycin SA are available from Creative BioLabs (Shirley, NY). Various antibody drug conjugation services are also available commercially from companies such as Lonza Inc. (Allendale, NJ), Abzena PLC (Cambridge, UK), ADC Biotechnology (St. Asaph, UK), Baxter BioPharma Solutions (Baxter Healthcare Corporation, Deerfield, IL) and Piramal Pharma Solutions (Grangemouth, UK).

[00191] The ADCs, once prepared, may be purified by standard techniques such as chromatography (for example, HPLC, size-exclusion, adsorption, ion exchange and/or affinity capture), dialysis and/or tangential flow filtration.

METHODS OF USE

[00192] Certain aspects of the present disclosure relate to the therapeutic or diagnostic use of the anti-FRα antibody constructs and ADCs. FRα is overexpressed in a wide variety of cancers and

certain embodiments of the present disclosure thus relate to the methods of using the anti-FRα antibody constructs and ADCs in the treatment or diagnosis of an FRα-positive cancer.

[00193] Certain embodiments relate to methods of inhibiting the growth of FRα-positive tumor cells comprising contacting the cells with an anti-FRα antibody construct or ADC described herein. The cells may be *in vitro* or *in vivo*. In certain embodiments, the anti-FRα antibody constructs and ADCs may be used in methods of treating an FRα-positive cancer or tumor in a subject.

[00194] Cancers that overexpress FRα are typically solid tumors. Examples include, but are not limited to, ovarian cancer, endometrial cancer, lung cancers (such as non-small cell lung cancer (NSCLC)), mesothelioma, breast cancer (including triple negative breast cancer (TNBC)), colorectal cancer, biliary tract cancer, pancreatic cancer and esophageal cancer. Certain embodiments of the present disclosure relate to methods of treating a FRα-positive cancer with an anti-FRα antibody construct or ADC as described herein, where the cancer is ovarian cancer, endometrial cancer, lung cancer (such as non-small cell lung cancer (NSCLC)), mesothelioma, breast cancer, colorectal cancer, biliary tract cancer, pancreatic or esophageal cancer. In some embodiments, the anti-FRα antibody construct or ADC as described herein may be useful in treating triple negative breast cancer (TNBC).

[00195] Treatment of an FRα-positive cancer may result in one or more of alleviation of symptoms, shrinking the size of the tumor, inhibiting growth of the tumor, diminishing one or more direct or indirect pathological consequences of the disease, preventing metastasis, decreasing the rate of disease progression, amelioration or palliation of the disease state, improving survival, increasing progression-free survival, remission and/or improving prognosis.

[00196] In certain embodiments, when used in the treatment of cancer, the anti-FRα antibody constructs or ADCs may be administered systemically to the subject to be treated, for example, by bolus injection or continuous infusion into the subject's bloodstream. In certain embodiments, when used in the treatment of cancer, the anti-FRα antibody constructs or ADCs may be administered to the subject locally at the site to be treated.

[00197] It is contemplated that the the anti-FRα antibody constructs or ADCs may be used alone or in combination with one or more known chemotherapeutic or immunotherapeutic agents

typically used in the treatment of cancer. Combinations of the anti-FRα antibody constructs or ADCs with standard chemotherapeutics or immunotherapeutics may act to improve the efficacy of the chemotherapeutic or immunotherapeutic and, therefore, may improve standard cancer therapies. This application can be important in the treatment of drug-resistant cancers which are not responsive to standard treatment. When used in conjunction with one or more known chemotherapeutic or immunotherapeutic agents, the anti-FRα antibody construct or ADC may be administered prior to, or after, administration of the chemotherapeutic or immunotherapeutic agents, or they may be administered concomitantly.

[00198] The dosage of the the anti-FRα antibody construct or ADC to be administered is not subject to defined limits, but it will a therapeutically effective amount. A "therapeutically effective amount" refers to that amount of an anti-FRα antibody construct or ADC described herein which, when administered to a subject, is sufficient to effect a treatment of the particular indication. A therapeutically effective amount of anti-FRα antibody construct or ADC in respect of cancer treatment may, for example, have one or more of the following effects: reduce the number of cancer cells, reduce the tumor size, inhibit cancer cell infiltration into peripheral organs, inhibit tumor metastasis, inhibit tumor growth; increase survival time and/or relieve to some extent one or more of the symptoms associated with the cancer. For cancer therapy, efficacy may alternatively be measured, for example, by assessing the time to disease progression (TTP) and/or determining the response rate (RR).

[00199] Certain embodiments relate to methods of detecting the presence of FRα in a biological sample, such as a sample comprising cells or tissue, using an anti-FRα antibody construct described herein. In some embodiments, the biological sample may have been taken from a patient, for example, a patient known or suspected to have a cancer. Some embodiments relate to methods of detecting the presence of FRα in a biological sample that comprise contacting the sample with an anti-FRα antibody construct described herein.

[00200] Certain embodiments relate to methods of diagnosing a disorder associated with increased expression of FRα, such as a cancer, using an anti-FRα antibody construct described herein. The method of diagnosis may be an *in vivo* method in which the anti-FRα antibody construct is administered to the subject, or it may be an *in vitro* method in which a sample taken from the

subject is contacted with the anti-FRα antibody construct. For *in vivo* methods, administration may be systemic or local.

[00201] In methods of detecting the presence of FRα or diagnosing a disorder associated with increased expression of FRα, the anti-FRα antibody construct may be labelled with a detectable label, such as a fluorescent, luminescent, chromophoric, chemiluminescent, radioactive or enzymatic label as is known in the art.

PHARMACEUTICAL COMPOSITIONS

[00202] For therapeutic use, the anti-FRα antibody constructs and ADCs may be provided in the form of pharmaceutical compositions comprising the anti-FRα antibody construct or ADC and a pharmaceutically acceptable carrier or diluent. The compositions may be prepared by known procedures using well-known and readily available ingredients.

[00203] Pharmaceutical compositions may be formulated for administration to a subject by, for example, parenteral, oral (including, for example, buccal or sublingual), topical, rectal or vaginal routes, or by inhalation or spray. "Parenteral" administration may be subcutaneous injection, or intradermal, intra-articular, intravenous, intramuscular, intravascular, intrasternal or intrathecal injection or infusion. The pharmaceutical composition will typically be formulated in a format suitable for administration to the subject, for example, as a syrup, elixir, tablet, troche, lozenge, hard or soft capsule, pill, suppository, oily or aqueous suspension, dispersible powder or granule, emulsion, injectable or solution. Pharmaceutical compositions may be provided as unit dosage formulations.

[00204] In certain embodiments, pharmaceutical compositions comprising the anti-FRα antibody constructs or ADCs may be formulated for parenteral administration by infusion or in a unit dosage injectable form, for example as lyophilized formulations or aqueous solutions.

[00205] Pharmaceutically acceptable carriers are generally nontoxic to recipients at the dosages and concentrations employed. Examples of such carriers include, but are not limited to, buffers such as phosphate, citrate, and other organic acids; antioxidants such as ascorbic acid and methionine; preservatives such as octadecyldimethylbenzyl ammonium chloride, hexamethonium chloride, benzalkonium chloride, benzethonium chloride, phenol, butyl alcohol, benzyl alcohol,

alkyl parabens (such as methyl or propyl paraben), catechol, resorcinol, cyclohexanol, 3-pentanol and m-cresol; low molecular weight (less than about 10 residues) polypeptides; proteins such as serum albumin or gelatin; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates such as glucose, mannose or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes such as Zn-protein complexes, and non-ionic surfactants such as polyethylene glycol (PEG).

[00206] In certain embodiments, pharmaceutical compositions comprising the anti-FRα antibody constructs or ADCs may be in the form of a sterile injectable aqueous or oleaginous solution or suspension. Such suspensions may be formulated using suitable dispersing or wetting agents and/or suspending agents that are known in the art. The sterile injectable solution or suspension may comprise the anti-FRα antibody construct or ADC in a non-toxic parentally acceptable diluent or solvent. Acceptable diluents and solvents that may be employed include, for example, 1,3-butanediol, water, Ringer's solution or isotonic sodium chloride solution. In addition, sterile, fixed oils may be employed as a solvent or suspending medium. For this purpose, various bland fixed oils may be employed, including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables. Adjuvants such as local anesthetics, preservatives and/or buffering agents may also be included in the injectable solution or suspension.

[00207] In certain embodiments, pharmaceutical compositions comprising the anti-FRα antibody constructs or ADCs may be formulated for intravenous administration to a subject, for example a human. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and/or a local anaesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water-free concentrate in a hermetically sealed container such as an ampoule or sachet indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an

ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

[00208] Other pharmaceutical compositions and methods of preparing pharmaceutical compositions are known in the art and are described, for example, in "*Remington: The Science and Practice of Pharmacy*" (formerly "*Remingtons Pharmaceutical Sciences*"); Gennaro, A., Lippincott, Williams & Wilkins, Philadelphia, PA (2000).

PHARMACEUTICAL KITS

[00209] Certain embodiments relate to pharmaceutical kits comprising an anti-FRα antibody construct or ADC as described herein.

[00210] The kit typically will comprise a container holding the anti-FRα antibody construct or ADC and a label and/or package insert on or associated with the container. The label or package insert contains instructions customarily included in commercial packages of therapeutic products, providing information about the indications, usage, dosage, administration, contraindications and/or warnings concerning the use of such therapeutic products. The label or package insert may further include a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, for use or sale for human or animal administration. In some embodiments, the container may have a sterile access port. For example, the container may be an intravenous solution bag or a vial having a stopper that may be pierced by a hypodermic injection needle.

[00211] In addition to the container holding the anti-FRα antibody construct or ADC, the kit may optionally comprise one or more additional containers comprising other components of the kit. For example, a pharmaceutically acceptable buffer (such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution or dextrose solution), other buffers or diluents.

[00212] Suitable containers include, for example, bottles, vials, syringes, intravenous solution bags, and the like. The containers may be formed from a variety of materials such as glass or plastic. If appropriate, one or more components of the kit may be lyophilized or provided in a dry

form, such as a powder or granules, and the kit can additionally contain a suitable solvent for reconstitution of the lyophilized or dried component(s).

[00213] The kit may further include other materials desirable from a commercial or user standpoint, such as filters, needles, and syringes.

[00214] The following Examples are provided for illustrative purposes and are not intended to limit the scope of the invention in any way.

EXAMPLES

GENERAL

[00215] ***Biological Assays:*** Expression levels of FRα in the cell lines and CDX models was assessed in-house using a research level IHC assay and assigned a relative expression level (high/mid/low or strong/moderate/weak). PDX models were assessed similarly using archival tumor samples.

EXAMPLE 1: PREPARATION OF ANTI-FOLATE RECEPTOR ALPHA ANTIBODIES

[00216] Antibodies that specifically bind folate receptor alpha (FRα) were generated by immunizing rabbits with human FRα antigen, isolated and sequenced as described below.

1.1 Immunizations

[00217] Antibodies to FRα were raised in rabbits immunized with soluble HIS-tagged human folate receptor 1 antigen (FRα-HIS) (ACROBiosystems, Newark, DE; Cat# FO1-H82E2). Briefly, two New Zealand White rabbits were immunized with a primary boost consisting of 200 µg of FRα-HIS antigen mixed with Alum (5 mg/injection)/CpG (10 µg/injection) administered subcutaneously at 3 sites (0.333 mL/site) along the rabbit's dorsal body. These were followed by 4 immunizations with 100 µg of FRα-HIS antigen mixed with Alum (5mg/injection)/CpG (10 µg/injection). Each of the immunizations were separated by 14 days. The animals were bled prior to the fourth immunization for serology.

1.2 Selection of Animals for Harvest

[00218] Anti-human FRα antibody titers were determined by flow cytometry using CHO cells expressing human FRα. Briefly, CHO cells were transiently transfected with a pTT5-based expression plasmid (National Research Council of Canada) encoding human FRα according to manufacturer's instructions for Lipofectamine™ 2000 (Thermo Fisher Scientific Corp., Waltham, MA). A dilution of immunized rabbit sera starting at 1:400 and serially diluted 1:2 over 11 points was incubated with 50,000 CHO cells transiently expressing human FRα for 30 minutes. Samples were then washed, and antibody binding was detected with a goat anti-rabbit secondary antibody conjugated to Alexa Fluor-647 (Jackson Immuno Research Labs, West Grove, PA) by flow cytometry. Titers were determined by identifying the highest dilution sample that showed at least 2 times fluorescent signal above background.

1.3 Recovery of B Cells and Discovery of Anti-Human FRα Antibodies

[00219] Immunized rabbits with desired titers above 100,000 were sacrificed, and the spleens harvested. The lymphoid cells were dissociated by grinding in FACS buffer (PBS, 2% v/v FBS) to release the cells from the tissues. The cells were pelleted and then suspended for 1 minute in 5 mL of Pharm Lyse™ (Becton, Dickinson & Co., Franklin Lakes, NJ) to lyse red blood cells. Equal volume of FACS buffer was added to neutralize the Pharm Lyse™ and the resultant lymphocyte sample was pelleted and resuspended in FACS buffer.

[00220] The lymphocyte suspension was then stained with goat anti-rabbit IgG Alexa Fluor-647 (Jackson Immuno Research Labs, West Grove, PA) to identify IgG+ B cells. After 30 minutes of staining, IgG+ B cells were sorted on a FACSAria™ (Becton, Dickinson & Co., Franklin Lakes, NJ) and counted. Using the Selected Lymphocyte Antibody Method (SLAM) (Babcook *et al.*, 1996, *Proc Natl Acad Sci USA,* 93(15):7843–7848), B cells were plated at different densities ranging from single cell up to 50 cells in a 384 well plate, expanded in culture for 7 days and the supernatants harvested for detection of anti-human FRα antibodies. The 384 well plates were stored at -80°C.

[00221] Supernatants were screened for human FRα specific monoclonal antibodies by ELISA. 384 well ELISA plates were coated with 25 µL/well of human FRα-HIS (2 µg/mL) in PBS, then incubated at 4°C overnight. After incubation, the plates were washed twice with water, 90 µL/well

Blocking Buffer (2% skim milk, PBS) was added and the plates incubated at room temperature for 1 hour. After incubation, the plates were washed and 12.5 µL/well of antibody-containing supernatants + 12.5 µL Blocking Buffer, and positive and negative controls were added and the plates incubated at room temperature for 2 hours.

[00222] After incubation, the plates were washed, 25 µL of 0.4 µg/mL goat anti-rabbit IgG Fc-HRP detection antibody (Jackson Immuno Research Labs, West Grove, PA) was added to each well and the plates were incubated at room temperature for 1 hour. After the incubation, the plates were washed and 25 µL of tetramethylbenzidine (TMB) was added and the plates allowed to develop for about 10 minutes (until negative control wells started to show signal). Then 25 µL stop solution (1 N HCL) was added to each well and the plates read on a Synergy™ H1 microplate (BioTek Instruments, Winooski, VT) at wavelength 450nm.

1.4 Sequencing Anti-Human FRα Antibodies

[00223] Total RNA was extracted from wells containing antibodies having desired characteristics using RNeasy (Qiagen, Hilden, Germany) according to manufacturer's protocols. Resulting total RNA was used as template with SuperScript™ III (Thermo Fisher Scientific Corp., Waltham, MA) and oligo-dT20 (Integrated DNA Technologies, Inc., Coralville, IA) to transcribe cDNA from mRNA. cDNA was subsequently treated with RNaseH (New England Biolabs, Ipswich, MA). Initial PCR of heavy and light chain antibody-coding sequences was performed using primers and methods modified from Babcook *et al.*, 1996, *Proc Natl Acad Sci USA,* 93(15):7843–7848 and von Boehmer *et al.*, 2016, *Nat Protoc.,* 11(10):1908, with cDNA as the nucleic acid template. PCR products were cloned into pCRTOPO4 using the Zero Blunt™ TOPO™ PCR Cloning kit (Thermo Fisher Scientific Corp., Waltham, MA) and transformed into E. cloni® cells (Lucigen Corporation, Middleton, WI). Antibiotic-resistant clones were Sanger sequenced and analyzed for unique antibody-coding sequences.

[00224] A subsequent PCR reaction was then performed on these unique sequences using V-segment family and J-segment family-specific primers. The resulting amplicons were cloned into pTT5-based expression plasmids (National Research Council of Canada). Unique heavy chain sequences and light chain sequences emerging from a single well sample were co-expressed in HEK293-6E cells (National Research Council of Canada) in all possible combinations to

determine the correct heavy and light chain pairing. Antibodies produced were assayed for binding to antigen that was transiently expressed on HEK293 cells.

1.5 Generation of Chimeric Antibodies

[00225] Coding sequences for antibody variable regions were cloned in frame into a huIgG1 expression and a huCK expression vector (based on the pTT5 vector). The huIgG1 constant region starts at alanine Kabat-118 and huCK constant region starts at arginine Kabat-108. The activities of the resultant recombinant chimeric antibodies were confirmed in specificity binding assays and were found comparable to the parental ones.

EXAMPLE 2: HUMANIZATION OF ANTI-FRα ANTIBODY

[00226] One of the chimeric anti-human FRα (anti-hFRα), variant v23924, generated as described in Example 1, was selected for humanization. The CDR sequences of v23924 are provided in Table 2.1, and the VH and VL sequences are provided in Table 2.2. Humanization was conducted as described below.

Table 2.1: CDR Sequences of the Anti-hFRα Antibody v23924

Numbering System	**Sequence**					
	Heavy Chain CDR1	**SEQ ID NO**	**Heavy Chain CDR2**	**SEQ ID NO**	**Heavy Chain CDR3**	**SEQ ID NO**
IMGT	GFSLSSYG	20	VNSGGSA	21	ARSGSGYPMDYLAI	22
Kabat	SYGVS	26	AVNSGGSAY YANWAKS	27	SGSGYPMDYLAI	25
Chothia	GFSLSSY	23	NSGGS	24	SGSGYPMDYLAI	25
AbM	GFSLSSYGVS	31	AVNSGGSAY	32	SGSGYPMDYLAI	25
Contact	SSYGVS	28	WIGAVNSGG SAY	29	ARSGSGYPMDYLA	30
	Light Chain CDR1	**SEQ ID NO**	**Light Chain CDR2**	**SEQ ID NO**	**Light Chain CDR3**	**SEQ ID NO**
IMGT	QSIGDW	40	EAS	41	QQGYGRSNVDNI	42
Kabat	QASQSIGDWLA	43	EASTLAS	44	QQGYGRSNVDNI	42

Chothia	QASQSIGDWLA	43	EASTLAS	44	QQGYGRSNVDNI	42
AbM	QASQSIGDWLA	43	EASTLAS	44	QQGYGRSNVDNI	42
Contact	GDWLAWY	45	LLIYEASTLA	46	QQGYGRSNVDN	47

Table 2.2: VH and VL Sequences of the Anti-hFRα Antibody v23924

	Sequence	SEQ ID NO
VH	QSVKESEGGLFKPTDTLTLTCTVSGFSLSSYGVSWVRQAPGNGLE WIGAVNSGGSAYYANWAKSRSTITRNTNLFTVTLKMTSLAVADTA TYFCARSGSGYPMDYLAIWGPGTLVTVSS	19
VL	AYDMTQTPASVEVAVGGTVTIKCQASQSIGDWLAWYQQKPGQPP RLLIYEASTLASGVPSRFSGSGSGTQFTLTISGVECADAATYYCQQG YGRSNVDNIFGGGTEVVVK	39

2.1 Humanization

[00227] Sequence alignment of the rabbit VH and VL sequences of v23924 to respective human germline sequences identified IGHV3-23*01 and IGKVI-39*01 as the closest, as well as most frequent, human germline sequences. CDR sequences according to the AbM definition (see Table 2.1) were ported onto the framework of these selected human germline sequences as shown in Fig. 1. Back mutations to rabbit residues in the resultant sequences at positions judged likely to be important for the retention of binding affinity to antigen, hFRα, were included creating several humanized sequences in which generated sequences for the most part built on the previous sequence, and where the first humanized sequence contained the minimal number of back mutations. None of the variants modified the CDRs of the parent antibody as defined by the AbM method.

[00228] This process was carried out in two cycles, in which the first cycle ("cycle one") resulted in six variable heavy chain humanized sequences and five variable light chain humanized sequences. The second cycle ("cycle two") expanded to an additional 5 variable heavy chain humanized sequences in pursuit of more closer parental-like affinity of the humanized antibody to hFRα. Full heavy chain sequences containing humanized heavy chain variable domain (VH) and hIgG1 heavy chain constant domains (CH1, hinge, CH2, CH3), and full light chain sequence containing humanized light chain variable domain (VL) and human kappa light chain constant

domain (kappa CL) were assembled. Monoclonal antibody (mAb) variants were then assembled such that in cycle one, each of the humanized heavy chains was paired with each of the humanized light chains to provide 30 humanized variants and in cycle two, additional humanized heavy chains were paired with select humanized light chains to give an additional 15 humanized variants, for a total of 45 humanized variants to be evaluated experimentally.

2.2 Production of Humanized Antibodies

[00229] Each of the 45 humanized mAb constructs, as well as the parental v23924 mAb construct, were produced in full-size antibody (FSA) format containing either two identical full-length heavy chains (parental v23924 and 15 humanized variants) resulting in a homodimeric Fc region (HomoFc), or heterodimeric full-length heavy chains comprising complementary mutations in CH3 region to drive exclusive heavy chain pairing (30 humanized variants) resulting in a heterodimeric Fc region (HetFc). A version of v23924 that contained a HetFc instead of a HomoFc was also produced (variant v30618). All constructs included two identical kappa light chains.

[00230] The two identical full-length heavy chains comprised by the HomoFc region contained the human CH1-hinge-CH2-CH3 domain sequence of IGHG1*01 (SEQ ID NO:146; see Table 2.3). The heterodimeric full-length heavy chains comprised by the HetFc region (HetFc-A and HetFc-B) contained the human CH1-hinge-CH2-CH3 domain sequence of IGHG1*01 with the following mutations in the Fc region:

[00231] HetFc-A: T350V_L351Y_F405A_Y407V

[00232] HetFc-B: T350V_T366L_K392L_T394W

[00233] The sequences of HetFc-A (SEQ ID NO:148) and HetFc-B (SEQ ID NO:149) are provided in Table 2.3. The human kappa CL sequence of IGKC*01 (SEQ ID NO:147; see Table 2.3) was used for all constructs.

Table 2.3: HomoFc and HetFc Sequences

	Sequence	SEQ ID NO
CH1-hinge-CH2-CH3 (IGHG1*01)	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK KVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTV LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRD ELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSF FLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG	146
CL (kappa) (IGKC*01)	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQ SGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSS PVTKSFNRGEC	147
CH1-hinge-CH2-CH3 (HetFc-A) (IGHG1*01)	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK KVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTV LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYVYPPSRD ELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSF ALVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG	148
CH1-hinge-CH2-CH3 (HetFc-B) (IGHG1*01)	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK KVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTV LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYVLPPSRD ELTKNQVSLLCLVKGFYPSDIAVEWESNGQPENNYLTWPPVLDSDGSF FLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG	149

[00234] Each of the humanized VH domain sequences from cycle one was appended to the human CH1-hinge-CH2-CH3 (HetFc-A and HetFc-B) domain sequence of IGHG1*01 to provide twelve humanized full heavy chain sequences (six humanized VH x2). Rabbit VH and each of the additional five humanized VH domain sequences from cycle two were appended to the human CH1-hinge-CH2-CH3 domain sequence of IGHG1*01, to provide the parental rabbit-human chimeric full heavy chain sequence and five additional humanized full heavy chain sequences. Each of the VL domain sequences was appended to the human kappa CL sequence of IGKC*01 to provide five humanized light chain sequences. All sequences were reverse translated to DNA, codon optimized for mammalian expression and gene synthesized.

[00235] Heavy chain vector inserts comprising a signal peptide (artificially designed sequence: MRPTWAWWLFLVLLLALWAPARG (SEQ ID NO:150) (Barash *et al.*, 2002, *Biochem and Biophys Res. Comm.*, 294:835–842)) and the heavy chain clone terminating at residue G446 (EU numbering) of the CH3 domain were ligated into a pTT5 vector to produce heavy chain expression vectors. Light chain vector inserts comprising the same signal peptide were ligated into a pTT5 vector to produce light chain expression vectors. The resulting heavy and light chain expression vectors were sequenced to confirm correct reading frame and sequence of the coding DNA.

[00236] The heavy and light chains of each of the humanized antibody variants were expressed in 200 ml cultures of CHO-3E7 cells. Briefly, CHO-3E7 cells, at a density of 1.7-2 x 10^6 cells /ml, viability >95%, were cultured at 37°C in FreeStyle™ F17 medium (Thermo Fisher Scientific, Waltham, MA) supplemented with 4 mM glutamine (GE Life Sciences, Marlborough, MA) and 0.1% Pluronic® F-68 (Gibco/ Thermo Fisher Scientific, Waltham, MA). A total volume of 200 ml CHO-3E7 cells + 1x antibiotic/antimycotics (GE Life Sciences, Marlborough, MA) was transfected with a total of 200 ug DNA (100 ug of antibody DNA and 100 ug of GFP/AKT/stuffer DNA) using PEI-MAX® (Polyscience, Inc., Philadelphia, PA) at a DNA:PEI ratio of 1:4 (w/w). Twenty-four hours after the addition of the DNA-PEI mixture, 0.5mM valproic acid (final concentration) + 1% w/v Tryptone (final concentration) were added to the cells, which were then transferred to 32°C and incubated for 6 more days prior to harvesting.

[00237] Protein A purification was performed in batch mode or using 1mL HiTrap™ MabSelect™ SuRe™ columns (Cytiva, Marlborough, MA). In batch mode, clarified supernatant samples were incubated in batch with mAb Select SuRe™ resin (GE Healthcare, Chicago, IL) cleaned-in-place (CIP'd) with NaOH and equilibrated in Dulbecco's PBS (DPBS). Resin was poured into CIP'd columns, the columns were washed with DPBS. In both purification modes, protein was eluted with 100 mM sodium citrate buffer pH 3.0. The eluted fractions were pH adjusted by adding 10% (v/v) 1M HEPES (pH ~10.6-10.7) to yield a final pH of 6-7. Samples were buffer exchanged into DPBS. Protein was quantitated based on absorbance at 280nm (A280 nm). Parental rabbit-human antibody chimera variants (v23924 and v30618) were further purified by preparatory SEC chromatography on a Superdex 200 Increase 10/30 column (GE Healthcare, Chicago, IL) in DPBS mobile phase following Protein A purification.

[00238] Following purification, purity of samples was assessed by electrophoresis under non-reducing and reducing conditions using the High Throughput Protein Express assay and Caliper LabChip® GXII or GXII Touch HT (Perkin Elmer, Waltham, MA). Procedures were carried out according to HT Protein Express LabChip® User Guide version 2 with the following modifications. Antibody samples, at either 2µl or 5µl (concentration range 5-2000 ng/µl), were added to separate wells in 96 well plates (BioRad, Hercules, CA) along with 7µl of HT Protein Express Sample Buffer (Perkin Elmer, Cat # 760328). Antibody samples were then denatured at 70°C for 15 mins. The LabChip® instrument was operated using the HT Protein Express Chip (Perkin Elmer, Waltham, MA) and the Ab-200 assay setting.

[00239] The yield (post protein-A purification) across the forty-five humanized antibody variants and the parental chimeric antibody, v23924, ranged from ~ 9-17 mg (or 45-85 mg/L). Fig. 2A and 2C show the Caliper electrophoresis results for the parental chimeric antibody v23924 and a representative humanized variant, v30384. As can be seen from Fig. 2, for the representative humanized antibody sample, non-reducing (NR) and reducing (R) Caliper reflected a single species corresponding to full-size antibody and intact heavy and light chains. This was also the case for the other humanized variants.

2.3 Quality Assessment of Humanized Antibodies

[00240] Species homogeneity of the humanized antibody variants was assessed by UPLC-SEC after Protein A purification and after preparatory SEC purification for the parental chimera antibody, v23924.

[00241] UPLC-SEC was performed using a Waters Acquity BEH200 SEC column (2.5 mL, 4.6 x 150 mm, stainless steel, 1.7 µm particles) (Waters LTD, Mississauga, ON) set to 30°C and mounted on a Waters Acquity UPLC™ H-Class Bio system with a photodiode array (PDA) detector. The mobile phase was Dulbecco's phosphate buffered saline (DPBS) with 0.02% Tween 20 pH 7.4 and the flow rate was 0.4 ml/min. Total run time for each injection was 7 min with a total mobile phase volume of 2.8 mL. Elution was monitored by UV absorbance in the range 210-500 nm, and chromatograms were extracted at 280 nm. Peak integration was performed using Waters Empower® 3 software employing the Apex Track™ and detect shoulders features.

[00242] Fig. 2B and 2D show the UPLC-SEC profiles for the parental chimeric antibody v23924 (post SEC purification) and for a representative humanized antibody v30384 (post Protein A purification, respectively. The UPLC-SEC profile for the representative humanized antibody sample reflected high species homogeneity, comparable to the parental chimeric antibody sample. The samples from the remaining humanized antibody variants had similar profiles to that shown for the representative humanized antibody sample.

EXAMPLE 3: BINDING OF HUMANIZED ANTIBODIES TO hFRα

3.1 Affinity Assessment of the Humanized Antibodies for hFRα

[00243] To determine whether the humanization process affected the affinity of the humanized variants for their target, the ability of the 45 purified humanized antibody variants to bind the hFRα antigen was assessed by Bio-layer interferometry (BLI) as follows.

[00244] hFRα antigen binding was assessed using the Octet® RED96 system (ForteBio, Fremont, CA) by cycling through the following steps: loading of antibodies (0.9 μg/mL) onto anti-human IgG Fc capture (AHC) biosensors over 200s; stabilization of baseline for 60s; association to recombinant His-tagged human FRα (ACROBiosystems, Newark, DE) at multiple relevant concentrations spanning the expected K_D for 400-500s; recordation of dissociation for 500-1000s; and regeneration performed by cycling 3 times between 10 mM glycine pH 1.5 (15s) and the assay buffer (1s) before proceeding to the next antibody. The assay buffer used was KB buffer (kinetics buffer, composed of PBS pH 7.4, 0.1 % BSA, 0.02 % Tween 20, 0.05% sodium azide) supplemented with 0.06% Tween 20 and in some instances also with 1% BSA. The experiment was conducted at 30°C with a shake speed of 1000 rpm.

[00245] Data analysis was performed using Data Analysis Software 9.0 (ForteBio, Fremont CA). The reference-subtracted binding curves were globally fitted to the 1:1 interaction model to generate the binding kinetic parameters k_{on}, k_{off}, and the dissociation constant K_D.

[00246] Nine of the 45 humanized antibody variants were found to bind hFRα with K_D values ranging from ~63nM to 210nM (see Table 3.1). The K_D of the parental chimera antibody (v23924) was determined to be 27nM. Humanized antibody variants that exhibited binding to hFRα were characterized by an ~2-fold to ~8-fold reduced affinity compared to that of the parental chimera

antibody. As can be seen from Table 3.1, all successful humanized variants shared the same variable light chain (4L) but differed in variable heavy chain composition. The sequences of light chain and heavy chains of the successful humanized variants are provided in Table 3.2. Fig. 3 shows the BLI sensorgrams for the parental chimeric antibody v23924, and a representative humanized antibody v30384.

Table 3.1: Antigen Binding Assessment of Humanized Variants by Octet®

Variant	Composition (VH + VL)	Fc Region[1]	Mean K_D (M)[2]	Std Dev K_D (M)
v23924 (parental)	--	HomoFc	2.7E-08	8.4E-09
Cycle One Variants				
v30384	3H4L	HetFc	1.1E-07	4.2E-09
v30389	3bH4L	HetFc	1.0E-07	1.4E-09
v30394	4H4L	HetFc	6.3E-08	7.1E-11
v30399	4bH4L	HetFc	6.9E-08	2.5E-09
Cycle Two Variants				
v31422	3cH4L	HomoFc	1.8E-07	2.8E-08
v31423	3dH4L	HomoFc	1.1E-07	1.2E-08
v31424	3eH4L	HomoFc	1.1E-07	1.9E-08
v31425	3fH4L	HomoFc	1.6E-07	4.2E-10
v31426	3gH4L	HomoFc	2.1E-07	3.5E-10

[1] HetFc = heterodimeric Fc region; HomoFc = homodimeric Fc region (see Example 2)

[2] n=2

Table 3.2: Amino Acid Sequences of Humanized VH and VL Chains

Name	Amino Acid Sequence	SEQ ID NO
3H_VH	EVQLLESGGGLVQPGGSLRLSCAVSGFSLSSYGVSWVRQAPGKG LEWIGAVNSGGSAYYADSVKGRSTISRDNSKNTVYLQMNSLRAE DTAVYYCARSGSGYPMDYLAIWGQGTLVTVSS	50
3bH_VH	EVQVLESGGGLVQPGGSLRLSCAVSGFSLSSYGVSWVRQAPGKG LEWIGAVNSGGSAYYADSVKGRSTISRDNSKNTVYLQMNSLRAE DTAVYYCARSGSGYPMDYLAIWGQGTLVTVSS	54

Name	Amino Acid Sequence	SEQ ID NO
3cH_VH	EVQLLESGGGLFQPGGSLRLSCAVSGFSLSSYGVSWVRQAPGKG LEWIGAVNSGGSAYYADSVKGRSTISRDNSKNTVYLQMNSLRAE DTAVYYCARSGSGYPMDYLAIWGQGTLVTVSS	76
3dH_VH	EVQLLESGGGLVQPGGSLRLSCAVSGFSLSSYGVSWVRQAPGKG LEWIGAVNSGGSAYYADSVKGRSTISRDTSKNTVYLQMNSLRAE DTAVYYCARSGSGYPMDYLAIWGQGTLVTVSS	79
3eH_VH	EVQLLESGGGLVQPGGSLRLSCAVSGFSLSSYGVSWVRQAPGKG LEWIGAVNSGGSAYYADSVKGRSTISRDTSKFTVYLQMNSLRAE DTAVYYCARSGSGYPMDYLAIWGQGTLVTVSS	82
3fH_VH	EVQLLESGGGLFQPGGSLRLSCAVSGFSLSSYGVSWVRQAPGKG LEWIGAVNSGGSAYYADSVKGRSTISRDTSKFTVYLQMNSLRAE DTAVYYCARSGSGYPMDYLAIWGQGTLVTVSS	85
3gH_VH	QQLLESGGGLFQPGGSLRLSCAVSGFSLSSYGVSWVRQAPGKGL EWIGAVNSGGSAYYADSVKGRSTISRDTSKFTVYLQMNSLRAED TAVYYCARSGSGYPMDYLAIWGQGTLVTVSS	88
4H_VH	EVQLLESGGGLVQPGGSLRLSCAVSGFSLSSYGVSWVRQAPGKG LEWIGAVNSGGSAYYADWAKGRSTISRDNSKNTVYLQMNSLRA EDTAVYYCARSGSGYPMDYLAIWGQGTLVTVSS	57
4bH_VH	EVQVLESGGGLVQPGGSLRLSCAVSGFSLSSYGVSWVRQAPGKG LEWIGAVNSGGSAYYADWAKGRSTISRDNSKNTVYLQMNSLRA EDTAVYYCARSGSGYPMDYLAIWGQGTLVTVSS	61
4L_VL	DYQMTQSPSSVSASVGDRVTITCRASQSIGDWLAWYQQKPGKAP KLLIYEASTLASGVPSRFSGSGSGTDFTLTISSVQPEDAATYYCQQ GYGRSNVDNIFGGGTKVEVK	64

3.2 Avidity Assessment of the Humanized Antibodies for hFRα

[00247] The avidity of antigen binding for parental and selected humanized variants in FSA format was assessed by surface plasmon resonance (SPR) as described below.

[00248] The SPR assay for determination of hFRα affinity and avidity of the parental chimeric antibody (v23924) and the humanized variants was carried out on a Biacore™ T200 SPR system with PBS-T (PBS + 0.05% (v/v) Tween 20) running buffer (with 0.5 M EDTA stock solution added to 3.4 mM final concentration) at a temperature of 25°C. CM5 Series S sensor chip, Biacore™ amine coupling kit (NHS, EDC and 1 M ethanolamine), and 10 mM sodium acetate buffers were purchased from GE Healthcare Life Science (Mississauga, ON, Canada). PBS

running buffer with 0.05% Tween20 (PBST) was purchased from Teknova Inc. (Hollister, CA). Recombinant human FRα was purchased from ACRObiosystems (Newark, DE).

[00249] Screening of the variants for binding to hFRα antigen was conducted in two steps: capture of hFRα onto surface, followed by injection of three to five concentrations of variant. The hFRα surface was prepared on a CM5 Series S sensor chip by standard amine coupling methods as described by the manufacturer (GE Healthcare Life Science, Mississauga, ON, Canada). The immobilization of the hFRα was performed using Biacore™ T200 immobilization wizard with an amine coupling method aiming for resonance units (RUs) ranging from 5 to 200 RUs. Using multi-cycle kinetics, three to five concentrations of a two-fold dilution series of samples starting at 300nM with a blank buffer control were injected at 50 uL/min for 180s with a 600s dissociation phase, resulting in a set of sensorgrams with a buffer blank reference. The hFRα surface was regenerated to prepare for the next injection cycle by one pulse of 10mM glycine/HCl pH 1.5 for 30s at 30 uL/min. Blank-subtracted sensorgrams were analyzed using Biacore™ T200 Evaluation Software v3.0. The blank-subtracted sensorgrams were then fit to the 1:1 Langmuir binding model.

[00250] The results are shown in Table 3.3. Both parental antibody (v23924) and the two humanized variants (v30384 and v30399) in the regular bivalent antibody format (FSA) demonstrated avidity in binding to the hFRα antigen. Namely, ~17-fold lower K_D values in the case of parental and ~27-39-fold in the case of humanized samples were obtained in FSA compared to one-armed antibody (OAA) format at medium-low antigen density, which further decreased to ~108-fold and ~116-181-fold respectively, at high antigen density.

Table 3.3: Avidity Assessment of Selected Humanized Variants by SPR

Variant	Format[1]	Mean K_D (M)[2]		
		High Antigen Density	Medium Antigen Density	Medium-Low Antigen Density
v23924	FSA	2.78E-10	1.37E-09	2.29E-09
v32596	OAA v23924 equivalent	3E-08	3.03E-08	3.79E-08
v30384	FSA	7.9E-10	2.58E-09	3.76E-09

Variant	Format[1]	Mean K_D (M)[2]		
		High Antigen Density	Medium Antigen Density	Medium-Low Antigen Density
v32597	OAA v30384 equivalent	1.43E-07	1.39E-07	1.46E-07
v30399	FSA	7.09E-10	2.67E-09	3.71E-09
v32598	OAA v30399 equivalent	8.2E-08	8.08E-08	9.15E-08

[1] OAA = one armed antibody, FSA = full size antibody; OAA antibody samples are equivalents of respective FSA samples and were produced in the similar manner to FSA samples described in Example 2.
[2] n=2

EXAMPLE 4: PURITY OF HUMANIZED ANTI-FRα ANTIBODIES

[00251] The apparent purity of the humanized antibody variants from Example 3 was assessed using mass spectrometry after Protein A purification (Example 2) and non-denaturating deglycosylation.

[00252] As the antibody variant samples contained Fc N-linked glycans only, the samples were treated with N-glycosidase F (PNGase-F) only. The purified samples were de-glycosylated with PNGaseF as follows: 0.1U PNGaseF/µg of antibody in 50mM Tris-HCl pH 7.0, overnight incubation at 37°C, final protein concentration of 0.48 mg/mL. After deglycosylation, the samples were stored at 4°C prior to LC-MS analysis.

[00253] The deglycosylated protein samples were analyzed by intact LC-MS using an Agilent 1100 HPLC system coupled to an LTQ-Orbitrap™ XL mass spectrometer (ThermoFisher, Waltham, MA) (tuned for optimal detection of larger proteins (>50kDa)) via an Ion Max electrospray source. The samples were injected onto a 2.1 x 30 mm Poros R2 reverse phase column (Applied Biosystems Corp., Waltham, MA) and resolved using a 0.1% formic acid aq/acetonitrile (degassed) linear gradient consisting of increasing concentration (20-90%) of acetonitrile. The column was heated to 82.5°C and solvents were heated pre-column to 80°C to improve protein peak shape. The cone voltage (source fragmentation setting) was approximately 40 V, the FT resolution setting was 7,500 and the scan range was m/z 400-4,000. The LC-MS system was evaluated for IgG sample analysis using a deglycosylated IgG standard (Waters IgG standard) as

well as a deglycosylated mAb standard mix (25:75 half:full sized antibody). For each LC-MS analysis, the mass spectra acquired across the antibody peak (typically 3.6-4.1 minutes) were summed and the entire multiply charged ion envelope (m/z 1,200-4,000) was deconvoluted into a molecular weight profile using the MaxEnt 1 module of MassLynx™ data analysis software (Waters, Milford, MA). The apparent amount of each antibody species in each sample was determined from peak heights in the resulting molecular weight profiles.

[00254] The results are shown in Table 4.1. Almost all humanized variants were of high purity, ranging from ~89-100% desired species, with only v30389 showing a lower purity (82.6%). The slightly lower purity of four variants, v30384, v30389, v30394 and v30399, compared to that of the remaining variants is due to these variants comprising a heterodimeric CH3 region (see Example 2) which results in the presence of some half-antibodies. Fig. 4 depicts LC/MS profile for two representative humanized variants, v30384 and v31422. In the LC/MS profiles of all samples, a side peak of~+266Da was observed, which is likely an artifact of the analysis.

Table 4.1: Purity of Humanized Variants Determined by LC/MS

Variant	% Desired Species
v30384	93.2
v30389	82.6
v30394	89.1
v30399	91.8
v31422	100
v31423	100
v31424	100
v31425	100
v31426	100

EXAMPLE 5: THERMAL STABILITY OF HUMANIZED ANTI-FRα ANTIBODIES

[00255] The thermal stability of the humanized antibody variants was assessed by differential scanning calorimetry (DSC) as described below.

[00256] 400 µL of purified samples primarily at concentrations of 0.4 mg/mL in PBS were used for DSC analysis with a VP-Capillary DSC (GE Healthcare, Chicago, IL). At the start of each DSC run, 5 buffer blank injections were performed to stabilize the baseline, and a buffer injection was placed before each sample injection for referencing. Each sample was scanned from 20°C to 100°C at a 60°C/hr rate, with low feedback, 8 sec filter, 3 min pre-scan thermostat, and 70 psi nitrogen pressure. The resulting thermograms were referenced and analyzed using Origin 7 software (OriginLab Corporation, Northampton, MA) to determine melting temperature (Tm) as an indicator of thermal stability.

[00257] The Fab Tm values determined for the humanized variants are shown in Table 5.1. All humanized variants exhibited increased thermal stability compared to the parental antibody, v23924 (Fab Tm of ~72 °C), with Fab Tm values ranging from ~81-84°C. Of the humanized variants, v30394 showed the highest thermal stability.

Table 5.1: Thermal Stability of Humanized Variants

Variant	Fab Tm (°C)
v23924 (parental)	72.1
v30384	81.7
v30389	80.7
v30394	84.4
v30399	83.4
v31422	82.6
v31423	83.0
v31424	81.3
v31425	82.4
v31426	81.9

EXAMPLE 6: DETERMINATION OF ISOELECTRIC POINT FOR HUMANIZED ANTI-FRα ANTIBODIES

[00258] The isoelectric point of the humanized antibody variants was determined by capillary isoelectric focusing (cIEF) as described below.

[00259] cIEF was carried out using CE-UV Agilent 7100 Capillary Electrophoresis (CE) system. 5ug (or maximal 2.5uL) of sample was applied to the capillary (ampholytes range of 3.0-10.0). pI markers mix, 4.1, 4.22, 5.5, 7.0 and 10.0 for system suitability tests and 4.1 and 10.0 for sample analyses were used. The Agilent 7100 CE system equipped with an external water bath set to 6°C, the detector filter assembly (280nm) and 9 bar external pressure were used for all CE runs. The neutral coated capillary (fluorocarbon) was cut at both ends at a distance of 8.5 cm and 24.5 cm from the detection window, respectively, equipped with a green alignment interface and fitted into the Agilent capillary cassette. Once a day, capillaries were conditioned as follows: high pressure flush at 3.5 bar with 350 mM acetic acid for 5 minutes, with water for 2 minutes and with cIEF gel for 5 minutes. Prior to every run, capillaries were conditioned as follows: high pressure flush at 3.5 bar with 4.3 M urea solution for 3 minutes and with water for 2 minutes. Samples were injected by applying 2 bar high pressure for 100 seconds, followed by a water dip of both inlet and outlet electrode. Focusing was performed for 10 minutes at 25 kV with 200 mM phosphoric acid as anolyte and 300 mM NaOH as catholyte. Using chemical mobilization, the outlet vial was exchanged for 350 mM acetic acid and 30 kV was applied for 30 minutes. After each run, a high pressure flush at 3.5 bar with water was performed for 2 minutes. Manual integration for peaks RT and electropherograms were obtained using Agilent OpenLAB Intelligent Reporting A.01.06.111 software. Raw data (signal vs retention time) were exported to a CVS file and main isoform pI, pI range and pI at the center of mass were calculated (based on the internal pI markers) in Microsoft Excel.

[00260] The results are shown in Table 6.1. The pI values determined for the main isoform for the majority of the humanized variants ranged from 7.78 to 7.97, with one variant having pI of 8.25 (v31426), which all fall within the typical range for therapeutic antibodies and are relatively comparable to that of the parental chimeric antibody v23924 (pI of 7.65).

Table 6.1: Isoelectric Point for Humanized Variants

Variant	pI (main isoform)
v23924 (parental)	7.65
v30384	7.79
v30389	7.78
v30394	7.89
v30399	7.89
v31422	7.93
v31423	7.94
v31424	7.97
v31425	7.93
v31426	8.25

EXAMPLE 7: CHROMATOGRAPHIC ANALYSIS OF ANTI-FRα ANTIBODIES

[00261] Parental and humanized variants were analyzed by hydrophobic interaction chromatography (HIC) and size exclusion chromatography (SEC) as described below.

7.1 HIC Analysis

[00262] The hydrophobicity/hydrophilicity of the antibodies were assessed by HIC as described in *Antibody Drug Conjugates, Methods in Molecular Biology*, 2013, vol. 1045, pp. 275-284. L. Ducry, Ed. The experiments were performed on an Agilent Infinity II 1290 HPLC using a TSKgel® Butyl-NPR column (2.5µm, 4.6 x 35mm, TOSOH Bioscience GmbH, Griesheim, Germany) pre-equilibrated with 5 column volumes of Buffer A (1.5 M $(NH_4)_2SO_4$, 25 mM NaH_2PO_4, pH 6.95) at room temperature. Typically, 20-30 µg of sample at 2-3 mg/mL concentration was loaded on the column with 95% Buffer A and 5% Buffer B (75% 25 mM PO_4^{3-} plus 25% isopropanol, pH 6.95) and run for 15 mins at 0.5 mL/min using the gradient shown in Table 7.1. HIC chromatograms were integrated using appropriate parameters that provided complete, baseline-to-baseline integration.

Table 7.1: HIC Solvent Gradient

Time (min)	% Buffer A	% Buffer B
0	95	5
0.1	95	5
5	80	20
9.5	65	35
11.5	50	50
12.5	5	95
13.5	5	95
12.6	95	5
15	95	5

7.2 SEC Analysis

[00263] Analytical SEC was performed using an Agilent Infinity II 1260 HPLC with Advance Bio SEC column (300 Å, 2.7 µm, 7.8 × 150 mm) equilibrated with 5 column volumes of Mobile Phase A (150 mM Na_2PO_4, pH 6.95) at room temperature. Typically, 20-30 µg of sample at 2-3 mg/mL concentration was eluted isostatically for 7 mins at 1 mL/min and absorbance was monitored at A280. Chromatograms were integrated to provide complete, baseline-to-baseline integration of each peak, with reasonably placed separation between partially resolved peaks. The peak corresponding to the major component for IgG (approximate retention time 3.3 min) was reported as the monomer based on the SEC profile of control trastuzumab. Any peak occurring prior to 3.3 min was designated as high molecular weight species (HMWS), and any peak occurring after 3.3 min was designated as low molecular weight species (LMWS), excluding solvent peaks (over 5.2 min).

7.3 Results

[00264] The summary of HIC retention time (HIC-RT) and SEC monomer % of parental and humanized variants are provided in Table 7.2. Overall, HIC and SEC showed favourable biophysical behaviour of all humanized variants. Parental chimeric antibody v23924 eluted at 6.5 mins on HIC gradient, whereas all humanized variants eluted between 6.0-6.7 mins. SEC profiles

showed >90% monomer for all humanized variants, with variant v30389 having the lowest monomer % (94%). All these variants had <5% HMWS and <5% LMWS.

Table 7.2: HIC and SEC Analysis of Parental and Humanized Anti-FRα Antibodies

Variant	Antibody	HIC-RT (min)	SEC		
			Monomer %	HMWS %	LMWS %
v23924	Chimeric (parental)	6.5	100	0	0
v30618	Chimeric (HetFc)	6.7	94	6	0
v30384	Humanized Cycle one*	6.2	97	3	0
v30389		6.2	94	3	4
v30394		6.0	95	4	2
v30399		6.1	96	4	0
v31422	Humanized Cycle two	6.0	100	0	0
v31423		6.0	100	0	0
v31424		6.1	100	0	0
v31425		6.2	100	0	0
v31426		6.5	100	0	0

*No previous Prep-SEC

EXAMPLE 8: ADDITIONAL STABILITY STUDIES

[00265] In order to further investigate the stability of the humanized antibody variants, 40°C stability and acid stability studies were performed in which samples were characterized at specific timepoints by Caliper and UPLC-SEC, as described in Example 2 and in the case of 40°C stability study additionally by cIEF and Octet antigen binding, as described in Examples 6 and 3, respectively. Trastuzumab was used as a control in these studies.

[00266] Selected humanized variants (v30389, v30394, v30399, v31423 and v31424) underwent a 40°C stability study for a duration of 14 days at ~1mg/ml sample concentration in PBS pH 7.4 buffer. Samples were characterized at timepoints 0, 5, 7, 11 and 14 days. An acid stability study

was performed upon buffer exchange of the sample into acetate buffer of pH 3.6 at varied sample concentrations for a duration of 1hr at 25°C, with characterization at timepoints 0, 15, 30 and 60 min. Additionally, freeze-thaw (from -80°C to room temperature) constituting three cycles of 30 min per cycle was performed at ~1mg/ml sample concentration in PBS pH7.4.

[00267] The results are shown in Table 8.1. No significant issues with the stability of the humanized variants were identified in the studies. Freeze-thaw and acid stability studies did not reveal any change in sample composition over the course of the study, as determined by Caliper and UPLC-SEC. The 40°C stability study showed minor changes in Caliper and UPLC-SEC profiles as the study progressed (specifically, the appearance of some amounts of lower molecular weight species, ranging from ~10-17%). No changes to the antigen-binding affinity were observed. cIEF revealed expected minor increases in more acidic species to relative to the main isoform.

Table 8.1: Stability Assessment of Humanized Variants in 40°C, Acid Stability and Freeze-Thaw Studies

Variant	% Monomer by UPLC-SEC		
	40°C Study Day 0/7/14	**Acid Stability Study 0/30/60 min**	**Freeze-Thaw Study**
v6738[1]	99.93/99.71/99.43	Err[2]/100/100	ND
v30389	92.75/87.64/82.02	Err/94.91/93.83	ND
v30394	95.49/89.01/83.51	95.12/95.53/95.49	ND
v30399	96.13/89.25/81.68	Err/100/100	ND
v31423	100/90.92/82.58	Err/100/100	99.22
v31424	100/90.96	100/100/100	99.36

[1] Trastuzumab control

[2] Err = Erroneous baseline

EXAMPLE 9: FUNCTIONAL CHARACTERIZATION OF ANTI-FRα ANTIBODIES – FRα SPECIFICITY

[00268] The binding cross-reactivity of parental chimeric antibody v23924 to FRα (FOLR1), FOLR2, FOLR3 and FOLR4 was assessed by flow cytometry and ELISA. FRα, FOLR2 and

FOLR4 binding was assessed through flow cytometry using HEK293 transfected cells. FOLR3 binding was assessed through ELISA as FOLR3 is a soluble protein. Control anti-FOLR2 (mouse anti-human FOLR2; Nordic BioSite AB, Täby, Sweden; Cat. No. AFC-4544-2), anti-FOLR3 (mouse anti-human FOLR3; LS Bio, Seattle, WA; Cat. No. LS-C125621) and anti-FOLR4 (mouse anti-His DyLight™ 650; Novus Biologicals, Littleton, CO; Cat. No. NBP2-31055C) antibodies were included in these experiments.

[00269] FRα, FOLR2 and FOLR4 binding: Briefly, HEK293-6e cells were transfected for ~24 hours to transiently express human FRα (Cat. No. 13420), FOLR2 (Cat. No. 13481) and FOLR4 (Cat. No. 13483) (all from GenScript Biotech, Piscataway, NJ), 1 ug DNA per 1 million cells. Following transfection, 50,000 cells were seeded in V-bottom 96-well plates and incubated with 50 nM primary antibody for 45 min under standard culturing conditions. Following incubation, cells were washed and stained with anti-Human IgG Fc AF647 conjugate (Jackson Immuno Research Labs, West Grove, PA; Cat. No. 109-605-098) at RT for 45 min. Following incubation and washing, fluorescence was detected by flow cytometry on a BD LSRFortessa™ Cell Analyzer (BD Biosciences, Franklin Lake, NJ) with 1,000 minimum events collected per well.

[00270] FOLR3 binding: ELISA 96-well plate was coated with commercial purified FOLR3 protein (R&D Systems, Inc., Minneapolis, MN; Cat. No. 5319-FR) for 1 hr at 37°C. The plate was blocked with 1% milk in PBS, pH 7.4, for 1 hr at RT. Following blocking, primary antibodies were added at 7 nM for 1 hr at RT. HRP-conjugated secondary antibody (Jackson Immuno Research Labs, West Grove, PA; Cat. No. 109-035-098) was then added at 0.4 µg/ml, for 1 hr at RT. Plates were developed using tetramethylbenzidine (TMB) and HCl was used to stop reaction. Absorbance was read at 450 nm using a Synergy™ H1 microplate reader (BioTek Instruments, Winooski, VT).

Results

[00271] The results are shown in Tables 9.1 and 9.2.

[00272] Anti-FRα, anti-FOLR2, anti-FOLR3 and anti-FOLR4 control antibodies showed expected binding to the respective target proteins by flow cytometry or ELISA. By flow cytometry, live singlet cell population was gated using FlowJo™ v8 software (BD Biosciences, Franklin Lake, NJ), and AF647 GeoMean and % positive binding was determined in this population for each antibody. For ELISA, % positive binding of anti-FOLR3 and v23924 antibodies was determined

using raw absorbance values compared to negative control absorbance signal. v23924 showed expected binding to human FRα, and did not exhibit binding cross reactivity to FOLR2, FOLR3 or FOLR4, indicating FRα specificity.

Table 9.1: Binding to FRα, FOLR2 and FOLR4 Assessed by Flow Cytometry

Antibody	AF647/DyLight650 GeoMean Singlet Population			% Positive Binding Singlet Population		
	FRα	FOLR2	FOLR4	FRα	FOLR2	FOLR4
v23924	7663	7	27	71	0	0
anti-FRα Control	17971	227	188	86	1	0
anti-FOLR2 Control	17	4267	15	1	87	0
anti-FOLR4 Control	32	32	164	1	1	48
Human IgG	59	20	23	0	0	0

Table 9.2: Binding to FOLR3 Assessed by ELISA

Antibody	Absorbance 450 nm	% Binding
v23924	0.29	8.9
anti-FRα Control	0.24	7.4
anti-FOLR3 Control	3.27	100.0
Human IgG	0.15	4.5

EXAMPLE 10: FUNCTIONAL CHARACTERIZATION OF ANTI-FRα ANTIBODIES – BINDING TO CYNOMOLGUS FRα

[00273] The cross-reactivity of parental chimeric antibody v23924 to human and cynomolgus monkey FRα was assessed by flow cytometry using transfected CHO-S cells as described below. Palivizumab (anti-RSV) (v22277) was used as a negative control.

[00274] Briefly, CHO-S cells were transfected for ~24 hours to transiently express human or cynomolgus monkey FRα, 1 ug DNA per 1 million cells. Following transfection, cells were seeded at 50,000 cells/well in V-bottom 96-well plates and treated with antibody for 24 hours at 4°C to

prevent internalization. Following incubation, cells were washed and stained with anti-human IgG Fc AF647 conjugate (Jackson Immuno Research Labs, West Grove, PA; Cat. No. 109-605-098) at 4°C for 30 min. Following incubation and washing, fluorescence was detected by flow cytometry on a BD LSRFortessa™ Cell Analyzer (BD Biosciences, Franklin Lake, NJ).

[00275] The results are shown in Table 10.1. v23924 showed comparable binding to human and cynomolgus FRα on CHO-S transfected cells, with apparent Kd values of 83.89 pM and 121.60 pM on human FRα and cynomolgus monkey FRα transfected cells, respectively. No binding by control v22277 was observed, as expected.

Table 10.1: Binding to Cynomolgus Monkey FRα

Antibody	Cells	Bmax	Apparent Kd (nM)
v23924	CHO-S Human FRα	398.90	0.08
v22277		6.94	>50
v23924	CHO-S Cyno FRα	7626	0.12
v22277		79.62	>50

EXAMPLE 11: FUNCTIONAL CHARACTERIZATION OF ANTI-FRα ANTIBODIES – CELLULAR BINDING

[00276] The on-cell binding capabilities of the parental chimeric antibody v23924 and a representative humanized variant v30384 were assessed on JEG-3 and HEC-1-A endogenous FRα-expressing cell lines by flow cytometry as described below.

[00277] Briefly, cells were seeded at 50,000 cells/well in V-bottom 96-well plates and treated with antibody for 24 hours at 4°C to prevent internalization. Following incubation, cells were washed and stained with anti-Human IgG Fc AF647 conjugate (Jackson Immuno Research Labs, West Grove, PA; Cat. No. 109-605-098) at 4°C for 30 min. Following incubation and washing, fluorescence was detected by flow cytometry on a BD LSRFortessa™ Cell Analyzer (BD Biosciences, Franklin Lake, NJ) with 1,000 minimum events collected per well. AF647/APC-A GeoMean (fluorescence signal geometric mean, proportional to anti-Human AF647 binding) in

live cell population was plotted using GraphPad Prism Version 9 (GraphPad Software, San Diego, CA).

[00278] The results are shown in Table 11.1. Both the chimeric (v23924) and humanized (v30384) antibodies yielded comparable apparent Kd and Bmax values in both JEG-3 and HEC-1-A cell lines (high and moderate endogenous FRα expression, respectively).

Table 11.1: Cellular Binding

Antibody	JEG-3			HEC-1-A		
	Bmax	Curve Hill Slope (h)	Kd (nM)	Bmax	Curve Hill Slope (h)	Kd (nM)
v23924	20337	1.18	0.32	2490	1.38	0.05
v30384	18299	1.73	0.31	2125	1.31	0.07
Human IgG	NA	NA	NA	NA	NA	NA

EXAMPLE 12: FUNCTIONAL CHARACTERIZATION OF ANTI-FRα ANTIBODIES – INTERNALIZATION

[00279] The receptor-mediated internalization capabilities of the parental chimeric antibody v23924 and a representative humanized variant, v30384, in FRα-expressing cell lines (IGROV-1 and OVCAR-3) were determined by high content imaging as described below. The FRα-targeting antibodies mirvetuximab and farletuzumab were used as positive controls, and palivizumab (anti-RSV) (v22277) was used as a negative control.

[00280] Briefly, antibodies were fluorescently labeled by coupling to an anti-human IgG Fc Fab fragment pHAb dye conjugate (Promega Corporation, Madison, WI; Cat. No. G9841) (~3 dye molecules per Fab fragment) at a 5:1 molar excess for 24 hours at 4°C. Cells were seeded and incubated overnight at 37°C in 5% CO_2 in 96-well plates. Coupled antibodies were added to cells the following day and incubated at 37°C for 6-24 hours to allow for internalization. Following incubation, cells were stained with Dye Cycle Violet (ThermoFisher Scientific Corporation, Waltham, MA; Cat. No. V35003) for viable cell identification and internalized fluorescence in live

cells was analyzed by high content imaging using the CellInsight™ CX5 High Content Screening (HCS) Platform (ThermoFisher Scientific Corporation, Waltham, MA). Fold-over fluorescence of the chimeric antibody v23924 was plotted using GraphPad Prism, Version 9 (GraphPad Software, San Diego, CA).

Results

[00281] The results are shown in Fig. 5A & 5B (IGROV-1 cells) and Fig. 6A & 6B (OVCAR-3 cells). Chimeric antibody v23924 and the humanized variant v30384 showed comparable levels of internalization in both IGROV-1 cells (high FRα) and OVCAR-3 cells (moderate FRα). In both IGROV-1 and OVCAR cells, the chimeric antibody v23924 and the humanized variant v30384 showed increased internalization compared to mirvetuximab and farletuzumab positive controls across all tested concentrations (25 to 1 nM) and time points (6 and 24 hours). For example, following a 6-hour incubation in IGROV-1 cells, humanized variant v30384 showed 3.3 and 6.1-fold increase in internalized fluorescence compared to mirvetuximab and farletuzumab, respectively, at 25 nM, and 2.6 and 19.1-fold increase in internalized fluorescence compared to mirvetuximab and farletuzumab, respectively, at 5 nM (Fig. 5A). Similarly, following a 24-hour incubation in IGROV-1 cells, humanized variant v30384 showed 2.1 and 3.9-fold increase in internalized fluorescence compared to mirvetuximab and farletuzumab, respectively, at 25 nM, and 1.9 and 4.7-fold increase in internalized fluorescence compared to mirvetuximab and farletuzumab, respectively, at 5 nM (Fig. 5B).

EXAMPLE 13: EPITOPE MAPPING

[00282] High resolution epitope mapping of the parental chimeric antibody v23924 on human FRα antigen (hFRα) was conducted by hydrogen/deuterium exchange mass spectrometry (HDX-MS) at NovoAb Bioanalytics Inc. (Victoria, BC. Canada) as described below.

13.1 Sample preparation for HDX-MS

[00283] Lyophilized hFRα was purchased from ACROBiosystems (Newark, DE; Catalogue no: FO1-H5229) and dissolved to a concentration of 2.5 mg/ml. The antigen-antibody complex was prepared by mixing hFRα with the parental chimeric antibody v23924 at a molar ratio of 2:1. All samples had a pH of 7.4 and were clear (no precipitation was observed). For peptide identification, hFRα at a concentration of 10 μM was reduced with 100 mM of tris-(2-carboxyethyl) phosphine

(TCEP) in the presence of 2 M guanidine at pH 2.4, and then digested with pepsin at an enzyme-to-protein molar ratio of 1:1. HDX was initiated by mixing the protein samples with D_2O buffer at a ratio of 2:8 (v/v). The resulting solutions were incubated at 26°C, and aliquots were taken at 20 s, 7 min, 1 h and 4 h, and instantly quenched by adding a 200 mM TCEP solution containing 4 M guanidine. These samples were flash frozen in liquid nitrogen and stored at -80°C. During LC-MS experiments, the protein aliquots were quickly thawed and kept on ice for the reduction to proceed for 2 min, and then digested at 0°C with pepsin for 2 min.

13.2 LC-MS and LC-MS/MS

[00284] In the LC-MS experiments, 20 µL aliquots of each sample were instantly injected into a C18 analytical column and separated by reversed-phase liquid chromatography using a Dionex UHPLC system (Thermo Fisher Scientific, Bremen, Germany) at a flow rate of 100 µL/min. The UHPLC system was coupled to a Thermo Scientific Orbitrap Fusion™ mass spectrometer equipped with a heated electrospray ionization (HESI) II source. The column, accessories, injector and solvent delivery lines were embedded in an ice bucket to minimize H/D back-exchange. The syringe used for injection was chilled on ice. The mobile phase was 0.1% formic acid (A) and 100% acetonitrile/0.1% formic acid (B), and the peptides were separated by a 13-minute gradient. The MS survey scan was carried out within m/z 300-1600 range, with a mass resolution of 120,000 FWHM. The Orbitrap detector was calibrated to be < 3 ppm error by using Calibration Mix (Calmix; ThermoFisher Scientific Corporation, Waltham, MA). In the electron transfer dissociation (ETD) experiments, fluoranthene radical anions were introduced into the ion trap over 50 ms. Collision induced dissociation (CID) and ETD fragment ions were detected in the Orbitrap using a scan range of 150-2000 m/z.

[00285] For data analysis, raw bottom-up LC-MS/MS data were processed using the Proteome Discoverer™ software suite (Thermo Fisher Scientific). The generated peak lists were submitted to the Mascot 2.2 server in-house and searched against the sequence of hFRα. The peptides thus identified were used for HDX data analysis. ETD data were processed using Xcalibur™ software (ThermoFisher Scientific Corporation, Waltham, MA) and the generated ETD peak lists were searched against the sequence of hFRα using Protein Prospector (available online from the University of California, San Francisco website; http://prospector.ucsf.edu). Matched ions were also checked and confirmed by manual inspection. The mass shift of the peptides and the

deuteration status of individual amides were determined based on their centroid m/z values before and after H/D exchange. All HDX data were normalized to 100% D_2O content (80% D exchange-in buffer for all the time points). Percent deuterium incorporation values were obtained by comparing the number of acquired deuterium to the total number of amide hydrogens contained in each peptide. The amide level deuteration information was calculated based on the deuterium uptake of the ETD fragments.

13.3 Results

Protein sequence coverage and peptide identification

[00286] The presence of protein disulfide bonds has a significant impact on the pepsin digestion pattern and efficiency in the peptide-based HDX-MS analysis. Since both hFRα and the antibody v23924 contain multiple disulfide bridges, an optimized protocol for rapid protein disulfide reduction and pepsin digestion was first developed. The reduction time and digestion time was optimized to be 2 min for each, and these conditions were applicable to both hFRα and the hFRα -v23924 complex. The peptides thus identified covered 100% of the antigen sequence (see Fig. 7).

Peptide level HDX comparison

[00287] The deuterium incorporation levels of peptides from hFRα and the v23924 complex were plotted versus HDX time (20s, 7min, 1 h, and 4 h). The results are summarized and shown in Fig. 8. Most of the peptides have the same deuterium uptake behavior before and after antibody binding (Fig. 8A), suggesting the binding site (epitope) of the v23924 antibody is quite localized. In the differential plot shown in Fig. 8B, three peptides (numbers 14, 15 and 16) showed significant lowering in deuterium uptake after v23924 binding, indicating they are in the epitope region. The sequences of these peptides are WWEDCRTSY (118-126) (SEQ ID NO:151), WEDCRTSY (119-126) (SEQ ID NO:152), and WEDCRTSYTCKSNWHKGWNWTSGF (119-142) (SEQ ID NO:153), respectively.

Epitope Determination at Amino Acid Level

[00288] Although the three epitope peptides differ in sequence, their HDX differences are the same (Fig. 8), and they all contain the sequence WEDCRTSY (119-126) (SEQ ID NO:152). This indicates that all the epitope residues are included in the shortest peptide 119-126. The observation of multiple differential peptides in the same region provides additional confirmation that this

region of the protein is the binding site of the v23924 antibody. To further locate the HDX differences and pinpoint the epitope down to individual amino acids, MSMS was carried out on the peptide 119-126 using ETD. The HDX time point of 1 h was selected as this gave the largest difference. The ETD fragments provided single-residue resolution. The deuteration level of each amino acid was calculated and compared between hFRα and the v23924 complex (Fig. 9). Based on the results of the difference plot, the epitope residues were determined to be E120, D121, R123, T124, S125, and Y126 of SEQ ID NO: 15 (*i.e.* the epitope sequence is: EDRTSY; SEQ ID NO: 154).

EXAMPLE 14: AFFINITY MATURATION OF ANTI-FRα ANTIBODY

[00289] The humanized antibody v30384 (see Example 3) was affinity matured using the HuTarg™ system (Innovative Targeting Solutions, Vancouver, BC, Canada). Genetic recombination was applied to the variable regions of the humanized variant v30384, and high affinity mutants were identified using next-generation sequencing (NGS).

14.1 Design of library plasmid pool

[00290] The CDR loops of the humanized variant v30384 variable domains were interspersed with RAG1/2 recombination signal sequences (RSS). These variable domains were synthesized at Integrated DNA Technologies, Inc. (Coralville, IA) and cloned into plasmid E951 (Innovative Targeting Solutions, Vancouver, BC, Canada).

14.2 HuTarg™ library generation

[00291] The following steps were performed in accordance with Innovative Targeting System's protocols. E951-based plasmid pools were integrated into HuTarg™ cells, and RAG1/2 expression induced for 48 hours. HuTarg™ cells displaying successfully recombined antibodies, shown after staining with PE-conjugated goat anti-human kappa light chain antibody (Bio-Rad Laboratories, Hercules, CA; cat#206009) were then selected for further interrogation.

14.3 FACS-based selection of affinity mutants

[00292] HuTarg™ cells were subjected to multiple rounds of FACS-based sorting on a BD FACSAria™ Flow Cytometer (BD Biosciences, Franklin Lakes, NJ), with each round using a reduced amount of biotinylated soluble HIS-tagged FRα antigen (FRα-HIS; ACROBiosystems

Newark, DE; cat# FO1-H82E2), detected with streptavidin conjugated to AlexaFluor-647 (Thermo Fisher Scientific Corp., Waltham, MA; cat# S11223). HuTarg™ cells that exhibited increased binding to biotinylated FRα-HIS were sorted directly to RNAzol RT (Sigma-Aldrich, St. Louis, MI; cat# R4533) in preparation for next-generation sequencing.

14.4 Next-generation sequencing of affinity mutants

[00293] Total RNA from cells lysed in RNAzol was isolated as per manufacturer's instructions. RNA was then digested with ezDNase™ (Thermo Fisher Scientific Corp., Waltham, MA; cat# 11766051), and cDNA transcribed using Superscript™ IV (Thermo Fisher Scientific Corp., Waltham, MA; cat# 18090010) and a gene-specific primer. VH and VK domains were targeted for PCR amplification, and molecularly barcoded with NEBNext® Ultra™ DNA Library Prep Kit (New England Biolabs, Ipswich, MA; cat# E7370L). Samples were pooled and run on an Illumina MiSeq™ sequencer with a 500-cycle kit using v2 chemistry (Illumina, San Diego, CA; cat# MS-102-2003). Sequence analysis was performed to identify mutations within VH and VK sequences that exhibited high likelihood of conferring affinity increase.

14.5 Recombinant expression of affinity mutants

[00294] DNA sequences encoding mutated VH and VK domains were synthesized as "MiniGenes" (Integrated Technologies, Inc., Coralville, IA) and cloned into expression vectors to provide expression plasmids coding for complete human IgG1 heavy chains and human kappa light chains, respectively. Expression plasmids were matrixed with one another to pair every heavy chain plasmid with every light chain plasmid. This matrix was recombinantly expressed in Expi293™ cells (Thermo Fisher Scientific Corp., Waltham, MA; cat# A14635) according to manufacturer's instructions to create 64 samples.

14.6 Evaluation of affinity mutants

[00295] Protein G particles (Spherotech Inc., Lake Forest, IL) were coated with the humanized variant v30384 or affinity matured antibodies at a normalized concentration. Soluble human FRα was diluted to a limiting antigen concentration and incubated with antibody coated beads. FRα antigen binding and antibody capture was detected using AlexaFluor-647 conjugated streptavidin and AlexaFluor-488 conjugated goat anti-human IgG Fcγ (both from Jackson Laboratories, Bar Harbor, ME), respectively. Samples were analyzed by flow cytometry on a BD LSRFortessa™

Cell Analyzer (BD Biosciences, Franklin Lakes, NJ). Geometric mean for FRα binding and antibody capture was analyzed for each sample. Antibody capture was normalized to FRα binding to affinity rank humanized variant v30384 against affinity matured antibodies.

[00296] Single point affinity ranking was performed by measuring the ratio of antibody captured on beads to the amount of antigen captured by antibody. Variant v30384 binding was minimal (3x over background) using a human FRα concentration of 1.9 nM, while the majority of mutated variants exhibited higher binding ratios. Of 64 mutated variants, 3 variants showed a 4-fold increase in binding ratio, and 10 showed an equivalent binding ratio.

EXAMPLE 15: ASSESSMENT OF AFFINITY OF AFFINITY MATURED ANTIBODIES FOR FRα

[00297] Ten of the affinity matured variants described in Example 14 were produced in full size antibody (FSA) format at WuXi Biologics (Hong Kong) Limited, China, via transient transfection in CHO-K1 cells and affinity capture purification with a subsequent polishing step (where necessary) involving primarily preparatory SEC or CEX chromatography, yielding greater than 97% sample purity by HPLC-SEC. The FSA format was similar to that of parental humanized variant v30384 except these variants comprised a HomoFc rather than HetFc. The ten affinity matured variants were characterized for binding to hFRα using the Octet® RED96 system as described in Example 3.

Results

[00298] The results are shown in Table 15.1. Affinity maturation was successful in obtaining humanized variants with affinities to hFRα substantially higher than the parental humanized antibody v30384 (K_D 1.27E-07 M), ranging from ~12 to 58-fold improvement. The lowest K_D of 2.2E-09 M was observed for variant v35348. The gain in affinity was determined to be primarily achieved by lowering of the dissociation constant.

Table 15.1: Affinity Assessment of Affinity Matured Variants

Variant	Mean K_D (M)*	Std Dev K_D (M)
v30384 (parental humanized)	1.27E-07	2.49E-08

Variant	Mean K_D (M)*	Std Dev K_D (M)
v35305	1.08E-08	9.12E-10
v35342	4.1E-09	1.3E-10
v35347	8.65E-09	9.18E-10
v35348	2.2E-09	1.8E-10
v35350	3.55E-09	2.89E-10
v35354	3.13E-09	7.64E-11
v35356	4.98E-09	1.8E-10
v35358	7.22E-09	4.21E-10
v36167	7.29E-09	1E-10
v36168	4.97E-09	2.64E-10

* n=2

EXAMPLE 16: CHROMATOGRAPHIC ANALYSIS OF AFFINITY MATURED ANTIBODIES

[00299] The ten affinity matured variants from Example 15 were analyzed by hydrophobic interaction chromatography (HIC) and size exclusion chromatography (SEC) as described in Example 7. The results are shown in Table 16.1. Affinity maturation of the antibody resulted in changes to the hydrophobicity/hydrophilicity as demonstrated by the variable HIC-RT. Antibody monomer content was above 97% in all cases and did not correlate with HIC-RT.

Table 16.1: HIC and SEC Analysis of Affinity Matured Anti-FRα Antibodies

Variant	HIC-RT (min)	SEC		
		Monomer %	HMWS %	LMWS %
v30384 (parental humanized)	6.2	97	3	0
v36167	8.4	98.8	1.2	0
v36168	10.0	99.4	0.6	0
v35305	9.2	100.0	0.0	0
v35342	10.1	100.0	0.0	0

Variant	HIC-RT (min)	SEC		
		Monomer %	HMWS %	LMWS %
v35347	4.9	97.3	2.7	0
v35348	5.6	98.4	1.6	0
v35350	9.9	100.0	0.0	0
v35354	9.6	100.0	0.0	0
v35356	5.0	98.7	1.3	0
v35358	8.7	98.7	1.3	0

EXAMPLE 17: FUNCTIONAL CHARACTERIZATION OF AFFINITY MATURED ANTIBODIES – CELLULAR BINDING

[00300] The on-cell binding capabilities of a representative affinity matured variant v35356 were assessed on IGROV-1 and JEG-3 endogenous FRα-expressing cell lines by flow cytometry as described in Example 11.

Results

[00301] The results are shown in Table 17.1. Parental humanized variant v30384 and affinity matured variant v35356 yielded comparable apparent Kd and Bmax values in both IGROV-1 and JEG-3 cell lines (high and moderate endogenous FRα expression, respectively).

Table 17.1: Cellular Binding

Antibody	IGROV-1			JEG-3		
	Bmax	Curve Hill Slope (h)	Kd (nM)	Bmax	Curve Hill Slope (h)	Kd (nM)
v30384 (parental humanized)	42,611	1.442	1.38	35,942	1.598	0.50
v35356 (affinity matured)	43,842	1.537	1.31	40,110	2.119	0.44
Human IgG	208	NA	NA	144	NA	NA

EXAMPLE 18: FUNCTIONAL CHARACTERIZATION OF AFFINITY MATURED ANTIBODIES – INTERNALIZATION

[00302] The receptor-mediated internalization capabilities of the parental humanized variant, v30384, and a representative affinity matured variant, v35356, in FRα-expressing cell lines (IGROV-1 and JEG-3) were determined by flow cytometry as described below. Palivizumab (anti-RSV) (v22277) was used as a negative control.

[00303] Briefly, antibodies were fluorescently labeled by coupling to Fab-AF488 anti-Human IgG Fc labelling reagent (Jackson Immuno Research Labs, West Grove, PA; Cat. No. 109-547-008) at a 1:1 molar ratio for 24 hours at 4°C. Cells were seeded and incubated overnight at 37°C in 5% CO_2 in 48-well plates. Coupled antibodies were added to cells the following day and incubated at 37°C for 24 hours to allow for internalization. Following incubation, cells were dissociated, washed and surface AF488 fluorescence was quenched using an anti-488 antibody at 100nM incubated at 4°C for 45 min. Quenched AF488 fluorescence (internalized fluorescence) was analyzed by flow cytometry for all samples on a BD LSRFortessa™ Cell Analyzer (BD Biosciences, Franklin Lake, NJ), with 1,000 minimum events collected per well. AF488/FITC-A GeoMean in live cell population was plotted using GraphPad Prism Version 9 (GraphPad Software, San Diego, CA).

Results

[00304] The results are shown in Fig. 10. Parental humanized variant v30384 and the affinity matured variant v35356 showed comparable internalization in IGROV-1 (Fig. 10(A)) and JEG-3 (Fig. 10(B)) cells, when administered at 20 nM, at both 5 hour and 24 hour exposure.

EXAMPLE 19: PREPARATION OF ANTIBODY-DRUG CONJUGATES – CYSTEINE CONJUGATION

[00305] Antibody-drug conjugates (ADCs) comprising chimeric parental antibody v23924, humanized antibody variants or affinity matured antibody variants conjugated to maleimide containing drug-linkers were prepared at various drug-to-antibody ratios (DARs). Exemplary protocols are provided below. The drug-linkers employed are listed in Table 19.1. The ADCs prepared and the conjugation conditions employed are summarized in Table 19.2.

19.1 Conjugation of v23924 by partial reduction of interchain disulfide bonds (DAR 4)

[00306] A solution (5.14 mL) of the chimeric antibody v23924 (25 mg) was diluted to 3.57 mg/mL with a 5 mM solution of DTPA (diethylenetriamine pentaacetic acid) in PBS (pH 7.4), and to this solution was added 10 mM tris(2-carboxyethyl) phosphine (TCEP) (1.78 eq, 31 µL). Following incubation for 2 h in a 37°C water bath, drug-linker DL1 (10 eq, 87 µL) as 20 mM DMSO stock solution was added. The conjugation reaction was mixed thoroughly by pipetting and reaction was allowed to proceed on ice for up to 1 h followed by quenching of excess drug-linker with 20 mM aqueous stock of N-acetyl cysteine (9 eq, 78 µL) for 30 mins prior to purification of the ADC from small molecules. v23924 was also conjugated to drug-linkers DL2 and DL3 at DAR 4 using same conjugation procedure.

19.2 Conjugation of v23924 after complete reduction of interchain disulfide bonds (DAR 10)

[00307] A solution (3.1 mL) of the chimeric antibody v23924 (15 mg) was diluted to 3.57 mg/mL with a 5 mM solution of DTPA in PBS (pH 7.4). 10 mM TCEP (18 eq, 188 µL) was added and the reaction mixture was incubated for 3 h in a 37°C water bath before removal of excess TCEP using 10 mL 40 kD Zeba™ Spin Desalting Column (Thermo Fisher Scientific, Waltham, MA) pre-equilibrated with PBS, pH 7.4. Fully reduced antibody was conjugated to 20 mM DMSO stock of drug-linker DL1 (18 eq, 94 µL) on ice for up to 1 h followed by quenching of excess drug-linker with 20 mM aqueous stock of N-acetyl cysteine (12 eq, 63 µL) for 30 mins prior to purification of the ADC from small molecules.

19.3 Conjugation of v30384 by partial reduction of interchain disulfide bonds (DAR 4)

[00308] A solution (2.1 mL) of the humanized antibody variant v30384 (10 mg) was diluted to 3.52 mg/mL with a 5 mM solution of DTPA in PBS (pH 7.4). 1 mM TCEP (2.37 eq, 164.5µL) was added and the reaction mixture was incubated for 2 h in a 37°C water bath before conjugation to 20 mM DMSO stock of drug-linker DL1 (10 eq, 34.7 µL) on ice for up to 1 h followed by quenching of excess drug-linker with 20 mM aqueous stock of N-acetyl cysteine (9 eq, 31.2 µL) for 30 mins prior to purification of the ADC from small molecules.

19.4 Conjugation of v30384 after complete reduction of interchain disulfide bonds (DAR 8)

[00309] A solution (958.3 µL) of the humanized antibody variant v30384 (20 mg) was diluted to 5 mg/mL with a 5 mM solution of DTPA in PBS (pH 7.4). 10 mM TCEP (12 eq, 167 µL) was

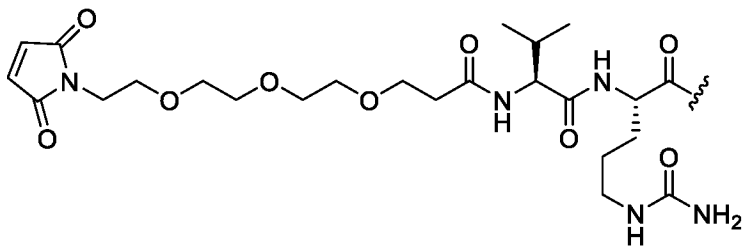
added and the reaction mixture was incubated for 3 h in a 37°C water bath before removal of excess TCEP using 10 mL 40 kD Zeba™ Spin Desalting Column (Thermo Fisher Scientific, Waltham, MA) pre-equilibrated with 10 mM Na-acetate, pH 5.5. Fully reduced antibody was conjugated to 10 mM DMSO stock of drug-linker DL5 (15 eq, 208 µL) in presence of 10% DMSO (vol/vol) for up to 2 h at RT with continuous stirring in the dark followed by quenching of excess drug-linker with 10 mM aqueous stock of N-acetyl cysteine (20 eq, 274 µL) for 30 mins prior to purification of the ADC from small molecules.

Table 19.1: Drug-Linkers used in the Preparation of Cysteine-Conjugated ADCs

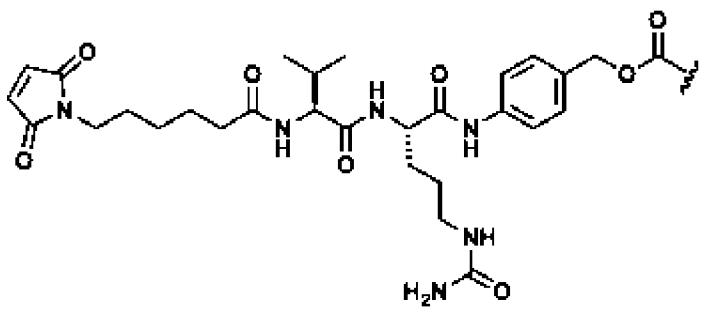
Drug-Linker	Linker[1]	Payload
DL1	MTvc	Compound **1**[1]
DL2	MTvc	Compound **2**[1]
DL3	MCvc-PABC	MMAE
DL4	MCvc-PABC	Eribulin
DL5	MC-GGFG	Dxd
DL7	MTvk	Compound **2**[1]

[1] Structures shown below.

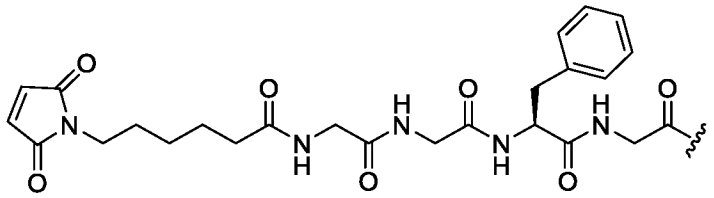
MTvc:



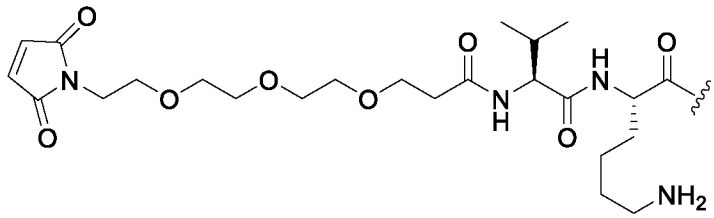
MCvcPABC:



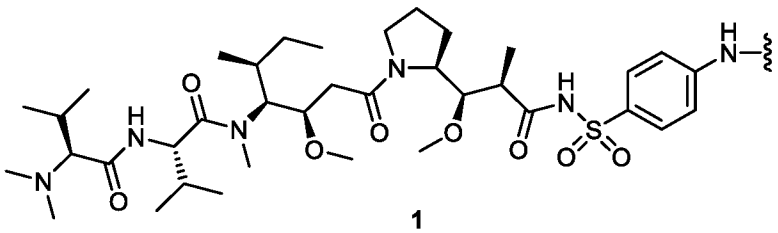
MC-GGFG:



MTvk:



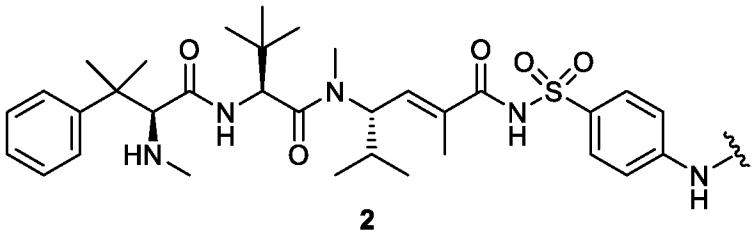
Compound **1**:



1

(see International Patent Application Publication No. WO 2016/041082)

Compound **2**:



2

(see International Patent Application Publication No. WO 2014/144871)

Table 19.2: Summary of the Preparation of Cysteine-Conjugated ADCs

Variant	Antibody Type	Fc Type[1]	Drug-Linker	Target DAR	Scale (mg)	TCEP molar excess (eq)	Antibody Conc. In Reaction (mg/mL)	Conjugation Buffer
v23924	Chimeric	HomoFc	DL1	2	0.5	0.9	3.6	PBS, pH 7.4
v23924	Chimeric	HomoFc	DL1	4	25.0	1.8	3.6	PBS, pH 7.4
v23924	Chimeric	HomoFc	DL1	10	15.0	18.0	3.6	PBS, pH 7.4
v30384	Humanized	HetFc	DL1	4	0.3	2.3	3.1	PBS, pH 7.4
v30389	Humanized	HetFc	DL1	4	0.3	2.3	3.1	PBS, pH 7.4
v30394	Humanized	HetFc	DL1	4	0.3	2.3	3.1	PBS, pH 7.4
v30399	Humanized	HetFc	DL1	4	0.3	2.3	2.5	PBS, pH 7.4
v30618	Chimeric with Cys	HetFc	DL1	4	0.3	1.8	3.1	PBS, pH 7.4
v31422	Humanized	HomoFc	DL1	4	0.3	2.3	3.5	PBS, pH 7.4
v31423	Humanized	HomoFc	DL1	4	0.3	2.3	3.5	PBS, pH 7.4
v31424	Humanized	HomoFc	DL1	4	0.3	2.3	3.5	PBS, pH 7.4
v31425	Humanized	HomoFc	DL1	4	0.3	2.3	3.5	PBS, pH 7.4
v31426	Humanized	HomoFc	DL1	4	0.3	2.3	3.5	PBS, pH 7.4
v30384	Humanized	HomoFc	DL1	4	10.0	2.4	3.5	PBS, pH 7.4
v30399	Humanized	HomoFc	DL1	4	10.0	2.4	3.5	PBS, pH 7.4
v31423	Humanized	HomoFc	DL1	4	10.0	2.3	3.3	PBS, pH 7.4
v31424	Humanized	HomoFc	DL1	4	9.1	2.3	3.3	PBS, pH 7.4
v36167	Affinity matured	HomoFc	DL1	4	0.15	2.3	2.0	PBS, pH 7.4
v36168	Affinity matured	HomoFc	DL1	4	0.15	2.5	2.0	PBS, pH 7.4
v35305	Affinity matured	HomoFc	DL1	4	0.22	2.1	2.2	PBS, pH 7.4
v35342	Affinity matured	HomoFc	DL1	4	0.22	2.1	2.2	PBS, pH 7.4
v35347	Affinity matured	HomoFc	DL1	4	0.22	2.1	2.2	PBS, pH 7.4
v35348	Affinity matured	HomoFc	DL1	4	0.22	2.1	2.2	PBS, pH 7.4

Variant	Antibody Type	Fc Type[1]	Drug-Linker	Target DAR	Scale (mg)	TCEP molar excess (eq)	Antibody Conc. In Reaction (mg/mL)	Conjugation Buffer
v35350	Affinity matured	HomoFc	DL1	4	0.22	2.1	2.2	PBS, pH 7.4
v35354	Affinity matured	HomoFc	DL1	4	0.22	2.1	2.2	PBS, pH 7.4
v35356	Affinity matured	HomoFc	DL1	4	0.22	2.1	2.2	PBS, pH 7.4
v35358	Affinity matured	HomoFc	DL1	4	0.22	2.1	2.2	PBS, pH 7.4
v23924	Chimeric	HomoFc	DL2	10	0.7	12.0	3.5	PBS, pH 7.4
v23924	Chimeric	HomoFc	DL3	4	0.5	12.0	3.3	PBS, pH 7.4
v30384	Humanized	HetFc	DL3	4	1.0	2.8	3.0	PBS, pH 7.4
v30384	Humanized	HetFc	DL4	4	1.0	2.4	3.2	PBS, pH 7.4 with 50% Propylene Glycol (v/v)
v30384	Humanized	HetFc	DL5	8	20.0	12.0	4.1	10 mM Na-Acetate, pH 5.5 with 10 % DMSO (v/v)
v36167	Affinity matured	HomoFc	DL5	8	1.8	10	2.5	10 mM Na-Acetate, pH 5.5 with 10 % DMSO (v/v)
v36168	Affinity matured	HomoFc	DL5	8	1.8	10	2.5	10 mM Na-Acetate, pH 5.5 with 10 % DMSO (v/v)
v35347	Affinity matured	HomoFc	DL5	8	1.8	10	3.0	10 mM Na-Acetate, pH 5.5 with 10 % DMSO (v/v)
v35348	Affinity matured	HomoFc	DL5	8	1.8	10	3.0	10 mM Na-Acetate, pH 5.5 with 10 % DMSO (v/v)
v35356	Affinity matured	HomoFc	DL5	8	1.8	10	3.0	10 mM Na-Acetate, pH 5.5 with 10 % DMSO (v/v)

Variant	Antibody Type	Fc Type[1]	Drug-Linker	Target DAR	Scale (mg)	TCEP molar excess (eq)	Antibody Conc. In Reaction (mg/mL)	Conjugation Buffer
v35358	Affinity matured	HomoFc	DL5	8	1.8	10	3.0	10 mM Na-Acetate, pH 5.5 with 10 % DMSO (v/v)
v36675[2]	Humanized	HomoFc	DL5	8	1500	12	5	10 mM Na-Acetate, pH 5.5 with 7 % DMSO (v/v)
v23924	Chimeric	HomoFc	DL7	10	0.7	12.0	3.5	PBS, pH 7.4
v23924	Chimeric	HomoFc	DL7	4	20.0	1.8	3.6	PBS, pH 7.4

[1] See Example 2

[2] Variant v36675 is the same as v30384 but comprises HomoFc rather than HetFc

EXAMPLE 20: PREPARATION OF ANTIBODY-DRUG CONJUGATES – LYSINE CONJUGATION

[00310] Antibody-drug conjugates (ADCs) comprising the humanized antibody variant v30384 conjugated to drug-linker DL6 or DL8 (see Table 20.1) at a target DAR of 3.3 or 4, respectively, were prepared as described below and summarized in Table 20.2.

[00311] ***DL6:*** *A* solution (207 µL) of the humanized antibody v30384 (1 mg) was reacted with 10 mM DMSO stock of drug-linker DL6 (14 eq, 9.7 µL) in PBS, pH 7.4. The conjugation reaction was mixed thoroughly by pipetting and the reaction allowed to proceed for up to 17 h at room temperature.

[00312] ***DL8:*** A solution (5.9 mL) of the humanized antibody v30384 (30 mg) was reacted with 20 mM DMSO stock of drug-linker DL8 (9.5 eq, 99 µL) in PBS, pH 7.4. The conjugation reaction was mixed thoroughly by pipetting and the reaction allowed to proceed for up to 18 hrs at room temperature.

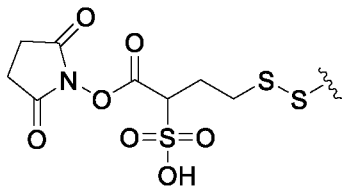
Table 20.2: Drug-Linkers used in the Preparation of Lysine-Conjugated ADCs

Drug-Linker	Linker[1]	Payload
DL6	sSPDB	DM4
DL8	NHS-ADvc	Compound 1[2]

[1] Structures shown below.

[2] See footnote to Table 19.1

sSPDB:



NHS-ADvc:

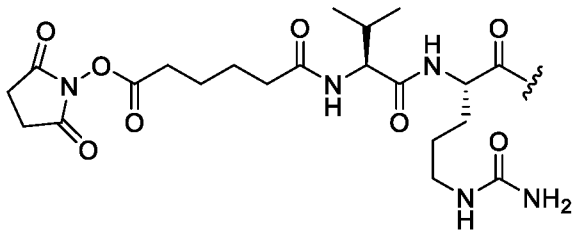


Table 20.2: Summary of the Preparation of Lysine-Conjugated ADCs

Variant	Antibody Type	Fc Type*	Drug-Linker	Target DAR	Scale (mg)	TCEP molar excess (eq)	Antibody Conc. In Reaction (mg/mL)	Conjugation Buffer
v30384	Humanized	HetFc	DL6	3.3	1.0	14.0	4.8	PBS, pH 7.4
v30384	Humanized	HetFc	DL8	4	30	9.5	5.1	PBS, pH 7.4

* See Example 2

EXAMPLE 21: PURIFICATION AND CHARACTERIZATION OF ADCs

[00313] ADCs prepared as described in Examples 19 and 20 were purified using an appropriate size 40 kD Zeba™ Spin Desalting Column (Thermo Fisher Scientific, Waltham, MA) pre-

equilibrated with PBS, pH 7.4, or 10 mM Na-Acetate, pH 5.5. ADCs produced at >1mg scale, were sterile filtered (0.22 μm).

[00314] The purified ADCs were stored at 4°C and analyzed for total protein content using absorbance at 280 nm or using a bicinchonic acid (BCA) assay with reference to a standard curve generated from trastuzumab 1 mg/mL. ADCs were also characterized by HPLC-HIC, SEC, CE-SDS and RP-HPLC-MS as described below. The average DAR and DAR distribution for the ADCs were derived from HIC and LC-MS data. Endotoxin levels were assessed using the ToxinSensor™ Single Test Kit (Genescript BioTech, Piscataway, NJ; Cat# L00450) with a threshold set at 0.5 EU/mg. Residual free drug and drug-linker levels (%FD) were assessed by RP-HPLC-MS and calculated based on the following equation with a threshold set at 1 mol%DAR:

$$\%FD = \frac{[Free\ drug] + [Free\ drug - linker] + [Drug - linker\ TCEP\ adduct]}{[ADC] \times DAR}$$

[00315] The biophysical properties determined for the ADCs are summarized in Table 21.4.

21.1 DAR Determination by HIC for Cysteine-Conjugated ADCs

[00316] The average DAR by HIC was assessed as described in *Antibody Drug Conjugates, Methods in Molecular Biology*, 2013, vol. 1045, pp. 275-284. L. Ducry, Ed. The experiments were performed on an Agilent Infinity II 1290 HPLC (Agilent Technologies, Santa Clara, CA) using a TSKgel® Butyl-NPR column (2.5μm, 4.6 x 35mm; TOSOH Bioscience GmbH, Griesheim, Germany) pre-equilibrated with 5 column volumes of Buffer A (1.5 M $(NH_4)_2SO_4$, 25 mM NaH_2PO_4, pH = 6.95) and at room temperature. Typically, 20-30 μg of sample at 2-3 mg/mL concentration was loaded on the column with 95% Buffer A and 5% Buffer B (75% 25 mM NaH_2PO_4 plus 25% isopropanol, pH 6.95) and run for 15 mins at 0.5 mL/min using the gradient shown in Table 21.1. HIC chromatograms were integrated using appropriate parameters that provided complete, baseline-to-baseline integration of each peak, followed by integration of each peak showing reasonable separation. As a reference, unconjugated naked antibodies were run on the same gradient to obtain the HIC retention time of DAR = 0 species.

Table 21.1: HIC Gradient

Time (min)	% Buffer A	% Buffer B
0	95	5
0.1	95	5
5	80	20
9.5	65	35
11.5	50	50
12.5	5	95
13.5	5	95
12.6	95	5
15	95	5

21.2 DAR Determination by RP-HPLC-MS for Lysine-Conjugated ADCs

[00317] ADC samples were deglycosylated using Endo S for 1 hour at RT and injected onto an Agilent 1290 Infinity II LC coupled with an Agilent 6545 Quadrupole Time of Flight (Q-TOF) mass spectrometer (Agilent Technologies, Santa Clara, CA). Protein species were separated using a PLRP-S column (1000 Å, 8 uM, 50 × 2.1 mm) at a flow rate of 0.3 ml/min using the gradient shown in Table 21.2. Buffer A: 0.1% formic acid (FA), 0.025% trifluoracetic acid (TFA) and 10% isopropyl alcohol (IPA) in water. Buffer B: 0.1% FA and 10% IPA in acetonitrile (ACN).

Table 21.2: RP-HPLC-MS Gradient

Time (min)	% Buffer A	% Buffer B
0	80	20
20	60	40
22	10	90
22.5	1	99
24	1	99

[00318] The MS source conditions are shown in Table 21.3 and the acquisition parameters were as follows:

[00319] *Mode*: MS; *Mass Range*: 500 to 7000 m/z; *Acquisition Rate*: 1 spectra/s and 1000 ms/spectrum, 3354 transients/spectrum.

Table 21.3: MS Source Conditions

Gas Temp: 300°C	VCap: 5000V
Drying Gas: 13 l/min	Nozzle Voltage: 2000V
Nebulizer: 45 psig	Fragmentor: 170V
Sheath Gas Temp: 400°C	Skimmer: 65V
Sheath Gas Flow: 12 l/min	Oct RF Vpp: 750V

[00320] Qualitive analysis using MassHunter software (Agilent Technologies, Santa Clara, CA) was employed for deconvolution and data analysis. Deconvolution parameters were as follows:

[00321] *Deconvolution Algorithm:* Maximum Entropy; *Mass Range:* 70000-160000; *Mass Step:* 1.0; *Used limited m/z range:* 1000-7000; *Subtract baseline:* 7.0; *Adduct:* Proton; *Isotope width:* Automatic; *Height Filter:* Peak signal to noise >=30.0; *Maximum number of peaks:* Limited by height 100.

[00322] Average DAR was the calculated from the deconvoluted spectrum using the following equation:

$$\textbf{average DAR} = \sum_{i=0}^{n} \frac{Drug\ load_i \cdot peak\ intensity_i}{Total\ peak\ intensity}$$

21.3 SEC-HPLC Analysis of ADCs

[00323] Analytical SEC was performed using an Agilent Infinity II 1260 HPLC (Agilent Technologies, Santa Clara, CA) with Advance Bio SEC column (300 Å, 2.7 µm, 7.8 × 150 mm; Agilent Technologies) equilibrated with 5 column volumes of Buffer (150 mM Na_2PO_4, pH 6.95) at room temperature. Typically, 20-30 µg of sample at 2-3 mg/mL concentration was eluted isostatically for 7 mins at 1 mL/min and absorbance monitored at A280. Chromatograms were integrated to provide complete, baseline-to-baseline integration of each peak, with reasonably placed separation between partially resolved peaks. The peak corresponding to the major

component for IgG (approximate retention time 3.3 min) was reported as the monomer based on the SEC profile of unmodified chimeric antibody v23924. Any peak occurring prior to 3.3 min was designated as high molecular weight species (HMWS), and any peak occurring after 3.3 min was designated as low molecular weight species (LMWS), excluding solvent peaks (over 5.2 min).

21.4 CE-SDS Analysis of ADCs

[00324] Initially, all samples were diluted to 1 mg/mL before preparing the samples in a 96-well PCR plate following manufacturer's protocol (Protein Express Assay LabChip™; PerkinElmer, Inc., Waltham, MA). Briefly, 2 µg of ADC was mixed with 7 uL Protein Express buffer in the presence (reducing) or absence (non-reducing) of 400 mM dithiothreitol (DTT), followed by heat denaturation at 95°C for 5 minutes. Samples were then diluted in dH_2O at a 1:2 ratio before data acquisition. After each CE-SDS run, the gel and corresponding electropherogram were analyzed using LabChip™ Reviewer (PerkinElmer, Inc., Waltham, MA).

Table 21.4: Biophysical Properties of ADCs

ADC[1]	Target DAR	Reported DAR		Monomer % (HPLC-SEC)	%FD (mol%/DAR)	Endotoxin (EU/mg)
		HPLC-HIC	LC-MS			
v23924-DL1	2	2.1	1.9	100	ND[2]	ND
v23924-DL1	4	4.0	4.0	99	0.4	<0.15
v23924-DL1	10	9.4	9.7	99	0.4	<0.23
v30384-DL1[3]	4	3.8	4.0	98	ND	ND
v30384-DL1[4]	4	3.8	4.1	97	<0.1	<0.23
v30389-DL1	4	3.8	3.9	93	ND	ND
v30394-DL1	4	3.9	3.7	96	ND	ND
v30399-DL1[3]	4	3.9	4.1	97	ND	ND
v30399-DL1[4]	4	3.8	4.1	98	<0.1	<0.21
v30618-DL1	4	4.2	4.2	97	ND	ND
v31422-DL1	4	3.9	4.2	99	ND	ND
v31423-DL1[3]	4	4.1	4.6	99	ND	ND
v31423-DL1[4]	4	3.9	4.3	99	<0.1	<0.42
v31424-DL1[3]	4	4.0	4.3	100	ND	ND

ADC[1]	Target DAR	Reported DAR		Monomer % (HPLC-SEC)	%FD (mol%/DAR)	Endotoxin (EU/mg)
		HPLC-HIC	LC-MS			
v31424-DL1[4]	4	3.8	4.4	100	<0.1	<0.5
v31425-DL1	4	3.9	4.3	100	ND	ND
v31426-DL1	4	3.9	4.4	99	ND	ND
v36167-DL1	4	4.0	ND	100	ND	ND
v36168-DL1	4	4.0	ND	100	ND	ND
v35305-DL1	4	4.0	ND	100	ND	ND
v35342-DL1	4	4.2	ND	100	ND	ND
v35347-DL1	4	3.5	ND	98	ND	ND
v35348-DL1	4	3.3	ND	99	ND	ND
v35350-DL1	4	3.3	ND	100	ND	ND
v35354-DL1	4	4.2	ND	100	ND	ND
v35356-DL1	4	3.7	ND	100	ND	ND
v35358-DL1	4	3.8	ND	99	ND	ND
v23924-DL2	10	9.5	9.7	98	ND	ND
v23924-DL3	4	3.5	2.9	100	ND	ND
v30384-DL3	4	4.0	ND	91	ND	ND
v30384-DL4	4	3.8	ND	93	ND	ND
v30384-DL5	8	ND	8	96	<1.0	<0.25
v36167-DL5	8	8.0	ND	100	ND	ND
v36168-DL5	8	8.0	ND	100	ND	ND
v35347-DL5	8	8.0	ND	100	ND	ND
v35348-DL5	8	8.0	ND	100	ND	ND
v35356-DL5	8	8.0	ND	100	ND	ND
v35358-DL5	8	8.0	ND	100	ND	ND
v36675-DL5	8	8.0	8.0	99	0.8	<0.02
v30384-DL6	3.3	ND	3.3	100	ND	ND
v23924-DL7	10	9.5	9.8	100	ND	ND
v23924-DL7	4	4.0	3.6	100	<0.1	<0.19
v30384-DL8	4.0	ND	3.9	97	0.4	<0.25

[1] See Examples 19 & 20 for details of drug-linkers (DL)

[2] ND = not determined

[3] Small scale preparation, %FD and endotoxin not determined

[4] Large scale preparation

EXAMPLE 22: *IN VITRO* CYTOTOXICITY OF ADCs

[00325] The cell growth inhibition (cytotoxicity) capabilities of ADCs comprising the humanized variant v30384 or the affinity-matured variant v35356 each conjugated to drug-linker DL1 (see Example 19) were determined in a panel of FRα-expressing cell lines as described below.

[00326] Briefly, cells were seeded in 384-well plates at 1,000 cells/well density and treated with a titration of test article, generated in complete cell growth medium. Treated cells were incubated for 4 days under standard culturing conditions (37°C/5% CO_2). After incubation, CellTiter-Glo® reagent (Promega Corporation, Madison, WI; Cat. No. G7570) was spiked in all wells and luminescence corresponding to ATP present in each well was measured using a Synergy™ H1 plate reader (BioTek Instruments, Winooski, VT). Based on blank wells (no test article added, only blank media), % cytotoxicity values were calculated using ATP measurement RLU values (Relative Light Units) and plotted against test article concentration using GraphPad Prism 9 software (GraphPad Software, San Diego, CA).

Results

[00327] The results are shown in Table 22.1 and Fig. 19. Both the ADC comprising the humanized variant v30384 and the ADC comprising the affinity-matured variant v35356 showed comparable *in vitro* cytotoxicity in the FRα-expressing cells KB-Hela, IGROV-1, JEG-3 and SKOV-3. Neither of the ADCs showed any non-specific cytotoxicity in the FRα-negative MDA-MB-468 cell line.

Table 22.1: *In vitro* Cytotoxicity

ADC	DAR	EC50 (nM) DAR-corrected				
		KB-HeLa	IGROV-1	JEG-3	SKOV-3	MDA-MB-468
v30384-DL1	3.9	0.0528	0.169	0.102	0.1767	>15
v35356-DL1	3.7	0.0307	0.0395	0.0409	0.0545	>15

EXAMPLE 23: INTRACELLULAR PAYLOAD RELEASE AND QUANTITATION

[00328] The intracellular payload delivery capabilities of representative ADCs in the cell-lines JEG-3 (high FRα), Caov-3 (moderate FRα), HEC-1-A (moderate FRα), and H2110 (moderate/low FRα) were assessed using mass spectrometry as described below. The ADCs tested comprised the humanized variant v30384 or the affinity-matured variant v35356 each conjugated to drug-linker DL1 (see Example 19).

[00329] Briefly, cells were seeded in 12-well plates at 80,000 cells/well and treated with the ADCs at various concentrations for 24 hours under standard culturing conditions. After incubation, cells were washed using PBS pH 7.4, collected, counted and frozen down at -80°C. After thawing, cells were lysed using pure acetonitrile. Cell lysate supernatant was injected into an Agilent 1290 Infinity II LC coupled with an Agilent 6470 Triple Quadrupole (QQQ) mass spectrometer (Agilent Technologies, Santa Clara, CA) and free payload (Compound 1) in cell lysate samples was quantified using free payload standards of known concentration.

Results

[00330] The results are shown in Fig. 11. In the high FRα-expressing cell line JEG-3, both ADCs showed comparable intracellular payload (Compound 1) delivery. In the lower FRα-expressing cell lines Caov-3, H2110 and HEC-1-A, the ADC comprising the affinity matured variant v35356 showed higher payload delivery at 24 hours compared to the ADC comprising the parental humanized variant v30384.

EXAMPLE 24: *IN VIVO* EFFICACY STUDIES – CHIMERIC ANTI-FRα ANTIBODY ADCs

[00331] *In vivo* anti-tumor activities of the parental chimeric antibody v23924 conjugated to drug-linker DL1 or DL7 were assessed in a number of xenograft models expressing varying levels of FRα as described below. Activities of control ADCs comprising a known FRα-targeted antibody, mirvetuximab, were assessed for comparison. The control ADCs were v17717 (mirvetuximab Fab with HetFc) conjugated to drug-linker DL1 and v17717 conjugated to drug-linker DL6. For statistical analyses, a linear mixed effects model was fit to log-transformed tumor volumes, followed by F-test for the null hypothesis that mean growth rates are equal and post-hoc pairwise comparisons.

[00332] The ADCs, xenograft models, dosages and study durations employed in each xenograft study are summarized in Table 24.1. For each xenograft study, tumor volume and body weight of the animals were measured twice weekly.

Table 24.1: Study Parameters

Xenograft Model	Cancer Type	FRα Expression	ADC	Dose level(s) mg/kg	Study Duration
CTG0848	Non-small cell lung	Mid/High	v23924-DL1	5, 10	28 days
OV90	Ovarian	Mid	v23924-DL1	9, 18	60 days
			v17717-DL6	9	
OVCAR3	Ovarian	Mid/High	v23924-DL1	9	60 days
			v17717-DL6	9	
LXFA737	Lung	Mid	v23924-DL1	5	45 days
			v17717-DL6	5	
			v17717-DL1	5	
JEG3	Choriocarcinoma	Not determined	v23924-DL1	3	25 days
			v17717-DL6	3	
			v17717-DL1	3	
HCC1954	Breast	Low	v23924-DL1	10	49 days
			v17717-DL6	10	
			v17717-DL1	10	
SKOV3	Ovarian	Low	v23924-DL1	10	40 days
			v17716-DL6	10	
			v17717-DL1	10	
KB	Endocervical	Mid	v23924 DL1	5, 10	28 days
			v23924-DL7	5, 10	
			v17717-DL6	5, 10	

[00333] For the CTG-0848 PDX model, tumor fragments were implanted subcutaneously to female nude mice. When mean tumor volume reached ~100-250 mm^3, the animals were matched

by tumor size and assigned into treatment groups (n=5 per group) and treated on Day 0 with a single IV dose of ADC as shown in Table 24.1.

[00334] For the OV90 model, tumor cell suspensions (1 x10^7 cells in 0.1 ml 50% Matrigel®) were implanted subcutaneously into female CB.17 SCID mice. When mean tumor volume reached 100-150 mm^3, the animals were assigned to groups (n=9 per group) and treated on study day 1 with a single IV dose of ADC as shown in Table 24.1.

[00335] For the OVCAR3 model, tumor fragments (~1 mm^3) were implanted subcutaneously into female CB.17 SCID mice. When mean tumor volume reached ~100-150 mm^3, the animals were assigned to groups (n=8 per group) and treated on study day 1 with a single IV dose of ADC as shown in Table 24.1.

[00336] For the LXFA737 PDX model, tumor fragments (3-4 mm edge length) were implanted subcutaneously into female nude mice. When mean tumor volume reached ~80-200 mm^3, the animals were assigned to groups (n=6 per group) and treated on study day 0 with a single IV dose of ADC as shown in Table 24.1.

[00337] For the JEG3 CDX model, tumor cell suspensions (1 x10^7 cells in 0.1 ml 50% Matrigel®) were implanted subcutaneously into NOD/SCID mice. When mean tumor volume reached ~100 mm^3, the animals were assigned to groups (n=5 per group) and treated with a single IV dose of ADC on study day 0 as shown in Table 24.1.

[00338] For the HCC1954 CDX model, tumor cell suspensions (5 x10^6 cells in 0.1 ml 50% Matrigel®) were implanted subcutaneously into nude mice. When mean tumor volume reached ~150 mm^3, the animals were assigned to groups (n=5 per group) and treated on study day 0 with a single IV dose of ADC as shown in Table 24.1.

[00339] For the SKOV3 CDX model, tumor cell suspensions (1 x10^7 cells in 0.1 ml 50% Matrigel®) were implanted subcutaneously into nude mice. When mean tumor volume reached ~170 mm^3, the animals were assigned to groups (n=5 per group) and treated with a single IV dose of ADC on study day 0 as shown in Table 24.1.

[00340] For the KB CDX model, tumor cell suspensions (3 x10^6 cells in 0.1 ml PBS) were implanted subcutaneously into nude mice. When mean tumor volume reached ~100-150 mm^3, the animals were assigned to groups (n=5 per group) and treated with a single IV dose of ADC on study day 1 as shown in Table 24.1.

[00341] For all models except the HCC1954 and KB CDX models, serum was collected at a number of timepoints for pharmacokinetic analysis as described in Example 28.

Results

[00342] The results are shown in Fig. 12.

[00343] In the CTG-0848 PDX model, when dosed at 5 and 10 mg/kg, v23924-DL1 resulted in a sustained regression of tumor growth (Fig. 12A). In the OV90 model, when dosed at 9 and 18 mg/kg, v23924-DL1 resulted in a moderate inhibition of tumor growth rate (Fig. 12B), but this was not statistically significantly different from vehicle control (mixed effects model). In the OVCAR3 model, when dosed at 9 mg/kg, both v23924-DL1 and v17717-DL6 resulted in statistically significant inhibition of the tumor growth rate ($p<0.001$ mixed effects model). v23924-DL1 resulted in a sustained tumor regression, with a significantly greater inhibition of tumor growth rate than v17717-DL6 ($p<0.001$) (Fig. 12C).

[00344] In the LXFA737 PDX model, when dosed at 5 mg/kg, v23924-DL1, v17717-DL6 and v17717-DL1 resulted in tumor growth rate inhibitions of 198%, 144% and 165%, respectively (each tumor growth rate significantly different from control, $p<0.001$) (Fig. 12D). In the JEG3 CDX model, when dosed at 3 mg/kg, v23924-DL1, v17717-DL6 and v17717-DL1 resulted in transient tumor regressions, with tumor growth rate inhibitions of 132%, 148% and 139%, respectively (each tumor growth rate significantly different from control, $p<0.001$) (Fig. 12E). In the HCC1954 CDX model, when dosed at 10 mg/kg, v23924-DL1, v17717-DL6 and v17717-DL1 resulted in transient tumor regressions, with tumor growth rate inhibitions of 287%, 278% and 242%, respectively (each tumor growth rate significantly different from control, $p<0.01$) (Fig. 12F).

[00345] In the SKOV3 CDX model, when dosed at 10 mg/kg, v23924-DL1, v17716-DL6 and v17717-DL1 resulted in transient tumor regressions, with tumor growth rate inhibitions of 287%,

243% and 278%, respectively (each tumor growth rate significantly different from control, $p<0.01$) (Fig. 12G). In the KB CDX model, when dosed at 5 mg/kg, v23924-DL1, v23924-DL7 and v17717-DL6 resulted in tumor growth rate inhibitions of 289%, 171% and -1%, respectively, with both v23924 ADCs significantly superior to the vehicle control and v17717-DL6 ($p<0.01$). When dosed at 10 mg/kg, all ADCs resulted in sustained tumor regressions in this model (Fig. 12H).

EXAMPLE 25: *IN VIVO* EFFICACY STUDIES – CHIMERIC AND HUMANIZED ANTI-FRα ANTIBODY ADCs

[00346] *In vivo* anti-tumor activities of the chimeric antibody v23924 and the humanized variants v30384 and v30399 (see Example 3) conjugated to the drug-linker DL1 (see Example 19) were assessed in the mid/high-level FRα expressing OVCAR3 ovarian cancer model as described below. For statistical analyses, a linear mixed effects model was fit to log-transformed tumor volumes, followed by F-test for the null hypothesis that mean growth rates are equal and post-hoc pairwise comparisons.

[00347] Tumor fragments (~1 mm^3) were implanted subcutaneously into female CB.17 SCID mice. When mean tumor volume reached ~100-150 mm^3, the animals were assigned to groups (n=8 per group) and treated with a single IV dose of either 4 mg/kg or 9 mg/kg ADC on study day 1. Tumor volume and body weight were measured twice weekly with a study duration of 60 days.

Results

[00348] The results are shown in Fig. 13. When dosed at 4 mg/kg, v23924-DL1 and humanized v30384-DL1 and v30399-DL1 resulted in similar tumor regressions followed by regrowth. When dosed at 9 mg/kg, all ADCs resulted in sustained tumor regressions.

EXAMPLE 26: *IN VIVO* EFFICACY STUDIES - HUMANIZED ANTI-FRα ANTIBODY ADCs #1

[00349] *In vivo* anti-tumor activities of the humanized variant v30384 (see Example 3) conjugated to the drug-linker DL1 or DL8 (see Examples 19 and 20) were assessed in a number of xenograft models expressing varying levels of FRα as described below. Activities of control ADCs comprising a known FRα-targeted antibody, mirvetuximab, were assessed in select models for comparison. The control ADCs were v17717 (mirvetuximab Fab with HetFc) conjugated to drug-

linker DL6 (v17717-DL6) and v17716 (mirvetuximab Fab with HomoFc) conjugated to drug-linker DL6 (v17716-DL6). In the H2110 CDX model, v31629 (farletuzumab) conjugated to drug-linker DL4 (v31629-DL4) was also included as a control. For statistical analyses, a linear mixed effects model was fit to log-transformed tumor volumes, followed by F-test for the null hypothesis that mean growth rates are equal and post-hoc pairwise comparisons.

[00350] The ADCs, xenograft models, dosages and study durations employed in each xenograft study are summarized in Table 26.1. For each xenograft study, tumor volume and body weight of the animals were measured twice weekly.

Table 26.1: Study Parameters

Xenograft Model	Cancer Type	FRα Expression	ADC	Dose level(s) mg/kg	Study Duration
H2110	Lung	Mid/Low	v30384-DL1	1.25, 2.5, 5	28 days
			v17716-DL6	1.24, 2.5, 5	
			v31629-DL4	2.5	
SKOV3	Ovarian	Low	v30384-DL1	8	42 days
				4 and 8, q10d x4	
			v17717-DL6	8	
				4 and 8, q15d x3	
IGROV-1	Ovarian	High	v30384-DL1	4 and 8, q10d x4	60 days
			v17716-DL6	4 and 8, q15d x3	
LXFA737	Lung	Mid	v30384-DL1	4, 8	45 days
			v17717-DL6	4, 8	

[00351] For the H2110 CDX model, tumor cell suspensions (1 x10^7 cells in 0.1 ml 50% Matrigel®) were implanted subcutaneously into CB.17 SCID mice. When mean tumor volume reached ~155 mm^3, the animals were assigned to groups (n=8 per group) and treated with a single IV dose of ADC on study day 0 as shown in Table 26.1. Serum was collected at a number of timepoints for pharmacokinetic analysis as described in Example 28.

[00352] For the SKOV3 CDX model, tumor cell suspensions (1 x10^7 cells in 0.1 ml 50% Matrigel®) were implanted subcutaneously into nude mice. When mean tumor volume reached ~175 mm^3, the animals were assigned to groups (n=9 per group for repeat dose groups, n=7 for single dose groups) and treated with ADC starting on study day 0 according to dose levels and schedules as shown in Table 26.1. Serum was collected at a number of timepoints for pharmacokinetic analysis as described in Example 28.

[00353] For the IGROV-1 model, tumor cell suspensions (1 x10^7 cells in 0.1 ml 50% Matrigel®) were implanted subcutaneously into female nude mice. When mean tumor volume reached ~100-150 mm^3, the animals were assigned to groups (n=12 per group) and treated with ADC according to dose levels and schedules as shown in Table 26.1.

[00354] For the LXFA737 CDX model, tumor fragments (3-4 mm edge length) were implanted subcutaneously into nude mice. When mean tumor volume reached ~100 mm^3, the animals were assigned to groups (n=10 per group) and treated with a single IV dose of ADC on study day 0 as shown in Table 26.1.

Results

[00355] The results are shown in Fig. 14.

[00356] In the H2110 CDX model, when dosed at 1.25, 2.5 and 5 mg/kg, v30384-DL1 and v17716-DL6 both resulted in minor to moderate tumor growth inhibition across shallow dose responses, but these were not statistically significant (Fig. 14A). v31629-DL4 dosed at 2.5 mg/kg resulted in a minor inhibition of tumor growth rate comparable to v17716-DL6 and v30384-DL1.

[00357] In the SKOV3 CDX model, single administration of 8 mg/kg of v30384-DL1, v30384-DL8 and v17716-DL6 resulted in significant inhibitions of tumor growth rates of 203%, 178% and 232% ($p<0.01$), respectively, with a moderate but statistically significant greater tumor growth rate inhibition by v17716-DL6 ($p<0.01$) (Fig. 14B). Repeat dosing (v30384-DL1 every 10 days for a total of 4 doses, v17716-DL6 every 15 days for a total of 3 doses) had minor effects on tumor growth compared to single dose administration (Fig. 14C).

[00358] In the IGROV-1 model, when dosed at 8 mg/kg, both v30384-DL1 (every 10 days for a total of 4 doses) and v17716-DL6 (every 15 days for a total of 3 doses) resulted in significant

inhibition of tumor growth compared to control ($p<0.001$). When dosed at 4 mg/kg, only v30384-DL1 (every 10 days for a total of 4 doses) resulted in significant inhibition of tumor growth compared to control ($p<0.001$). v30384-DL1 resulted in significantly greater tumor growth rate inhibition than v17716-DL6 at both the 4 and 8 mg/kg dose levels ($p<0.001$ and $p=0.014$, respectively) (Fig. 14D).

[00359] In the LXFA737 CDX model, when dosed at 8 mg/kg, v30384-DL8 and v17716-DL6 resulted in a significant inhibition of tumor growth rate of 154% and 313%, respectively ($p<0.01$). v17716-DL6 resulted in significantly greater tumor growth rate inhibition compared to v30384-DL8 at both 4 mg/kg (132% and 21%) and 8 mg/kg (313% and 154%) dose levels ($p<0.01$) (Fig. 14E).

EXAMPLE 27: *IN VIVO* EFFICACY STUDIES - HUMANIZED ANTI-FRα ANTIBODY ADCs #2

[00360] *In vivo* anti-tumor activities of the humanized variant v30384 (see Example 3) conjugated to the drug-linker DL5 (see Example 19) were assessed in a number of xenograft models expressing varying levels of FRα as described below. For statistical analyses, a linear mixed effects model was fit to log-transformed tumor volumes, followed by F-test for the null hypothesis that mean growth rates are equal and post-hoc pairwise comparisons.

[00361] The ADCs, xenograft models, dosages and study durations employed in each xenograft study are summarized in Table 27.1. For each xenograft study, tumor volume and body weight of the animals were measured twice weekly.

Table 27.1: Study Parameters

Xenograft Model	Cancer Type	FRα Expression	Dose level(s) mg/kg	Study Duration
OV90	Ovarian	Mid	1, 3, 10	61 days
H2110	Lung	Mid/Low	0.3, 1, 3, 10	28 days
OVCAR3	Ovarian	Mid/High	0.25, 0.75, 1.5, 3	61 days
OVCAR3	Ovarian	Mid/High	6	61 days

[00362] For the OV90 CDX model, tumor cell suspensions (1 x10^7 cells in 0.1 ml 50% Matrigel®) were implanted subcutaneously into CB.17 SCID mice. When mean tumor volume reached ~100-150 mm^3, the animals were assigned to groups (n=6 per group) and treated with a single IV dose of ADC on study day 1 as shown in Table 27.1.

[00363] For the H2110 CDX model, tumor cell suspensions (1 x10^7 cells in 0.1 ml 50% Matrigel®) were implanted subcutaneously into CB.17 SCID mice. When mean tumor volume reached ~150 mm^3, the animals were assigned to groups (n=5 per group) and treated with a single IV dose of ADC as shown in Table 27.1.

[00364] For the OVCAR3 CDX model, tumor fragments (~1 mm^3) were implanted subcutaneously into CB.17 SCID mice. When mean tumor volume reached ~100-150 mm^3, the animals were assigned to groups (n=5 per group) and treated with a single IV dose of ADC on study day 1 as shown in Table 27.1.

[00365] For each model, serum was collected at a number of timepoints for pharmacokinetic analysis as described in Example 28.

Results

[00366] The results are shown in Fig. 15. In all three models, v30384-DL5 resulted in a dose response across the dosages tested.

[00367] In the OV90 CDX model, v30384-DL5 resulted in a dose response across 1, 3 and 10 mg/kg (25%, 47% and 185% inhibition of tumor growth rate, respectively, significant ($p<0.01$) at 3 and 10 mg/kg dose levels) (Fig. 15A). In the H2110 CDX model, v30384-DL5 resulted in a dose response across 0.3, 1, 3 and 10 mg/kg dose levels (Fig. 15B). In the OVCAR3 CDX model, v30384-DL5 resulted in a dose response across 0.25, 0.75, 1.5 and 3 mg/kg dose levels, with the higher dose groups leading to sustained regressions with regrowth at ~5 weeks post dose (Fig. 15C). In an additional study in the OVCAR3 CDX model, v30384-DL5 was dosed at 6 mg/kg, leading to lasting regressions (Fig. 15D).

EXAMPLE 28: PHARMACOKINETICS STUDIES

[00368] From a subset of the xenograft studies described in Examples 24, 26 and 27, serum was collected as noted and analyzed for the pharmacokinetics (PK) of the ADCs under assessment as described below. Overall, these studies in immunocompromised tumor-bearing mice demonstrate that ADCs based on v30384 have favorable PK properties.

[00369] Test article (total antibody and/or intact ADC) concentrations were measured from mouse serum by 384 well plate ELISA. For detection of total IgG in serum from mice dosed with chimeric v23924, humanized v30384 and comparator v17717 and v17716 based ADCs, ELISA plates were coated with goat anti-human IgG Fc capture antibody (Jackson Immuno Research Laboratories, West Grove, PA). For ELISA of DL1 intact ADCs, a rabbit anti-Compound 1 capture antibody was used. For ELISA of DL6 intact ADCs, a mouse anti-DM1/4 capture antibody was used (Levena Biopharma, San Diego, CA). Serum samples were applied after blocking, followed by application of detection antibody: goat anti-human IgG F(ab')2 conjugated to horseradish peroxidase (HRP) (Jackson Immuno Research Laboratories, West Grove, PA). Absorbance at 450 nm was measured after application of 3,3′,5,5′-tetramethylbenzidine (TMB) and HCl quenching. Concentrations were determined with reference to standards (GraphPad Prism software (GraphPad Software, San Diego, CA)).

[00370] The results are shown in Fig. 16. Analysis across the multiple studies demonstrated the total IgG and ADC PK of each of v23924-DL1 and v17717-DL1 to be comparable, with typical antibody-like prolonged exposures (see Fig. 16A-F). v17717-DL6 and v17716-DL6 ADC PK consistently demonstrated lower exposure and greater clearance compared to their respective total IgG PK.

[00371] v30384-DL5 demonstrated proportional PK across 1, 3 and 10 mg/kg doses in the OV90 model (Fig.16G). In the H2110 model, v30384-DL5 demonstrated prolonged exposure at 10 mg/kg and greater clearance at lower doses (Fig. 16H).

EXAMPLE 29: PENETRATION OF ANTI-FRα ANTIBODIES IN MULTICELLULAR TUMOR SPHEROIDS

[00372] The ability of anti-FRα antibodies to penetrate FRα-expressing cell line spheroids was assessed according to the method described below. Spheroids provide a three-dimensional organization of cells with layers of distinct cell populations and the formation of different gradients from the outer to the inner regions. Cell signaling is more complex in spheroids than in two-dimensional cell cultures. As a result of these features, spheroids have the potential to recapitulate drug resistance and metabolic adaptation.

[00373] The spheroid penetration capability of humanized variant v36675 was compared to mirvetuximab (v17716) and non-FRα targeting control palivizumab (anti-RSV) (v22277). Variant v36675 is the same as variant v30384 but comprises HomoFc rather than HetFc. The cell line used was high FRα-expressing JEG-3 (placental choriocarcinoma).

[00374] Antibodies were fluorescently labeled by coupling to a Fab fragment AF488 conjugate targeting anti-Human IgG Fc (Jackson Immuno Research Labs, West Grove, PA; Cat. No. 109-547-008) at a 1:1 molar ratio in PBS pH 7.4 (Thermo Fisher Scientific, Waltham, MA; Cat. No. 10010-023), for 24 hours at 4°C.

[00375] JEG-3 cells were detached from culture vessels with TrypLE™ Express Enzyme (1X) (Thermo Fisher Scientific, Waltham, MA) and counted using the Cellaca® MX high-throughput automated cell counter (Nexcelom Bioscience LLC, Lawrence, MA). Cells were diluted in complete growth medium (Minimum Essential Media supplemented with 10% Fetal Bovine Serum (both from Thermo Fisher Scientific, Waltham, MA)) and seeded at 3,000 cells/well into 96-well CellCarrier Spheroid ultra-low attachment plates (Perkin Elmer, Waltham, MA), centrifuged, and incubated for 3 days under standard culturing conditions to allow for spheroid formation and growth.

[00376] After spheroid formation, Fab-AF488 coupled antibodies were added to spheroids at a final concentration of 25 nM and incubated under standard culturing conditions for 4-96 hours. Following incubation, excess antibody was removed by adding 100 µL complete growth medium and removing 100 µL medium from the well, to a total three washes. Spheroids were treated with

a solution of 1 µM Hoechst 33342 (Thermo Fisher Scientific, Waltham, MA) and 100 nM anti-Alexa Fluor 488 antibody (Thermo Fisher Scientific, Waltham, MA; Cat. No. A-11094), and incubated at 37°C/5% CO_2 for 2 hours.

[00377] Imaging was performed using an Operetta CLS™ high content analysis system (Perkin Elmer, Waltham, MA) with confocal acquisition and 10x magnification air objective. A Z stack of 15 planes separated by 15 µm was acquired and the slice with greatest diameter representing the spheroid center slice was selected for 2D analysis. Image analysis was performed using Harmony® 4.5 software (Perkin Elmer, Waltham, MA). Briefly, spheroid identification was performed by applying a mask around Hoechst 33342 positive objects to one spheroid per well. The spheroid region was divided into subregions of concentric bands, each representing 10% area of the spheroid region. Mean AF488 fluorescence within each subregion band was quantified, corrected by subtracting the inner 10% mean AF488 fluorescence, and plotted using GraphPad Prism Version 9 (GraphPad Software, San Diego, CA).

Results

[00378] The results are summarized in Table 29.1 and Fig. 20. The humanized antibody variant v36675 showed a greater degree of penetration than mirvetuximab into JEG-3 spheroids by AF488 intensity at each subregion band and by distance from spheroid edge to which AF488 fluorescence was detectable at all time points assessed. Non-binding control palivizumab showed lesser AF488 signal throughout the spheroid compared to both anti-FRα antibodies at all time points assessed.

Table 29.1: Penetration of Anti-FRα Antibodies in JEG-3 Spheroids

Time (h)	Sample	Average Corrected AF488 Mean Intensity									
		Band 1	**Band 2**	**Band 3**	**Band 4**	**Band 5**	**Band 6**	**Band 7**	**Band 8**	**Band 9**	**Spheroid Center**
4	v36675	-11.2	97.5	129.6	107.1	56.1	33.0	23.9	14.6	7.8	0.0
	Mirvetuximab	9.8	100.1	57.1	17.0	0.1	-13.5	-15.9	-13.7	-7.1	0.0
	Palivizumab	-16.0	5.1	31.7	31.2	21.6	25.4	23.5	14.8	7.5	0.0
24	v36675	-133.1	576.7	643.2	370.2	199.2	95.3	25.7	6.4	-1.7	0.0
	Mirvetuximab	-134.5	129.0	427.8	374.6	187.3	71.3	17.9	10.1	0.3	0.0
	Palivizumab	-18.2	18.0	50.9	62.1	53.5	37.4	21.5	7.3	1.8	0.0
48	v36675	303.6	1017.6	648.1	418.6	324.6	214.1	106.6	47.2	17.5	0.0
	Mirvetuximab	153.2	542.7	332.1	206.6	146.2	112.5	71.7	28.9	4.2	0.0
	Palivizumab	17.8	136.1	133.9	148.9	141.8	125.0	69.6	12.8	4.0	0.0
96	v36675	-60.5	1519.5	1213.0	815.5	507.5	305.5	196.5	121.5	48.5	0.0
	Mirvetuximab	-24.8	698.9	495.9	261.9	134.9	44.7	5.8	-12.1	-11.6	0.0
	Palivizumab	-147.8	-25.7	-0.1	-20.1	-28.2	-18.8	-13.6	-9.9	-5.3	0.0

EXAMPLE 30: SPECIFICITY ASSESSMENT OF ANTI-FRα ANTIBODIES

[00379] The Retrogenix Cell Microarray Technology (Charles River Laboratories, Wilmington, MA) was used to screen for any specific off-target binding interactions of the following anti-FRα antibodies: humanized variant v36675 and affinity matured variant v35356.

[00380] Retrogenix Cell Microarray Technology identifies interactions both with cell surface receptors and secreted proteins by screening test ligands for binding against a library of cDNA clones representing over 6,300 human proteins. These proteins include plasma membrane monomers, heterodimers (formed by co-expression of the separate subunits) and secreted proteins (expressed with an inert cell membrane tether). Each cDNA is spotted in duplicate onto specialized slides and overlaid with HEK293 cells. These cells become reverse-transfected, resulting in clusters of cells each overexpressing a different, individual protein (or heterodimeric complex).

[00381] The study was carried out at Charles River Laboratories consisted of three phases: pre-screen, library screen and confirmation screen. A pre-screen was first performed to determine a suitable concentration of the test antibodies for the library screen (*i.e.* the concentration at which a low level of background binding to fixed untransfected HEK293 cells and strong binding to cells over-expressing FRα was observed). In the library screen phase, the test antibodies were screened as a pool against fixed HEK293 cells, individually expressing the ~6,300 human proteins. In the confirmation screen phase, each library hit was re-expressed and re-tested with each test antibody individually using both fixed and live HEK293 cells.

[00382] In all three phases, slides were individually spotted with expression vectors encoding both ZsGreen1 (for assessing transfection efficiency) and (1) in the pre-screen phase: human FRα or control receptors (EGFR and CD20); (2) in the library screen phase: the above described protein library individually arrayed in duplicate across a number of microarray slides ("slide sets") with two replicate slides screened for each of the slide sets; or (3) in the confirmation screen phase: protein hits identified in the library screen or control receptors (EGFR and CD20) arrayed in duplicate.

[00383] In the pre-screen phase, each of the selected antibodies at 2, 5 or 20 μg/mL, control antibody (Rituximab biosimilar, which binds CD20) at 1μg/mL or PBS were added to the slides

after cell fixation. In the library screen phase, a pool of the two antibodies (variant v36675 at 20 μg/mL and variant v35356 at 5 μg/mL) was added to each slide after cell fixation. In the confirmation screen phase, slides were treated with individual antibodies: variant v36675 at 20 μg/mL, variant v35356 at 5 μg/mL or control antibody (Rituximab biosimilar) at 1μg/mL, or no test article, in the absence of fixation (live cells; n=1 slide per treatment) and after cell fixation (n=2 slides per treatment).

[00384] Binding was detected using an AlexaFluor® 647 labelled anti-human IgG H+L (AF647 anti-hIgG H+L), followed by fluorescence imaging. Fluorescent images were analysed and quantitated (for transfection) using ImageQuant™ software (Version 8.2; GE Healthcare, Chicago, IL). A protein hit was defined as a duplicate spot showing an increased signal compared to background levels and was identified by visual inspection using the images gridded on the ImageQuant™ software. Hits were classified as strong, medium, weak or very weak by visual inspection by two experienced scientists based on the intensity of the duplicate spots.

Results

[00385] The majority of the initial hits identified in the library screen, with spot intensities ranging from very weak to strong, were confirmed to be hits in the confirmation screens for both variant v36675 and variant v35356. However, aside from hits that were reflective of the expected strong interactions with FRα, the other hits were deemed to be non-specific as these hits were either 1) also observed for the control antibody (Rituximab biosimilar) as they included FcgR receptors (signal due to Fc-domain mediated interactions) or 2) included various immunoglobulins that were recognized by the detection antibody.

[00386] For the humanized variant v36675, no other interactions were identified (see Fig. 21), indicating the high specificity of variant v36675 for its primary target, FRα. The weak interaction observed for this variant with the heterodimer FCGR3A + CD247, which was not observed in the control (compare Fig. 21A & B), was not considered strong enough to be a hit.

[00387] For the affinity matured variant v35356, a signal of weak intensity was detected for the protein COL6A2 isoform 2C2A in the library screen. This "hit" was confirmed in the fixed cell screen but not in the live cell screen, indicating that observed discrepancy was likely due to a fixation artifact.

EXAMPLE 31: COMPETITION ASSAY

[00388] Competition binding to the FRα target between the parental chimeric antibody v23924 and the anti-FRα antibodies mirvetuximab (v17716) and farletuzumab (v31629) was assessed in the moderate FRα-expressing tumor cell line H2110 by flow cytometry as follows.

[00389] Briefly, antibodies were fluorescently labelled with Alexa Fluor 647 (AF647; ThermoFisher Scientific, Waltham, MA; Cat. No. A20006) according to manufacturer's specifications prior to cell treatment. Tumor cells were seeded at 50,000 cells/well in V-bottom 96-well plates and treated with unlabelled antibodies for 1 hour at 4°C to prevent internalization. Following incubation, cells were washed and fluorescently labelled antibodies were added for 1 hour at 4°C. Following incubation and washing, fluorescence was detected by flow cytometry on a BD LSRFortessa™ Cell Analyzer (BD Biosciences, Franklin Lake, NJ), with 1,000 minimum events collected per well. AF647/APC-A GeoMean (fluorescence signal geometric mean, proportional to anti-Human AF647 binding) in live cell population was used to calculate % competition binding relative to untreated control and data was plotted using GraphPad Prism Version 9 (GraphPad Software, San Diego, CA).

Results

[00390] The results are shown in Fig. 22 and Table 31.1. Chimeric antibody v23924 was found to compete with farletuzumab for FRα binding in the H2110 cell line, yielding close to 100% competition binding in both binding orientations (primary antibody farletuzumab and secondary fluorescently labelled antibody variant v23924, and primary antibody variant v23924 and secondary fluorescently labelled antibody farletuzumab). Chimeric antibody v23924 was found not to compete for FRα binding with mirvetuximab in the H2110 cell line. These antibodies showed low levels of % competition in both binding orientations. In contrast, farletuzumab showed competition binding with mirvetuximab. Chimeric antibody v23924 thus demonstrates a binding profile that is distinct from both mirvetuximab and farletuzumab.

Table 31.1: Competition Binding with Farletuzumab and Mirvetuximab on H2110 Cells

	% Competition Binding			
	v23924	**Mirvetuximab**	**Farletuzumab**	**Untreated**

v23924	98	99	11	21	99	98	0	0
Mirvetuximab	13	7	100	98	99	100	0	0
Farletuzumab	99	72	91	75	98	100	0	0
Untreated	1	1	0	0	0	0	0	0

EXAMPLE 32: ADDITIONAL FUNCTIONAL CHARACTERIZATION OF ANTI-FRα ANTIBODIES

[00391] Functional activities of the humanized antibody variants v30384 (HetFc) or v36675 (HomoFc) were assessed in comparison to the biparatopic anti-FRα antibody B5327A (IMGN151; v36264) and the anti-FRα antibody mirvetuximab (v17716) by cellular binding, antigen-mediated antibody internalization, antibody penetration into tumor cell spheroids, and 3D cell growth inhibition of spheroids, using FRα-expressing tumor cell lines.

32.1 Cellular Binding

[00392] The ability of the humanized antibody variant v30384 (HetFc), v36264 (B5327A) and v17716 (mirvetuximab) to bind to FRα-expressing tumor cell lines was assessed on the endogenous FRα-expressing tumor cell line IGROV-1 (ovarian adenocarcinoma; FRα-high) by flow cytometry following the same general procedure as described in Example 11.

[00393] The results are shown in Table 32.1. The humanized variant v30384 and mirvetuximab (v17716) demonstrated similar on-cell binding capabilities, with comparable Bmax values (16,599 and 19,505 intensity geometric mean, respectively) and apparent Kd values (0.43 and 0.69 nM, respectively). The biparatopic antibody B5327A (v36264) demonstrated increased Bmax compared to the other two antibodies (26,267 intensity geometric mean) as expected of a biparatopic antibody.

Table 32.1 Cellular Binding of Anti-FRα Antibodies on IGROV-1 Cell Line

Sample	Bmax	Kd (nM)	Hillslope (h)
v30384	16,599	0.43	1.2
v36264 (B5327A)	26,267	0.70	1.4

Sample	Bmax	Kd (nM)	Hillslope (h)
v17716 (mirvetuximab)	19,505	0.69	1.4

32.2 Internalization

[00394] The receptor-mediated internalization capabilities of the humanized antibody variant v30384 (HetFc), v36264 (B5327A) and v17716 (mirvetuximab) were assessed by uptake into the endogenous FRα-expressing tumor cell line IGROV-1 (ovarian adenocarcinoma) by flow cytometry as follows.

[00395] Antibodies were fluorescently labeled by coupling to an anti-human IgG Fc Fab fragment AF488 conjugate (Jackson Immuno Research Labs, West Grove, PA; Cat. No. 109-547-008) at a 1:1 molar ratio in PBS pH 7.4 (Thermo Fisher Scientific, Waltham, MA; Cat. No. 10010-023) for 24 hours at 4°C. Cells were seeded at 50,000 cells/well in 48-well plates and incubated overnight under standard culturing conditions (37°C/5% CO_2) to allow attachment. Coupled antibodies were added to cells the following day at 25 nM and incubated under standard culturing conditions for 5 hours to allow for internalization. Following incubation, cells were dissociated, washed, and surface AF488 fluorescence was quenched using an anti-AF488 antibody (Life Technologies, Carlsbad, CA; Cat. No. A-11094) at 100 nM for 30 min at 4°C. Quenched AF488 fluorescence (internalized fluorescence) was detected by flow cytometry on a BD LSRFortessa™ Cell Analyzer (BD Biosciences, Franklin Lake, NJ) with 1,000 minimum events collected per well. The AF488/FITC-A GeoMean (fluorescence signal geometric mean, proportional to anti-Human Fab AF488 labelling) was calculated for the live single cell population using FlowJo™ Version 10.8.1 (BD Biosciences, Franklin Lake, NJ) and plotted using GraphPad Prism Version 9 (GraphPad Software, San Diego, CA).

[00396] The results are shown in Fig. 23. Following a 5-hour incubation of IGROV-1 cells with 25 nM test antibody, the humanized antibody variant v30384 showed comparable internalized fluorescence to the biparatopic antibody v36264 (1.1-fold vs. v30384), while mirvetuximab (v17716) showed a markedly lower degree of internalized fluorescence (0.5-fold vs. v30384).

32.3 Antibody Penetration into 3D Spheroids

[00397] The ability of the humanized antibody variant v36675 (HomoFc), v36264 (B5327A) and v17716 (mirvetuximab) to penetrate FRα-expressing cell line spheroids was assessed by treatment of spheroids formed from high FRα-expressing cell line JEG-3 (placental choriocarcinoma) with fluorescently labeled antibodies following the same general procedure as described in Example 29.

[00398] The results are shown in Fig. 24. Following a 96-hour incubation of JEG-3 spheroids with 25 nM fluorescently-labeled antibody, the humanized antibody variant v36675 showed greater fluorescence intensity than the biparatopic antibody B5327A (v36264) or mirvetuximab (v17716) from the second layer inwards, indicative of a greater degree of cellular uptake for v36675 at all layers and overall greater distance penetrated into the spheroid. In contrast, the biparatopic antibody B5327A (v36264) showed greater fluorescence intensity than v36675 or v17716 on the outermost layer, indicative of antibody accumulation on the spheroid surface.

32.4 3D Spheroid Growth Inhibition

[00399] The 3D cytotoxicity capabilities of the humanized antibody variant v30384 (HetFc), v36264 (B5327A), and v17716 (mirvetuximab), each conjugated to drug-linker DL1 were assessed in FRα-expressing IGROV-1 (ovarian adenocarcinoma) and JEG-3 (placental choriocarcinoma) cell line spheroids as follows.

[00400] Briefly, cells were seeded in Ultra-Low Attachment 384-well plates (Corning, New York, NY) at 3,000 cells/well, centrifuged, and incubated for 3 days under standard culturing conditions to allow for spheroid formation and growth. Spheroids were then treated with a titration of test article, generated in cell growth medium. Spheroids were incubated for 6 days under standard culturing conditions. After incubation, CellTiter-Glo® 3D reagent (Promega Corporation, Madison, WI) was added to all wells. Plates were incubated in the dark at room temperature for 1 hour and luminescence was quantified using a BioTek Cytation 5 Cell Imaging Multi-Mode Reader (Agilent Technologies, Inc., Santa Clara, CA). Percent cytotoxicity values were calculated based on blank wells (no test article added) and plotted against test article concentration using GraphPad Prism 9 software (GraphPad Software, San Diego, CA). EC_{50} values were calculated based on a non-linear regression log(agonist) versus response, variable slope (four parameters) by GraphPad Prism 9.

[00401] The results are shown in Table 32.2. Against IGROV-1 spheroids, all three antibody-drug conjugates (ADCs) demonstrated comparable cell growth inhibition capability. Against JEG-3 spheroids, v30384-DL1 was similar in potency to v17716-DL1 (EC_{50} 0.18 and 0.17 nM, respectively), while the biparatopic ADC, v36264-DL1, demonstrated slightly lower potency (0.35 nM).

Table 32.4 3D *In vitro* Cell Growth Inhibition

ADC	DAR	EC_{50} (nM)	
		IGROV-1 Spheroids	JEG-3 Spheroids
v30384-DL1	3.9	0.22	0.18
v36264-DL1	3.8	0.34	0.35
v17716-DL1	3.9	0.23	0.17

[00402] The disclosures of all patents, patent applications, publications and database entries referenced in this specification are hereby specifically incorporated by reference in their entirety to the same extent as if each such individual patent, patent application, publication and database entry were specifically and individually indicated to be incorporated by reference.

[00403] Modifications of the specific embodiments described herein that would be apparent to those skilled in the art are intended to be included within the scope of the following claims.

SEQUENCE TABLES

Table A: Clone Numbers for Variants

Variant	Clone Numbers			
	H1	H2	L1	L2
23924	18894	18894	18915	18915
30618	18908	18901	18915	18915
30384	22046	22057	22054	22054
30389	22047	22058	22054	22054
30394	22048	22059	22054	22054
30399	22049	22060	22054	22054
31422	22874	22874	22054	22054
31423	22875	22875	22054	22054
31424	22876	22876	22054	22054
31425	22877	22877	22054	22054
31426	22878	22878	22054	22054
35305	26434	26434	26449	26449
35342	26443	26443	26450	26450
35347	26445	26445	27276	27276
35348	26445	26445	26448	26448
35350	26445	26445	26450	26450
35354	26446	26446	26450	26450
35356	26447	26447	26448	26448
35358	26447	26447	26450	26450
36167	27272	27272	27274	27274
36168	27273	27273	27274	27274
36675	27275	27275	22054	22054

Table B: Clone Sequences

Clone ID	Region	Sequence	SEQ ID NO
18894	Full	QSVKESEGGLFKPTDTLTLTCTVSGFSLSSYGVSWVRQAPGN GLEWIGAVNSGGSAYYANWAKSRSTITRNTNLFTVTLKMTS LAVADTATYFCARSGSGYPMDYLAIWGPGTLVTVSSASTKG PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSN TKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR EEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSD IAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLSPG	17
	Full	CAGAGCGTGAAGGAGTCCGAGGGCGGCCTGTTCAAGCCCA CCGACACACTGACCCTGACATGCACCGTGTCTGGCTTTAGC CTGAGCTCCTACGGCGTGTCTTGGGTGCGGCAGGCCCCCG GAAACGGACTGGAGTGGATCGGAGCCGTGAACAGCGGCG GCTCTGCCTACTATGCCAACTGGGCCAAGTCCCGGTCTACA ATCACCAGAAACACAAATCTGTTCACAGTGACCCTGAAGA TGACCTCCCTGGCCGTGGCAGACACAGCAACCTATTTTTGT GCAAGGAGCGGCTCCGGATACCCAATGGATTATCTGGCCA TCTGGGGCCCTGGCACACTGGTGACCGTGTCTAGCGCTAG CACTAAGGGGCCTTCCGTGTTTCCACTGGCTCCCTCTAGTA AATCCACCTCTGGAGGCACAGCTGCACTGGGATGTCTGGT GAAGGATTACTTCCCTGAACCAGTCACAGTGAGTTGGAAC TCAGGGGCTCTGACAAGTGGAGTCCATACTTTTCCCGCAGT GCTGCAGTCAAGCGGACTGTACTCCCTGTCCTCTGTGGTCA CCGTGCCTAGTTCAAGCCTGGGCACCCAGACATATATCTG CAACGTGAATCACAAGCCATCAAATACAAAAGTCGACAAG AAAGTGGAGCCCAAGAGCTGTGATAAAACTCATACCTGCC CACCTTGTCCGGCGCCAGAACTGCTGGGAGGACCAAGCGT GTTCCTGTTTCCACCCAAGCCTAAAGACACCCTGATGATTT CCCGGACTCCTGAGGTCACCTGCGTGGTCGTGGACGTGTCT CACGAGGACCCCGAAGTCAAGTTCAACTGGTACGTGGATG GCGTCGAAGTGCATAATGCCAAGACCAAACCCCGGGAGG AACAGTACAACTCTACCTATAGAGTCGTGAGTGTCCTGAC AGTGCTGCACCAGGACTGGCTGAATGGGAAGGAGTATAAG TGTAAAGTGAGCAACAAAGCCCTGCCCGCCCCAATCGAAA AAACAATCTCTAAAGCAAAAGGACAGCCTCGCGAACCACA GGTGTACACTCTGCCTCCATCTCGGGACGAGCTGACTAAG AACCAGGTCAGTCTGACCTGTCTGGTGAAAGGATTCTATC CCAGCGATATCGCTGTGGAGTGGGAATCCAATGGCCAGCC TGAGAACAATTACAAGACCACACCCCCTGTGCTGGACTCT GATGGCAGTTTCTTTCTGTATAGTAAGCTGACCGTCGATAA ATCACGATGGCAGCAGGGGAACGTGTTCAGCTGTTCAGTG ATGCACGAAGCCCTGCACAACCATTACACCCAGAAGAGCC TGAGCCTGTCTCCCGGC	18

Clone ID	Region	Sequence	SEQ ID NO
	VH	QSVKESEGGLFKPTDTLTLTCTVSGFSLSSYGVSWVRQAPGN GLEWIGAVNSGGSAYYANWAKSRSTITRNTNLFTVTLKMTS LAVADTATYFCARSGSGYPMDYLAIWGPGTLVTVSS	19
	IMGT CDR1	GFSLSSYG	20
	IMGT CDR2	VNSGGSA	21
	IMGT CDR3	ARSGSGYPMDYLAI	22
	Chothia CDR1	GFSLSSY	23
	Chothia CDR2	NSGGS	24
	Chothia CDR3	SGSGYPMDYLAI	25
	Kabat CDR1	SYGVS	26
	Kabat CDR2	AVNSGGSAYYANWAKS	27
	Kabat CDR3	SGSGYPMDYLAI	25
	Contact CDR1	SSYGVS	28
	Contact CDR2	WIGAVNSGGSAY	29
	Contact CDR3	ARSGSGYPMDYLA	30
	AbM CDR1	GFSLSSYGVS	31
	AbM CDR2	AVNSGGSAY	32
	AbM CDR3	SGSGYPMDYLAI	25
18901	Full	QSVKESEGGLFKPTDTLTLTCTVSGFSLSSYGVSWVRQAPGN GLEWIGAVNSGGSAYYANWAKSRSTITRNTNLFTVTLKMTS LAVADTATYFCARSGSGYPMDYLAIWGPGTLVTVSSASTKG PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSN TKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR EEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE KTISKAKGQPREPQVYVYPPSRDELTKNQVSLTCLVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFALVSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPG	33

Clone ID	Region	Sequence	SEQ ID NO
	Full	CAGAGCGTGAAGGAGTCCGAGGGCGGCCTGTTCAAGCCCA CCGACACACTGACCCTGACATGCACCGTGTCTGGCTTTAGC CTGAGCTCCTACGGCGTGTCTTGGGTGCGGCAGGCCCCCG GAAACGGACTGGAGTGGATCGGAGCCGTGAACAGCGGCG GCTCTGCCTACTATGCCAACTGGGCCAAGTCCCGGTCTACA ATCACCAGAAACACAAATCTGTTCACAGTGACCCTGAAGA TGACCTCCCTGGCCGTGGCAGACACAGCAACCTATTTTTGT GCAAGGAGCGGCTCCGGATACCCAATGGATTATCTGGCCA TCTGGGGCCCTGGCACACTGGTGACCGTGTCTAGCGCTAG CACTAAGGGGCCTTCCGTGTTTCCACTGGCTCCCTCTAGTA AATCCACCTCTGGAGGCACAGCTGCACTGGGATGTCTGGT GAAGGATTACTTCCCTGAACCAGTCACAGTGAGTTGGAAC TCAGGGGCTCTGACAAGTGGAGTCCATACTTTTCCCGCAGT GCTGCAGTCAAGCGGACTGTACTCCCTGTCCTCTGTGGTCA CCGTGCCTAGTTCAAGCCTGGGCACCCAGACATATATCTG CAACGTGAATCACAAGCCATCAAATACAAAAGTCGACAAG AAAGTGGAGCCCAAGAGCTGTGATAAAACTCATACCTGCC CACCTTGTCCGGCGCCAGAACTGCTGGGAGGACCAAGCGT GTTCCTGTTTCCACCCAAGCCTAAAGACACCCTGATGATTT CCCGGACTCCTGAGGTCACCTGCGTGGTCGTGGACGTGTCT CACGAGGACCCCGAAGTCAAGTTCAACTGGTACGTGGATG GCGTCGAAGTGCATAATGCCAAGACCAAACCCCGGGAGG AACAGTACAACTCTACCTATAGAGTCGTGAGTGTCCTGAC AGTGCTGCACCAGGACTGGCTGAATGGGAAGGAGTATAAG TGTAAAGTGAGCAACAAAGCCCTGCCCGCCCCAATCGAAA AAACAATCTCTAAAGCAAAAGGACAGCCTCGCGAACCACA GGTCTACGTCTACCCCCCATCAAGAGATGAACTGACAAAA AATCAGGTCTCTCTGACATGCCTGGTCAAAGGATTCTACCC TTCCGACATCGCCGTGGAGTGGGAAAGTAACGGCCAGCCC GAGAACAATTACAAGACCACACCCCCTGTCCTGGACTCTG ATGGGAGTTTCGCTCTGGTGTCAAAGCTGACCGTCGATAA AAGCCGGTGGCAGCAGGGCAATGTGTTTAGCTGCTCCGTC ATGCACGAAGCCCTGCACAATCACTACACACAGAAGTCCC TGAGCCTGAGCCCTGGC	34
	VH	QSVKESEGGLFKPTDTLTLTCTVSGFSLSSYGVSWVRQAPGN GLEWIGAVNSGGSAYYANWAKSRSTITRNTNLFTVTLKMTS LAVADTATYFCARSGSGYPMDYLAIWGPGTLVTVSS	19
	IMGT CDR1	GFSLSSYG	20
	IMGT CDR2	VNSGGSA	21
	IMGT CDR3	ARSGSGYPMDYLAI	22
	Chothia CDR1	GFSLSSY	23
	Chothia CDR2	NSGGS	24
	Chothia CDR3	SGSGYPMDYLAI	25
	Kabat CDR1	SYGVS	26
	Kabat CDR2	AVNSGGSAYYANWAKS	27

Clone ID	Region	Sequence	SEQ ID NO
	Kabat CDR3	SGSGYPMDYLAI	25
	Contact CDR1	SSYGVS	28
	Contact CDR2	WIGAVNSGGSAY	29
	Contact CDR3	ARSGSGYPMDYLA	30
	AbM CDR1	GFSLSSYGVS	31
	AbM CDR2	AVNSGGSAY	32
	AbM CDR3	SGSGYPMDYLAI	25
18908	Full	QSVKESEGGLFKPTDTLTLTCTVSGFSLSSYGVSWVRQAPGN GLEWIGAVNSGGSAYYANWAKSRSTITRNTNLFTVTLKMTS LAVADTATYFCARSGSGYPMDYLAIWGPGTLVTVSSASTKG PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSN TKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR EEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE KTISKAKGQPREPQVYVLPPSRDELTKNQVSLLCLVKGFYPS DIAVEWESNGQPENNYLTWPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPG	35
	Full	CAGAGCGTGAAGGAGTCCGAGGGCGGCCTGTTCAAGCCCA CCGACACACTGACCCTGACATGCACCGTGTCTGGCTTTAGC CTGAGCTCCTACGGCGTGTCTTGGGTGCGGCAGGCCCCCG GAAACGGACTGGAGTGGATCGGAGCCGTGAACAGCGGCG GCTCTGCCTACTATGCCAACTGGGCCAAGTCCCGGTCTACA ATCACCAGAAACACAAATCTGTTCACAGTGACCCTGAAGA TGACCTCCCTGGCCGTGGCAGACACAGCAACCTATTTTTGT GCAAGGAGCGGCTCCGGATACCCAATGGATTATCTGGCCA TCTGGGGCCCTGGCACACTGGTGACCGTGTCTAGCGCTAG CACTAAGGGGCCTTCCGTGTTTCCACTGGCTCCCTCTAGTA AATCCACCTCTGGAGGCACAGCTGCACTGGGATGTCTGGT GAAGGATTACTTCCCTGAACCAGTCACAGTGAGTTGGAAC TCAGGGGCTCTGACAAGTGGAGTCCATACTTTTCCCGCAGT GCTGCAGTCAAGCGGACTGTACTCCCTGTCCTCTGTGGTCA CCGTGCCTAGTTCAAGCCTGGGCACCCAGACATATATCTG CAACGTGAATCACAAGCCATCAAATACAAAAGTCGACAAG AAAGTGGAGCCCAAGAGCTGTGATAAAACTCATACCTGCC CACCTTGTCCGGCGCCAGAACTGCTGGGAGGACCAAGCGT GTTCCTGTTTCCACCCAAGCCTAAAGACACCCTGATGATTT CCCGGACTCCTGAGGTCACCTGCGTGGTCGTGGACGTGTCT CACGAGGACCCCGAAGTCAAGTTCAACTGGTACGTGGATG GCGTCGAAGTGCATAATGCCAAGACCAAACCCCGGGAGG AACAGTACAACTCTACCTATAGAGTCGTGAGTGTCCTGAC AGTGCTGCACCAGGACTGGCTGAATGGGAAGGAGTATAAG TGTAAAGTGAGCAACAAAGCCCTGCCCGCCCCAATCGAAA AAACAATCTCTAAAGCAAAAGGACAGCCTCGCGAACCACA GGTCTACGTGCTGCCCCCTAGCCGCGACGAACTGACTAAA AATCAGGTCTCTCTGCTGTGTCTGGTCAAAGGATTCTACCC	36

Clone ID	Region	Sequence	SEQ ID NO
		TTCCGACATCGCCGTGGAGTGGGAAAGTAACGGCCAGCCC GAGAACAATTACCTGACCTGGCCCCCTGTGCTGGACTCTG ATGGGAGTTTCTTTCTGTATTCAAAGCTGACAGTCGATAAA AGCCGGTGGCAGCAGGGCAATGTGTTCAGCTGCTCCGTCA TGCACGAAGCACTGCACAACCATTACACTCAGAAGTCCCT GTCCCTGTCACCTGGC	
	VH	QSVKESEGGLFKPTDTLTLTCTVSGFSLSSYGVSWVRQAPGN GLEWIGAVNSGGSAYYANWAKSRSTITRNTNLFTVTLKMTS LAVADTATYFCARSGSGYPMDYLAIWGPGTLVTVSS	19
	IMGT CDR1	GFSLSSYG	20
	IMGT CDR2	VNSGGSA	21
	IMGT CDR3	ARSGSGYPMDYLAI	22
	Chothia CDR1	GFSLSSY	23
	Chothia CDR2	NSGGS	24
	Chothia CDR3	SGSGYPMDYLAI	25
	Kabat CDR1	SYGVS	26
	Kabat CDR2	AVNSGGSAYYANWAKS	27
	Kabat CDR3	SGSGYPMDYLAI	25
	Contact CDR1	SSYGVS	28
	Contact CDR2	WIGAVNSGGSAY	29
	Contact CDR3	ARSGSGYPMDYLA	30
	AbM CDR1	GFSLSSYGVS	31
	AbM CDR2	AVNSGGSAY	32
	AbM CDR3	SGSGYPMDYLAI	25
18915	Full	AYDMTQTPASVEVAVGGTVTIKCQASQSIGDWLAWYQQKP GQPPRLLIYEASTLASGVPSRFSGSGSGTQFTLTISGVECADAA TYYCQQGYGRSNVDNIFGGGTEVVVKRTVAAPSVFIFPPSDE QLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVT EQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVT KSFNRGEC	37
	Full	GCCTACGACATGACCCAGACACCCGCCTCCGTGGAGGTGG CAGTGGGAGGAACCGTGACAATCAAGTGCCAGGCCAGCC AGTCCATCGGCGATTGGCTGGCCTGGTACCAGCAGAAGCC TGGACAGCCACCTCGGCTGCTGATCTATGAGGCCTCTACCC TGGCCAGCGGAGTGCCATCCAGATTCTCTGGCAGCGGCTC CGGCACACAGTTTACCCTGACAATCTCTGGAGTGGAGTGC GCAGACGCAGCAACCTACTATTGTCAGCAGGGCTATGGCA GGAGCAACGTGGATAATATCTTTGGAGGAGGAACCGAGGT GGTGGTGAAGAGGACAGTGGCGGCGCCCAGTGTCTTCATT TTTCCCCCTAGCGACGAACAGCTGAAGTCTGGGACAGCCA GTGTGGTCTGTCTGCTGAACAACTTCTACCCTAGAGAGGCT AAAGTGCAGTGGAAGGTCGATAACGCACTGCAGTCCGGAA	38

Clone ID	Region	Sequence	SEQ ID NO
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	VL	AYDMTQTPASVEVAVGGTVTIKCQASQSIGDWLAWYQQKP GQPPRLLIYEASTLASGVPSRFSGSGSGTQFTLTISGVECADAA TYYCQQGYGRSNVDNIFGGGTEVVVK	39
	IMGT CDR1	QSIGDW	40
	IMGT CDR2	EAS	41
	IMGT CDR3	QQGYGRSNVDNI	42
	Chothia CDR1	QASQSIGDWLA	43
	Chothia CDR2	EASTLAS	44
	Chothia CDR3	QQGYGRSNVDNI	42
	Kabat CDR1	QASQSIGDWLA	43
	Kabat CDR2	EASTLAS	44
	Kabat CDR3	QQGYGRSNVDNI	42
	Contact CDR1	GDWLAWY	45
	Contact CDR2	LLIYEASTLA	46
	Contact CDR3	QQGYGRSNVDN	47
	AbM CDR1	QASQSIGDWLA	43
	AbM CDR2	EASTLAS	44
	AbM CDR3	QQGYGRSNVDNI	42
22046	Full	EVQLLESGGGLVQPGGSLRLSCAVSGFSLSSYGVSWVRQAPG KGLEWIGAVNSGGSAYYADSVKGRSTISRDNSKNTVYLQMN SLRAEDTAVYYCARSGSGYPMDYLAIWGQGTLVTVSSASTK GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKP SNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP IEKTISKAKGQPREPQVYVYPPSRDELTKNQVSLTCLVKGFYP SDIAVEWESNGQPENNYKTTPPVLDSDGSFALVSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPG	48

Clone ID	Region	Sequence	SEQ ID NO
	Full	GAGGTGCAGCTGCTGGAGAGCGGAGGAGGACTGGTGCAG CCAGGAGGCAGCCTGCGGCTGTCCTGCGCCGTGAGCGGCT TTTCCCTGAGCTCCTACGGCGTGTCCTGGGTGAGACAGGCC CCCGGCAAGGGACTGGAGTGGATCGGAGCCGTGAACTCTG GCGGCAGCGCCTACTATGCCGACTCTGTGAAGGGCCGGTC CACCATCTCTAGAGATAACTCTAAGAATACAGTGTATCTG CAGATGAATAGCCTGAGGGCCGAGGACACAGCCGTGTACT ATTGTGCACGCTCCGGCTCTGGCTACCCCATGGATTATCTG GCCATCTGGGGACAGGGCACCCTGGTGACAGTGTCTAGCG CTAGCACTAAGGGGCCTTCCGTGTTTCCACTGGCTCCCTCT AGTAAATCCACCTCTGGAGGCACAGCTGCACTGGGATGTC TGGTGAAGGATTACTTCCCTGAACCAGTCACAGTGAGTTG GAACTCAGGGGCTCTGACAAGTGGAGTCCATACTTTTCCC GCAGTGCTGCAGTCAAGCGGACTGTACTCCCTGTCCTCTGT GGTCACCGTGCCTAGTTCAAGCCTGGGCACCCAGACATAT ATCTGCAACGTGAATCACAAGCCATCAAATACAAAAGTCG ACAAGAAAGTGGAGCCCAAGAGCTGTGATAAAACTCATAC CTGCCCACCTTGTCCGGCGCCAGAACTGCTGGGAGGACCA AGCGTGTTCCTGTTTCCACCCAAGCCTAAAGACACCCTGAT GATTTCCCGGACTCCTGAGGTCACCTGCGTGGTCGTGGAC GTGTCTCACGAGGACCCCGAAGTCAAGTTCAACTGGTACG TGGATGGCGTCGAAGTGCATAATGCCAAGACCAAACCCCG GGAGGAACAGTACAACTCTACCTATAGAGTCGTGAGTGTC CTGACAGTGCTGCACCAGGACTGGCTGAATGGGAAGGAGT ATAAGTGTAAAGTGAGCAACAAAGCCCTGCCCGCCCCAAT CGAAAAAACAATCTCTAAAGCAAAAGGACAGCCTCGCGA ACCACAGGTCTACGTCTACCCCCCATCAAGAGATGAACTG ACAAAAAATCAGGTCTCTCTGACATGCCTGGTCAAAGGAT TCTACCCTTCCGACATCGCCGTGGAGTGGGAAAGTAACGG CCAGCCCGAGAACAATTACAAGACCACACCCCCTGTCCTG GACTCTGATGGGAGTTTCGCTCTGGTGTCAAAGCTGACCGT CGATAAAAGCCGGTGGCAGCAGGGCAATGTGTTTAGCTGC TCCGTCATGCACGAAGCCCTGCACAATCACTACACACAGA AGTCCCTGAGCCTGAGCCCTGGC	49
	VH	EVQLLESGGGLVQPGGSLRLSCAVSGFSLSSYGVSWVRQAPG KGLEWIGAVNSGGSAYYADSVKGRSTISRDNSKNTVYLQMN SLRAEDTAVYYCARSGSGYPMDYLAIWGQGTLVTVSS	50
	IMGT CDR1	GFSLSSYG	20
	IMGT CDR2	VNSGGSA	21
	IMGT CDR3	ARSGSGYPMDYLAI	22
	Chothia CDR1	GFSLSSY	23
	Chothia CDR2	NSGGS	24
	Chothia CDR3	SGSGYPMDYLAI	25
	Kabat CDR1	SYGVS	26
	Kabat CDR2	AVNSGGSAYYADSVKG	51

Clone ID	Region	Sequence	SEQ ID NO
	Kabat CDR3	SGSGYPMDYLAI	25
	Contact CDR1	SSYGVS	28
	Contact CDR2	WIGAVNSGGSAY	29
	Contact CDR3	ARSGSGYPMDYLA	30
	AbM CDR1	GFSLSSYGVS	31
	AbM CDR2	AVNSGGSAY	32
	AbM CDR3	SGSGYPMDYLAI	25
22047	Full	EVQVLESGGGLVQPGGSLRLSCAVSGFSLSSYGVSWVRQAPG KGLEWIGAVNSGGSAYYADSVKGRSTISRDNSKNTVYLQMN SLRAEDTAVYYCARSGSGYPMDYLAIWGQGTLVTVSSASTK GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKP SNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP IEKTISKAKGQPREPQVYVYPPSRDELTKNQVSLTCLVKGFYP SDIAVEWESNGQPENNYKTTPPVLDSDGSFALVSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPG	52
	Full	GAGGTGCAGGTGCTGGAGAGCGGAGGAGGACTGGTGCAG CCAGGAGGCAGCCTGCGGCTGTCCTGCGCCGTGAGCGGCT TTTCCCTGAGCTCCTACGGCGTGTCCTGGGTGAGACAGGCC CCCGGCAAGGGACTGGAGTGGATCGGAGCCGTGAACTCTG GCGGCAGCGCCTACTATGCCGACTCTGTGAAGGGCCGGTC CACCATCTCTAGAGATAACTCTAAGAATACAGTGTATCTG CAGATGAATAGCCTGAGGGCCGAGGACACAGCCGTGTACT ATTGTGCACGCTCCGGCTCTGGCTACCCCATGGATTATCTG GCCATCTGGGGACAGGGCACCCTGGTGACAGTGTCTAGCG CTAGCACTAAGGGGCCTTCCGTGTTTCCACTGGCTCCCTCT AGTAAATCCACCTCTGGAGGCACAGCTGCACTGGGATGTC TGGTGAAGGATTACTTCCCTGAACCAGTCACAGTGAGTTG GAACTCAGGGGCTCTGACAAGTGGAGTCCATACTTTTCCC GCAGTGCTGCAGTCAAGCGGACTGTACTCCCTGTCCTCTGT GGTCACCGTGCCTAGTTCAAGCCTGGGCACCCAGACATAT ATCTGCAACGTGAATCACAAGCCATCAAATACAAAAGTCG ACAAGAAAGTGGAGCCCAAGAGCTGTGATAAAACTCATAC CTGCCCACCTTGTCCGGCGCCAGAACTGCTGGGAGGACCA AGCGTGTTCCTGTTTCCACCCAAGCCTAAAGACACCCTGAT GATTTCCCGGACTCCTGAGGTCACCTGCGTGGTCGTGGAC GTGTCTCACGAGGACCCCGAAGTCAAGTTCAACTGGTACG TGGATGGCGTCGAAGTGCATAATGCCAAGACCAAACCCCG GGAGGAACAGTACAACTCTACCTATAGAGTCGTGAGTGTC CTGACAGTGCTGCACCAGGACTGGCTGAATGGGAAGGAGT ATAAGTGTAAAGTGAGCAACAAAGCCCTGCCCGCCCCAAT CGAAAAAACAATCTCTAAAGCAAAAGGACAGCCTCGCGA ACCACAGGTCTACGTCTACCCCCCATCAAGAGATGAACTG ACAAAAAATCAGGTCTCTCTGACATGCCTGGTCAAAGGAT	53

Clone ID	Region	Sequence	SEQ ID NO
		TCTACCCTTCCGACATCGCCGTGGAGTGGGAAAGTAACGG CCAGCCCGAGAACAATTACAAGACCACACCCCCTGTCCTG GACTCTGATGGGAGTTTCGCTCTGGTGTCAAAGCTGACCGT CGATAAAAGCCGGTGGCAGCAGGGCAATGTGTTTAGCTGC TCCGTCATGCACGAAGCCCTGCACAATCACTACACACAGA AGTCCCTGAGCCTGAGCCCTGGC	
	VH	EVQVLESGGGLVQPGGSLRLSCAVSGFSLSSYGVSWVRQAPG KGLEWIGAVNSGGSAYYADSVKGRSTISRDNSKNTVYLQMN SLRAEDTAVYYCARSGSGYPMDYLAIWGQGTLVTVSS	54
	IMGT CDR1	GFSLSSYG	20
	IMGT CDR2	VNSGGSA	21
	IMGT CDR3	ARSGSGYPMDYLAI	22
	Chothia CDR1	GFSLSSY	23
	Chothia CDR2	NSGGS	24
	Chothia CDR3	SGSGYPMDYLAI	25
	Kabat CDR1	SYGVS	26
	Kabat CDR2	AVNSGGSAYYADSVKG	51
	Kabat CDR3	SGSGYPMDYLAI	25
	Contact CDR1	SSYGVS	28
	Contact CDR2	WIGAVNSGGSAY	29
	Contact CDR3	ARSGSGYPMDYLA	30
	AbM CDR1	GFSLSSYGVS	31
	AbM CDR2	AVNSGGSAY	32
	AbM CDR3	SGSGYPMDYLAI	25
22048	Full	EVQLLESGGGLVQPGGSLRLSCAVSGFSLSSYGVSWVRQAPG KGLEWIGAVNSGGSAYYADWAKGRSTISRDNSKNTVYLQM NSLRAEDTAVYYCARSGSGYPMDYLAIWGQGTLVTVSSAST KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA LTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHK PSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPK DTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVYVYPPSRDELTKNQVSLTCLVKGF YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFALVSKLTVDK SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG	55

Clone ID	Region	Sequence	SEQ ID NO
	Full	GAGGTGCAGCTGCTGGAGTCCGGAGGAGGACTGGTGCAGC CAGGAGGCAGCCTGCGGCTGTCCTGCGCCGTGAGCGGCTT TTCCCTGAGCTCCTACGGCGTGTCTTGGGTGAGACAGGCCC CCGGCAAGGGACTGGAGTGGATCGGAGCCGTGAACTCTGG AGGCAGCGCCTACTATGCAGACTGGGCAAAGGGCCGGTCC ACCATCTCTAGAGATAACTCTAAGAATACAGTGTATCTGC AGATGAATAGCCTGAGGGCCGAGGACACAGCCGTGTACTA TTGTGCACGCTCCGGCTCTGGCTACCCCATGGATTATCTGG CCATCTGGGGACAGGGCACCCTGGTGACAGTGTCTAGCGC TAGCACTAAGGGGCCTTCCGTGTTTCCACTGGCTCCCTCTA GTAAATCCACCTCTGGAGGCACAGCTGCACTGGGATGTCT GGTGAAGGATTACTTCCCTGAACCAGTCACAGTGAGTTGG AACTCAGGGGCTCTGACAAGTGGAGTCCATACTTTTCCCG CAGTGCTGCAGTCAAGCGGACTGTACTCCCTGTCCTCTGTG GTCACCGTGCCTAGTTCAAGCCTGGGCACCCAGACATATA TCTGCAACGTGAATCACAAGCCATCAAATACAAAAGTCGA CAAGAAAGTGGAGCCCAAGAGCTGTGATAAAACTCATACC TGCCCACCTTGTCCGGCGCCAGAACTGCTGGGAGGACCAA GCGTGTTCCTGTTTCCACCCAAGCCTAAAGACACCCTGATG ATTTCCCGGACTCCTGAGGTCACCTGCGTGGTCGTGGACGT GTCTCACGAGGACCCCGAAGTCAAGTTCAACTGGTACGTG GATGGCGTCGAAGTGCATAATGCCAAGACCAAACCCCGGG AGGAACAGTACAACTCTACCTATAGAGTCGTGAGTGTCCT GACAGTGCTGCACCAGGACTGGCTGAATGGGAAGGAGTAT AAGTGTAAAGTGAGCAACAAAGCCCTGCCCGCCCCAATCG AAAAAACAATCTCTAAAGCAAAAGGACAGCCTCGCGAAC CACAGGTCTACGTCTACCCCCCATCAAGAGATGAACTGAC AAAAAATCAGGTCTCTCTGACATGCCTGGTCAAAGGATTC TACCCTTCCGACATCGCCGTGGAGTGGGAAAGTAACGGCC AGCCCGAGAACAATTACAAGACCACACCCCCTGTCCTGGA CTCTGATGGGAGTTTCGCTCTGGTGTCAAAGCTGACCGTCG ATAAAAGCCGGTGGCAGCAGGGCAATGTGTTTAGCTGCTC CGTCATGCACGAAGCCCTGCACAATCACTACACACAGAAG TCCCTGAGCCTGAGCCCTGGC	56
	VH	EVQLLESGGGLVQPGGSLRLSCAVSGFSLSSYGVSWVRQAPG KGLEWIGAVNSGGSAYYADWAKGRSTISRDNSKNTVYLQM NSLRAEDTAVYYCARSGSGYPMDYLAIWGQGTLVTVSS	57
	IMGT CDR1	GFSLSSYG	20
	IMGT CDR2	VNSGGSA	21
	IMGT CDR3	ARSGSGYPMDYLAI	22
	Chothia CDR1	GFSLSSY	23
	Chothia CDR2	NSGGS	24
	Chothia CDR3	SGSGYPMDYLAI	25
	Kabat CDR1	SYGVS	26
	Kabat CDR2	AVNSGGSAYYADWAKG	58

Clone ID	Region	Sequence	SEQ ID NO
	Kabat CDR3	SGSGYPMDYLAI	25
	Contact CDR1	SSYGVS	28
	Contact CDR2	WIGAVNSGGSAY	29
	Contact CDR3	ARSGSGYPMDYLA	30
	AbM CDR1	GFSLSSYGVS	31
	AbM CDR2	AVNSGGSAY	32
	AbM CDR3	SGSGYPMDYLAI	25
22049	Full	EVQVLESGGGLVQPGGSLRLSCAVSGFSLSSYGVSWVRQAPG KGLEWIGAVNSGGSAYYADWAKGRSTISRDNSKNTVYLQM NSLRAEDTAVYYCARSGSGYPMDYLAIWGQGTLVTVSSAST KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA LTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHK PSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPK DTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVYVYPPSRDELTKNQVSLTCLVKGF YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFALVSKLTVDK SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG	59
	Full	GAGGTGCAGGTGCTGGAGTCCGGAGGAGGACTGGTGCAG CCAGGAGGCAGCCTGCGGCTGTCCTGCGCCGTGAGCGGCT TTTCCCTGAGCTCCTACGGCGTGTCTTGGGTGAGACAGGCC CCCGGCAAGGGACTGGAGTGGATCGGAGCCGTGAACTCTG GAGGCAGCGCCTACTATGCAGACTGGGCAAAGGGCCGGTC CACCATCTCTAGAGATAACTCTAAGAATACAGTGTATCTG CAGATGAATAGCCTGAGGGCCGAGGACACAGCCGTGTACT ATTGTGCACGCTCCGGCTCTGGCTACCCCATGGATTATCTG GCCATCTGGGGACAGGGCACCCTGGTGACAGTGTCTAGCG CTAGCACTAAGGGGCCTTCCGTGTTTCCACTGGCTCCCTCT AGTAAATCCACCTCTGGAGGCACAGCTGCACTGGGATGTC TGGTGAAGGATTACTTCCCTGAACCAGTCACAGTGAGTTG GAACTCAGGGGCTCTGACAAGTGGAGTCCATACTTTTCCC GCAGTGCTGCAGTCAAGCGGACTGTACTCCCTGTCCTCTGT GGTCACCGTGCCTAGTTCAAGCCTGGGCACCCAGACATAT ATCTGCAACGTGAATCACAAGCCATCAAATACAAAAGTCG ACAAGAAAGTGGAGCCCAAGAGCTGTGATAAAACTCATAC CTGCCCACCTTGTCCGGCGCCAGAACTGCTGGGAGGACCA AGCGTGTTCCTGTTTCCACCCAAGCCTAAAGACACCCTGAT GATTTCCCGGACTCCTGAGGTCACCTGCGTGGTCGTGGAC GTGTCTCACGAGGACCCCGAAGTCAAGTTCAACTGGTACG TGGATGGCGTCGAAGTGCATAATGCCAAGACCAAACCCCG GGAGGAACAGTACAACTCTACCTATAGAGTCGTGAGTGTC CTGACAGTGCTGCACCAGGACTGGCTGAATGGGAAGGAGT ATAAGTGTAAAGTGAGCAACAAAGCCCTGCCCGCCCCAAT CGAAAAAACAATCTCTAAAGCAAAAGGACAGCCTCGCGA ACCACAGGTCTACGTCTACCCCCCATCAAGAGATGAACTG ACAAAAAATCAGGTCTCTCTGACATGCCTGGTCAAAGGAT	60

Clone ID	Region	Sequence	SEQ ID NO
		TCTACCCTTCCGACATCGCCGTGGAGTGGGAAAGTAACGG CCAGCCCGAGAACAATTACAAGACCACACCCCCTGTCCTG GACTCTGATGGGAGTTTCGCTCTGGTGTCAAAGCTGACCGT CGATAAAAGCCGGTGGCAGCAGGGCAATGTGTTTAGCTGC TCCGTCATGCACGAAGCCCTGCACAATCACTACACACAGA AGTCCCTGAGCCTGAGCCCTGGC	
	VH	EVQVLESGGGLVQPGGSLRLSCAVSGFSLSSYGVSWVRQAPG KGLEWIGAVNSGGSAYYADWAKGRSTISRDNSKNTVYLQM NSLRAEDTAVYYCARSGSGYPMDYLAIWGQGTLVTVSS	61
	IMGT CDR1	GFSLSSYG	20
	IMGT CDR2	VNSGGSA	21
	IMGT CDR3	ARSGSGYPMDYLAI	22
	Chothia CDR1	GFSLSSY	23
	Chothia CDR2	NSGGS	24
	Chothia CDR3	SGSGYPMDYLAI	25
	Kabat CDR1	SYGVS	26
	Kabat CDR2	AVNSGGSAYYADWAKG	58
	Kabat CDR3	SGSGYPMDYLAI	25
	Contact CDR1	SSYGVS	28
	Contact CDR2	WIGAVNSGGSAY	29
	Contact CDR3	ARSGSGYPMDYLA	30
	AbM CDR1	GFSLSSYGVS	31
	AbM CDR2	AVNSGGSAY	32
	AbM CDR3	SGSGYPMDYLAI	25
22054	Full	DYQMTQSPSSVSASVGDRVTITCRASQSIGDWLAWYQQKPG KAPKLLIYEASTLASGVPSRFSGSGSGTDFTLTISSVQPEDAAT YYCQQGYGRSNVDNIFGGGTKVEVKRTVAAPSVFIFPPSDEQ LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTE QDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTK SFNRGEC	62
	Full	GACTACCAGATGACCCAGTCTCCAAGCTCCGTGTCCGCCTC TGTGGGCGACAGGGTGACCATCACATGCAGAGCCAGCCAG TCCATCGGCGATTGGCTGGCCTGGTATCAGCAGAAGCCCG GCAAGGCCCCTAAGCTGCTGATCTATGAGGCCTCTACCCT GGCCAGCGGCGTGCCTTCCCGGTTCTCTGGCAGCGGCTCC GGCACAGACTTTACCCTGACAATCTCTAGCGTGCAGCCAG AGGATGCCGCCACCTACTATTGTCAGCAGGGCTATGGCAG GTCCAACGTGGATAATATCTTTGGAGGAGGCACCAAGGTG GAGGTGAAGAGGACAGTGGCGGCGCCCAGTGTCTTCATTT TTCCCCCTAGCGACGAACAGCTGAAGTCTGGGACAGCCAG TGTGGTCTGTCTGCTGAACAACTTCTACCCTAGAGAGGCTA AAGTGCAGTGGAAGGTCGATAACGCACTGCAGTCCGGAAA	63

Clone ID	Region	Sequence	SEQ ID NO
		TTCTCAGGAGAGTGTGACTGAACAGGACTCAAAAGATAGC ACCTATTCCCTGTCAAGCACACTGACTCTGAGCAAGGCCG ACTACGAGAAGCATAAAGTGTATGCTTGTGAAGTCACCCA CCAGGGGCTGAGTTCACCAGTCACAAAATCATTCAACAGA GGGGAGTGC	
	VL	DYQMTQSPSSVSASVGDRVTITCRASQSIGDWLAWYQQKPG KAPKLLIYEASTLASGVPSRFSGSGSGTDFTLTISSVQPEDAAT YYCQQGYGRSNVDNIFGGGTKVEVK	64
	IMGT CDR1	QSIGDW	40
	IMGT CDR2	EAS	41
	IMGT CDR3	QQGYGRSNVDNI	42
	Chothia CDR1	RASQSIGDWLA	65
	Chothia CDR2	EASTLAS	44
	Chothia CDR3	QQGYGRSNVDNI	42
	Kabat CDR1	RASQSIGDWLA	65
	Kabat CDR2	EASTLAS	44
	Kabat CDR3	QQGYGRSNVDNI	42
	Contact CDR1	GDWLAWY	45
	Contact CDR2	LLIYEASTLA	46
	Contact CDR3	QQGYGRSNVDN	47
	AbM CDR1	RASQSIGDWLA	65
	AbM CDR2	EASTLAS	44
	AbM CDR3	QQGYGRSNVDNI	42
22057	Full	EVQLLESGGGLVQPGGSLRLSCAVSGFSLSSYGVSWVRQAPG KGLEWIGAVNSGGSAYYADSVKGRSTISRDNSKNTVYLQMN SLRAEDTAVYYCARSGSGYPMDYLAIWGQGTLVTVSSASTK GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKP SNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP IEKTISKAKGQPREPQVYVLPPSRDELTKNQVSLLCLVKGFYP SDIAVEWESNGQPENNYLTWPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPG	66

Clone ID	Region	Sequence	SEQ ID NO
	Full	GAGGTGCAGCTGCTGGAGAGCGGAGGAGGACTGGTGCAG CCAGGAGGCAGCCTGCGGCTGTCCTGCGCCGTGAGCGGCT TTTCCCTGAGCTCCTACGGCGTGTCCTGGGTGAGACAGGCC CCCGGCAAGGGACTGGAGTGGATCGGAGCCGTGAACTCTG GCGGCAGCGCCTACTATGCCGACTCTGTGAAGGGCCGGTC CACCATCTCTAGAGATAACTCTAAGAATACAGTGTATCTG CAGATGAATAGCCTGAGGGCCGAGGACACAGCCGTGTACT ATTGTGCACGCTCCGGCTCTGGCTACCCCATGGATTATCTG GCCATCTGGGGACAGGGCACCCTGGTGACAGTGTCTAGCG CTAGCACTAAGGGGCCTTCCGTGTTTCCACTGGCTCCCTCT AGTAAATCCACCTCTGGAGGCACAGCTGCACTGGGATGTC TGGTGAAGGATTACTTCCCTGAACCAGTCACAGTGAGTTG GAACTCAGGGGCTCTGACAAGTGGAGTCCATACTTTTCCC GCAGTGCTGCAGTCAAGCGGACTGTACTCCCTGTCCTCTGT GGTCACCGTGCCTAGTTCAAGCCTGGGCACCCAGACATAT ATCTGCAACGTGAATCACAAGCCATCAAATACAAAAGTCG ACAAGAAAGTGGAGCCCAAGAGCTGTGATAAAACTCATAC CTGCCCACCTTGTCCGGCGCCAGAACTGCTGGGAGGACCA AGCGTGTTCCTGTTTCCACCCAAGCCTAAAGACACCCTGAT GATTTCCCGGACTCCTGAGGTCACCTGCGTGGTCGTGGAC GTGTCTCACGAGGACCCCGAAGTCAAGTTCAACTGGTACG TGGATGGCGTCGAAGTGCATAATGCCAAGACCAAACCCCG GGAGGAACAGTACAACTCTACCTATAGAGTCGTGAGTGTC CTGACAGTGCTGCACCAGGACTGGCTGAATGGGAAGGAGT ATAAGTGTAAAGTGAGCAACAAAGCCCTGCCCGCCCCAAT CGAAAAAACAATCTCTAAAGCAAAAGGACAGCCTCGCGA ACCACAGGTCTACGTGCTGCCCCCTAGCCGCGACGAACTG ACTAAAAATCAGGTCTCTCTGCTGTGTCTGGTCAAAGGATT CTACCCTTCCGACATCGCCGTGGAGTGGGAAAGTAACGGC CAGCCCGAGAACAATTACCTGACCTGGCCCCCTGTGCTGG ACTCTGATGGGAGTTTCTTTCTGTATTCAAAGCTGACAGTC GATAAAAGCCGGTGGCAGCAGGGCAATGTGTTCAGCTGCT CCGTCATGCACGAAGCACTGCACAACCATTACACTCAGAA GTCCCTGTCCCTGTCACCTGGC	67
	VH	EVQLLESGGGLVQPGGSLRLSCAVSGFSLSSYGVSWVRQAPG KGLEWIGAVNSGGSAYYADSVKGRSTISRDNSKNTVYLQMN SLRAEDTAVYYCARSGSGYPMDYLAIWGQGTLVTVSS	50
	IMGT CDR1	GFSLSSYG	20
	IMGT CDR2	VNSGGSA	21
	IMGT CDR3	ARSGSGYPMDYLAI	22
	Chothia CDR1	GFSLSSY	23
	Chothia CDR2	NSGGS	24
	Chothia CDR3	SGSGYPMDYLAI	25
	Kabat CDR1	SYGVS	26
	Kabat CDR2	AVNSGGSAYYADSVKG	51

Clone ID	Region	Sequence	SEQ ID NO
	Kabat CDR3	SGSGYPMDYLAI	25
	Contact CDR1	SSYGVS	28
	Contact CDR2	WIGAVNSGGSAY	29
	Contact CDR3	ARSGSGYPMDYLA	30
	AbM CDR1	GFSLSSYGVS	31
	AbM CDR2	AVNSGGSAY	32
	AbM CDR3	SGSGYPMDYLAI	25
22058	Full	EVQVLESGGGLVQPGGSLRLSCAVSGFSLSSYGVSWVRQAPG KGLEWIGAVNSGGSAYYADSVKGRSTISRDNSKNTVYLQMN SLRAEDTAVYYCARSGSGYPMDYLAIWGQGTLVTVSSASTK GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKP SNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP IEKTISKAKGQPREPQVYVLPPSRDELTKNQVSLLCLVKGFYP SDIAVEWESNGQPENNYLTWPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPG	68
	Full	GAGGTGCAGGTGCTGGAGAGCGGAGGAGGACTGGTGCAG CCAGGAGGCAGCCTGCGGCTGTCCTGCGCCGTGAGCGGCT TTTCCCTGAGCTCCTACGGCGTGTCCTGGGTGAGACAGGCC CCCGGCAAGGGACTGGAGTGGATCGGAGCCGTGAACTCTG GCGGCAGCGCCTACTATGCCGACTCTGTGAAGGGCCGGTC CACCATCTCTAGAGATAACTCTAAGAATACAGTGTATCTG CAGATGAATAGCCTGAGGGCCGAGGACACAGCCGTGTACT ATTGTGCACGCTCCGGCTCTGGCTACCCCATGGATTATCTG GCCATCTGGGGACAGGGCACCCTGGTGACAGTGTCTAGCG CTAGCACTAAGGGGCCTTCCGTGTTTCCACTGGCTCCCTCT AGTAAATCCACCTCTGGAGGCACAGCTGCACTGGGATGTC TGGTGAAGGATTACTTCCCTGAACCAGTCACAGTGAGTTG GAACTCAGGGGCTCTGACAAGTGGAGTCCATACTTTTCCC GCAGTGCTGCAGTCAAGCGGACTGTACTCCCTGTCCTCTGT GGTCACCGTGCCTAGTTCAAGCCTGGGCACCCAGACATAT ATCTGCAACGTGAATCACAAGCCATCAAATACAAAAGTCG ACAAGAAAGTGGAGCCCAAGAGCTGTGATAAAACTCATAC CTGCCCACCTTGTCCGGCGCCAGAACTGCTGGGAGGACCA AGCGTGTTCCTGTTTCCACCCAAGCCTAAAGACACCCTGAT GATTTCCCGGACTCCTGAGGTCACCTGCGTGGTCGTGGAC GTGTCTCACGAGGACCCCGAAGTCAAGTTCAACTGGTACG TGGATGGCGTCGAAGTGCATAATGCCAAGACCAAACCCCG GGAGGAACAGTACAACTCTACCTATAGAGTCGTGAGTGTC CTGACAGTGCTGCACCAGGACTGGCTGAATGGGAAGGAGT ATAAGTGTAAAGTGAGCAACAAAGCCCTGCCCGCCCCAAT CGAAAAAACAATCTCTAAAGCAAAAGGACAGCCTCGCGA ACCACAGGTCTACGTGCTGCCCCCTAGCCGCGACGAACTG ACTAAAAATCAGGTCTCTCTGCTGTGTCTGGTCAAAGGATT	69

Clone ID	Region	Sequence	SEQ ID NO
		CTACCCTTCCGACATCGCCGTGGAGTGGGAAAGTAACGGC CAGCCCGAGAACAATTACCTGACCTGGCCCCCTGTGCTGG ACTCTGATGGGAGTTTCTTTCTGTATTCAAAGCTGACAGTC GATAAAAGCCGGTGGCAGCAGGGCAATGTGTTCAGCTGCT CCGTCATGCACGAAGCACTGCACAACCATTACACTCAGAA GTCCCTGTCCCTGTCACCTGGC	
	VH	EVQVLESGGGLVQPGGSLRLSCAVSGFSLSSYGVSWVRQAPG KGLEWIGAVNSGGSAYYADSVKGRSTISRDNSKNTVYLQMN SLRAEDTAVYYCARSGSGYPMDYLAIWGQGTLVTVSS	54
	IMGT CDR1	GFSLSSYG	20
	IMGT CDR2	VNSGGSA	21
	IMGT CDR3	ARSGSGYPMDYLAI	22
	Chothia CDR1	GFSLSSY	23
	Chothia CDR2	NSGGS	24
	Chothia CDR3	SGSGYPMDYLAI	25
	Kabat CDR1	SYGVS	26
	Kabat CDR2	AVNSGGSAYYADSVKG	51
	Kabat CDR3	SGSGYPMDYLAI	25
	Contact CDR1	SSYGVS	28
	Contact CDR2	WIGAVNSGGSAY	29
	Contact CDR3	ARSGSGYPMDYLA	30
	AbM CDR1	GFSLSSYGVS	31
	AbM CDR2	AVNSGGSAY	32
	AbM CDR3	SGSGYPMDYLAI	25
22059	Full	EVQLLESGGGLVQPGGSLRLSCAVSGFSLSSYGVSWVRQAPG KGLEWIGAVNSGGSAYYADWAKGRSTISRDNSKNTVYLQM NSLRAEDTAVYYCARSGSGYPMDYLAIWGQGTLVTVSSAST KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA LTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHK PSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPK DTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVYVLPPSRDELTKNQVSLLCLVKGF YPSDIAVEWESNGQPENNYLTWPPVLDSDGSFFLYSKLTVDK SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG	70

Clone ID	Region	Sequence	SEQ ID NO
	Full	GAGGTGCAGCTGCTGGAGTCCGGAGGAGGACTGGTGCAGC CAGGAGGCAGCCTGCGGCTGTCCTGCGCCGTGAGCGGCTT TTCCCTGAGCTCCTACGGCGTGTCTTGGGTGAGACAGGCCC CCGGCAAGGGACTGGAGTGGATCGGAGCCGTGAACTCTGG AGGCAGCGCCTACTATGCAGACTGGGCAAAGGGCCGGTCC ACCATCTCTAGAGATAACTCTAAGAATACAGTGTATCTGC AGATGAATAGCCTGAGGGCCGAGGACACAGCCGTGTACTA TTGTGCACGCTCCGGCTCTGGCTACCCCATGGATTATCTGG CCATCTGGGGACAGGGCACCCTGGTGACAGTGTCTAGCGC TAGCACTAAGGGGCCTTCCGTGTTTCCACTGGCTCCCTCTA GTAAATCCACCTCTGGAGGCACAGCTGCACTGGGATGTCT GGTGAAGGATTACTTCCCTGAACCAGTCACAGTGAGTTGG AACTCAGGGGCTCTGACAAGTGGAGTCCATACTTTTCCCG CAGTGCTGCAGTCAAGCGGACTGTACTCCCTGTCCTCTGTG GTCACCGTGCCTAGTTCAAGCCTGGGCACCCAGACATATA TCTGCAACGTGAATCACAAGCCATCAAATACAAAAGTCGA CAAGAAAGTGGAGCCCAAGAGCTGTGATAAAACTCATACC TGCCCACCTTGTCCGGCGCCAGAACTGCTGGGAGGACCAA GCGTGTTCCTGTTTCCACCCAAGCCTAAAGACACCCTGATG ATTTCCCGGACTCCTGAGGTCACCTGCGTGGTCGTGGACGT GTCTCACGAGGACCCCGAAGTCAAGTTCAACTGGTACGTG GATGGCGTCGAAGTGCATAATGCCAAGACCAAACCCCGGG AGGAACAGTACAACTCTACCTATAGAGTCGTGAGTGTCCT GACAGTGCTGCACCAGGACTGGCTGAATGGGAAGGAGTAT AAGTGTAAAGTGAGCAACAAAGCCCTGCCCGCCCCAATCG AAAAAACAATCTCTAAAGCAAAAGGACAGCCTCGCGAAC CACAGGTCTACGTGCTGCCCCCTAGCCGCGACGAACTGAC TAAAAATCAGGTCTCTCTGCTGTGTCTGGTCAAAGGATTCT ACCCTTCCGACATCGCCGTGGAGTGGGAAAGTAACGGCCA GCCCGAGAACAATTACCTGACCTGGCCCCCTGTGCTGGAC TCTGATGGGAGTTTCTTTCTGTATTCAAAGCTGACAGTCGA TAAAAGCCGGTGGCAGCAGGGCAATGTGTTCAGCTGCTCC GTCATGCACGAAGCACTGCACAACCATTACACTCAGAAGT CCCTGTCCCTGTCACCTGGC	71
	VH	EVQLLESGGGLVQPGGSLRLSCAVSGFSLSSYGVSWVRQAPG KGLEWIGAVNSGGSAYYADWAKGRSTISRDNSKNTVYLQM NSLRAEDTAVYYCARSGSGYPMDYLAIWGQGTLVTVSS	57
	IMGT CDR1	GFSLSSYG	20
	IMGT CDR2	VNSGGSA	21
	IMGT CDR3	ARSGSGYPMDYLAI	22
	Chothia CDR1	GFSLSSY	23
	Chothia CDR2	NSGGS	24
	Chothia CDR3	SGSGYPMDYLAI	25
	Kabat CDR1	SYGVS	26
	Kabat CDR2	AVNSGGSAYYADWAKG	58

Clone ID	Region	Sequence	SEQ ID NO
	Kabat CDR3	SGSGYPMDYLAI	25
	Contact CDR1	SSYGVS	28
	Contact CDR2	WIGAVNSGGSAY	29
	Contact CDR3	ARSGSGYPMDYLA	30
	AbM CDR1	GFSLSSYGVS	31
	AbM CDR2	AVNSGGSAY	32
	AbM CDR3	SGSGYPMDYLAI	25
22060	Full	EVQVLESGGGLVQPGGSLRLSCAVSGFSLSSYGVSWVRQAPG KGLEWIGAVNSGGSAYYADWAKGRSTISRDNSKNTVYLQM NSLRAEDTAVYYCARSGSGYPMDYLAIWGQGTLVTVSSAST KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA LTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHK PSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPK DTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVYVLPPSRDELTKNQVSLLCLVKGF YPSDIAVEWESNGQPENNYLTWPPVLDSDGSFFLYSKLTVDK SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG	72
	Full	GAGGTGCAGGTGCTGGAGTCCGGAGGAGGACTGGTGCAG CCAGGAGGCAGCCTGCGGCTGTCCTGCGCCGTGAGCGGCT TTTCCCTGAGCTCCTACGGCGTGTCTTGGGTGAGACAGGCC CCCGGCAAGGGACTGGAGTGGATCGGAGCCGTGAACTCTG GAGGCAGCGCCTACTATGCAGACTGGGCAAAGGGCCGGTC CACCATCTCTAGAGATAACTCTAAGAATACAGTGTATCTG CAGATGAATAGCCTGAGGGCCGAGGACACAGCCGTGTACT ATTGTGCACGCTCCGGCTCTGGCTACCCCATGGATTATCTG GCCATCTGGGGACAGGGCACCCTGGTGACAGTGTCTAGCG CTAGCACTAAGGGGCCTTCCGTGTTTCCACTGGCTCCCTCT AGTAAATCCACCTCTGGAGGCACAGCTGCACTGGGATGTC TGGTGAAGGATTACTTCCCTGAACCAGTCACAGTGAGTTG GAACTCAGGGGCTCTGACAAGTGGAGTCCATACTTTTCCC GCAGTGCTGCAGTCAAGCGGACTGTACTCCCTGTCCTCTGT GGTCACCGTGCCTAGTTCAAGCCTGGGCACCCAGACATAT ATCTGCAACGTGAATCACAAGCCATCAAATACAAAAGTCG ACAAGAAAGTGGAGCCCAAGAGCTGTGATAAAACTCATAC CTGCCCACCTTGTCCGGCGCCAGAACTGCTGGGAGGACCA AGCGTGTTCCTGTTTCCACCCAAGCCTAAAGACACCCTGAT GATTTCCCGGACTCCTGAGGTCACCTGCGTGGTCGTGGAC GTGTCTCACGAGGACCCCGAAGTCAAGTTCAACTGGTACG TGGATGGCGTCGAAGTGCATAATGCCAAGACCAAACCCCG GGAGGAACAGTACAACTCTACCTATAGAGTCGTGAGTGTC CTGACAGTGCTGCACCAGGACTGGCTGAATGGGAAGGAGT ATAAGTGTAAAGTGAGCAACAAAGCCCTGCCCGCCCCAAT CGAAAAAACAATCTCTAAAGCAAAAGGACAGCCTCGCGA ACCACAGGTCTACGTGCTGCCCCCTAGCCGCGACGAACTG ACTAAAAATCAGGTCTCTCTGCTGTGTCTGGTCAAAGGATT	73

Clone ID	Region	Sequence	SEQ ID NO
		CTACCCTTCCGACATCGCCGTGGAGTGGGAAAGTAACGGC CAGCCCGAGAACAATTACCTGACCTGGCCCCCTGTGCTGG ACTCTGATGGGAGTTTCTTTCTGTATTCAAAGCTGACAGTC GATAAAAGCCGGTGGCAGCAGGGCAATGTGTTCAGCTGCT CCGTCATGCACGAAGCACTGCACAACCATTACACTCAGAA GTCCCTGTCCCTGTCACCTGGC	
	VH	EVQVLESGGGLVQPGGSLRLSCAVSGFSLSSYGVSWVRQAPG KGLEWIGAVNSGGSAYYADWAKGRSTISRDNSKNTVYLQM NSLRAEDTAVYYCARSGSGYPMDYLAIWGQGTLVTVSS	61
	IMGT CDR1	GFSLSSYG	20
	IMGT CDR2	VNSGGSA	21
	IMGT CDR3	ARSGSGYPMDYLAI	22
	Chothia CDR1	GFSLSSY	23
	Chothia CDR2	NSGGS	24
	Chothia CDR3	SGSGYPMDYLAI	25
	Kabat CDR1	SYGVS	26
	Kabat CDR2	AVNSGGSAYYADWAKG	58
	Kabat CDR3	SGSGYPMDYLAI	25
	Contact CDR1	SSYGVS	28
	Contact CDR2	WIGAVNSGGSAY	29
	Contact CDR3	ARSGSGYPMDYLA	30
	AbM CDR1	GFSLSSYGVS	31
	AbM CDR2	AVNSGGSAY	32
	AbM CDR3	SGSGYPMDYLAI	25
22874	Full	EVQLLESGGGLFQPGGSLRLSCAVSGFSLSSYGVSWVRQAPG KGLEWIGAVNSGGSAYYADSVKGRSTISRDNSKNTVYLQMN SLRAEDTAVYYCARSGSGYPMDYLAIWGQGTLVTVSSASTK GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKP SNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP IEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYP SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPG	74

Clone ID	Region	Sequence	SEQ ID NO
	Full	GAGGTGCAGCTGCTGGAGAGCGGAGGAGGACTGTTCCAGC CAGGAGGCAGCCTGCGGCTGTCCTGCGCCGTGAGCGGCTT TTCCCTGAGCTCCTACGGCGTGTCCTGGGTGAGACAGGCC CCCGGCAAGGGACTGGAGTGGATCGGAGCCGTGAACTCTG GCGGCAGCGCCTACTATGCCGACTCTGTGAAGGGCCGGTC CACCATCTCTAGAGATAACTCTAAGAATACAGTGTATCTG CAGATGAATAGCCTGAGGGCCGAGGACACAGCCGTGTACT ATTGTGCACGCTCCGGCTCTGGCTACCCCATGGATTATCTG GCCATCTGGGGACAGGGCACCCTGGTGACAGTGTCTAGCG CTAGCACTAAGGGGCCTTCCGTGTTTCCACTGGCTCCCTCT AGTAAATCCACCTCTGGAGGCACAGCTGCACTGGGATGTC TGGTGAAGGATTACTTCCCTGAACCAGTCACAGTGAGTTG GAACTCAGGGGCTCTGACAAGTGGAGTCCATACTTTTCCC GCAGTGCTGCAGTCAAGCGGACTGTACTCCCTGTCCTCTGT GGTCACCGTGCCTAGTTCAAGCCTGGGCACCCAGACATAT ATCTGCAACGTGAATCACAAGCCATCAAATACAAAAGTCG ACAAGAAAGTGGAGCCCAAGAGCTGTGATAAAACTCATAC CTGCCCACCTTGTCCGGCGCCAGAACTGCTGGGAGGACCA AGCGTGTTCCTGTTTCCACCCAAGCCTAAAGACACCCTGAT GATTTCCCGGACTCCTGAGGTCACCTGCGTGGTCGTGGAC GTGTCTCACGAGGACCCCGAAGTCAAGTTCAACTGGTACG TGGATGGCGTCGAAGTGCATAATGCCAAGACCAAACCCCG GGAGGAACAGTACAACTCTACCTATAGAGTCGTGAGTGTC CTGACAGTGCTGCACCAGGACTGGCTGAATGGGAAGGAGT ATAAGTGTAAAGTGAGCAACAAAGCCCTGCCCGCCCCAAT CGAAAAAACAATCTCTAAAGCAAAAGGACAGCCTCGCGA ACCACAGGTGTACACTCTGCCTCCATCTCGGGACGAGCTG ACTAAGAACCAGGTCAGTCTGACCTGTCTGGTGAAAGGAT TCTATCCCAGCGATATCGCTGTGGAGTGGGAATCCAATGG CCAGCCTGAGAACAATTACAAGACCACACCCCCTGTGCTG GACTCTGATGGCAGTTTCTTTCTGTATAGTAAGCTGACCGT CGATAAATCACGATGGCAGCAGGGGAACGTGTTCAGCTGT TCAGTGATGCACGAAGCCCTGCACAACCATTACACCCAGA AGAGCCTGAGCCTGTCTCCCGGC	75
	VH	EVQLLESGGGLFQPGGSLRLSCAVSGFSLSSYGVSWVRQAPG KGLEWIGAVNSGGSAYYADSVKGRSTISRDNSKNTVYLQMN SLRAEDTAVYYCARSGSGYPMDYLAIWGQGTLVTVSS	76
	IMGT CDR1	GFSLSSYG	20
	IMGT CDR2	VNSGGSA	21
	IMGT CDR3	ARSGSGYPMDYLAI	22
	Chothia CDR1	GFSLSSY	23
	Chothia CDR2	NSGGS	24
	Chothia CDR3	SGSGYPMDYLAI	25
	Kabat CDR1	SYGVS	26
	Kabat CDR2	AVNSGGSAYYADSVKG	51

Clone ID	Region	Sequence	SEQ ID NO
	Kabat CDR3	SGSGYPMDYLAI	25
	Contact CDR1	SSYGVS	28
	Contact CDR2	WIGAVNSGGSAY	29
	Contact CDR3	ARSGSGYPMDYLA	30
	AbM CDR1	GFSLSSYGVS	31
	AbM CDR2	AVNSGGSAY	32
	AbM CDR3	SGSGYPMDYLAI	25
22875	Full	EVQLLESGGGLVQPGGSLRLSCAVSGFSLSSYGVSWVRQAPG KGLEWIGAVNSGGSAYYADSVKGRSTISRDTSKNTVYLQMN SLRAEDTAVYYCARSGSGYPMDYLAIWGQGTLVTVSSASTK GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKP SNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP IEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYP SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPG	77
	Full	GAGGTGCAGCTGCTGGAGAGCGGAGGAGGACTGGTGCAG CCAGGAGGCAGCCTGCGGCTGTCCTGCGCCGTGAGCGGCT TTTCCCTGAGCTCCTACGGCGTGTCCTGGGTGAGACAGGCC CCCGGCAAGGGACTGGAGTGGATCGGAGCCGTGAACTCTG GCGGCAGCGCCTACTATGCCGACTCTGTGAAGGGCCGGTC CACCATCTCTAGAGATACCTCTAAGAACACAGTGTATCTG CAGATGAATAGCCTGAGGGCCGAGGACACAGCCGTGTACT ATTGTGCACGCTCCGGCTCTGGCTACCCCATGGATTATCTG GCCATCTGGGGACAGGGCACCCTGGTGACAGTGTCTAGCG CTAGCACTAAGGGGCCTTCCGTGTTTCCACTGGCTCCCTCT AGTAAATCCACCTCTGGAGGCACAGCTGCACTGGGATGTC TGGTGAAGGATTACTTCCCTGAACCAGTCACAGTGAGTTG GAACTCAGGGGCTCTGACAAGTGGAGTCCATACTTTTCCC GCAGTGCTGCAGTCAAGCGGACTGTACTCCCTGTCCTCTGT GGTCACCGTGCCTAGTTCAAGCCTGGGCACCCAGACATAT ATCTGCAACGTGAATCACAAGCCATCAAATACAAAAGTCG ACAAGAAAGTGGAGCCCAAGAGCTGTGATAAAACTCATAC CTGCCCACCTTGTCCGGCGCCAGAACTGCTGGGAGGACCA AGCGTGTTCCTGTTTCCACCCAAGCCTAAAGACACCCTGAT GATTTCCCGGACTCCTGAGGTCACCTGCGTGGTCGTGGAC GTGTCTCACGAGGACCCCGAAGTCAAGTTCAACTGGTACG TGGATGGCGTCGAAGTGCATAATGCCAAGACCAAACCCCG GGAGGAACAGTACAACTCTACCTATAGAGTCGTGAGTGTC CTGACAGTGCTGCACCAGGACTGGCTGAATGGGAAGGAGT ATAAGTGTAAAGTGAGCAACAAAGCCCTGCCCGCCCCAAT CGAAAAAACAATCTCTAAAGCAAAAGGACAGCCTCGCGA ACCACAGGTGTACACTCTGCCTCCATCTCGGGACGAGCTG ACTAAGAACCAGGTCAGTCTGACCTGTCTGGTGAAAGGAT	78

Clone ID	Region	Sequence	SEQ ID NO
		TCTATCCCAGCGATATCGCTGTGGAGTGGGAATCCAATGG CCAGCCTGAGAACAATTACAAGACCACACCCCCTGTGCTG GACTCTGATGGCAGTTTCTTTCTGTATAGTAAGCTGACCGT CGATAAATCACGATGGCAGCAGGGGAACGTGTTCAGCTGT TCAGTGATGCACGAAGCCCTGCACAACCATTACACCCAGA AGAGCCTGAGCCTGTCTCCCGGC	
	VH	EVQLLESGGGLVQPGGSLRLSCAVSGFSLSSYGVSWVRQAPG KGLEWIGAVNSGGSAYYADSVKGRSTISRDTSKNTVYLQMN SLRAEDTAVYYCARSGSGYPMDYLAIWGQGTLVTVSS	79
	IMGT CDR1	GFSLSSYG	20
	IMGT CDR2	VNSGGSA	21
	IMGT CDR3	ARSGSGYPMDYLAI	22
	Chothia CDR1	GFSLSSY	23
	Chothia CDR2	NSGGS	24
	Chothia CDR3	SGSGYPMDYLAI	25
	Kabat CDR1	SYGVS	26
	Kabat CDR2	AVNSGGSAYYADSVKG	51
	Kabat CDR3	SGSGYPMDYLAI	25
	Contact CDR1	SSYGVS	28
	Contact CDR2	WIGAVNSGGSAY	29
	Contact CDR3	ARSGSGYPMDYLA	30
	AbM CDR1	GFSLSSYGVS	31
	AbM CDR2	AVNSGGSAY	32
	AbM CDR3	SGSGYPMDYLAI	25
22876	Full	EVQLLESGGGLVQPGGSLRLSCAVSGFSLSSYGVSWVRQAPG KGLEWIGAVNSGGSAYYADSVKGRSTISRDTSKFTVYLQMN SLRAEDTAVYYCARSGSGYPMDYLAIWGQGTLVTVSSASTK GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKP SNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP IEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYP SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPG	80

Clone ID	Region	Sequence	SEQ ID NO
	Full	GAGGTGCAGCTGCTGGAGAGCGGAGGAGGACTGGTGCAG CCAGGAGGCAGCCTGCGGCTGTCCTGCGCCGTGAGCGGCT TTTCCCTGAGCTCCTACGGCGTGTCCTGGGTGAGACAGGCC CCCGGCAAGGGACTGGAGTGGATCGGAGCCGTGAACTCTG GCGGCAGCGCCTACTATGCCGACTCTGTGAAGGGCCGGTC CACCATCTCTAGAGATACCTCTAAGTTCACAGTGTATCTGC AGATGAATAGCCTGAGGGCCGAGGACACAGCCGTGTACTA TTGTGCACGCTCCGGCTCTGGCTACCCCATGGATTATCTGG CCATCTGGGGACAGGGCACCCTGGTGACAGTGTCTAGCGC TAGCACTAAGGGGCCTTCCGTGTTTCCACTGGCTCCCTCTA GTAAATCCACCTCTGGAGGCACAGCTGCACTGGGATGTCT GGTGAAGGATTACTTCCCTGAACCAGTCACAGTGAGTTGG AACTCAGGGGCTCTGACAAGTGGAGTCCATACTTTTCCCG CAGTGCTGCAGTCAAGCGGACTGTACTCCCTGTCCTCTGTG GTCACCGTGCCTAGTTCAAGCCTGGGCACCCAGACATATA TCTGCAACGTGAATCACAAGCCATCAAATACAAAAGTCGA CAAGAAAGTGGAGCCCAAGAGCTGTGATAAAACTCATACC TGCCCACCTTGTCCGGCGCCAGAACTGCTGGGAGGACCAA GCGTGTTCCTGTTTCCACCCAAGCCTAAAGACACCCTGATG ATTTCCCGGACTCCTGAGGTCACCTGCGTGGTCGTGGACGT GTCTCACGAGGACCCCGAAGTCAAGTTCAACTGGTACGTG GATGGCGTCGAAGTGCATAATGCCAAGACCAAACCCCGGG AGGAACAGTACAACTCTACCTATAGAGTCGTGAGTGTCCT GACAGTGCTGCACCAGGACTGGCTGAATGGGAAGGAGTAT AAGTGTAAAGTGAGCAACAAAGCCCTGCCCGCCCCAATCG AAAAAACAATCTCTAAAGCAAAAGGACAGCCTCGCGAAC CACAGGTGTACACTCTGCCTCCATCTCGGGACGAGCTGAC TAAGAACCAGGTCAGTCTGACCTGTCTGGTGAAAGGATTC TATCCCAGCGATATCGCTGTGGAGTGGGAATCCAATGGCC AGCCTGAGAACAATTACAAGACCACACCCCCTGTGCTGGA CTCTGATGGCAGTTTCTTTCTGTATAGTAAGCTGACCGTCG ATAAATCACGATGGCAGCAGGGGAACGTGTTCAGCTGTTC AGTGATGCACGAAGCCCTGCACAACCATTACACCCAGAAG AGCCTGAGCCTGTCTCCCGGC	81
	VH	EVQLLESGGGLVQPGGSLRLSCAVSGFSLSSYGVSWVRQAPG KGLEWIGAVNSGGSAYYADSVKGRSTISRDTSKFTVYLQMN SLRAEDTAVYYCARSGSGYPMDYLAIWGQGTLVTVSS	82
	IMGT CDR1	GFSLSSYG	20
	IMGT CDR2	VNSGGSA	21
	IMGT CDR3	ARSGSGYPMDYLAI	22
	Chothia CDR1	GFSLSSY	23
	Chothia CDR2	NSGGS	24
	Chothia CDR3	SGSGYPMDYLAI	25
	Kabat CDR1	SYGVS	26
	Kabat CDR2	AVNSGGSAYYADSVKG	51

Clone ID	Region	Sequence	SEQ ID NO
	Kabat CDR3	SGSGYPMDYLAI	25
	Contact CDR1	SSYGVS	28
	Contact CDR2	WIGAVNSGGSAY	29
	Contact CDR3	ARSGSGYPMDYLA	30
	AbM CDR1	GFSLSSYGVS	31
	AbM CDR2	AVNSGGSAY	32
	AbM CDR3	SGSGYPMDYLAI	25
22877	Full	EVQLLESGGGLFQPGGSLRLSCAVSGFSLSSYGVSWVRQAPG KGLEWIGAVNSGGSAYYADSVKGRSTISRDTSKFTVYLQMN SLRAEDTAVYYCARSGSGYPMDYLAIWGQGTLVTVSSASTK GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKP SNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP IEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYP SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPG	83
	Full	GAGGTGCAGCTGCTGGAGAGCGGAGGAGGACTGTTCCAGC CAGGAGGCAGCCTGCGGCTGTCCTGCGCCGTGAGCGGCTT TTCCCTGAGCTCCTACGGCGTGTCCTGGGTGAGACAGGCC CCCGGCAAGGGACTGGAGTGGATCGGAGCCGTGAACTCTG GCGGCAGCGCCTACTATGCCGACTCTGTGAAGGGCCGGTC CACCATCTCTAGAGATACCTCTAAGTTTACAGTGTATCTGC AGATGAATAGCCTGAGGGCCGAGGACACAGCCGTGTACTA TTGTGCACGCTCCGGCTCTGGCTACCCCATGGATTATCTGG CCATCTGGGGACAGGGCACCCTGGTGACAGTGTCTAGCGC TAGCACTAAGGGGCCTTCCGTGTTTCCACTGGCTCCCTCTA GTAAATCCACCTCTGGAGGCACAGCTGCACTGGGATGTCT GGTGAAGGATTACTTCCCTGAACCAGTCACAGTGAGTTGG AACTCAGGGGCTCTGACAAGTGGAGTCCATACTTTTCCCG CAGTGCTGCAGTCAAGCGGACTGTACTCCCTGTCCTCTGTG GTCACCGTGCCTAGTTCAAGCCTGGGCACCCAGACATATA TCTGCAACGTGAATCACAAGCCATCAAATACAAAAGTCGA CAAGAAAGTGGAGCCCAAGAGCTGTGATAAAACTCATACC TGCCCACCTTGTCCGGCGCCAGAACTGCTGGGAGGACCAA GCGTGTTCCTGTTTCCACCCAAGCCTAAAGACACCCTGATG ATTTCCCGGACTCCTGAGGTCACCTGCGTGGTCGTGGACGT GTCTCACGAGGACCCCGAAGTCAAGTTCAACTGGTACGTG GATGGCGTCGAAGTGCATAATGCCAAGACCAAACCCCGGG AGGAACAGTACAACTCTACCTATAGAGTCGTGAGTGTCCT GACAGTGCTGCACCAGGACTGGCTGAATGGGAAGGAGTAT AAGTGTAAAGTGAGCAACAAAGCCCTGCCCGCCCCAATCG AAAAAACAATCTCTAAAGCAAAAGGACAGCCTCGCGAAC CACAGGTGTACACTCTGCCTCCATCTCGGGACGAGCTGAC TAAGAACCAGGTCAGTCTGACCTGTCTGGTGAAAGGATTC	84

Clone ID	Region	Sequence	SEQ ID NO
		TATCCCAGCGATATCGCTGTGGAGTGGGAATCCAATGGCC AGCCTGAGAACAATTACAAGACCACACCCCCTGTGCTGGA CTCTGATGGCAGTTTCTTTCTGTATAGTAAGCTGACCGTCG ATAAATCACGATGGCAGCAGGGGAACGTGTTCAGCTGTTC AGTGATGCACGAAGCCCTGCACAACCATTACACCCAGAAG AGCCTGAGCCTGTCTCCCGGC	
	VH	EVQLLESGGGLFQPGGSLRLSCAVSGFSLSSYGVSWVRQAPG KGLEWIGAVNSGGSAYYADSVKGRSTISRDTSKFTVYLQMN SLRAEDTAVYYCARSGSGYPMDYLAIWGQGTLVTVSS	85
	IMGT CDR1	GFSLSSYG	20
	IMGT CDR2	VNSGGSA	21
	IMGT CDR3	ARSGSGYPMDYLAI	22
	Chothia CDR1	GFSLSSY	23
	Chothia CDR2	NSGGS	24
	Chothia CDR3	SGSGYPMDYLAI	25
	Kabat CDR1	SYGVS	26
	Kabat CDR2	AVNSGGSAYYADSVKG	51
	Kabat CDR3	SGSGYPMDYLAI	25
	Contact CDR1	SSYGVS	28
	Contact CDR2	WIGAVNSGGSAY	29
	Contact CDR3	ARSGSGYPMDYLA	30
	AbM CDR1	GFSLSSYGVS	31
	AbM CDR2	AVNSGGSAY	32
	AbM CDR3	SGSGYPMDYLAI	25
22878	Full	QQLLESGGGLFQPGGSLRLSCAVSGFSLSSYGVSWVRQAPGK GLEWIGAVNSGGSAYYADSVKGRSTISRDTSKFTVYLQMNSL RAEDTAVYYCARSGSGYPMDYLAIWGQGTLVTVSSASTKGP SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSN TKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR EEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSD IAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLSPG	86

Clone ID	Region	Sequence	SEQ ID NO
	Full	CAGCAGCTGCTGGAGAGCGGCGGCGGCCTGTTCCAGCCTG GAGGCAGCCTGAGACTGTCCTGCGCCGTGAGCGGCTTTTC CCTGAGCTCCTACGGCGTGTCCTGGGTGAGGCAGGCCCCC GGCAAGGGCCTGGAGTGGATCGGAGCCGTGAACTCTGGCG GCAGCGCCTACTATGCCGACTCTGTGAAGGGCCGGTCCAC CATCTCTAGAGATACCTCTAAGTTTACAGTGTATCTGCAGA TGAATAGCCTGAGGGCCGAGGACACAGCCGTGTACTATTG TGCACGCTCCGGCTCTGGCTACCCCATGGATTATCTGGCCA TCTGGGGACAGGGCACCCTGGTGACAGTGTCTAGCGCTAG CACTAAGGGGCCTTCCGTGTTTCCACTGGCTCCCTCTAGTA AATCCACCTCTGGAGGCACAGCTGCACTGGGATGTCTGGT GAAGGATTACTTCCCTGAACCAGTCACAGTGAGTTGGAAC TCAGGGGCTCTGACAAGTGGAGTCCATACTTTTCCCGCAGT GCTGCAGTCAAGCGGACTGTACTCCCTGTCCTCTGTGGTCA CCGTGCCTAGTTCAAGCCTGGGCACCCAGACATATATCTG CAACGTGAATCACAAGCCATCAAATACAAAAGTCGACAAG AAAGTGGAGCCCAAGAGCTGTGATAAAACTCATACCTGCC CACCTTGTCCGGCGCCAGAACTGCTGGGAGGACCAAGCGT GTTCCTGTTTCCACCCAAGCCTAAAGACACCCTGATGATTT CCCGGACTCCTGAGGTCACCTGCGTGGTCGTGGACGTGTCT CACGAGGACCCCGAAGTCAAGTTCAACTGGTACGTGGATG GCGTCGAAGTGCATAATGCCAAGACCAAACCCCGGGAGG AACAGTACAACTCTACCTATAGAGTCGTGAGTGTCCTGAC AGTGCTGCACCAGGACTGGCTGAATGGGAAGGAGTATAAG TGTAAAGTGAGCAACAAAGCCCTGCCCGCCCCAATCGAAA AAACAATCTCTAAAGCAAAAGGACAGCCTCGCGAACCACA GGTGTACACTCTGCCTCCATCTCGGGACGAGCTGACTAAG AACCAGGTCAGTCTGACCTGTCTGGTGAAAGGATTCTATC CCAGCGATATCGCTGTGGAGTGGGAATCCAATGGCCAGCC TGAGAACAATTACAAGACCACACCCCCTGTGCTGGACTCT GATGGCAGTTTCTTTCTGTATAGTAAGCTGACCGTCGATAA ATCACGATGGCAGCAGGGGAACGTGTTCAGCTGTTCAGTG ATGCACGAAGCCCTGCACAACCATTACACCCAGAAGAGCC TGAGCCTGTCTCCCGGC	87
	VH	QQLLESGGGLFQPGGSLRLSCAVSGFSLSSYGVSWVRQAPGK GLEWIGAVNSGGSAYYADSVKGRSTISRDTSKFTVYLQMNSL RAEDTAVYYCARSGSGYPMDYLAIWGQGTLVTVSS	88
	IMGT CDR1	GFSLSSYG	20
	IMGT CDR2	VNSGGSA	21
	IMGT CDR3	ARSGSGYPMDYLAI	22
	Chothia CDR1	GFSLSSY	23
	Chothia CDR2	NSGGS	24
	Chothia CDR3	SGSGYPMDYLAI	25
	Kabat CDR1	SYGVS	26
	Kabat CDR2	AVNSGGSAYYADSVKG	51

Clone ID	Region	Sequence	SEQ ID NO
	Kabat CDR3	SGSGYPMDYLAI	25
	Contact CDR1	SSYGVS	28
	Contact CDR2	WIGAVNSGGSAY	29
	Contact CDR3	ARSGSGYPMDYLA	30
	AbM CDR1	GFSLSSYGVS	31
	AbM CDR2	AVNSGGSAY	32
	AbM CDR3	SGSGYPMDYLAI	25
26434	Full	EVQLLESGGGLVQPGGSLRLSCAVSGFSLSLYGVSWVRQAPG KGLEWIGAVNSGGSAYYADSVKGRSTISRDNSKNTVYLQMN SLRAEDTAVYYCARSGSGYPMDYLAIWGQGTLVTVSSASTK GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKP SNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP IEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYP SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPG	89
	Full	GAGGTCCAGTTGCTGGAATCAGGAGGTGGACTCGTGCAGC CTGGGGGTTCCCTGCGCTTGTCTTGCGCTGTCAGCGGTTTT AGCTTATCTTTATACGGTGTAAGTTGGGTTCGGCAGGCTCC TGGCAAAGGTCTGGAATGGATCGGGGCTGTAAACAGCGGG GGGTCCGCCTACTATGCTGATTCTGTTAAGGGGAGGTCCA CCATTAGCAGAGATAATTCCAAGAACACCGTTTATCTCCA GATGAACTCCTTGAGGGCGGAGGACACTGCTGTCTATTAT TGTGCGCGGTCAGGCAGCGGATACCCGATGGACTATCTGG CGATCTGGGGCCAGGGAACCCTTGTGACAGTCTCTTCCGCT AGCACCAAGGGACCTAGCGTATTTCCACTGGCTCCCTCTA GTAAATCCACCTCTGGAGGCACAGCTGCACTGGGATGTCT GGTGAAGGATTACTTCCCTGAACCAGTCACAGTGAGTTGG AACTCAGGGGCTCTGACAAGTGGAGTCCATACTTTTCCCG CAGTGCTGCAGTCAAGCGGACTGTACTCCCTGTCCTCTGTG GTCACCGTGCCTAGTTCAAGCCTGGGCACCCAGACATATA TCTGCAACGTGAATCACAAGCCATCAAATACAAAAGTCGA CAAGAAAGTGGAGCCCAAGAGCTGTGATAAAACTCATACC TGCCCACCTTGTCCGGCTCCAGAGCTGCTCGGCGGACCATC CGTGTTCCTGTTTCCTCCAAAGCCTAAAGATACACTGATGA TTAGCCGCACTCCCGAAGTCACCTGTGTGGTCGTGGACGT GTCCCACGAGGACCCCGAAGTCAAGTTCAACTGGTACGTG GACGGCGTCGAGGTGCATAATGCCAAGACTAAACCAAGA GAGGAACAGTACAATTCAACCTATAGGGTCGTGAGCGTCC TGACAGTGCTGCATCAGGATTGGCTGAACGGCAAGGAGTA TAAGTGCAAAGTGTCTAACAAGGCCCTGCCCGCTCCTATC GAGAAGACTATTAGCAAGGCAAAAGGGCAGCCACGGGAA CCCCAGGTCTACACTCTGCCCCCTAGCAGAGACGAGCTGA CCAAAAACCAGGTGTCCCTGACTTGTCTGGTGAAGGGCTT	90

Clone ID	Region	Sequence	SEQ ID NO
		TTATCCTAGTGATATCGCTGTGGAGTGGGAATCAAATGGG CAGCCAGAAAACAATTACAAAACAACTCCACCCGTGCTGG ACAGCGATGGGTCCTTCTTTCTGTATTCCAAACTGACTGTG GACAAGTCCAGATGGCAGCAGGGAAACGTCTTCAGCTGTT CCGTGATGCACGAGGCCCTGCACAATCATTACACCCAGAA GTCTCTGAGTCTGTCACCCGGC	
	VH	EVQLLESGGGLVQPGGSLRLSCAVSGFSLSLYGVSWVRQAPG KGLEWIGAVNSGGSAYYADSVKGRSTISRDNSKNTVYLQMN SLRAEDTAVYYCARSGSGYPMDYLAIWGQGTLVTVSS	91
	IMGT CDR1	GFSLSLYG	92
	IMGT CDR2	VNSGGSA	21
	IMGT CDR3	ARSGSGYPMDYLAI	22
	Chothia CDR1	GFSLSLY	93
	Chothia CDR2	NSGGS	24
	Chothia CDR3	SGSGYPMDYLAI	25
	Kabat CDR1	LYGVS	94
	Kabat CDR2	AVNSGGSAYYADSVKG	51
	Kabat CDR3	SGSGYPMDYLAI	25
	Contact CDR1	SLYGVS	95
	Contact CDR2	WIGAVNSGGSAY	29
	Contact CDR3	ARSGSGYPMDYLA	30
	AbM CDR1	GFSLSLYGVS	96
	AbM CDR2	AVNSGGSAY	32
	AbM CDR3	SGSGYPMDYLAI	25
26443	Full	EVQLLESGGGLVQPGGSLRLSCAVSGFSLSLYGVSWVRQAPG KGLEWIGSLNSGGSAYYADSVKGRSTISRDNSKNTVYLQMN SLRAEDTAVYYCARSGSGYPMDYLAIWGQGTLVTVSSASTK GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKP SNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP IEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYP SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPG	97

Clone ID	Region	Sequence	SEQ ID NO
	Full	GAGGTTCAGTTGCTTGAGTCTGGAGGTGGGTTGGTACAAC CAGGTGGATCTCTCCGCCTTTCTTGTGCAGTCTCAGGCTTC TCTCTCAGCCTGTACGGCGTGTCATGGGTTAGACAAGCTCC GGGTAAAGGCCTTGAGTGGATTGGAAGTCTTAATAGTGGG GGAAGCGCCTACTATGCAGATAGCGTAAAAGGAAGATCA ACTATATCCAGAGACAATTCTAAGAATACCGTCTACTTGC AGATGAACTCTCTGCGGGCAGAGGATACTGCGGTTTACTA TTGTGCGCGAAGCGGTAGTGGATACCCTATGGATTATCTTG CTATTTGGGGTCAAGGTACCCTCGTAACGGTCTCTAGTGCT AGCACCAAGGGACCTAGCGTATTTCCACTGGCTCCCTCTA GTAAATCCACCTCTGGAGGCACAGCTGCACTGGGATGTCT GGTGAAGGATTACTTCCCTGAACCAGTCACAGTGAGTTGG AACTCAGGGGCTCTGACAAGTGGAGTCCATACTTTTCCCG CAGTGCTGCAGTCAAGCGGACTGTACTCCCTGTCCTCTGTG GTCACCGTGCCTAGTTCAAGCCTGGGCACCCAGACATATA TCTGCAACGTGAATCACAAGCCATCAAATACAAAAGTCGA CAAGAAAGTGGAGCCCAAGAGCTGTGATAAAACTCATACC TGCCCACCTTGTCCGGCTCCAGAGCTGCTCGGCGGACCATC CGTGTTCCTGTTTCCTCCAAAGCCTAAAGATACACTGATGA TTAGCCGCACTCCCGAAGTCACCTGTGTGGTCGTGGACGT GTCCCACGAGGACCCCGAAGTCAAGTTCAACTGGTACGTG GACGGCGTCGAGGTGCATAATGCCAAGACTAAACCAAGA GAGGAACAGTACAATTCAACCTATAGGGTCGTGAGCGTCC TGACAGTGCTGCATCAGGATTGGCTGAACGGCAAGGAGTA TAAGTGCAAAGTGTCTAACAAGGCCCTGCCCGCTCCTATC GAGAAGACTATTAGCAAGGCAAAAGGGCAGCCACGGGAA CCCCAGGTCTACACTCTGCCCCCTAGCAGAGACGAGCTGA CCAAAAACCAGGTGTCCCTGACTTGTCTGGTGAAGGGCTT TTATCCTAGTGATATCGCTGTGGAGTGGGAATCAAATGGG CAGCCAGAAAACAATTACAAAACAACTCCACCCGTGCTGG ACAGCGATGGGTCCTTCTTTCTGTATTCCAAACTGACTGTG GACAAGTCCAGATGGCAGCAGGGAAACGTCTTCAGCTGTT CCGTGATGCACGAGGCCCTGCACAATCATTACACCCAGAA GTCTCTGAGTCTGTCACCCGGC	98
	VH	EVQLLESGGGLVQPGGSLRLSCAVSGFSLSLYGVSWVRQAPG KGLEWIGSLNSGGSAYYADSVKGRSTISRDNSKNTVYLQMN SLRAEDTAVYYCARSGSGYPMDYLAIWGQGTLVTVSS	99
	IMGT CDR1	GFSLSLYG	92
	IMGT CDR2	LNSGGSA	100
	IMGT CDR3	ARSGSGYPMDYLAI	22
	Chothia CDR1	GFSLSLY	93
	Chothia CDR2	NSGGS	24
	Chothia CDR3	SGSGYPMDYLAI	25
	Kabat CDR1	LYGVS	94
	Kabat CDR2	SLNSGGSAYYADSVKG	101

Clone ID	Region	Sequence	SEQ ID NO
	Kabat CDR3	SGSGYPMDYLAI	25
	Contact CDR1	SLYGVS	95
	Contact CDR2	WIGSLNSGGSAY	102
	Contact CDR3	ARSGSGYPMDYLA	30
	AbM CDR1	GFSLSLYGVS	96
	AbM CDR2	SLNSGGSAY	103
	AbM CDR3	SGSGYPMDYLAI	25
26445	Full	EVQLLESGGGLVQPGGSLRLSCAVSGFSLSLYGVSWVRQAPG KGLEWIGAVNSGGSAYYADSFKGRSTISRDNSKNTVYLQMN SLRAEDTAVYYCARSGSGYPMDYHPIWGQGTLVTVSSASTK GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKP SNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP IEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYP SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPG	104
	Full	GAGGTCCAGCTCTTGGAGTCAGGAGGCGGCCTGGTTCAGC CGGGGGGTTCACTCAGACTTAGTTGTGCGGTAAGCGGGTT CAGCCTTAGTCTGTATGGCGTATCATGGGTCCGGCAAGCG CCTGGTAAGGGCCTGGAGTGGATAGGTGCTGTCAATAGCG GTGGCTCCGCATACTACGCGGACTCTTTTAAGGGCAGATC CACCATCAGCCGGGACAACAGCAAGAACACTGTATATCTT CAGATGAACTCACTCAGGGCGGAGGATACTGCGGTATACT ACTGTGCACGAAGTGGTAGTGGTTACCCAATGGATTACCA TCCCATTTGGGGGCAAGGGACACTCGTTACAGTGAGTAGT GCTAGCACCAAGGGACCTAGCGTATTTCCACTGGCTCCCTC TAGTAAATCCACCTCTGGAGGCACAGCTGCACTGGGATGT CTGGTGAAGGATTACTTCCCTGAACCAGTCACAGTGAGTT GGAACTCAGGGGCTCTGACAAGTGGAGTCCATACTTTTCC CGCAGTGCTGCAGTCAAGCGGACTGTACTCCCTGTCCTCTG TGGTCACCGTGCCTAGTTCAAGCCTGGGCACCCAGACATA TATCTGCAACGTGAATCACAAGCCATCAAATACAAAAGTC GACAAGAAAGTGGAGCCCAAGAGCTGTGATAAAACTCAT ACCTGCCCACCTTGTCCGGCTCCAGAGCTGCTCGGCGGAC CATCCGTGTTCCTGTTTCCTCCAAAGCCTAAAGATACACTG ATGATTAGCCGCACTCCCGAAGTCACCTGTGTGGTCGTGG ACGTGTCCCACGAGGACCCCGAAGTCAAGTTCAACTGGTA CGTGGACGGCGTCGAGGTGCATAATGCCAAGACTAAACCA AGAGAGGAACAGTACAATTCAACCTATAGGGTCGTGAGCG TCCTGACAGTGCTGCATCAGGATTGGCTGAACGGCAAGGA GTATAAGTGCAAAGTGTCTAACAAGGCCCTGCCCGCTCCT ATCGAGAAGACTATTAGCAAGGCAAAAGGGCAGCCACGG GAACCCCAGGTCTACACTCTGCCCCCTAGCAGAGACGAGC TGACCAAAAACCAGGTGTCCCTGACTTGTCTGGTGAAGGG	105

Clone ID	Region	Sequence	SEQ ID NO
		CTTTTATCCTAGTGATATCGCTGTGGAGTGGGAATCAAATG GGCAGCCAGAAAACAATTACAAAACAACTCCACCCGTGCT GGACAGCGATGGGTCCTTCTTTCTGTATTCCAAACTGACTG TGGACAAGTCCAGATGGCAGCAGGGAAACGTCTTCAGCTG TTCCGTGATGCACGAGGCCCTGCACAATCATTACACCCAG AAGTCTCTGAGTCTGTCACCCGGC	
	VH	EVQLLESGGGLVQPGGSLRLSCAVSGFSLSLYGVSWVRQAPG KGLEWIGAVNSGGSAYYADSFKGRSTISRDNSKNTVYLQMN SLRAEDTAVYYCARSGSGYPMDYHPIWGQGTLVTVSS	106
	IMGT CDR1	GFSLSLYG	92
	IMGT CDR2	VNSGGSA	21
	IMGT CDR3	ARSGSGYPMDYHPI	107
	Chothia CDR1	GFSLSLY	93
	Chothia CDR2	NSGGS	24
	Chothia CDR3	SGSGYPMDYHPI	108
	Kabat CDR1	LYGVS	94
	Kabat CDR2	AVNSGGSAYYADSFKG	109
	Kabat CDR3	SGSGYPMDYHPI	108
	Contact CDR1	SLYGVS	95
	Contact CDR2	WIGAVNSGGSAY	29
	Contact CDR3	ARSGSGYPMDYHP	110
	AbM CDR1	GFSLSLYGVS	96
	AbM CDR2	AVNSGGSAY	32
	AbM CDR3	SGSGYPMDYHPI	108
26446	Full	EVQLLESGGGLVQPGGSLRLSCAVSGFSLSLYGVSWVRQAPG KGLEWIGAVNSGGSAYYADSFKGRSTISRDNSKNTVYLQMN SLRAEDTAVYYCARSGSGYPMDYLAIWGQGTLVTVSSASTK GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKP SNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP IEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYP SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPG	111

Clone ID	Region	Sequence	SEQ ID NO
	Full	GAGGTACAGCTTCTGGAAAGTGGTGGCGGTCTTGTCCAGC CTGGAGGAAGTCTCAGGTTGTCATGTGCAGTCTCTGGTTTT TCCCTCTCATTGTACGGCGTGAGTTGGGTTAGACAAGCGCC AGGTAAGGGGTTGGAGTGGATCGGGGCGGTCAATTCCGGC GGTTCTGCCTATTACGCCGACTCATTCAAAGGCCGCTCCAC GATTAGTCGAGATAATTCCAAAAATACTGTGTATCTCCAA ATGAATAGTCTCCGCGCGGAGGACACAGCTGTTTATTATT GTGCCCGGTCCGGAAGTGGCTATCCAATGGACTATCTCGC CATCTGGGGTCAGGGCACGTTGGTAACGGTGTCCTCCGCT AGCACCAAGGGACCTAGCGTATTTCCACTGGCTCCCTCTA GTAAATCCACCTCTGGAGGCACAGCTGCACTGGGATGTCT GGTGAAGGATTACTTCCCTGAACCAGTCACAGTGAGTTGG AACTCAGGGGCTCTGACAAGTGGAGTCCATACTTTTCCCG CAGTGCTGCAGTCAAGCGGACTGTACTCCCTGTCCTCTGTG GTCACCGTGCCTAGTTCAAGCCTGGGCACCCAGACATATA TCTGCAACGTGAATCACAAGCCATCAAATACAAAAGTCGA CAAGAAAGTGGAGCCCAAGAGCTGTGATAAAACTCATACC TGCCCACCTTGTCCGGCTCCAGAGCTGCTCGGCGGACCATC CGTGTTCCTGTTTCCTCCAAAGCCTAAAGATACACTGATGA TTAGCCGCACTCCCGAAGTCACCTGTGTGGTCGTGGACGT GTCCCACGAGGACCCCGAAGTCAAGTTCAACTGGTACGTG GACGGCGTCGAGGTGCATAATGCCAAGACTAAACCAAGA GAGGAACAGTACAATTCAACCTATAGGGTCGTGAGCGTCC TGACAGTGCTGCATCAGGATTGGCTGAACGGCAAGGAGTA TAAGTGCAAAGTGTCTAACAAGGCCCTGCCCGCTCCTATC GAGAAGACTATTAGCAAGGCAAAAGGGCAGCCACGGGAA CCCCAGGTCTACACTCTGCCCCCTAGCAGAGACGAGCTGA CCAAAAACCAGGTGTCCCTGACTTGTCTGGTGAAGGGCTT TTATCCTAGTGATATCGCTGTGGAGTGGGAATCAAATGGG CAGCCAGAAAACAATTACAAAACAACTCCACCCGTGCTGG ACAGCGATGGGTCCTTCTTTCTGTATTCCAAACTGACTGTG GACAAGTCCAGATGGCAGCAGGGAAACGTCTTCAGCTGTT CCGTGATGCACGAGGCCCTGCACAATCATTACACCCAGAA GTCTCTGAGTCTGTCACCCGGC	112
	VH	EVQLLESGGGLVQPGGSLRLSCAVSGFSLSLYGVSWVRQAPG KGLEWIGAVNSGGSAYYADSFKGRSTISRDNSKNTVYLQMN SLRAEDTAVYYCARSGSGYPMDYLAIWGQGTLVTVSS	113
	IMGT CDR1	GFSLSLYG	92
	IMGT CDR2	VNSGGSA	21
	IMGT CDR3	ARSGSGYPMDYLAI	22
	Chothia CDR1	GFSLSLY	93
	Chothia CDR2	NSGGS	24
	Chothia CDR3	SGSGYPMDYLAI	25
	Kabat CDR1	LYGVS	94
	Kabat CDR2	AVNSGGSAYYADSFKG	109

Clone ID	Region	Sequence	SEQ ID NO
	Kabat CDR3	SGSGYPMDYLAI	25
	Contact CDR1	SLYGVS	95
	Contact CDR2	WIGAVNSGGSAY	29
	Contact CDR3	ARSGSGYPMDYLA	30
	AbM CDR1	GFSLSLYGVS	96
	AbM CDR2	AVNSGGSAY	32
	AbM CDR3	SGSGYPMDYLAI	25
26447	Full	EVQLLESGGGLVQPGGSLRLSCAVSGFSLSSYGVSWVRQAPG KGLEWIGAVNSGGSAYYADSFKGRSTISRDNSKNTVYLQMN SLRAEDTAVYYCARSGSGYPMDYHPIWGQGTLVTVSSASTK GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKP SNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP IEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYP SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPG	114
	Full	GAGGTGCAACTGTTGGAATCCGGAGGTGGTTTGGTGCAGC CTGGCGGTAGTCTGCGGCTTAGTTGCGCTGTTTCAGGCTTT TCTCTCTCATCATACGGGGTATCATGGGTGAGACAGGCAC CAGGAAAGGGTCTTGAATGGATTGGGGCCGTAAATTCCGG AGGTAGTGCATACTATGCTGACTCATTCAAGGGCAGGTCC ACCATTAGTCGAGATAACTCAAAGAACACGGTTTATCTGC AGATGAATAGCCTTAGAGCGGAGGACACGGCAGTATATTA TTGCGCGAGAAGCGGGTCTGGATATCCGATGGACTACCAT CCAATCTGGGGCCAGGGAACACTCGTAACGGTGAGCAGCG CTAGCACCAAGGGACCTAGCGTATTTCCACTGGCTCCCTCT AGTAAATCCACCTCTGGAGGCACAGCTGCACTGGGATGTC TGGTGAAGGATTACTTCCCTGAACCAGTCACAGTGAGTTG GAACTCAGGGGCTCTGACAAGTGGAGTCCATACTTTTCCC GCAGTGCTGCAGTCAAGCGGACTGTACTCCCTGTCCTCTGT GGTCACCGTGCCTAGTTCAAGCCTGGGCACCCAGACATAT ATCTGCAACGTGAATCACAAGCCATCAAATACAAAAGTCG ACAAGAAAGTGGAGCCCAAGAGCTGTGATAAAACTCATAC CTGCCCACCTTGTCCGGCTCCAGAGCTGCTCGGCGGACCAT CCGTGTTCCTGTTTCCTCCAAAGCCTAAAGATACACTGATG ATTAGCCGCACTCCCGAAGTCACCTGTGTGGTCGTGGACG TGTCCCACGAGGACCCCGAAGTCAAGTTCAACTGGTACGT GGACGGCGTCGAGGTGCATAATGCCAAGACTAAACCAAG AGAGGAACAGTACAATTCAACCTATAGGGTCGTGAGCGTC CTGACAGTGCTGCATCAGGATTGGCTGAACGGCAAGGAGT ATAAGTGCAAAGTGTCTAACAAGGCCCTGCCCGCTCCTAT CGAGAAGACTATTAGCAAGGCAAAAGGGCAGCCACGGGA ACCCCAGGTCTACACTCTGCCCCCTAGCAGAGACGAGCTG ACCAAAAACCAGGTGTCCCTGACTTGTCTGGTGAAGGGCT	115

Clone ID	Region	Sequence	SEQ ID NO
		TTTATCCTAGTGATATCGCTGTGGAGTGGGAATCAAATGG GCAGCCAGAAAACAATTACAAAACAACTCCACCCGTGCTG GACAGCGATGGGTCCTTCTTTCTGTATTCCAAACTGACTGT GGACAAGTCCAGATGGCAGCAGGGAAACGTCTTCAGCTGT TCCGTGATGCACGAGGCCCTGCACAATCATTACACCCAGA AGTCTCTGAGTCTGTCACCCGGC	
	VH	EVQLLESGGGLVQPGGSLRLSCAVSGFSLSSYGVSWVRQAPG KGLEWIGAVNSGGSAYYADSFKGRSTISRDNSKNTVYLQMN SLRAEDTAVYYCARSGSGYPMDYHPIWGQGTLVTVSS	116
	IMGT CDR1	GFSLSSYG	20
	IMGT CDR2	VNSGGSA	21
	IMGT CDR3	ARSGSGYPMDYHPI	107
	Chothia CDR1	GFSLSSY	23
	Chothia CDR2	NSGGS	24
	Chothia CDR3	SGSGYPMDYHPI	108
	Kabat CDR1	SYGVS	26
	Kabat CDR2	AVNSGGSAYYADSFKG	109
	Kabat CDR3	SGSGYPMDYHPI	108
	Contact CDR1	SSYGVS	28
	Contact CDR2	WIGAVNSGGSAY	29
	Contact CDR3	ARSGSGYPMDYHP	110
	AbM CDR1	GFSLSSYGVS	31
	AbM CDR2	AVNSGGSAY	32
	AbM CDR3	SGSGYPMDYHPI	108
26448	Full	DYQMTQSPSSVSASVGDRVTITCRASQSIGDWLAWYQQKPG KAPKLLIYEASTLASGVPSRFSGSGSGTDFTLTISSVQPEDAAT YYCQQGYGRWHILNIFGGGTKVEVKRTVAAPSVFIFPPSDEQ LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTE QDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTK SFNRGEC	117
	Full	GACTATCAAATGACGCAGTCCCCGTCCTCCGTTTCCGCCTC AGTTGGTGACCGGGTTACAATCACTTGCAGAGCATCTCAG TCTATTGGTGATTGGCTGGCGTGGTATCAACAGAAGCCCG GCAAAGCCCCTAAACTCCTGATATACGAAGCATCAACGCT TGCTTCTGGAGTACCCTCCCGGTTTTCTGGGTCAGGAAGCG GCACGGATTTTACCCTGACTATCTCTTCTGTTCAGCCTGAG GATGCCGCAACCTATTATTGCCAACAGGGGTATGGCAGAT GGCATATCCTAAATATTTTCGGTGGCGGGACCAAAGTTGA GGTTAAACGTACGGTAGCAGCTCCCAGCGTATTTATTTTTC CCCCTAGCGACGAACAGCTGAAGTCTGGGACAGCCAGTGT GGTCTGTCTGCTGAACAACTTCTACCCTAGAGAGGCTAAA GTGCAGTGGAAGGTCGATAACGCACTGCAGTCCGGAAATT	118

Clone ID	Region	Sequence	SEQ ID NO
		CTCAGGAGAGTGTGACTGAACAGGACTCAAAAGATAGCAC CTATTCCCTGTCAAGCACACTGACTCTGAGCAAGGCCGAC TACGAGAAGCATAAAGTGTATGCTTGTGAAGTCACCCACC AGGGGCTGAGTTCACCAGTCACAAAATCATTCAACAGAGG GGAGTGC	
	VL	DYQMTQSPSSVSASVGDRVTITCRASQSIGDWLAWYQQKPG KAPKLLIYEASTLASGVPSRFSGSGSGTDFTLTISSVQPEDAAT YYCQQGYGRWHILNIFGGGTKVEVK	119
	IMGT CDR1	QSIGDW	40
	IMGT CDR2	EAS	41
	IMGT CDR3	QQGYGRWHILNI	120
	Chothia CDR1	RASQSIGDWLA	65
	Chothia CDR2	EASTLAS	44
	Chothia CDR3	QQGYGRWHILNI	120
	Kabat CDR1	RASQSIGDWLA	65
	Kabat CDR2	EASTLAS	44
	Kabat CDR3	QQGYGRWHILNI	120
	Contact CDR1	GDWLAWY	45
	Contact CDR2	LLIYEASTLA	46
	Contact CDR3	QQGYGRWHILN	121
	AbM CDR1	RASQSIGDWLA	65
	AbM CDR2	EASTLAS	44
	AbM CDR3	QQGYGRWHILNI	120
26449	Full	DYQMTQSPSSVSASVGDRVTITCRASQSIWYWLAWYQQKPG KAPKLLIYEASTLASGVPSRFSGSGSGTDFTLTISSVQPEDAAT YYCQQGYGRSNVDNIFGGGTKVEVKRTVAAPSVFIFPPSDEQ LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTE QDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTK SFNRGEC	122
	Full	GACTATCAAATGACGCAGTCCCCGTCCTCCGTTTCCGCCTC AGTTGGTGACCGGGTTACAATCACTTGCAGAGCATCTCAG TCTATTTGGTATTGGCTGGCGTGGTATCAACAGAAGCCCG GCAAAGCCCCTAAACTCCTGATATACGAAGCATCAACGCT TGCTTCTGGAGTACCCTCCCGGTTTTCTGGGTCAGGAAGCG GCACGGATTTTACCCTGACTATCTCTTCTGTTCAGCCTGAG GATGCCGCAACCTATTATTGCCAACAGGGGTATGGCAGAT CAAACGTGGATAATATTTTCGGTGGCGGGACCAAAGTTGA GGTTAAACGTACGGTAGCAGCTCCCAGCGTATTTATTTTC CCCCTAGCGACGAACAGCTGAAGTCTGGGACAGCCAGTGT GGTCTGTCTGCTGAACAACTTCTACCCTAGAGAGGCTAAA GTGCAGTGGAAGGTCGATAACGCACTGCAGTCCGGAAATT	123

Clone ID	Region	Sequence	SEQ ID NO
		CTCAGGAGAGTGTGACTGAACAGGACTCAAAAGATAGCAC CTATTCCCTGTCAAGCACACTGACTCTGAGCAAGGCCGAC TACGAGAAGCATAAAGTGTATGCTTGTGAAGTCACCCACC AGGGGCTGAGTTCACCAGTCACAAAATCATTCAACAGAGG GGAGTGC	
	VL	DYQMTQSPSSVSASVGDRVTITCRASQSIWYWLAWYQQKPG KAPKLLIYEASTLASGVPSRFSGSGSGTDFTLTISSVQPEDAAT YYCQQGYGRSNVDNIFGGGTKVEVK	124
	IMGT CDR1	QSIWYW	125
	IMGT CDR2	EAS	41
	IMGT CDR3	QQGYGRSNVDNI	42
	Chothia CDR1	RASQSIWYWLA	126
	Chothia CDR2	EASTLAS	44
	Chothia CDR3	QQGYGRSNVDNI	42
	Kabat CDR1	RASQSIWYWLA	126
	Kabat CDR2	EASTLAS	44
	Kabat CDR3	QQGYGRSNVDNI	42
	Contact CDR1	WYWLAWY	127
	Contact CDR2	LLIYEASTLA	46
	Contact CDR3	QQGYGRSNVDN	47
	AbM CDR1	RASQSIWYWLA	126
	AbM CDR2	EASTLAS	44
	AbM CDR3	QQGYGRSNVDNI	42
26450	Full	DYQMTQSPSSVSASVGDRVTITCRASQSIWYWLAWYQQKPG KAPKLLIYEASTLASGVPSRFSGSGSGTDFTLTISSVQPEDAAT YYCQQGYGRWHILNIFGGGTKVEVKRTVAAPSVFIFPPSDEQ LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTE QDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTK SFNRGEC	128
	Full	GATTATCAGATGACACAGAGTCCTTCCTCAGTCAGCGCAT CTGTGGGAGATCGGGTTACAATTACCTGCCGAGCATCTCA ATCAATCTGGTATTGGCTGGCCTGGTACCAGCAAAAACCA GGAAAAGCACCTAAACTGTTGATTTATGAGGCCAGCACTC TTGCTAGTGGGGTCCCAAGTAGGTTCAGCGGCAGCGGTTC AGGGACGGATTTTACCTTGACCATAAGTTCCGTTCAGCCA GAAGACGCGGCGACCTATTATTGCCAACAAGGGTACGGTA GATGGCACATTTTGAACATATTCGGTGGAGGTACTAAGGT CGAGGTGAAGCGTACGGTAGCAGCTCCCAGCGTATTTATT TTTCCCCCTAGCGACGAACAGCTGAAGTCTGGGACAGCCA GTGTGGTCTGTCTGCTGAACAACTTCTACCCTAGAGAGGCT AAAGTGCAGTGGAAGGTCGATAACGCACTGCAGTCCGGAA	129

Clone ID	Region	Sequence	SEQ ID NO
		ATTCTCAGGAGAGTGTGACTGAACAGGACTCAAAAGATAG CACCTATTCCCTGTCAAGCACACTGACTCTGAGCAAGGCC GACTACGAGAAGCATAAAGTGTATGCTTGTGAAGTCACCC ACCAGGGGCTGAGTTCACCAGTCACAAAATCATTCAACAG AGGGGAGTGC	
	VL	DYQMTQSPSSVSASVGDRVTITCRASQSIWYWLAWYQQKPG KAPKLLIYEASTLASGVPSRFSGSGSGTDFTLTISSVQPEDAAT YYCQQGYGRWHILNIFGGGTKVEVK	130
	IMGT CDR1	QSIWYW	125
	IMGT CDR2	EAS	41
	IMGT CDR3	QQGYGRWHILNI	120
	Chothia CDR1	RASQSIWYWLA	126
	Chothia CDR2	EASTLAS	44
	Chothia CDR3	QQGYGRWHILNI	120
	Kabat CDR1	RASQSIWYWLA	126
	Kabat CDR2	EASTLAS	44
	Kabat CDR3	QQGYGRWHILNI	120
	Contact CDR1	WYWLAWY	127
	Contact CDR2	LLIYEASTLA	46
	Contact CDR3	QQGYGRWHILN	121
	AbM CDR1	RASQSIWYWLA	126
	AbM CDR2	EASTLAS	44
	AbM CDR3	QQGYGRWHILNI	120
27272	Full	EVQLLESGGGLVQPGGSLRLSCAVSGFSLSSYGVSWVRQAPG KGLEWIGAVNSGGSAYYADSFKGRSTISRDNSKNTVYLQMN SLRAEDTAVYYCARSGSGYPMDYLAIWGQGTLVTVSSASTK GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKP SNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP IEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYP SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	131

Clone ID	Region	Sequence	SEQ ID NO
	Full	GAAGTGCAGCTCCTGGAGAGTGGAGGCGGCCTAGTCCAAC CCGGTGGATCTCTGAGGCTCTCCTGTGCTGTTAGTGGTTTT TCACTCTCTTCCTATGGAGTCAGCTGGGTTAGACAGGCACC CGGCAAGGGCTTGGAGTGGATCGGCGCTGTTAATTCCGGC GGTTCCGCCTACTATGCTGACTCTTTCAAAGGAAGAAGTA CTATTTCCCGCGACAACTCTAAGAACACAGTGTATCTCCAA ATGAACTCCCTGCGTGCCGAGGACACCGCCGTCTACTACT GCGCCCGGAGCGGCTCTGGCTATCCAATGGATTACCTGGC CATCTGGGGCCAAGGTACCCTGGTGACAGTCTCAAGCGCT AGCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTC CAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTG GTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGA ACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGC CGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGG TGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACAT CTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGAC AAGAAGGTTGAGCCCAAATCTTGTGACAAAACTCACACAT GCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTC AGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGA TCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGT GAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTG GACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGG GAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCC TCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTA CAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATC GAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAA CCACAGGTGTACACCCTGCCCCCATCCCGGGACGAGCTGA CCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTT CTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGG CAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGG ACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTG GACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCT CCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAA GAGCCTCTCCCTGTCTCCGGGCAAA	132
	VH	EVQLLESGGGLVQPGGSLRLSCAVSGFSLSSYGVSWVRQAPG KGLEWIGAVNSGGSAYYADSFKGRSTISRDNSKNTVYLQMN SLRAEDTAVYYCARSGSGYPMDYLAIWGQGTLVTVSS	133
	IMGT CDR1	GFSLSSYG	20
	IMGT CDR2	VNSGGSA	21
	IMGT CDR3	ARSGSGYPMDYLAI	22
	Chothia CDR1	GFSLSSY	23
	Chothia CDR2	NSGGS	24
	Chothia CDR3	SGSGYPMDYLAI	25
	Kabat CDR1	SYGVS	26
	Kabat CDR2	AVNSGGSAYYADSFKG	109

Clone ID	Region	Sequence	SEQ ID NO
	Kabat CDR3	SGSGYPMDYLAI	25
	Contact CDR1	SSYGVS	28
	Contact CDR2	WIGAVNSGGSAY	29
	Contact CDR3	ARSGSGYPMDYLA	30
	AbM CDR1	GFSLSSYGVS	31
	AbM CDR2	AVNSGGSAY	32
	AbM CDR3	SGSGYPMDYLAI	25
27273	Full	EVQLLESGGGLVQPGGSLRLSCAVSGFSLSLYGVSWVRQAPG KGLEWIGSVNSGGSAYYADSVKGRSTISRDNSKNTVYLQMN SLRAEDTAVYYCARSGSGYPMDYLAIWGQGTLVTVSSASTK GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKP SNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP IEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYP SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	134
	Full	GAGGTTCAGCTTCTCGAGTCTGGCGGTGGCCTGGTTCAACC TGGTGGTAGTCTCCGACTGAGCTGTGCTGTTTCGGGCTTCA GCCTGAGCCTTTACGGCGTGTCCTGGGTCAGGCAGGCTCC AGGCAAGGGCCTGGAGTGGATCGGCTCCGTGAACAGCGGT GGAAGTGCCTACTATGCTGATTCTGTGAAGGGCCGAAGCA CGATTTCTCGCGATAACAGCAAGAATACTGTGTATTTGCA AATGAACTCTCTGCGTGCCGAGGATACCGCTGTGTACTACT GCGCTCGATCGGGGTCCGGTTACCCTATGGATTACCTGGCC ATCTGGGGTCAGGGTACCCTTGTTACTGTATCCTCTGCTAG CACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCA AGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGT CAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAAC TCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCCG TCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTG ACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCT GCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACA AGAAGGTTGAGCCCAAATCTTGTGACAAAACTCACACATG CCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCA GTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGAT CTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTG AGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGG ACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGG AGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCT CACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTAC AAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCG AGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAAC CACAGGTGTACACCCTGCCCCCATCCCGGGACGAGCTGAC CAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTC	135

Clone ID	Region	Sequence	SEQ ID NO
		TATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGC AGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGA CTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGG ACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTC CGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAG AGCCTCTCCCTGTCTCCGGGCAAA	
	VH	EVQLLESGGGLVQPGGSLRLSCAVSGFSLSLYGVSWVRQAPG KGLEWIGSVNSGGSAYYADSVKGRSTISRDNSKNTVYLQMN SLRAEDTAVYYCARSGSGYPMDYLAIWGQGTLVTVSS	136
	IMGT CDR1	GFSLSLYG	92
	IMGT CDR2	VNSGGSA	21
	IMGT CDR3	ARSGSGYPMDYLAI	22
	Chothia CDR1	GFSLSLY	93
	Chothia CDR2	NSGGS	24
	Chothia CDR3	SGSGYPMDYLAI	25
	Kabat CDR1	LYGVS	94
	Kabat CDR2	SVNSGGSAYYADSVKG	137
	Kabat CDR3	SGSGYPMDYLAI	25
	Contact CDR1	SLYGVS	95
	Contact CDR2	WIGSVNSGGSAY	138
	Contact CDR3	ARSGSGYPMDYLA	30
	AbM CDR1	GFSLSLYGVS	96
	AbM CDR2	SVNSGGSAY	139
	AbM CDR3	SGSGYPMDYLAI	25
27274	Full	DYQMTQSPSSVSASVGDRVTITCRASQSIWYWLAWYQQKPG KAPKLLIYEASTLASGVPSRFSGSGSGTDFTLTISSVQPEDAAT YYCQQGYGRWHILNIFGGGTKVEVKRTVAAPSVFIFPPSDEQ LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTE QDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTK SFNRGEC	140
	Full	GACTACCAGATGACCCAGAGCCCAAGTTCAGTATCGGCCT CCGTTGGTGACAGAGTCACCATCACCTGCCGGGCCAGCCA ATCCATCTGGTACTGGCTGGCCTGGTATCAACAAAAGCCG GGCAAAGCACCCAAGCTCCTCATCTACGAGGCTAGCACAC TGGCCTCCGGCGTTCCTTCGCGATTCAGTGGTTCCGGGTCT GGAACAGACTTCACTTTGACCATTTCTTCTGTGCAACCCGA GGATGCTGCAACATATTACTGCCAACAAGGCTATGGCCGG TGGCACATTCTGAACATCTTTGGCGGCGGAACTAAGGTTG AAGTCAAACGTACGGTGGCTGCACCATCTGTCTTCATCTTC CCGCCATCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGT TGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAA GTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACT	141

Clone ID	Region	Sequence	SEQ ID NO
		CCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCA CCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGA CTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCAT CAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGG GAGAGTGT	
	VL	DYQMTQSPSSVSASVGDRVTITCRASQSIWYWLAWYQQKPG KAPKLLIYEASTLASGVPSRFSGSGSGTDFTLTISSVQPEDAAT YYCQQGYGRWHILNIFGGGTKVEVK	130
	IMGT CDR1	QSIWYW	125
	IMGT CDR2	EAS	41
	IMGT CDR3	QQGYGRWHILNI	120
	Chothia CDR1	RASQSIWYWLA	126
	Chothia CDR2	EASTLAS	44
	Chothia CDR3	QQGYGRWHILNI	120
	Kabat CDR1	RASQSIWYWLA	126
	Kabat CDR2	EASTLAS	44
	Kabat CDR3	QQGYGRWHILNI	120
	Contact CDR1	WYWLAWY	127
	Contact CDR2	LLIYEASTLA	46
	Contact CDR3	QQGYGRWHILN	121
	AbM CDR1	RASQSIWYWLA	126
	AbM CDR2	EASTLAS	44
	AbM CDR3	QQGYGRWHILNI	120
27275	Full	EVQLLESGGGLVQPGGSLRLSCAVSGFSLSSYGVSWVRQAPG KGLEWIGAVNSGGSAYYADSVKGRSTISRDNSKNTVYLQMN SLRAEDTAVYYCARSGSGYPMDYLAIWGQGTLVTVSSASTK GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKP SNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP IEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYP SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPG	142
	Full + C-terminal Lys	EVQLLESGGGLVQPGGSLRLSCAVSGFSLSSYGVSWVRQAPG KGLEWIGAVNSGGSAYYADSVKGRSTISRDNSKNTVYLQMN SLRAEDTAVYYCARSGSGYPMDYLAIWGQGTLVTVSSASTK GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKP SNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK	157

Clone ID	Region	Sequence	SEQ ID NO
		CCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCA CCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGA CTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCAT CAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGG GAGAGTGT	
	VL	DYQMTQSPSSVSASVGDRVTITCRASQSIWYWLAWYQQKPG KAPKLLIYEASTLASGVPSRFSGSGSGTDFTLTISSVQPEDAAT YYCQQGYGRWHILNIFGGGTKVEVK	130
	IMGT CDR1	QSIWYW	125
	IMGT CDR2	EAS	41
	IMGT CDR3	QQGYGRWHILNI	120
	Chothia CDR1	RASQSIWYWLA	126
	Chothia CDR2	EASTLAS	44
	Chothia CDR3	QQGYGRWHILNI	120
	Kabat CDR1	RASQSIWYWLA	126
	Kabat CDR2	EASTLAS	44
	Kabat CDR3	QQGYGRWHILNI	120
	Contact CDR1	WYWLAWY	127
	Contact CDR2	LLIYEASTLA	46
	Contact CDR3	QQGYGRWHILN	121
	AbM CDR1	RASQSIWYWLA	126
	AbM CDR2	EASTLAS	44
	AbM CDR3	QQGYGRWHILNI	120
27275	Full	EVQLLESGGGLVQPGGSLRLSCAVSGFSLSSYGVSWVRQAPG KGLEWIGAVNSGGSAYYADSVKGRSTISRDNSKNTVYLQMN SLRAEDTAVYYCARSGSGYPMDYLAIWGQGTLVTVSSASTK GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKP SNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP IEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYP SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPG	142
	Full + C-terminal Lys	EVQLLESGGGLVQPGGSLRLSCAVSGFSLSSYGVSWVRQAPG KGLEWIGAVNSGGSAYYADSVKGRSTISRDNSKNTVYLQMN SLRAEDTAVYYCARSGSGYPMDYLAIWGQGTLVTVSSASTK GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKP SNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK	155

Clone ID	Region	Sequence	SEQ ID NO
		PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP IEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYP SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	
	Full	GAGGTCCAGTTGCTGGAATCAGGAGGTGGACTCGTGCAGC CTGGGGGTTCCCTGCGCTTGTCTTGCGCTGTCAGCGGTTTT AGCTTATCTTCATACGGTGTAAGTTGGGTTCGGCAGGCTCC TGGCAAAGGTCTGGAATGGATCGGGGCTGTAAACAGCGGG GGGTCCGCCTACTATGCTGATTCTGTTAAGGGGAGGTCCA CCATTAGCAGAGATAATTCCAAGAACACCGTTTATCTCCA GATGAACTCCTTGAGGGCGGAGGACACTGCTGTCTATTAT TGTGCGCGGTCAGGCAGCGGATACCCGATGGACTATCTGG CGATCTGGGGCCAGGGAACCCTTGTGACAGTCTCTTCCGCT AGCACTAAGGGGCCTTCCGTGTTTCCACTGGCTCCCTCTAG TAAATCCACCTCTGGAGGCACAGCTGCACTGGGATGTCTG GTGAAGGATTACTTCCCTGAACCAGTCACAGTGAGTTGGA ACTCAGGGGCTCTGACAAGTGGAGTCCATACTTTTCCCGC AGTGCTGCAGTCAAGCGGACTGTACTCCCTGTCCTCTGTGG TCACCGTGCCTAGTTCAAGCCTGGGCACCCAGACATATAT CTGCAACGTGAATCACAAGCCATCAAATACAAAAGTCGAC AAGAAAGTGGAGCCCAAGAGCTGTGATAAAACTCATACCT GCCCACCTTGTCCGGCTCCAGAGCTGCTCGGCGGACCATC CGTGTTCCTGTTTCCTCCAAAGCCTAAAGATACACTGATGA TTAGCCGCACTCCCGAAGTCACCTGTGTGGTCGTGGACGT GTCCCACGAGGACCCCGAAGTCAAGTTCAACTGGTACGTG GACGGCGTCGAGGTGCATAATGCCAAGACTAAACCAAGA GAGGAACAGTACAATTCAACCTATAGGGTCGTGAGCGTCC TGACAGTGCTGCATCAGGATTGGCTGAACGGCAAGGAGTA TAAGTGCAAAGTGTCTAACAAGGCCCTGCCCGCTCCTATC GAGAAGACTATTAGCAAGGCAAAAGGGCAGCCACGGGAA CCCCAGGTCTACACTCTGCCCCCTAGCAGAGACGAGCTGA CCAAAAACCAGGTGTCCCTGACTTGTCTGGTGAAGGGCTT TTATCCTAGTGATATCGCTGTGGAGTGGGAATCAAATGGG CAGCCAGAAAACAATTACAAAACAACTCCACCCGTGCTGG ACAGCGATGGGTCCTTCTTTCTGTATTCCAAACTGACTGTG GACAAGTCCAGATGGCAGCAGGGAAACGTCTTCAGCTGTT CCGTGATGCACGAGGCCCTGCACAATCATTACACCCAGAA GTCTCTGAGTCTGTCACCCGGC	143
	VH	EVQLLESGGGLVQPGGSLRLSCAVSGFSLSSYGVSWVRQAPG KGLEWIGAVNSGGSAYYADSVKGRSTISRDNSKNTVYLQMN SLRAEDTAVYYCARSGSGYPMDYLAIWGQGTLVTVSS	50
	IMGT CDR1	GFSLSSYG	20
	IMGT CDR2	VNSGGSA	21
	IMGT CDR3	ARSGSGYPMDYLAI	22
	Chothia CDR1	GFSLSSY	23

Clone ID	Region	Sequence	SEQ ID NO
	Chothia CDR2	NSGGS	24
	Chothia CDR3	SGSGYPMDYLAI	25
	Kabat CDR1	SYGVS	26
	Kabat CDR2	AVNSGGSAYYADSVKG	51
	Kabat CDR3	SGSGYPMDYLAI	25
	Contact CDR1	SSYGVS	28
	Contact CDR2	WIGAVNSGGSAY	29
	Contact CDR3	ARSGSGYPMDYLA	30
	AbM CDR1	GFSLSSYGVS	31
	AbM CDR2	AVNSGGSAY	32
	AbM CDR3	SGSGYPMDYLAI	25
27276	Full	DYQMTQSPSSVSASVGDRVTITCRASQSIGDWLAWYQQKPG KAPKLLIYEASTLASGVPSRFSGSGSGTDFTLTISSVQPEDAAT YYCQQGYGRSNVDNIFGGGTKVEVKRTVAAPSVFIFPPSDEQ LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTE QDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTK SFNRGEC	144
	Full	GACTATCAAATGACGCAGTCCCCGTCCTCCGTTTCCGCCTC AGTTGGTGACCGGGTTACAATCACTTGCAGAGCATCTCAG TCTATTGGTGATTGGCTGGCGTGGTATCAACAGAAGCCCG GCAAAGCCCCTAAACTCCTGATATACGAAGCATCAACGCT TGCTTCTGGAGTACCCTCCCGGTTTTCTGGGTCAGGAAGCG GCACGGATTTTACCCTGACTATCTCTTCTGTTCAGCCTGAG GATGCCGCAACCTATTATTGCCAACAGGGGTATGGCAGAT CAAACGTGGATAATATTTTCGGTGGCGGGACCAAAGTTGA GGTTAAACGTACGGTGGCGGCGCCCAGTGTCTTCATTTTTC CCCCTAGCGACGAACAGCTGAAGTCTGGGACAGCCAGTGT GGTCTGTCTGCTGAACAACTTCTACCCTAGAGAGGCTAAA GTGCAGTGGAAGGTCGATAACGCACTGCAGTCCGGAAATT CTCAGGAGAGTGTGACTGAACAGGACTCAAAAGATAGCAC CTATTCCCTGTCAAGCACACTGACTCTGAGCAAGGCCGAC TACGAGAAGCATAAAGTGTATGCTTGTGAAGTCACCCACC AGGGGCTGAGTTCACCAGTCACAAAATCATTCAACAGAGG GGAGTGC	145
	VL	DYQMTQSPSSVSASVGDRVTITCRASQSIGDWLAWYQQKPG KAPKLLIYEASTLASGVPSRFSGSGSGTDFTLTISSVQPEDAAT YYCQQGYGRSNVDNIFGGGTKVEVK	64
	IMGT CDR1	QSIGDW	40
	IMGT CDR2	EAS	41
	IMGT CDR3	QQGYGRSNVDNI	42
	Chothia CDR1	RASQSIGDWLA	65
	Chothia CDR2	EASTLAS	44
	Chothia CDR3	QQGYGRSNVDNI	42

Clone ID	Region	Sequence	SEQ ID NO
	Kabat CDR1	RASQSIGDWLA	65
	Kabat CDR2	EASTLAS	44
	Kabat CDR3	QQGYGRSNVDNI	42
	Contact CDR1	GDWLAWY	45
	Contact CDR2	LLIYEASTLA	46
	Contact CDR3	QQGYGRSNVDN	47
	AbM CDR1	RASQSIGDWLA	65
	AbM CDR2	EASTLAS	44
	AbM CDR3	QQGYGRSNVDNI	42

WE CLAIM:

1. An antibody construct comprising an antigen-binding domain that specifically binds to human folate receptor alpha (hFRα), wherein the antibody construct competes for binding to hFRα with a reference antibody that specifically binds to an epitope within hFRα comprising amino acid residues E120, D121, R123, T124, S125 and Y126 of SEQ ID NO: 15.

2. The antibody construct according to claim 1, wherein the epitope is a discontinuous epitope.

3. The antibody construct according to claim 1 or 2, wherein the reference antibody comprises an HCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 20, 23, 26, 28 or 31; an HCDR2 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 21, 24, 27, 29 or 32; an HCDR3 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 22, 25 or 30; a LCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 40, 43 or 45; a LCDR2 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 41, 44 or 46, and a LCDR3 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 42 or 47.

4. An antibody construct comprising an antigen-binding domain that specifically binds to an epitope within human folate receptor alpha (hFRα) comprising amino acid residues E120, D121, R123, T124, S125 and Y126 of SEQ ID NO: 15.

5. The antibody construct according to claim 4, wherein the epitope is a discontinuous epitope.

6. An antibody construct comprising an antigen-binding domain that specifically binds to human folate receptor alpha (hFRα), the antigen-binding domain comprising heavy chain CDR amino acid sequences (HCDR1, HCDR2 and HCDR3) comprising the sequences as set forth in SEQ ID NOs: 3, 4 and 5, and light chain CDR amino acid sequences (LCDR1, LCDR2 and LCDR3) comprising the sequences as set forth in SEQ ID NOs: 6, 7 and 8.

7. The antibody construct according to claim 4 or 6, wherein the antigen-binding domain comprises the CDR sequences of the VH domain having a sequence as set forth in any one of SEQ ID NOs: 19, 50, 54, 57, 61, 76, 79, 82, 85, 88, 91, 99, 106, 113, 116, 133 or 136.

8. The antibody construct according to any one of claims 4, 6 and 7, wherein the antigen-binding domain comprises the CDR sequences of the VL domain having a sequence as set forth in any one of SEQ ID NOs: 39, 64, 119, 124 or 130.

9. The antibody construct according to claim 4 or 6, wherein the antigen-binding domain comprises:

(i) an HCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 20, 23, 26, 28, 31, 92, 93, 94, 95 or 96; an HCDR2 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 21, 24, 27, 29, 32, 51, 58, 100, 101, 102, 103, 109, 137, 138 or 139, and an HCDR3 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 22, 25, 30, 107, 108 or 110, and

(ii) a LCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 40, 43, 45, 65, 125, 126 or 127; a LCDR2 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 41, 44 or 46, and a LCDR3 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 42, 47, 120 or 121.

10. The antibody construct according to claim 4 or 6, wherein the antigen-binding domain comprises:

(a) an HCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 20, 23, 26, 28 or 31; an HCDR2 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 21, 24, 27, 29 or 32; an HCDR3 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 22, 25 or 30; a LCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 40, 43 or 45; a LCDR2 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 41, 44 or 46, and a LCDR3 amino

acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 42 or 47, or

(b) an HCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 20, 23, 26, 28 or 31; an HCDR2 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 21, 24, 29, 32 or 51; an HCDR3 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 22, 25 or 30; a LCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 40, 45 or 65; a LCDR2 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 41, 44 or 46, and a LCDR3 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 42 or 47, or

(c) an HCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 20, 23, 26, 28 or 31; an HCDR2 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 21, 24, 29, 32 or 58; an HCDR3 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 22, 25 or 30; a LCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 40, 45 or 65; a LCDR2 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 41, 44 or 46, and a LCDR3 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 42 or 47, or

(d) an HCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 92, 93, 94, 95 or 96; an HCDR2 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 21, 24, 29, 32 or 51; an HCDR3 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 22, 25 or 30; a LCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 125, 126 or 127; a LCDR2 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 41, 44 or 46, and a LCDR3 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 42 or 47, or

(e) an HCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 92, 93, 94, 95 or 96; an HCDR2 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 24, 100, 101, 102 or 103; an HCDR3 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 22, 25 or 30; a LCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 125, 126 or 127; a LCDR2 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 41, 44 or 46, and a LCDR3 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 120 or 121, or

(f) an HCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 92, 93, 94, 95 or 96; an HCDR2 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 21, 24, 29, 32 or 109; an HCDR3 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 107, 108 or 110; a LCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 40, 45 or 65; a LCDR2 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 41, 44 or 46, and a LCDR3 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 42 or 47, or

(g) an HCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 92, 93, 94, 95 or 96; an HCDR2 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 21, 24, 29, 32 or 109; an HCDR3 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 107, 108 or 110; a LCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 40, 45 or 65; a LCDR2 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 41, 44 or 46, and a LCDR3 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 120 or 121, or

(h) an HCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 92, 93, 94, 95 or 96; an HCDR2 amino acid sequence selected from the

amino acid sequences as set forth in any one of SEQ ID NOs: 21, 24, 29, 32 or 109; an HCDR3 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 107, 108 or 110; a LCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 125, 126 or 127; a LCDR2 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 41, 44 or 46, and a LCDR3 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 120 or 121, or

(i) an HCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 92, 93, 94, 95 or 96; an HCDR2 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 21, 24, 29, 32 or 109; an HCDR3 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 22, 25 or 30; a LCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 125, 126 or 127; a LCDR2 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 41, 44 or 46, and a LCDR3 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 120 or 121, or

(j) an HCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 20, 23, 26, 28 or 31; an HCDR2 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 21, 24, 29, 32 or 109; an HCDR3 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 107, 108 or 110; a LCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 40, 45 or 65; a LCDR2 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 41, 44 or 46, and a LCDR3 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 120 or 121, or

(k) an HCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 20, 23, 26, 28 or 31; an HCDR2 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 21, 24, 29, 32 or 109; an HCDR3 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID

NOs: 107, 108 or 110; a LCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 125, 126 or 127; a LCDR2 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 41, 44 or 46, and a LCDR3 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 120 or 121, or

(l) an HCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 20, 23, 26, 28 or 31; an HCDR2 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 21, 24, 29, 32 or 109; an HCDR3 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 22, 25 or 30; a LCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 125, 126 or 127; a LCDR2 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 41, 44 or 46, and a LCDR3 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 120 or 121, or

(m) an HCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 92, 93, 94, 95 or 96; an HCDR2 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 21, 24, 137, 138 or 139; an HCDR3 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 22, 25 or 30; a LCDR1 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 125, 126 or 127; a LCDR2 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 41, 44 or 46, and a LCDR3 amino acid sequence selected from the amino acid sequences as set forth in any one of SEQ ID NOs: 120 or 121.

11. The antibody construct according to claim 4 or 6, wherein the antigen-binding domain comprises a VH amino acid sequence selected from the VH amino acid sequences as set forth in any one of SEQ ID NOs: 19, 50, 54, 57, 61, 76, 79, 82, 85, 88, 91, 99, 106, 113, 116, 133 or 136.

12. The antibody construct according to any one of claims 4, 6 and 11, wherein the antigen-binding domain comprises a VL amino acid sequence selected from the VL amino acid sequences as set forth in any one of SEQ ID NOs: 39, 64, 119, 124 or 130.

13. The antibody construct according to claim 4 or 6, wherein the antigen-binding domain comprises:

(i) a VH amino acid as set forth in SEQ ID NO: 19, and a VL amino acid sequence as set forth in SEQ ID NO: 39, or

(ii) a VH amino acid sequence as set forth in SEQ ID NO: 50, and a VL amino acid sequence as set forth in SEQ ID NO: 64, or

(iii) a VH amino acid sequence as set forth in SEQ ID NO: 54, and a VL amino acid sequence as set forth in SEQ ID NO: 64, or

(iv) a VH amino acid sequence as set forth in SEQ ID NO: 57, and a VL amino acid sequence as set forth in SEQ ID NO: 64, or

(v) a VH amino acid sequence as set forth in SEQ ID NO: 61, and a VL amino acid sequence as set forth in SEQ ID NO: 64, or

(vi) a VH amino acid sequence as set forth in SEQ ID NO: 76, and a VL amino acid sequence as set forth in SEQ ID NO: 64, or

(vii) a VH amino acid sequence as set forth in SEQ ID NO: 79, and a VL amino acid sequence as set forth in SEQ ID NO: 64, or

(viii) a VH amino acid sequence as set forth in SEQ ID NO: 82, and a VL amino acid sequence as set forth in SEQ ID NO: 64, or

(ix) a VH amino acid sequence as set forth in SEQ ID NO: 85, and a VL amino acid sequence as set forth in SEQ ID NO: 64, or

(x) a VH amino acid sequence as set forth in SEQ ID NO: 88, and a VL amino acid sequence as set forth in SEQ ID NO: 64, or

(xi) a VH amino acid sequence as set forth in SEQ ID NO: 91, and a VL amino acid sequence as set forth in SEQ ID NO: 124, or

(xii) a VH amino acid sequence as set forth in SEQ ID NO: 99, and a VL amino acid sequence as set forth in SEQ ID NO: 130, or

(xiii) a VH amino acid sequence as set forth in SEQ ID NO: 106, and a VL amino acid sequence as set forth in SEQ ID NO: 64, or

(xiv) a VH amino acid sequence as set forth in SEQ ID NO: 106, and a VL amino acid sequence as set forth in SEQ ID NO: 119, or

(xv) a VH amino acid sequence as set forth in SEQ ID NO: 106, and a VL amino acid sequence as set forth in SEQ ID NO: 130, or

(xvi) a VH amino acid sequence as set forth in SEQ ID NO: 113, and a VL amino acid sequence as set forth in SEQ ID NO: 130, or

(xvii) a VH amino acid sequence as set forth in SEQ ID NO: 116, and a VL amino acid sequence as set forth in SEQ ID NO: 119 or

(xviii) a VH amino acid sequence as set forth in SEQ ID NO: 116, and a VL amino acid sequence as set forth in SEQ ID NO: 130, or

(xix) a VH amino acid sequence as set forth in SEQ ID NO: 133, and a VL amino acid sequence as set forth in SEQ ID NO: 130, or

(xx) a VH amino acid sequence as set forth in SEQ ID NO: 136, and a VL amino acid sequence as set forth in SEQ ID NO: 130.

14. The antibody construct according to any one of claims 1 to 13 further comprising a scaffold, wherein the antigen-binding domain is operably linked to the scaffold.

15. The antibody construct according to claim 14, wherein the scaffold comprises an IgG Fc region.

16. The antibody construct according to any one of claims 1 to 13, wherein the antibody construct further comprises a second antigen-binding domain.

17. The antibody construct according to claim 16, wherein the second antigen-binding domain specifically binds to hFRα.

18. The antibody construct according to claim 16, wherein the second antigen-binding domain binds to an antigen other than hFRα.

19. The antibody construct according to any one of claims 16 to 18, further comprising one or more additional antigen-binding domains.

20. The antibody construct according to any one of claims 16 to 19 further comprising a scaffold, wherein at least one antigen-binding domain is operably linked to the scaffold.

21. The antibody construct according to claim 20, wherein the scaffold comprises an IgG Fc region.

22. An antibody construct comprising two antigen-binding domains operably linked to an IgG Fc region, wherein each of the antigen-binding domains specifically binds to human folate receptor alpha (hFRα) and comprises:

(a) a VL amino acid sequence as set forth in SEQ ID NO: 39, and a VH amino acid sequence as set forth in SEQ ID NO: 19; or

(b) a VL amino acid sequence as set forth in SEQ ID NO: 124, and a VH amino acid sequence as set forth in SEQ ID NO: 91; or

(c) a VL amino acid sequence as set forth in SEQ ID NO: 64, and

(i) a VH amino acid sequence as set forth in SEQ ID NO: 50, or

(ii) a VH amino acid sequence as set forth in SEQ ID NO: 54, or

(iii) a VH amino acid sequence as set forth in SEQ ID NO: 57, or

(iv) a VH amino acid sequence as set forth in SEQ ID NO: 61, or

(v) a VH amino acid sequence as set forth in SEQ ID NO: 76, or

(vi) a VH amino acid sequence as set forth in SEQ ID NO: 79, or

(vii) a VH amino acid sequence as set forth in SEQ ID NO: 82, or

(viii) a VH amino acid sequence as set forth in SEQ ID NO: 85, or

(ix) a VH amino acid sequence as set forth in SEQ ID NO: 88, or

(x) a VH amino acid sequence as set forth in SEQ ID NO: 106; or

(d) a VL amino acid sequence as set forth in SEQ ID NO: 130, and

(i) a VH amino acid sequence as set forth in SEQ ID NO: 99, or

(ii) a VH amino acid sequence as set forth in SEQ ID NO: 106, or

(iii) a VH amino acid sequence as set forth in SEQ ID NO: 113, or

(iv) a VH amino acid sequence as set forth in SEQ ID NO: 116, or

(v) a VH amino acid sequence as set forth in SEQ ID NO: 133, or

(vi) a VH amino acid sequence as set forth in SEQ ID NO: 136; or

(e) a VL amino acid sequence as set forth in SEQ ID NO: 119, and

(i) a VH amino acid sequence as set forth in SEQ ID NO: 106, or

(ii) a VH amino acid sequence as set forth in SEQ ID NO: 116.

23. A polynucleotide or set of polynucleotides encoding the antibody construct according to any one of claims 1 to 22.

24. An expression vector or set of expression vectors comprising the polynucleotide or set of polynucleotides according to claim 23.

25. A host cell comprising the expression vector or set of expression vectors according to claim 24.

26. An antibody-drug conjugate comprising the antibody construct according to any one of claims 1 to 22 conjugated to one or more drug moieties.

27. The antibody-drug conjugate according to claim 26, wherein the antibody conjugate is conjugated to between 1 and about 8 drug moieties.

28. An antibody-drug conjugate having general Formula I:

$$A\text{-}(L\text{-}(D)_m)_n \quad \textbf{(I)}$$

wherein:

A is the antibody construct according to any one of claims 1 to 22;

L is a linker;

D is a drug moiety;

m is between 1 and about 8, and

n is 1 and about 12.

29. The antibody-drug conjugate according to claim 28, wherein m is 1 or 2.

30. The antibody-drug conjugate according to claim 28 or 29, wherein n is between about 2 and about 8.

31. The antibody-drug conjugate according to any one of claims 26 to 30, wherein the drug moiety is a maytansinoid, maytansinoid analogue, benzodiazepine, pyrrolobenzodiazepine, duocarmycin, calicheamicin, calicheamicin analogue, auristatin, auristatin analogue, hemiasterlin, hemiasterlin analogue, tubulysin, tubulysin analogue, amatoxin, amatoxin analogue, camptothecin, camptothecin analogue, eribulin, TLR agonist or STING agonist.

32. The antibody-drug conjugate according to any one of claims 26 to 30, wherein the drug moiety an auristatin, auristatin analogue, hemiasterlin, hemiasterlin analogue, camptothecin, camptothecin analogue or eribulin.

33. A pharmaceutical composition comprising the antibody construct according to any one of claims 1 to 22, and a pharmaceutically acceptable carrier or diluent.

34. A pharmaceutical composition comprising the antibody-drug conjugate according to any one of claims 26 to 32, and a pharmaceutically acceptable carrier or diluent.

35. An antibody construct according to any one of claims 1 to 22 for use in therapy.

36. The antibody construct for use according to claim 35, wherein the therapy comprises treatment of cancer.

37. An antibody-drug conjugate according to any one of claims 26 to 32 for use in therapy.

38. The antibody-drug conjugate for use according to claim 37, wherein the therapy comprises treatment of cancer.

39. Use of an antibody construct according to any one of claims 1 to 22 in the manufacture of a medicament for the treatment of cancer.

40. Use of an antibody-drug conjugate according to any one of claims 26 to 32 in the manufacture of a medicament for the treatment of cancer.

41. A method of inhibiting the growth of FRα-positive tumor cells comprising contacting the cells with an antibody construct according to any one of claims 1 to 22.

42. A method of inhibiting the growth of FRα-positive tumor cells comprising contacting the cells with an antibody-drug conjugate according to any one of claims 26 to 32.

43. A method of treating a subject having a cancer comprising administering to the subject an effective amount of the antibody construct according to any one of claims 1 to 22.

44. A method of treating a subject having a cancer comprising administering to the subject an effective amount of the antibody-drug conjugate according to any one of claims 26 to 32.

1A Heavy Chain

EVQLLESGGGLVQPGGSLRLSCAAS ***GFSLSSYGVS*** WVRQAPGKGLEWVS ***AVNSGGSAY***
YADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK ***SGSGYPMDYLAI*** WGQGTLVTVSS

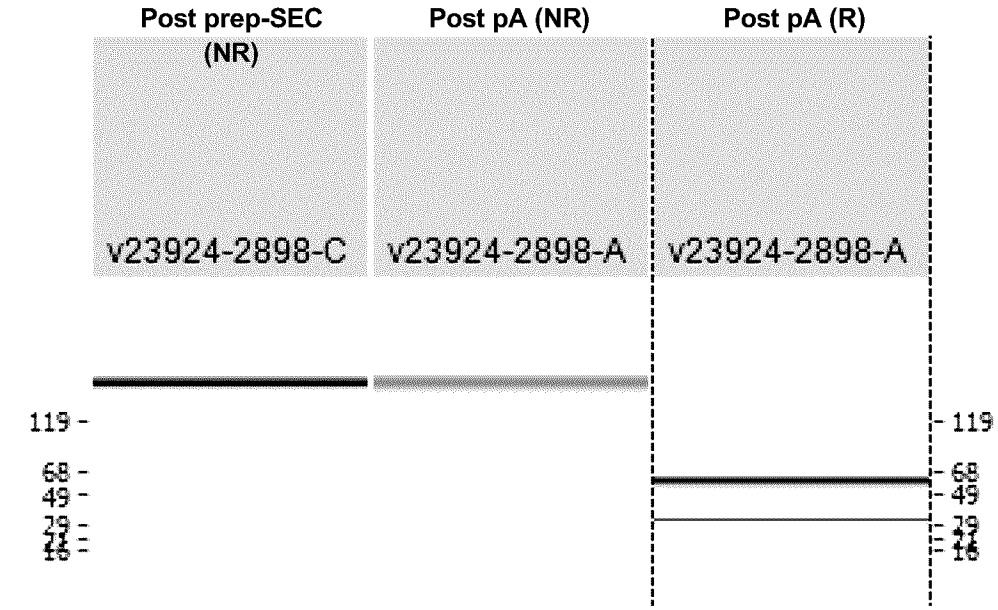
FIG. 1A

1B Light Chain

DIQMTQSPSSLSASVGDRVTITC ***QASQSIGDWLA*** WYQQKPGKAPKLLIY ***EASTLAS***
GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC ***QQGYGRSNVDNI*** FGGGTKVEIK

FIG. 1B

A.


Post prep-SEC (NR)
Post pA (NR)
Post pA (R)
v23924-2898-C
v23924-2898-A
v23924-2898-A
119
68
49
119
68
49

B.

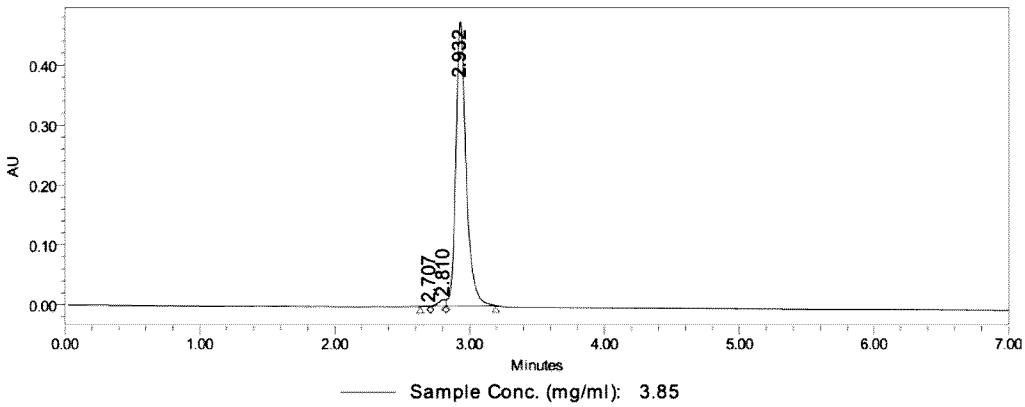
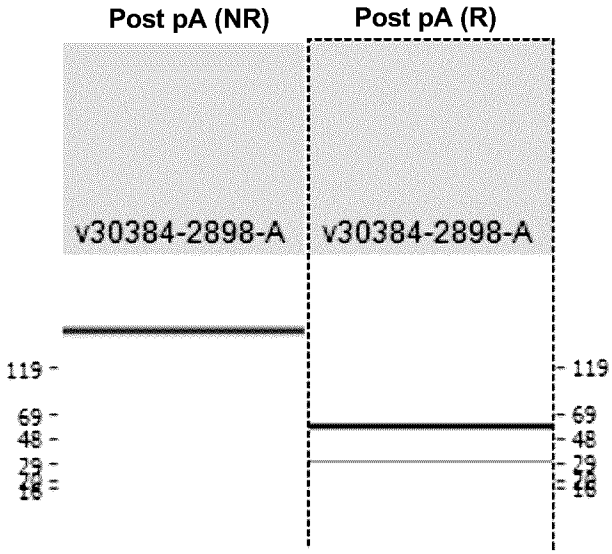

2.932
2.707
2.810
AU
0.40
0.30
0.20
0.10
0.00
0.00
1.00
2.00
3.00
4.00
5.00
6.00
7.00
Minutes
Sample Conc. (mg/ml): 3.85

FIG. 2

C.


Post pA (NR)
Post pA (R)
v30384-2898-A
v30384-2898-A
119
69
48
29
16

D.

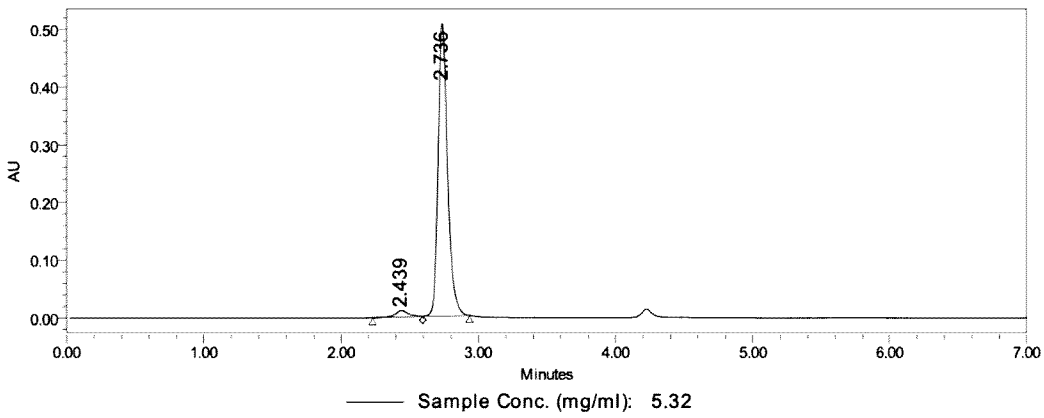

0.50
0.40
0.30
0.20
0.10
0.00
AU
2.736
2.439
0.00
1.00
2.00
3.00
4.00
5.00
6.00
7.00
Minutes
Sample Conc. (mg/ml): 5.32

FIG. 2 (cont.)

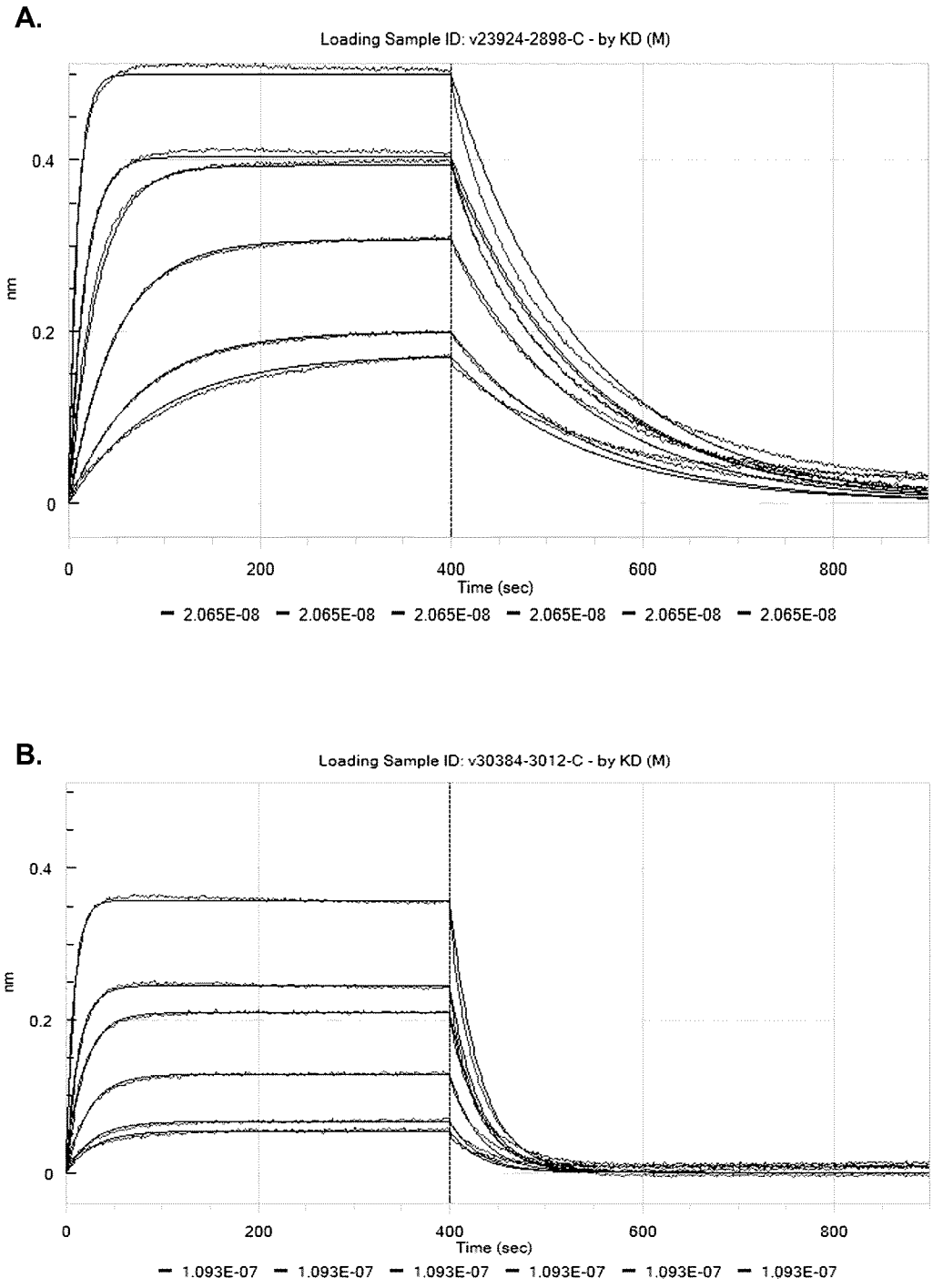
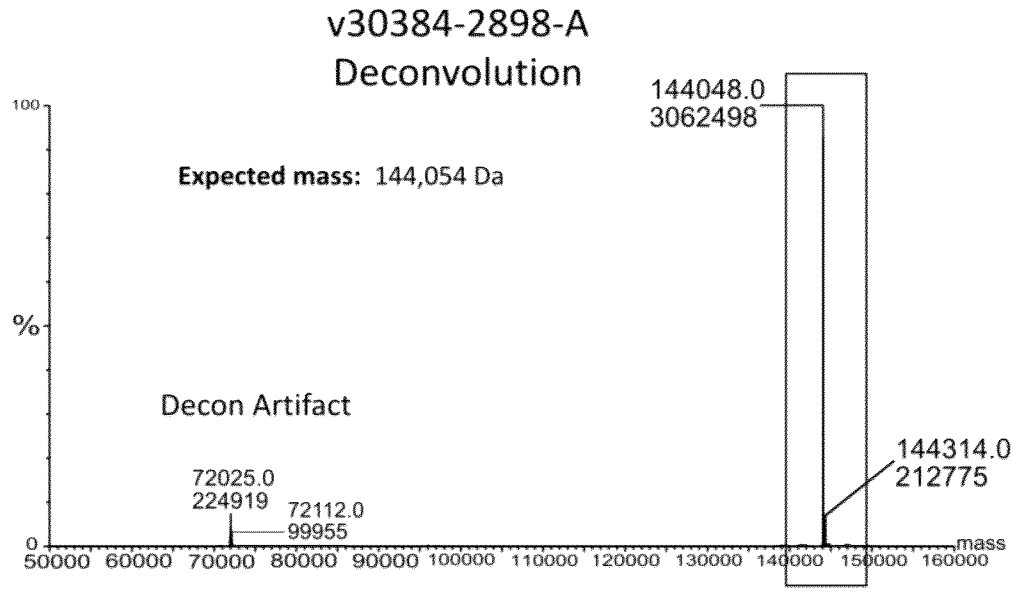

A.
Loading Sample ID: v23924-2898-C - by KD (M)
nm
0.4
0.2
0
0
200
400
600
800
Time (sec)
2.065E-08 2.065E-08 2.065E-08 2.065E-08 2.065E-08 2.065E-08
B.
Loading Sample ID: v30384-3012-C - by KD (M)
nm
0.4
0.2
0
0
200
400
600
800
Time (sec)
1.093E-07 1.093E-07 1.093E-07 1.093E-07 1.093E-07 1.093E-07

FIG. 3

A.


v30384-2898-A
Deconvolution
144048.0
3062498
Expected mass: 144,054 Da
100
%
Decon Artifact
72025.0
224919
72112.0
99955
144314.0
212775
0
mass
50000 60000 70000 80000 90000 100000 110000 120000 130000 140000 150000 160000

B.

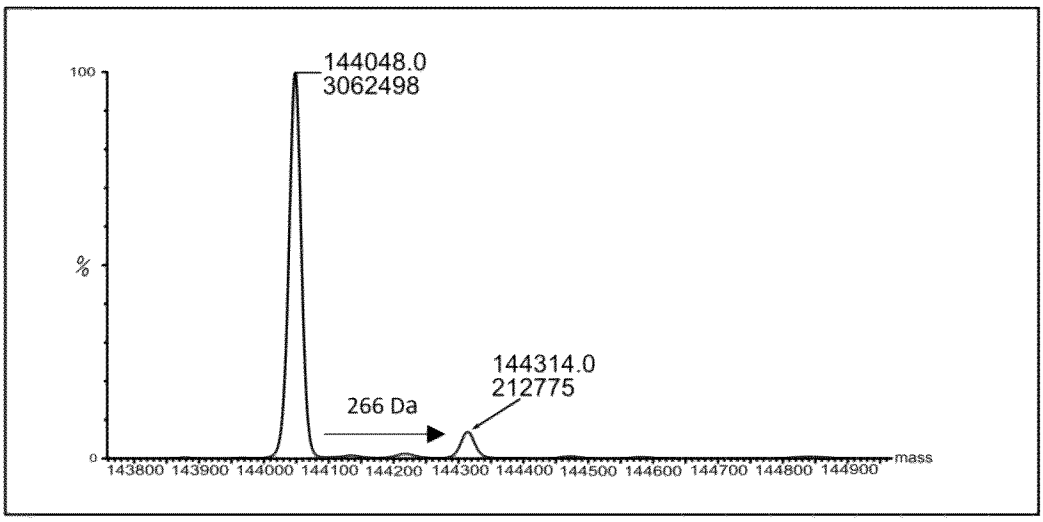
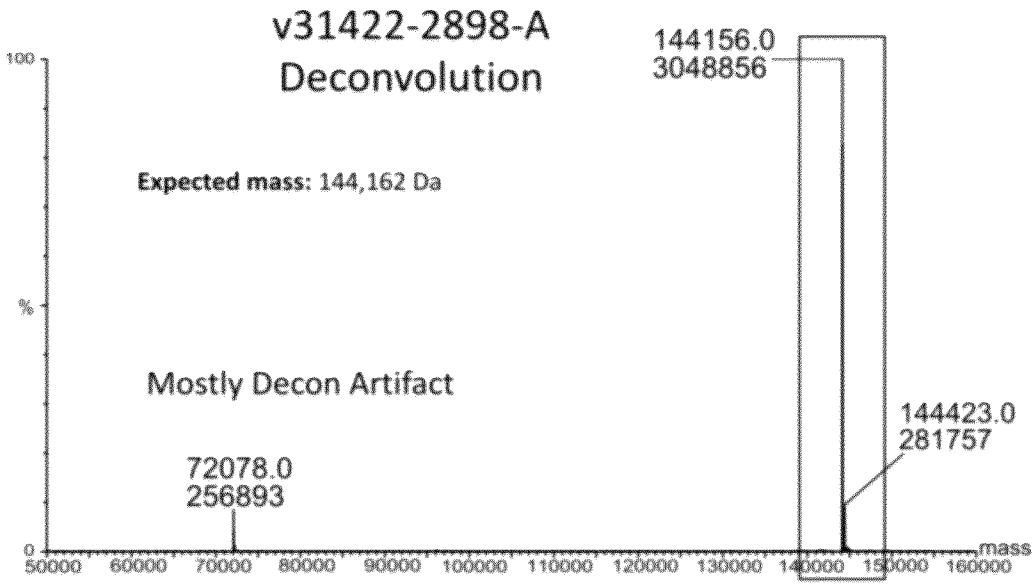

144048.0
3062498
100
%
144314.0
212775
266 Da
0
mass
143800 143900 144000 144100 144200 144300 144400 144500 144600 144700 144800 144900

FIG. 4

C.


v31422-2898-A
Deconvolution
144156.0
3048856
Expected mass: 144,162 Da
Mostly Decon Artifact
144423.0
281757
72078.0
256893
100
%
0
mass
50000 60000 70000 80000 90000 100000 110000 120000 130000 140000 150000 160000

D.

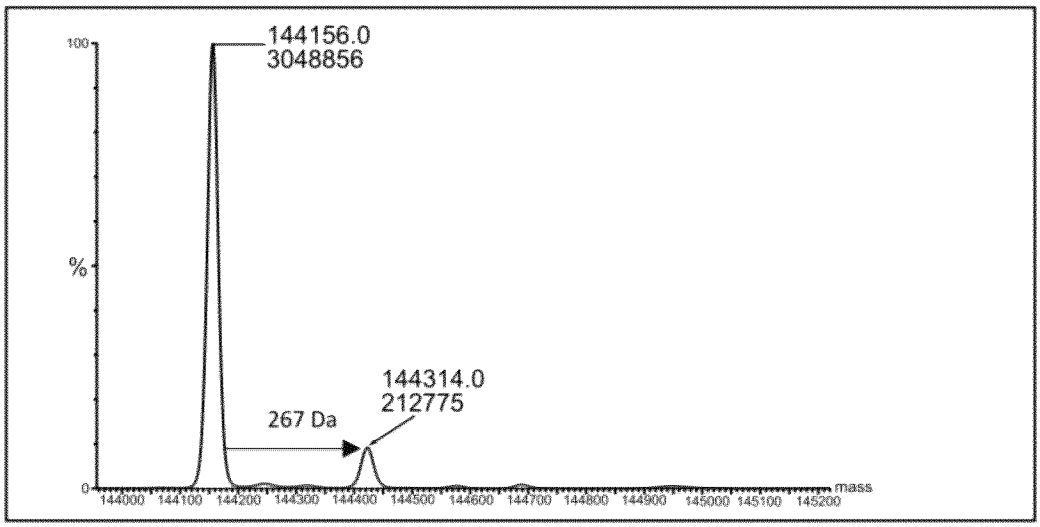

144156.0
3048856
144314.0
212775
267 Da
100
%
0
mass
144000 144100 144200 144300 144400 144500 144600 144700 144800 144900 145000 145100 145200

FIG. 4 (cont.)

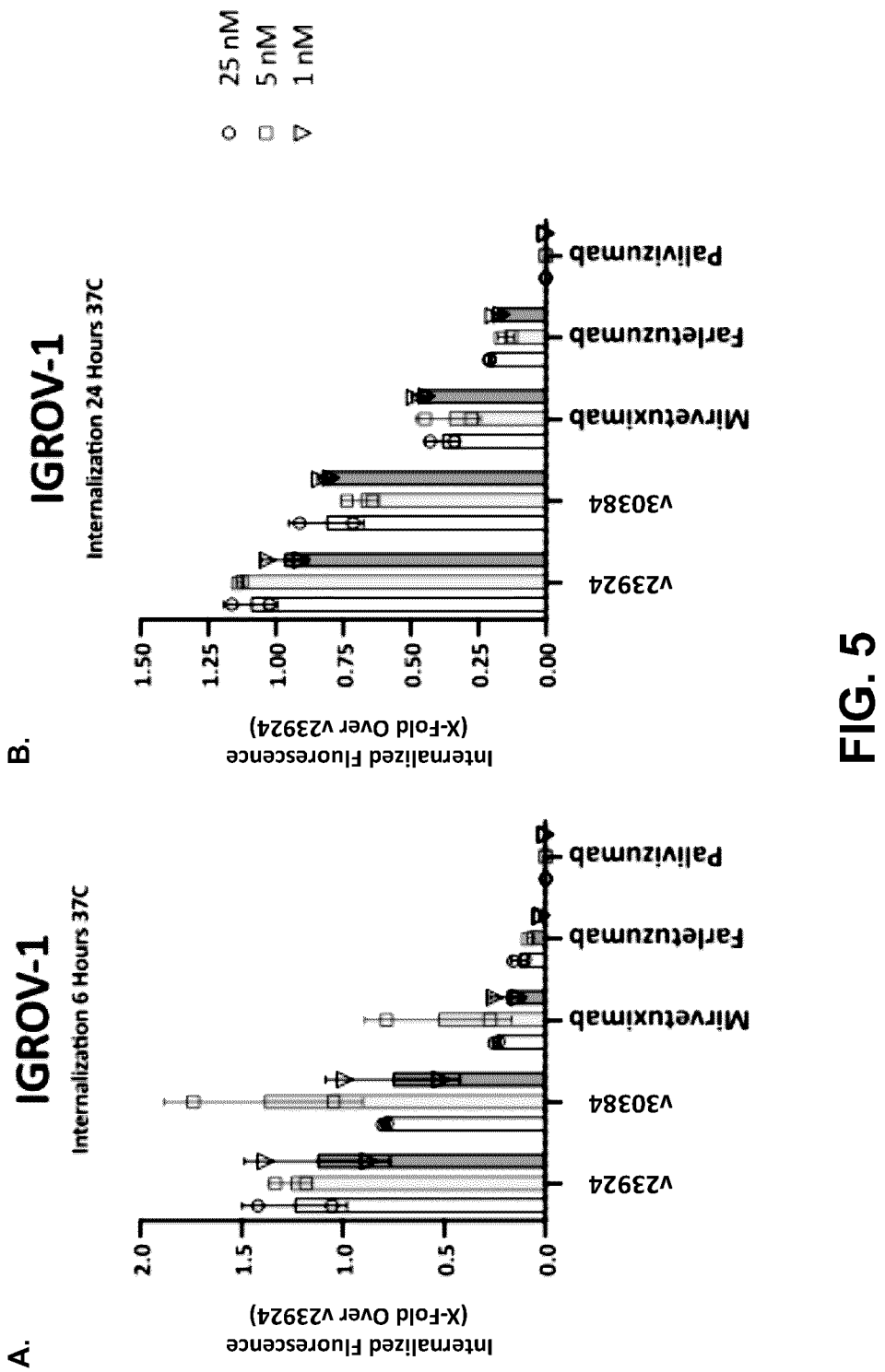

A.
IGROV-1
Internalization 6 Hours 37C
Internalized Fluorescence
(X-Fold Over v23924)
2.0
1.5
1.0
0.5
0.0
v23924
v30384
Mirvetuximab
Farletuzumab
Palivizumab
B.
IGROV-1
Internalization 24 Hours 37C
25 nM
5 nM
1 nM
Internalized Fluorescence
(X-Fold Over v23924)
1.50
1.25
1.00
0.75
0.50
0.25
0.00
v23924
v30384
Mirvetuximab
Farletuzumab
Palivizumab

FIG. 5

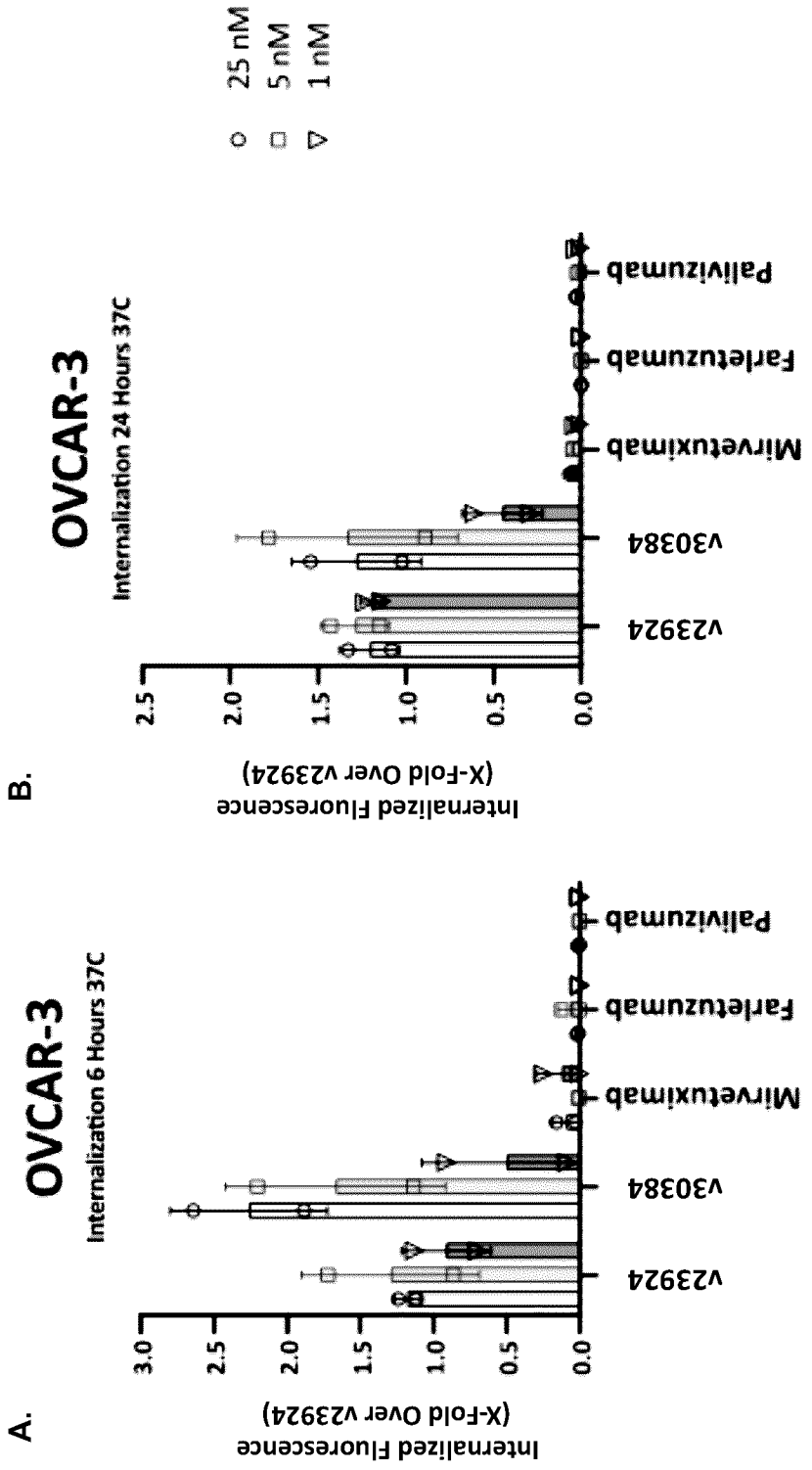

A.
OVCAR-3
Internalization 6 Hours 37C
Internalized Fluorescence
(X-Fold Over v23924)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
v23924
v30384
Mirvetuximab
Farletuzumab
Palivizumab
B.
OVCAR-3
Internalization 24 Hours 37C
Internalized Fluorescence
(X-Fold Over v23924)
2.5
2.0
1.5
1.0
0.5
0.0
v23924
v30384
Mirvetuximab
Farletuzumab
Palivizumab
25 nM
5 nM
1 nM

FIG. 6

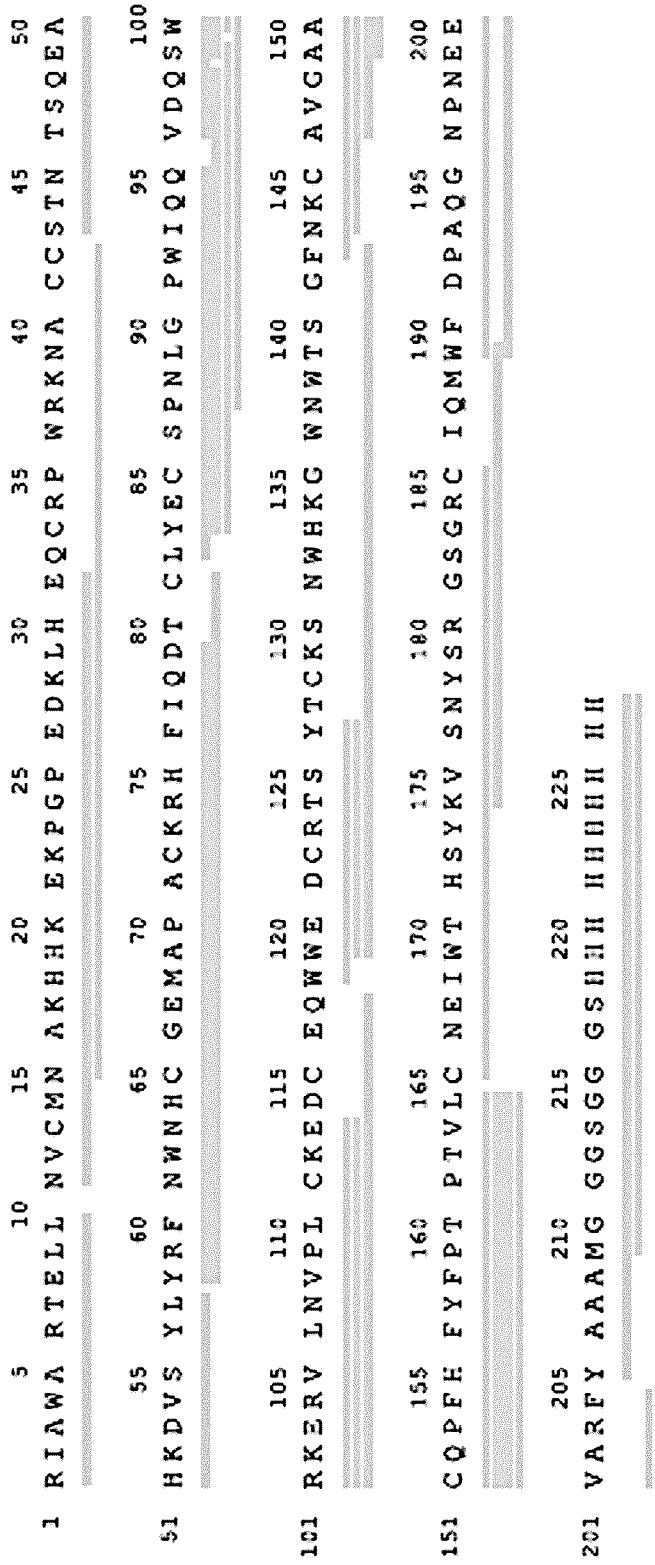


FIG. 7

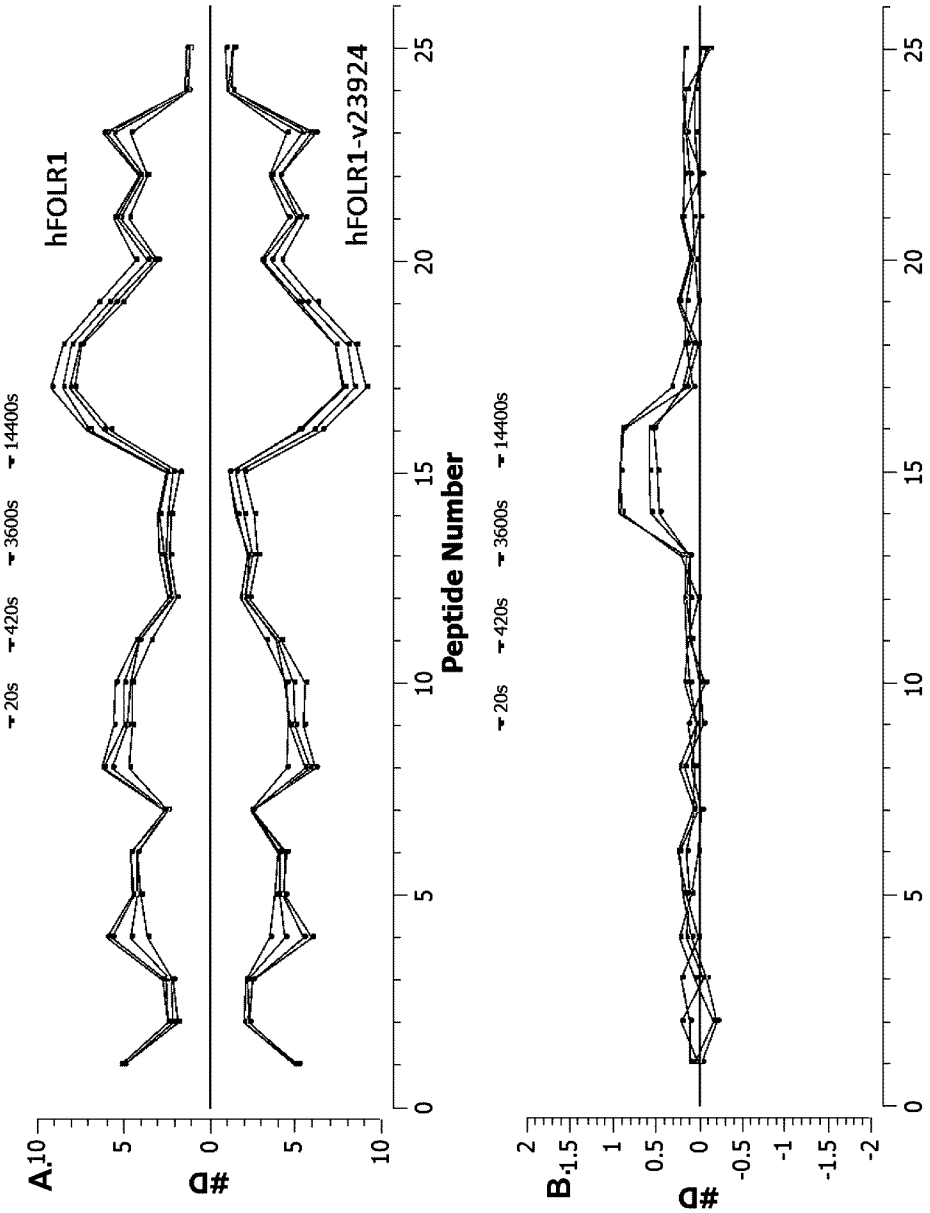

hFOLR1
hFOLR1-v23924
20s
420s
3600s
14400s
A.10
B.1.5
#D
Peptide Number
0
5
10
15
20
25
2
1
0.5
0
-0.5
-1
-1.5
-2

FIG. 8

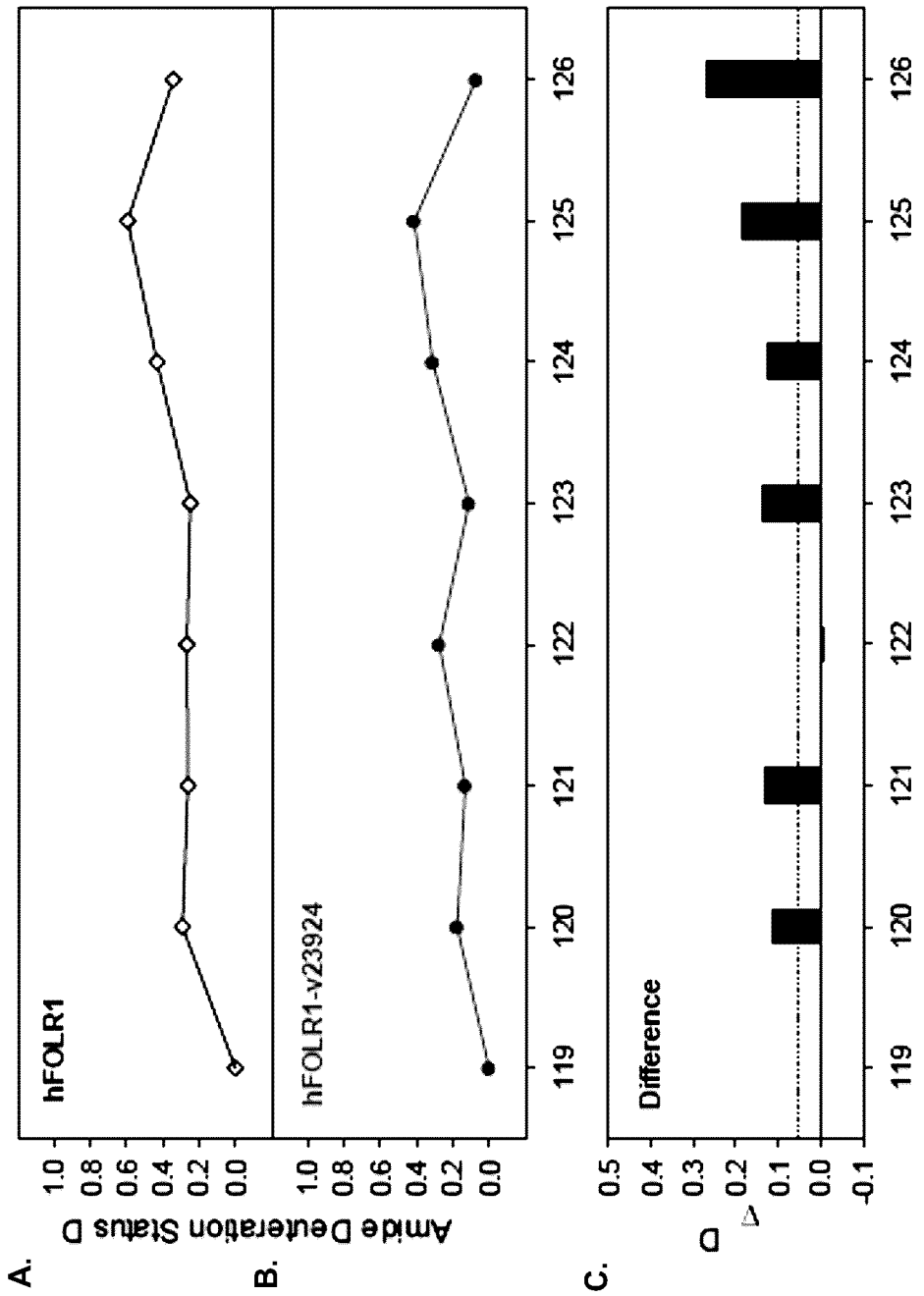

A.
hFOLR1
B.
hFOLR1-v23924
Amide Deuteration Status D
1.0
0.8
0.6
0.4
0.2
0.0
C.
Difference
Δ D
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
119
120
121
122
123
124
125
126

FIG. 9

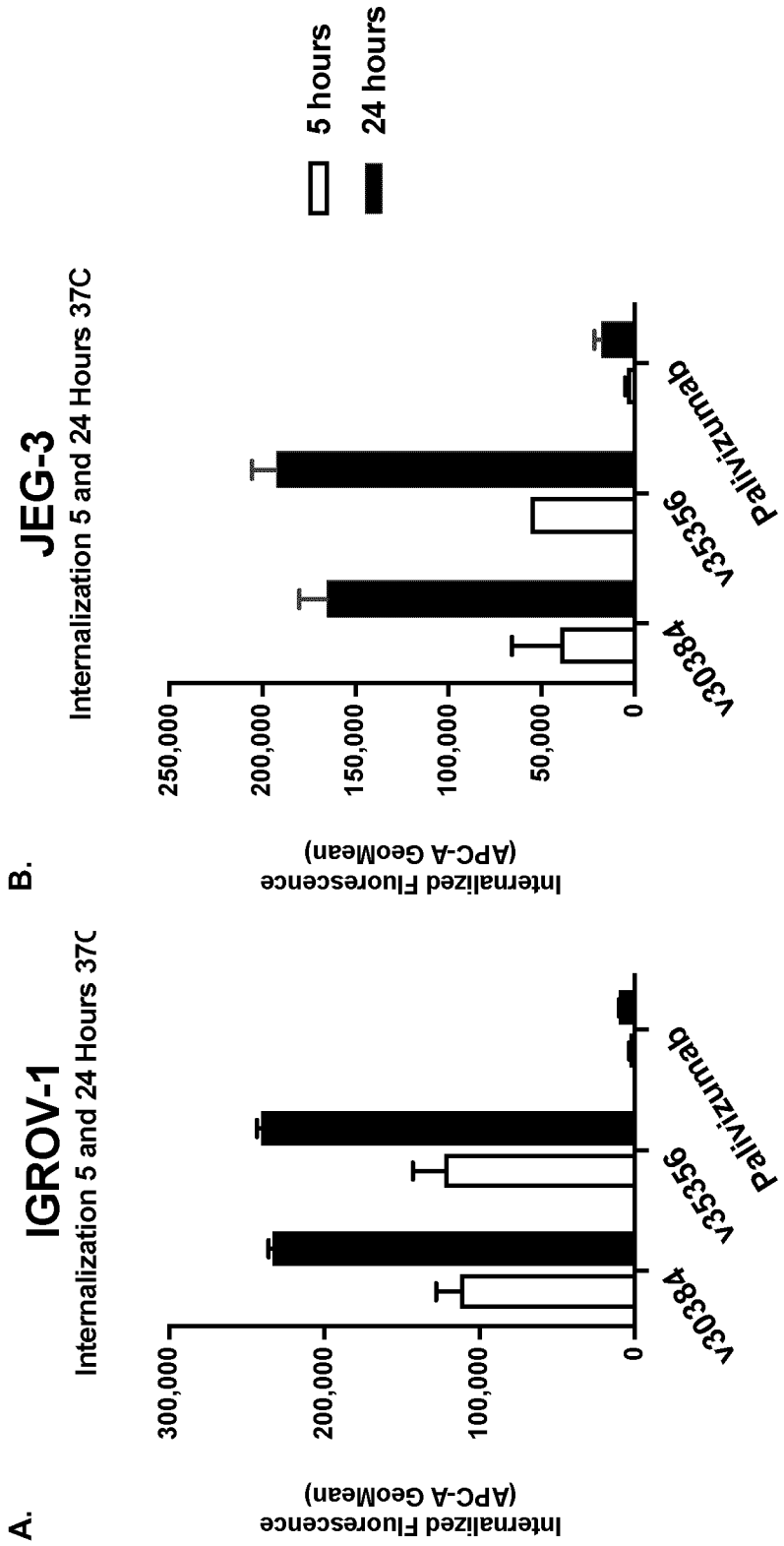

A.
IGROV-1
Internalization 5 and 24 Hours 37C
Internalized Fluorescence
(APC-A GeoMean)
300,000
200,000
100,000
0
V30384
V35356
Palivizumab
B.
JEG-3
Internalization 5 and 24 Hours 37C
5 hours
24 hours
Internalized Fluorescence
(APC-A GeoMean)
250,000
200,000
150,000
100,000
50,000
0
V30384
V35356
Palivizumab

FIG. 10

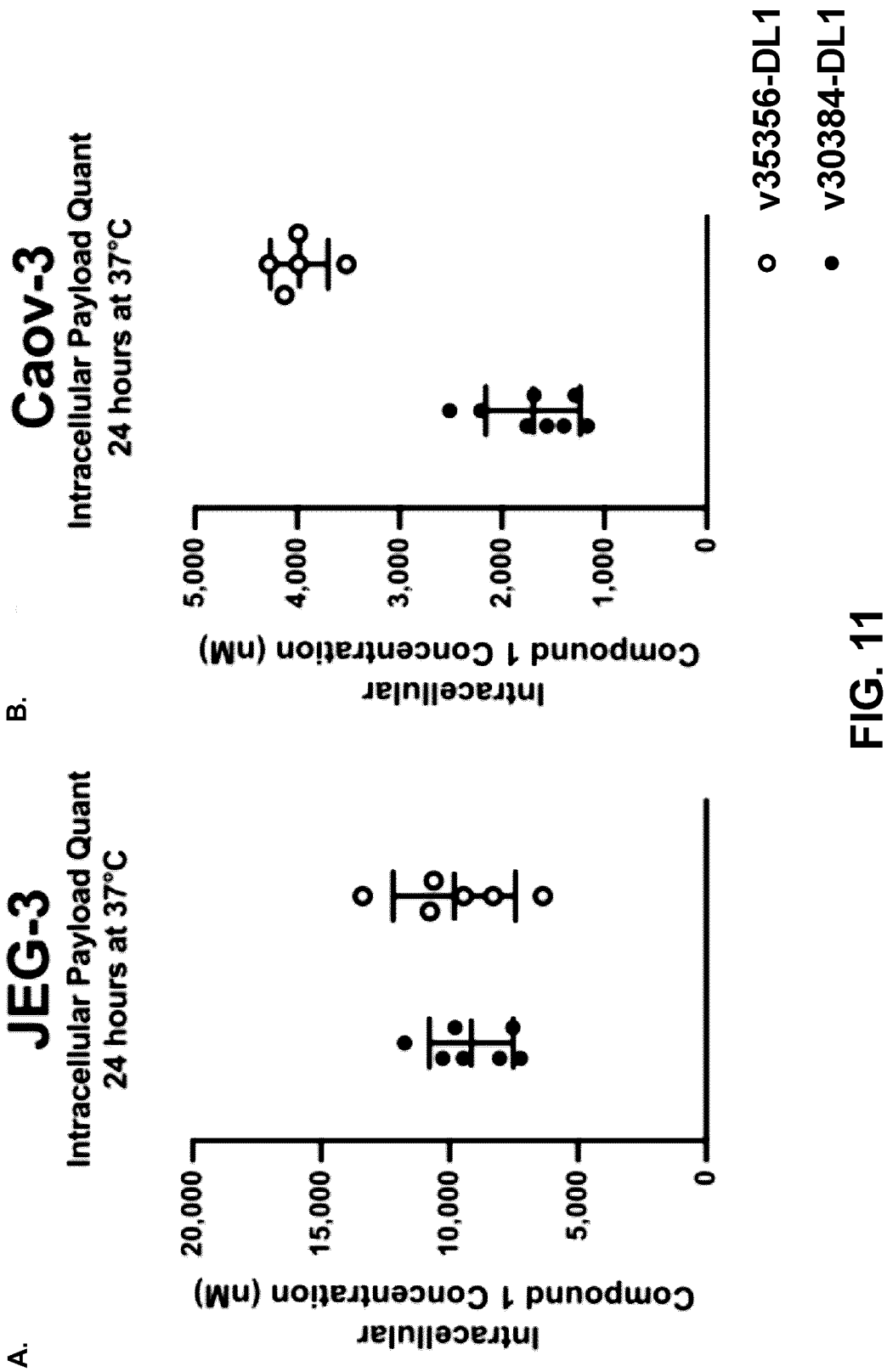

A.
JEG-3
Intracellular Payload Quant
24 hours at 37°C
Intracellular
Compound 1 Concentration (nM)
20,000
15,000
10,000
5,000
0
B.
Caov-3
Intracellular Payload Quant
24 hours at 37°C
Intracellular
Compound 1 Concentration (nM)
5,000
4,000
3,000
2,000
1,000
0
v35356-DL1
v30384-DL1

FIG. 11

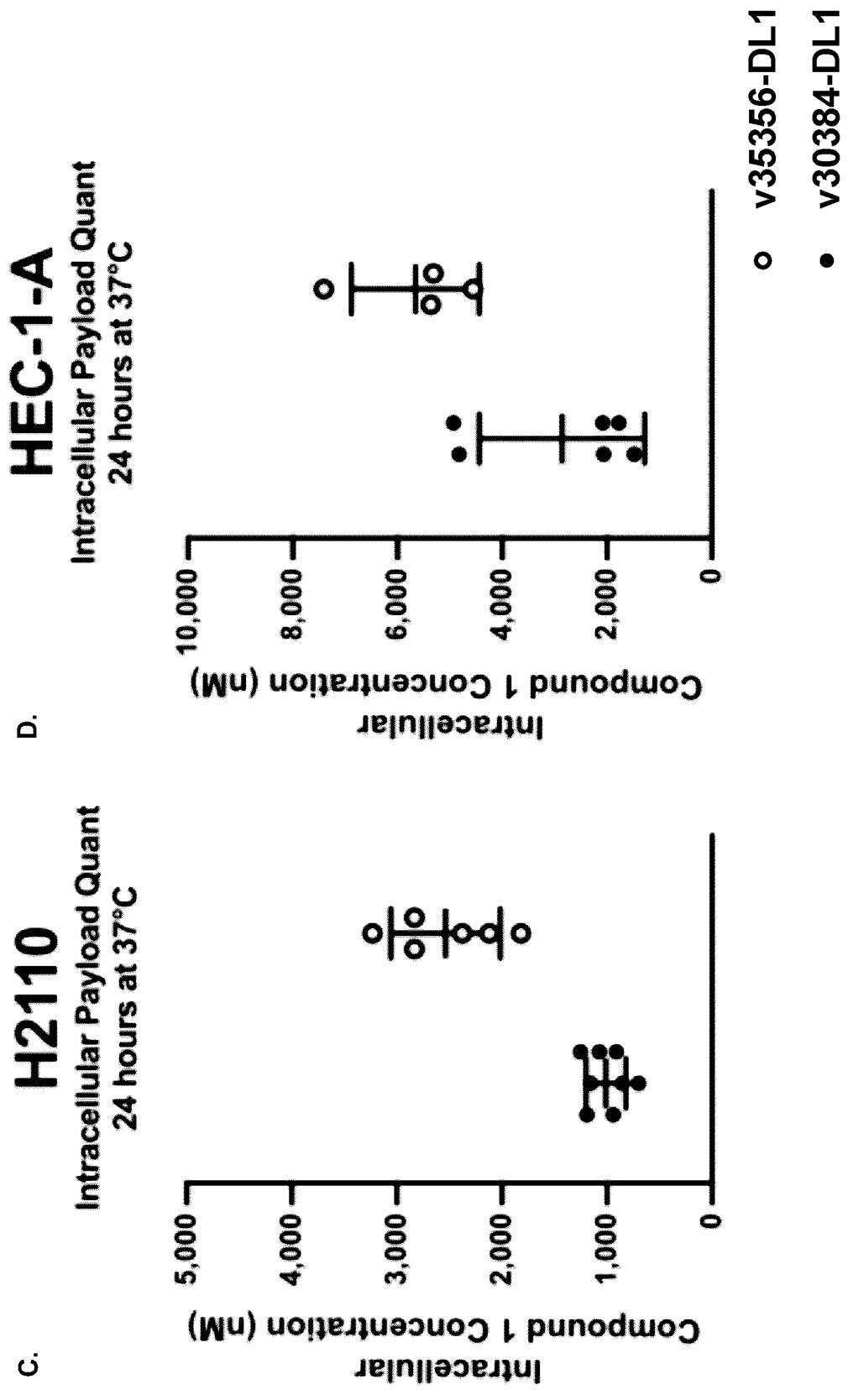
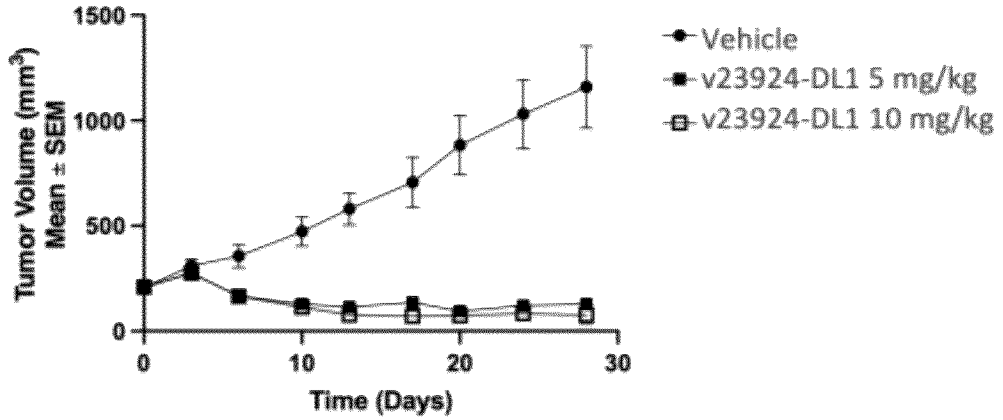

C.
H2110
Intracellular Payload Quant
24 hours at 37°C
Intracellular
Compound 1 Concentration (nM)
5,000
4,000
3,000
2,000
1,000
0
D.
HEC-1-A
Intracellular Payload Quant
24 hours at 37°C
Intracellular
Compound 1 Concentration (nM)
10,000
8,000
6,000
4,000
2,000
0
v35356-DL1
v30384-DL1

FIG. 11 (cont.)

A.


CTG-0848
Tumor Volume (mm3) Mean ± SEM
1500
1000
500
0
0
10
20
30
Time (Days)
Vehicle
v23924-DL1 5 mg/kg
v23924-DL1 10 mg/kg

B.

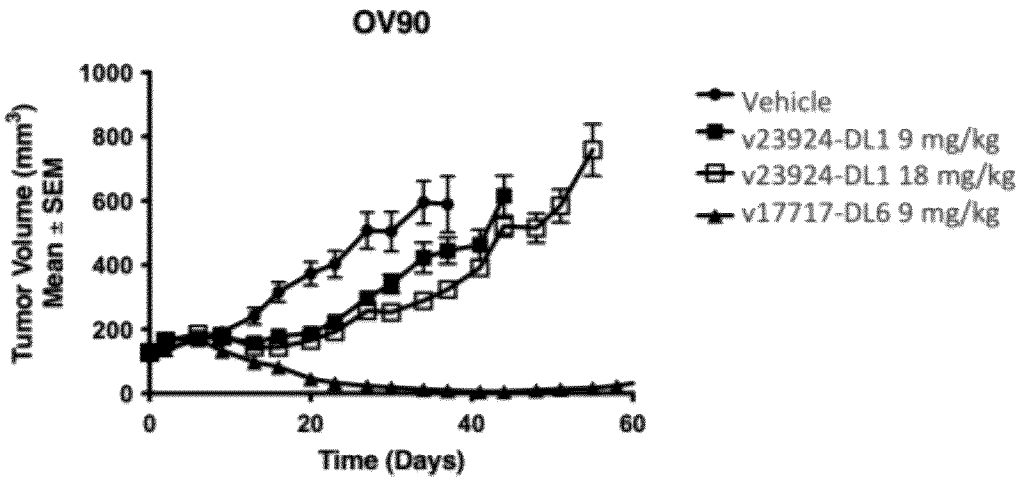
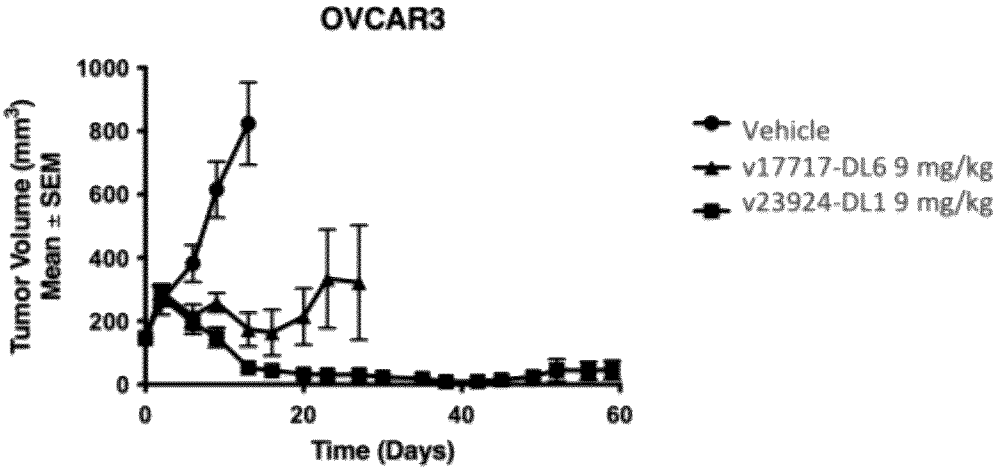

OV90
Tumor Volume (mm3) Mean ± SEM
1000
800
600
400
200
0
0
20
40
60
Time (Days)
Vehicle
v23924-DL1 9 mg/kg
v23924-DL1 18 mg/kg
v17717-DL6 9 mg/kg

FIG. 12

C.



D.

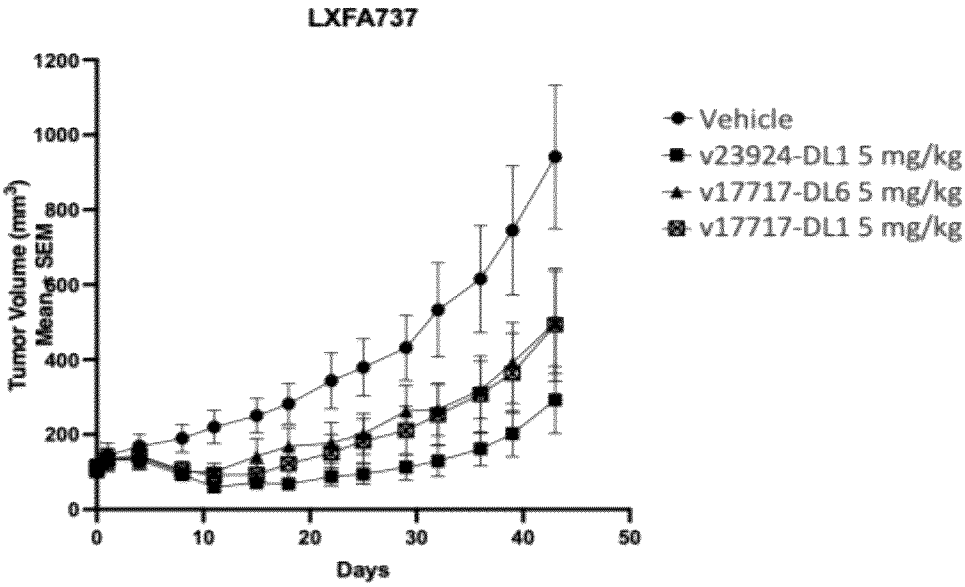


FIG. 12 (cont.)

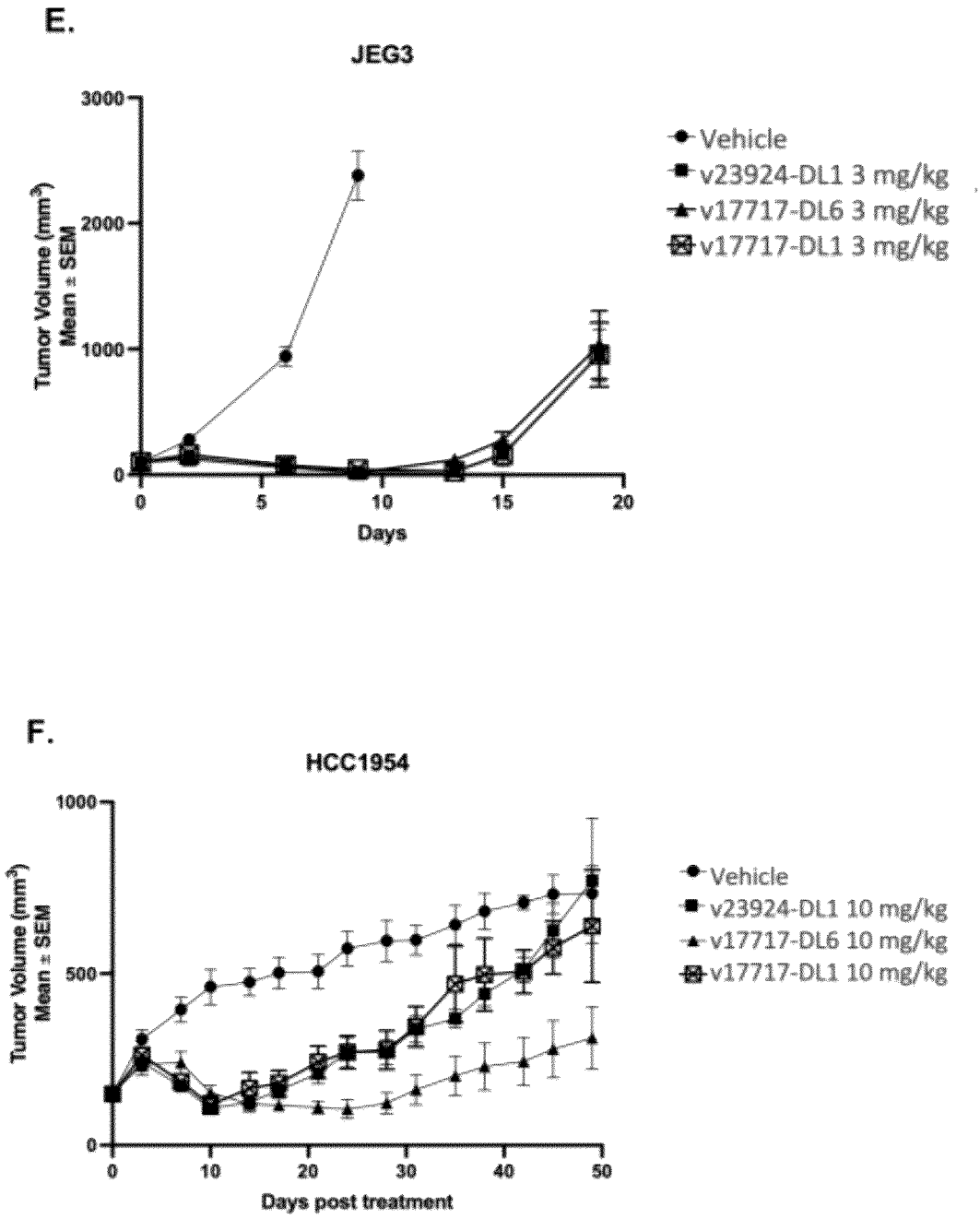

E.
JEG3
Tumor Volume (mm³)
Mean ± SEM
3000
2000
1000
0
0
5
10
15
20
Days
Vehicle
v23924-DL1 3 mg/kg
v17717-DL6 3 mg/kg
v17717-DL1 3 mg/kg
F.
HCC1954
Tumor Volume (mm³)
Mean ± SEM
1000
500
0
0
10
20
30
40
50
Days post treatment
Vehicle
v23924-DL1 10 mg/kg
v17717-DL6 10 mg/kg
v17717-DL1 10 mg/kg

FIG. 12 (cont.)

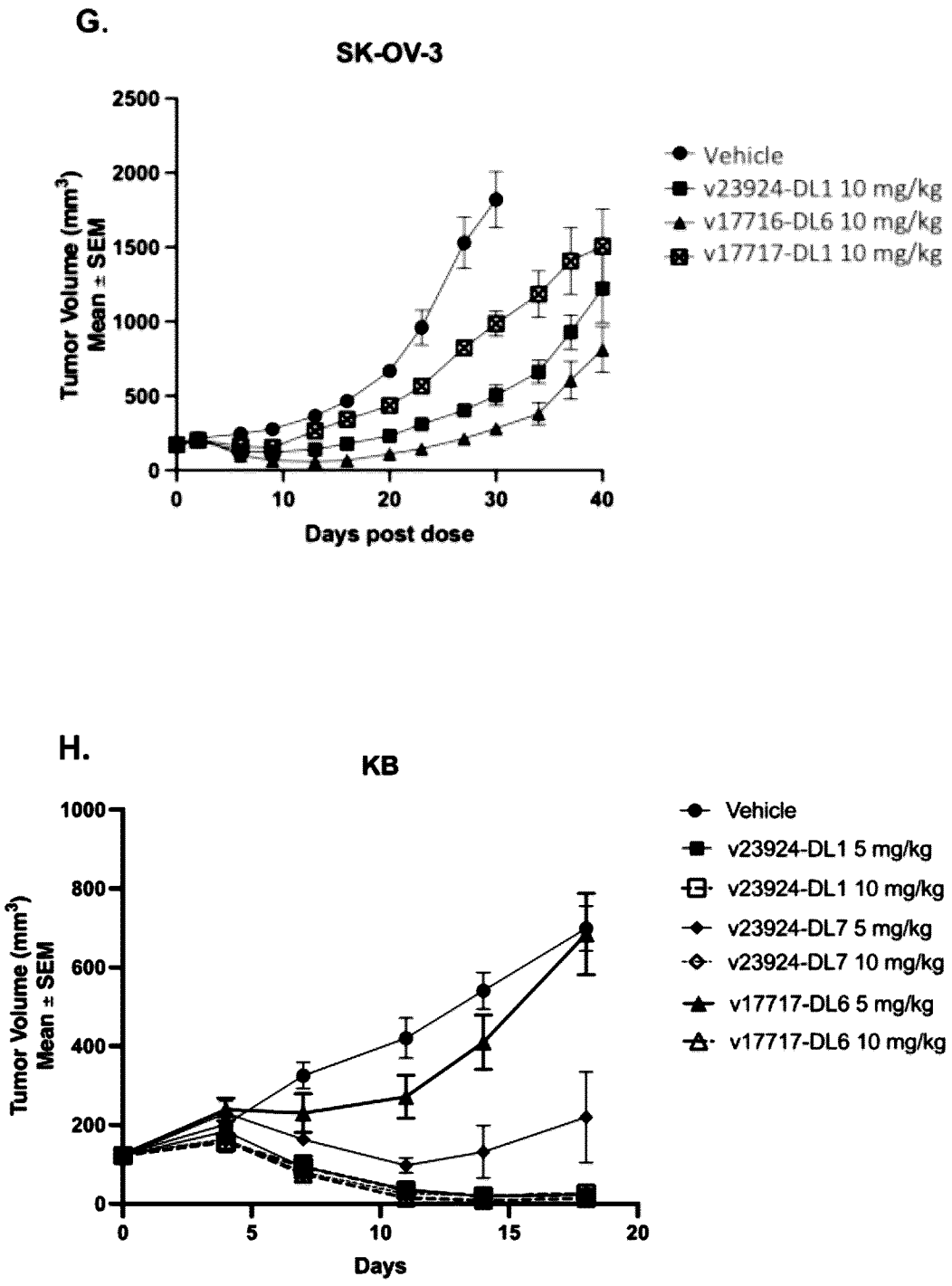

G.
SK-OV-3
Tumor Volume (mm3) Mean ± SEM
2500
2000
1500
1000
500
0
0
10
20
30
40
Days post dose
Vehicle
v23924-DL1 10 mg/kg
v17716-DL6 10 mg/kg
v17717-DL1 10 mg/kg
H.
KB
Tumor Volume (mm3) Mean ± SEM
1000
800
600
400
200
0
0
5
10
15
20
Days
Vehicle
v23924-DL1 5 mg/kg
v23924-DL1 10 mg/kg
v23924-DL7 5 mg/kg
v23924-DL7 10 mg/kg
v17717-DL6 5 mg/kg
v17717-DL6 10 mg/kg

FIG. 12 (cont.)

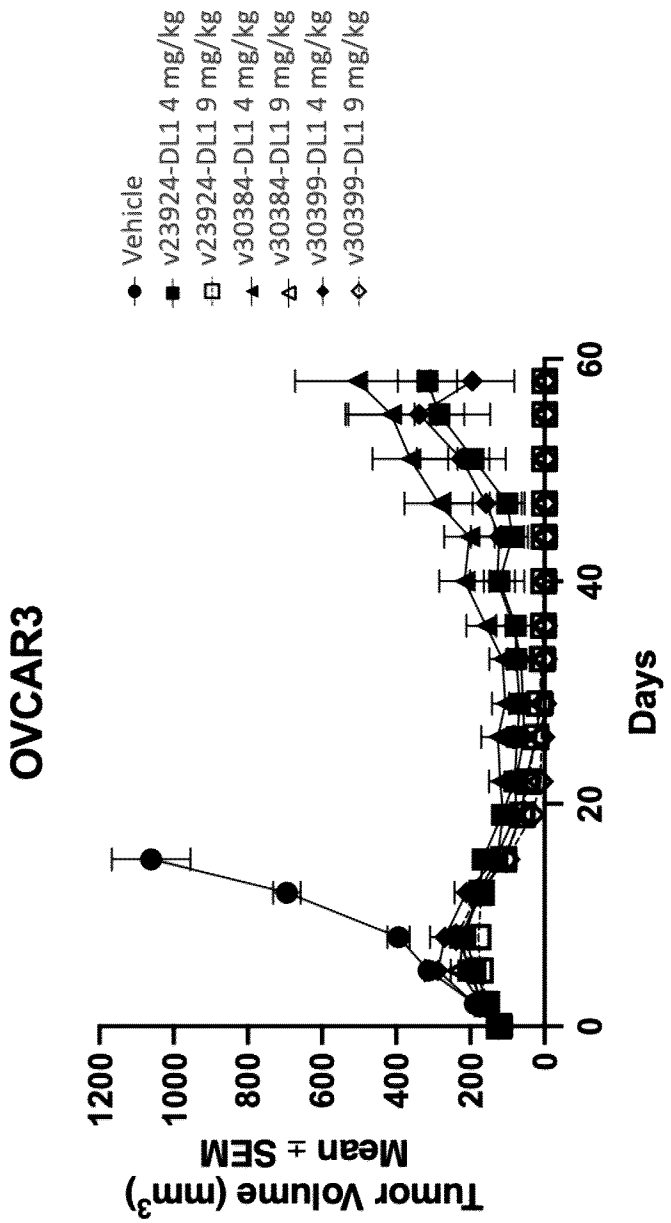
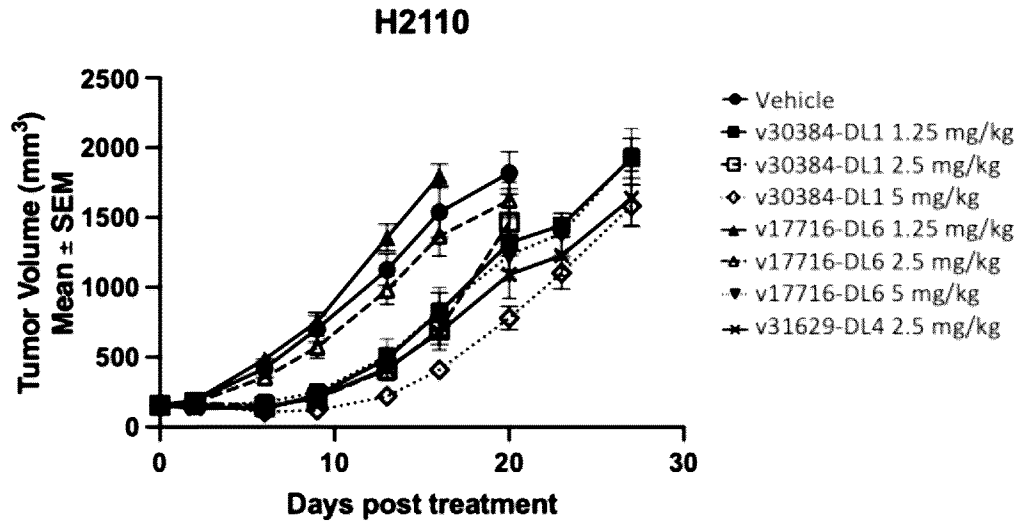

OVCAR3
Tumor Volume (mm3)
Mean ± SEM
1200
1000
800
600
400
200
0
0
20
40
60
Days
Vehicle
v23924-DL1 4 mg/kg
v23924-DL1 9 mg/kg
v30384-DL1 4 mg/kg
v30384-DL1 9 mg/kg
v30399-DL1 4 mg/kg
v30399-DL1 9 mg/kg

FIG. 13

A.


H2110
Tumor Volume (mm3) Mean ± SEM
2500
2000
1500
1000
500
0
0
10
20
30
Days post treatment
Vehicle
v30384-DL1 1.25 mg/kg
v30384-DL1 2.5 mg/kg
v30384-DL1 5 mg/kg
v17716-DL6 1.25 mg/kg
v17716-DL6 2.5 mg/kg
v17716-DL6 5 mg/kg
v31629-DL4 2.5 mg/kg

B.

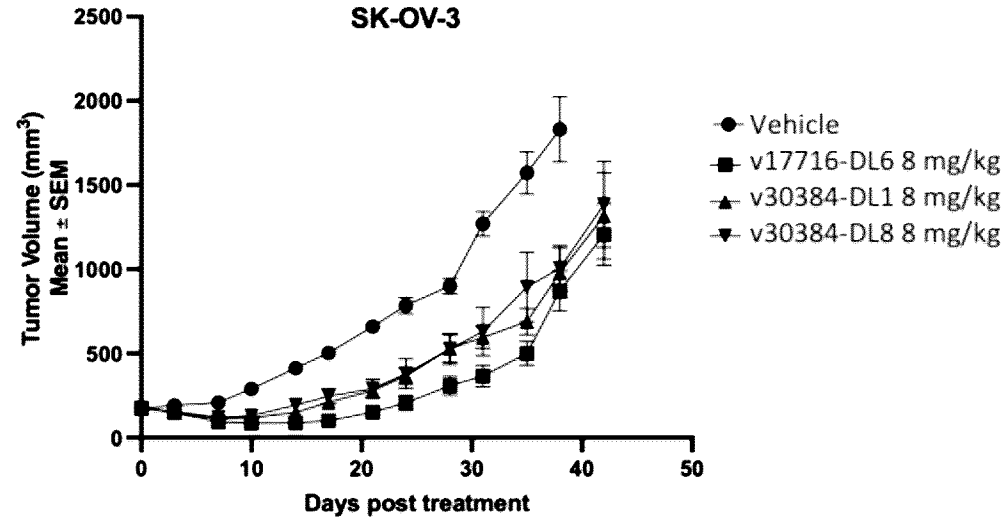
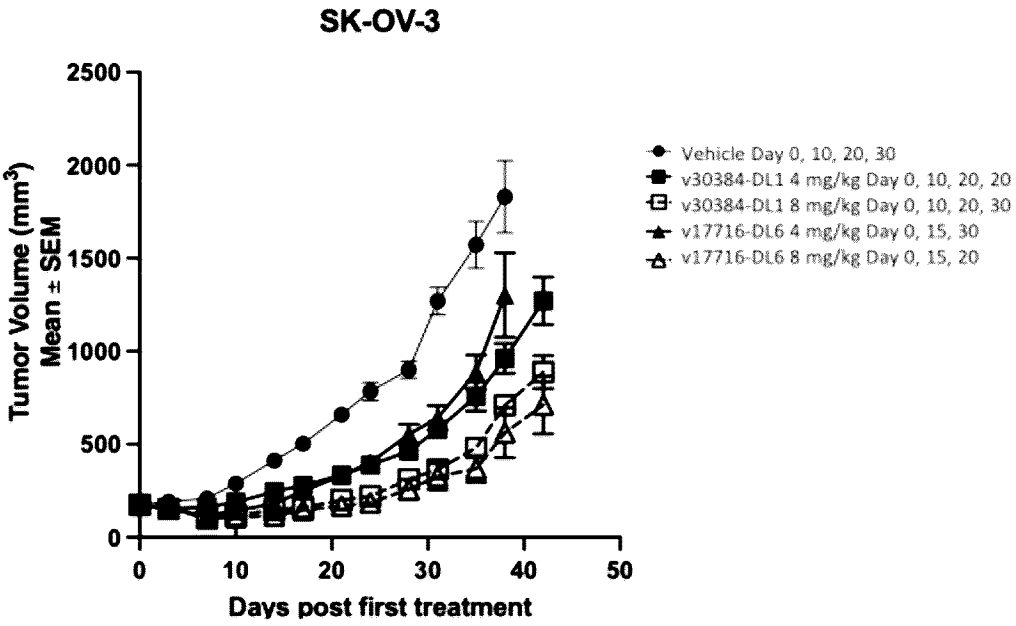

SK-OV-3
Tumor Volume (mm3) Mean ± SEM
2500
2000
1500
1000
500
0
0
10
20
30
40
50
Days post treatment
Vehicle
v17716-DL6 8 mg/kg
v30384-DL1 8 mg/kg
v30384-DL8 8 mg/kg

FIG. 14

C.



D.

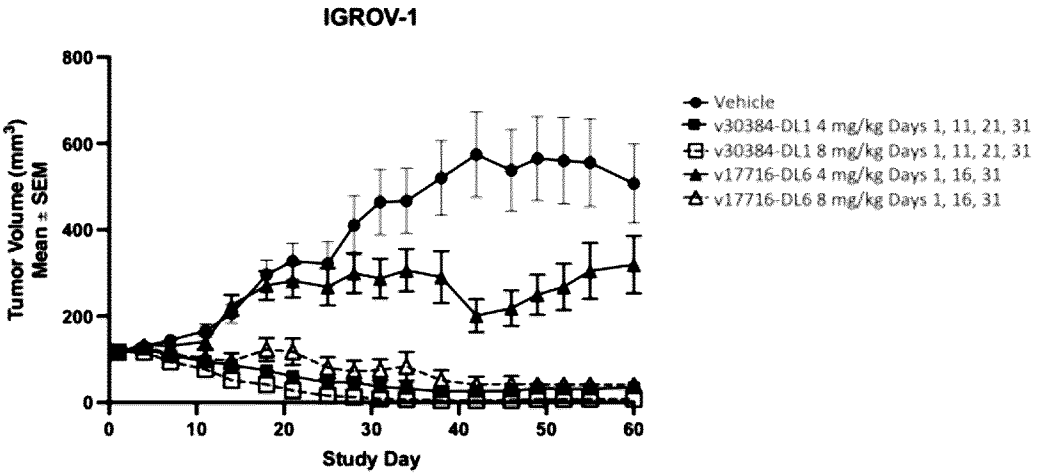


FIG. 14 (cont.)

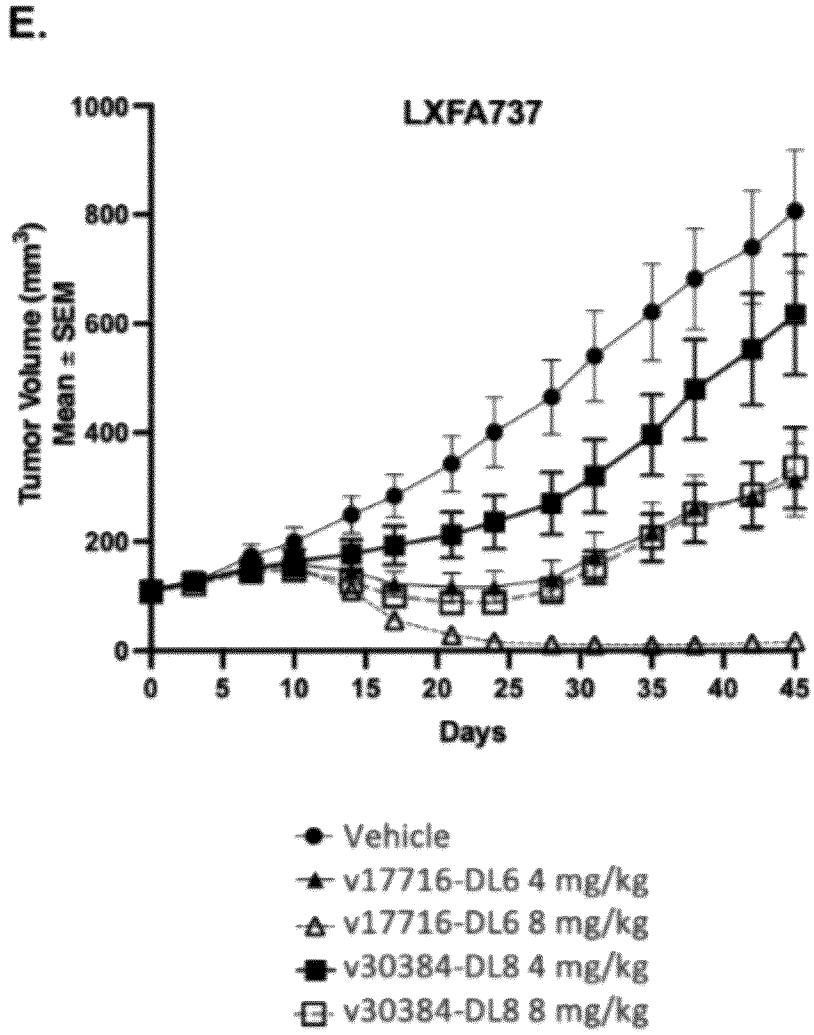

E.
LXFA737
Tumor Volume (mm3)
Mean ± SEM
1000
800
600
400
200
0
0
5
10
15
20
25
30
35
40
45
Days
Vehicle
v17716-DL6 4 mg/kg
v17716-DL6 8 mg/kg
v30384-DL8 4 mg/kg
v30384-DL8 8 mg/kg

FIG. 14 (cont.)

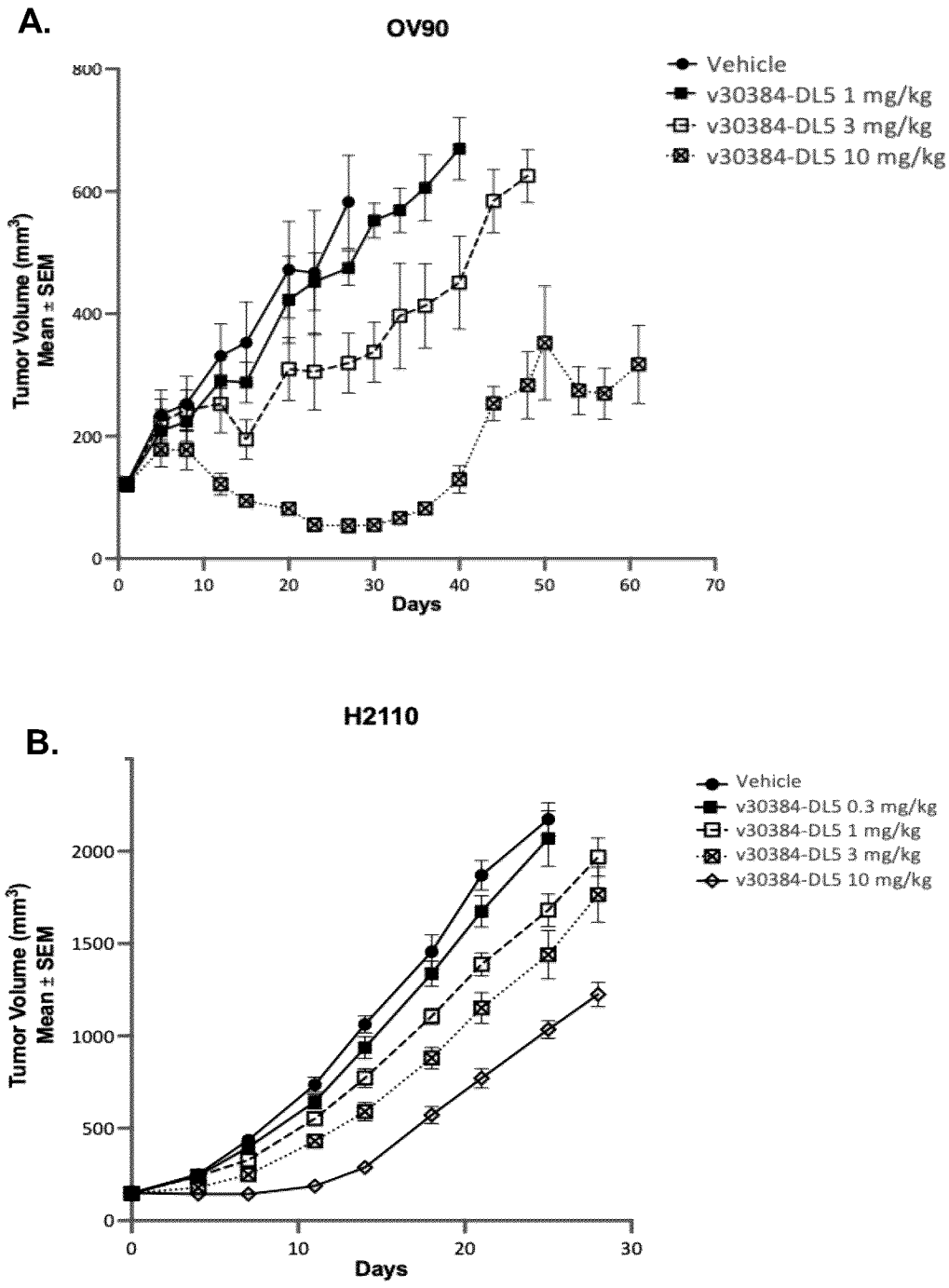

A.
OV90
Vehicle
v30384-DL5 1 mg/kg
v30384-DL5 3 mg/kg
v30384-DL5 10 mg/kg
Tumor Volume (mm3)
Mean ± SEM
Days
B.
H2110
Vehicle
v30384-DL5 0.3 mg/kg
v30384-DL5 1 mg/kg
v30384-DL5 3 mg/kg
v30384-DL5 10 mg/kg
Tumor Volume (mm3)
Mean ± SEM
Days

FIG. 15

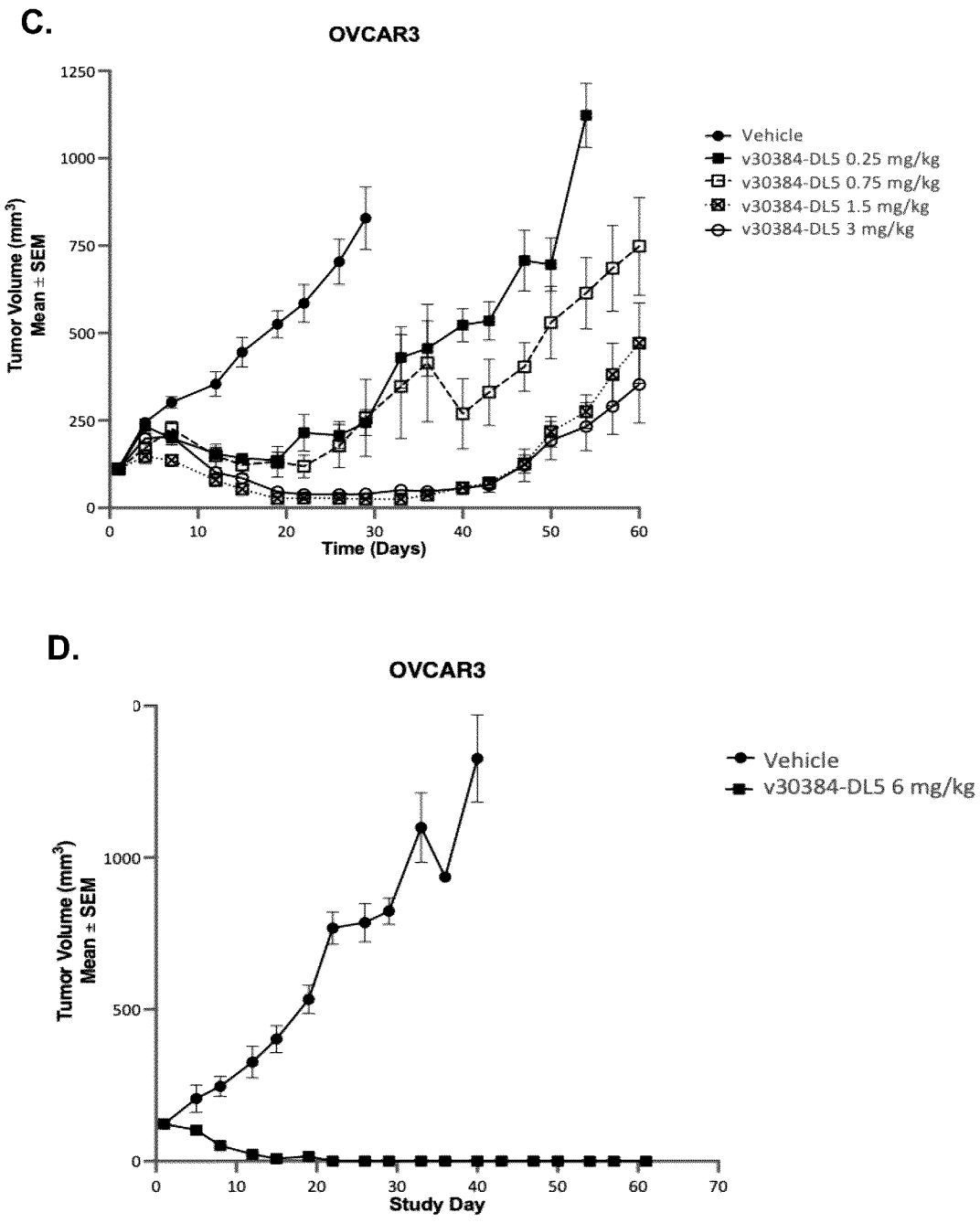
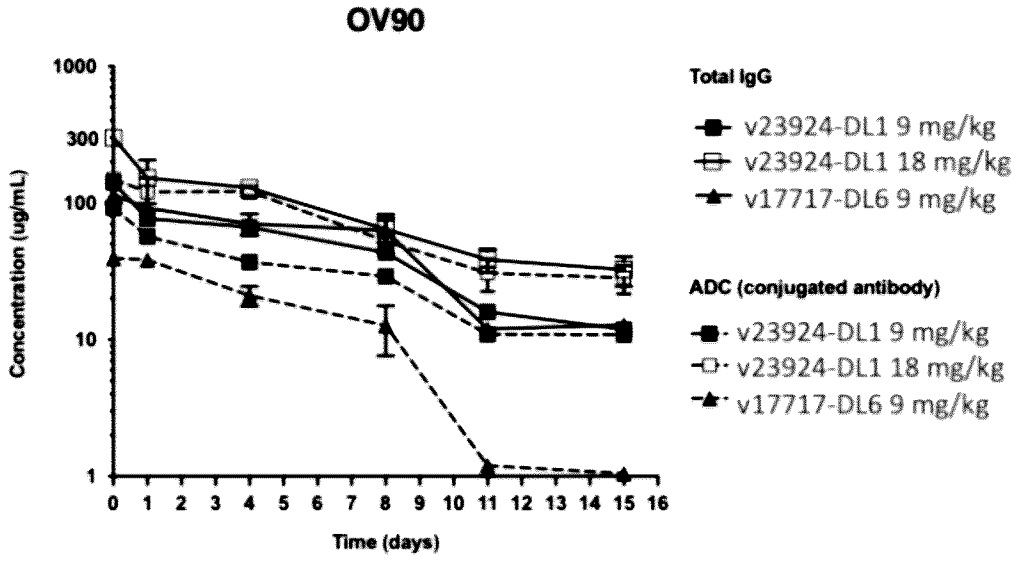

C.
OVCAR3
Tumor Volume (mm3) Mean ± SEM
Time (Days)
Vehicle
v30384-DL5 0.25 mg/kg
v30384-DL5 0.75 mg/kg
v30384-DL5 1.5 mg/kg
v30384-DL5 3 mg/kg
D.
OVCAR3
Tumor Volume (mm3) Mean ± SEM
Study Day
Vehicle
v30384-DL5 6 mg/kg

FIG. 15 (cont.)

A.


OV90
Concentration (ug/mL)
1000
300
100
10
1
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Time (days)
Total IgG
v23924-DL1 9 mg/kg
v23924-DL1 18 mg/kg
v17717-DL6 9 mg/kg
ADC (conjugated antibody)
v23924-DL1 9 mg/kg
v23924-DL1 18 mg/kg
v17717-DL6 9 mg/kg

B.

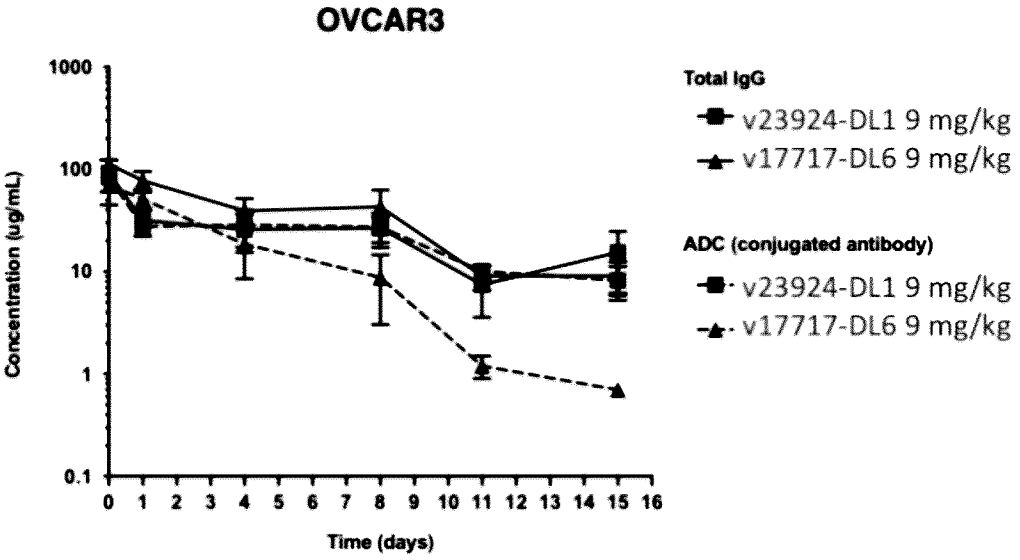
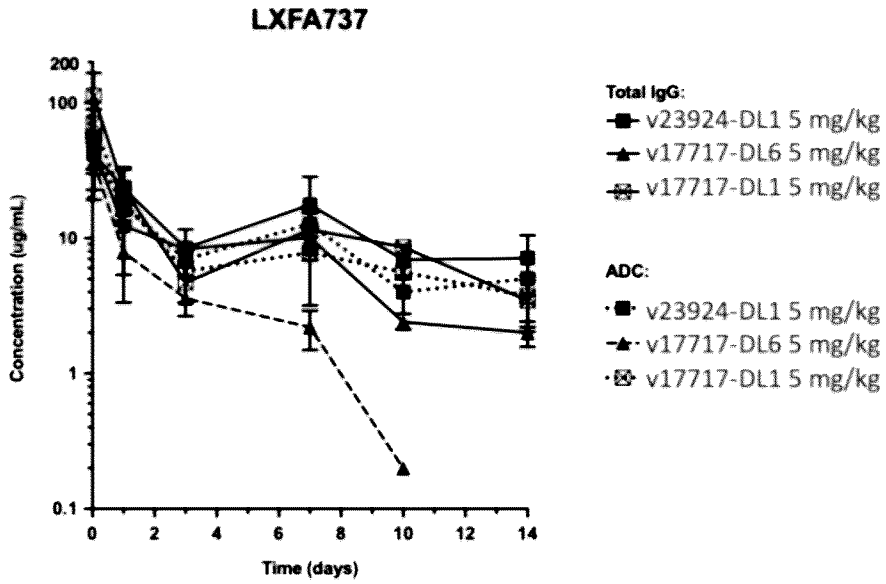

OVCAR3
Concentration (ug/mL)
1000
100
10
1
0.1
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Time (days)
Total IgG
v23924-DL1 9 mg/kg
v17717-DL6 9 mg/kg
ADC (conjugated antibody)
v23924-DL1 9 mg/kg
v17717-DL6 9 mg/kg

FIG. 16

C.



D.

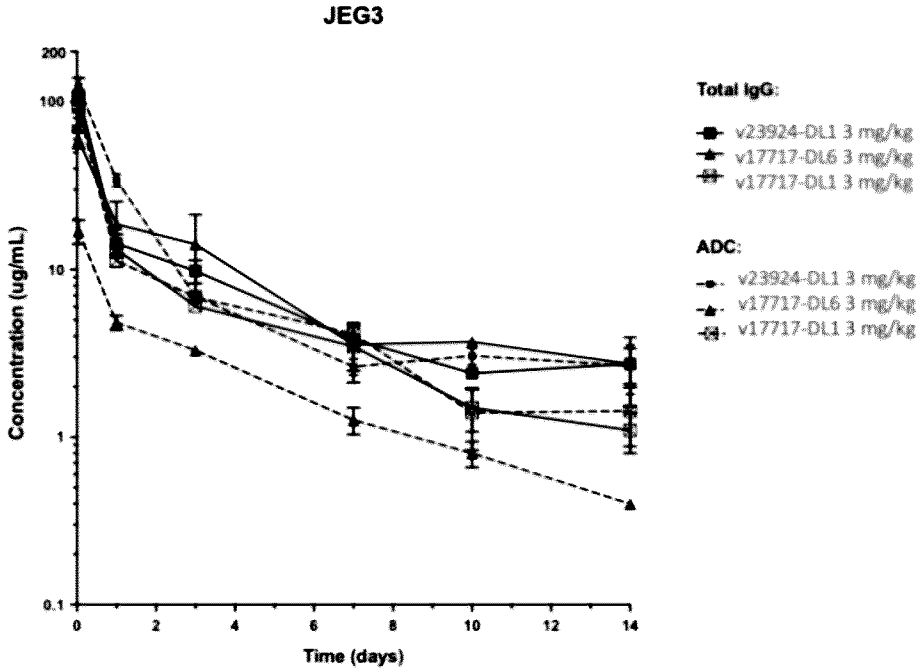


FIG. 16 (cont.)

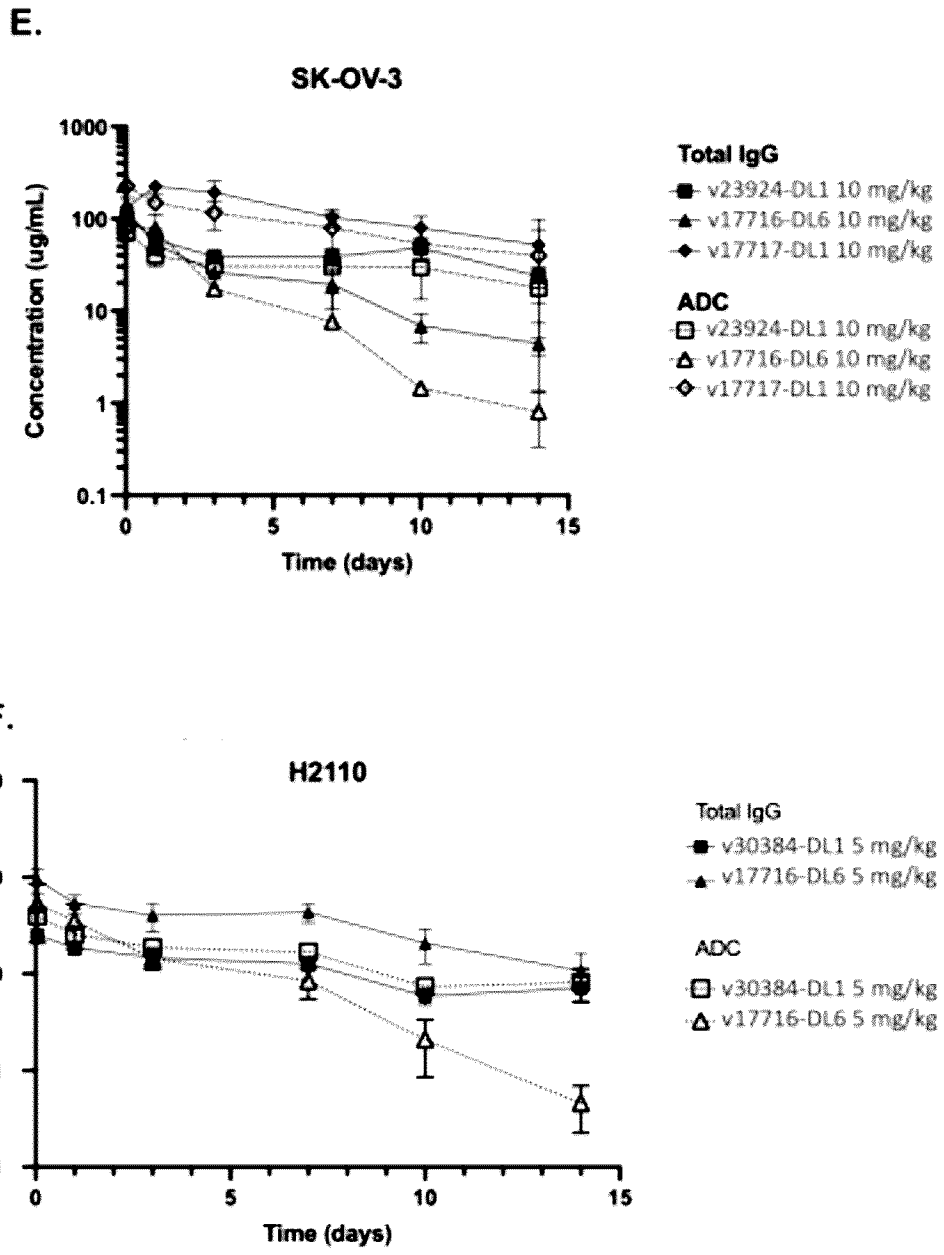
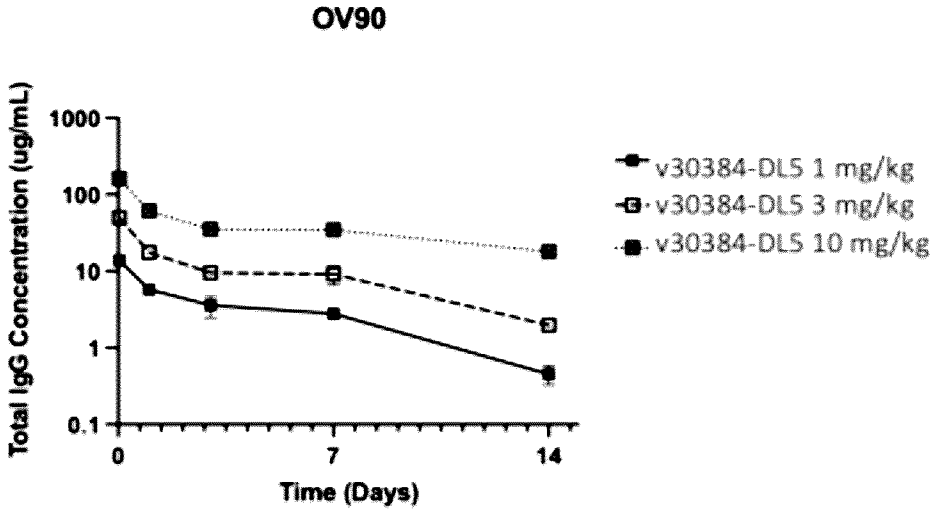

E.
SK-OV-3
Concentration (ug/mL)
1000
100
10
1
0.1
0
5
10
15
Time (days)
Total IgG
v23924-DL1 10 mg/kg
v17716-DL6 10 mg/kg
v17717-DL1 10 mg/kg
ADC
v23924-DL1 10 mg/kg
v17716-DL6 10 mg/kg
v17717-DL1 10 mg/kg
F.
H2110
Concentration (ug/mL)
1000
100
10
1
0.1
0
5
10
15
Time (days)
Total IgG
v30384-DL1 5 mg/kg
v17716-DL6 5 mg/kg
ADC
v30384-DL1 5 mg/kg
v17716-DL6 5 mg/kg

FIG. 16 (cont.)

G



H.

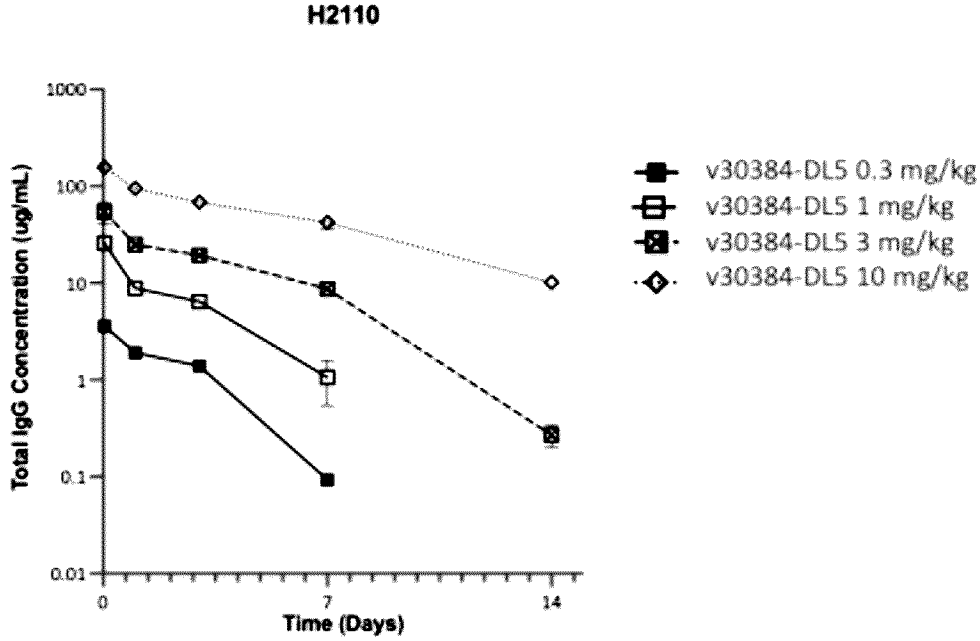


FIG. 16 (cont.)

CDR Sequences of Anti-FRα Antibodies

Variant	Numbering System	Heavy Chain CDR	Sequence	SEQ ID NO	Light Chain CDR	Sequence	SEQ ID NO
v23924 v30618	IMGT	HCDR1	GFSLSSYG	20	LCDR1	QSIGDW	40
		HCDR2	VNSGGSA	21	LCDR2	EAS	41
		HCDR3	ARSGSGYPMDYLAI	22	LCDR3	QQGYGRSNVDNI	42
	Chothia	HCDR1	GFSLSSY	23	LCDR1	QASQSIGDWLA	43
		HCDR2	NSGGS	24	LCDR2	EASTLAS	44
		HCDR3	SGSGYPMDYLAI	25	LCDR3	QQGYGRSNVDNI	42
	Kabat	HCDR1	SYGVS	26	LCDR1	QASQSIGDWLA	43
		HCDR2	AVNSGGSAYYANWAKS	27	LCDR2	EASTLAS	44
		HCDR3	SGSGYPMDYLAI	25	LCDR3	QQGYGRSNVDNI	42
	Contact	HCDR1	SSYGVS	28	LCDR1	GDWLAWY	45
		HCDR2	WIGAVNSGGSAY	29	LCDR2	LLIYEASTLA	46
		HCDR3	ARSGSGYPMDYLA	30	LCDR3	QQGYGRSNVDN	47
	AbM	HCDR1	GFSLSSYGVS	31	LCDR1	QASQSIGDWLA	43
		HCDR2	AVNSGGSAY	32	LCDR2	EASTLAS	44
		HCDR3	SGSGYPMDYLAI	25	LCDR3	QQGYGRSNVDNI	42
v30384 v30389 v31422 v31423 v31424 v31425 v31426 v36675	IMGT	HCDR1	GFSLSSYG	20	LCDR1	QSIGDW	40
		HCDR2	VNSGGSA	21	LCDR2	EAS	41
		HCDR3	ARSGSGYPMDYLAI	22	LCDR3	QQGYGRSNVDNI	42
	Chothia	HCDR1	GFSLSSY	23	LCDR1	RASQSIGDWLA	65
		HCDR2	NSGGS	24	LCDR2	EASTLAS	44
		HCDR3	SGSGYPMDYLAI	25	LCDR3	QQGYGRSNVDNI	42
	Kabat	HCDR1	SYGVS	26	LCDR1	RASQSIGDWLA	65
		HCDR2	AVNSGGSAYYADSVKG	51	LCDR2	EASTLAS	44
		HCDR3	SGSGYPMDYLAI	25	LCDR3	QQGYGRSNVDNI	42
	Contact	HCDR1	SSYGVS	28	LCDR1	GDWLAWY	45
		HCDR2	WIGAVNSGGSAY	29	LCDR2	LLIYEASTLA	46
		HCDR3	ARSGSGYPMDYLA	30	LCDR3	QQGYGRSNVDN	47
	AbM	HCDR1	GFSLSSYGVS	31	LCDR1	RASQSIGDWLA	65

FIG. 17

Variant	Numbering System	Heavy Chain CDR	Sequence	SEQ ID NO	Light Chain CDR	Sequence	SEQ ID NO
		HCDR2	AVNSGGSAY	32	LCDR2	EASTLAS	44
		HCDR3	SGSGYPMDYLAI	25	LCDR3	QQGYGRSNVDNI	42
v30394 v30399	IMGT	HCDR1	GFSLSSYG	20	LCDR1	QSIGDW	40
		HCDR2	VNSGGSA	21	LCDR2	EAS	41
		HCDR3	ARSGSGYPMDYLAI	22	LCDR3	QQGYGRSNVDNI	42
	Chothia	HCDR1	GFSLSSY	23	LCDR1	RASQSIGDWLA	65
		HCDR2	NSGGS	24	LCDR2	EASTLAS	44
		HCDR3	SGSGYPMDYLAI	25	LCDR3	QQGYGRSNVDNI	42
	Kabat	HCDR1	SYGVS	26	LCDR1	RASQSIGDWLA	65
		HCDR2	AVNSGGSAYYADWAKG	58	LCDR2	EASTLAS	44
		HCDR3	SGSGYPMDYLAI	25	LCDR3	QQGYGRSNVDNI	42
	Contact	HCDR1	SSYGVS	28	LCDR1	GDWLAWY	45
		HCDR2	WIGAVNSGGSAY	29	LCDR2	LLIYEASTLA	46
		HCDR3	ARSGSGYPMDYLA	30	LCDR3	QQGYGRSNVDN	47
	AbM	HCDR1	GFSLSSYGVS	31	LCDR1	RASQSIGDWLA	65
		HCDR2	AVNSGGSAY	32	LCDR2	EASTLAS	44
		HCDR3	SGSGYPMDYLAI	25	LCDR3	QQGYGRSNVDNI	42
v35305	IMGT	HCDR1	GFSLSLYG	92	LCDR1	QSIWYW	125
		HCDR2	VNSGGSA	21	LCDR2	EAS	41
		HCDR3	ARSGSGYPMDYLAI	22	LCDR3	QQGYGRSNVDNI	42
	Chothia	HCDR1	GFSLSLY	93	LCDR1	RASQSIWYWLA	126
		HCDR2	NSGGS	24	LCDR2	EASTLAS	44
		HCDR3	SGSGYPMDYLAI	25	LCDR3	QQGYGRSNVDNI	42
	Kabat	HCDR1	LYGVS	94	LCDR1	RASQSIWYWLA	126
		HCDR2	AVNSGGSAYYADSVKG	51	LCDR2	EASTLAS	44
		HCDR3	SGSGYPMDYLAI	25	LCDR3	QQGYGRSNVDNI	42
	Contact	HCDR1	SLYGVS	95	LCDR1	WYWLAWY	127
		HCDR2	WIGAVNSGGSAY	29	LCDR2	LLIYEASTLA	46
		HCDR3	ARSGSGYPMDYLA	30	LCDR3	QQGYGRSNVDN	47

FIG. 17 (cont.)

Variant	Numbering System	Heavy Chain CDR	Sequence	SEQ ID NO	Light Chain CDR	Sequence	SEQ ID NO
	AbM	HCDR1	GFSLSLYGVS	96	LCDR1	RASQSIWYWLA	126
		HCDR2	AVNSGGSAY	32	LCDR2	EASTLAS	44
		HCDR3	SGSGYPMDYLAI	25	LCDR3	QQGYGRSNVDNI	42
v35342	IMGT	HCDR1	GFSLSLYG	92	LCDR1	QSIWYW	125
		HCDR2	LNSGGSA	100	LCDR2	EAS	41
		HCDR3	ARSGSGYPMDYLAI	22	LCDR3	QQGYGRWHILNI	120
	Chothia	HCDR1	GFSLSLY	93	LCDR1	RASQSIWYWLA	126
		HCDR2	NSGGS	24	LCDR2	EASTLAS	44
		HCDR3	SGSGYPMDYLAI	25	LCDR3	QQGYGRWHILNI	120
	Kabat	HCDR1	LYGVS	94	LCDR1	RASQSIWYWLA	126
		HCDR2	SLNSGGSAYYADSVKG	101	LCDR2	EASTLAS	44
		HCDR3	SGSGYPMDYLAI	25	LCDR3	QQGYGRWHILNI	120
	Contact	HCDR1	SLYGVS	95	LCDR1	WYWLAWY	127
		HCDR2	WIGSLNSGGSAY	102	LCDR2	LLIYEASTLA	46
		HCDR3	ARSGSGYPMDYLA	30	LCDR3	QQGYGRWHILN	121
	AbM	HCDR1	GFSLSLYGVS	96	LCDR1	RASQSIWYWLA	126
		HCDR2	SLNSGGSAY	103	LCDR2	EASTLAS	44
		HCDR3	SGSGYPMDYLAI	25	LCDR3	QQGYGRWHILNI	120
v35347	IMGT	HCDR1	GFSLSLYG	92	LCDR1	QSIGDW	40
		HCDR2	VNSGGSA	21	LCDR2	EAS	41
		HCDR3	ARSGSGYPMDYHPI	107	LCDR3	QQGYGRSNVDNI	42
	Chothia	HCDR1	GFSLSLY	93	LCDR1	RASQSIGDWLA	65
		HCDR2	NSGGS	24	LCDR2	EASTLAS	44
		HCDR3	SGSGYPMDYHPI	108	LCDR3	QQGYGRSNVDNI	42
	Kabat	HCDR1	LYGVS	94	LCDR1	RASQSIGDWLA	65
		HCDR2	AVNSGGSAYYADSFKG	109	LCDR2	EASTLAS	44
		HCDR3	SGSGYPMDYHPI	108	LCDR3	QQGYGRSNVDNI	42
	Contact	HCDR1	SLYGVS	95	LCDR1	GDWLAWY	45
		HCDR2	WIGAVNSGGSAY	29	LCDR2	LLIYEASTLA	46

FIG. 17 (cont.)

Variant	Numbering System	Heavy Chain CDR	Sequence	SEQ ID NO	Light Chain CDR	Sequence	SEQ ID NO
		HCDR3	ARSGSGYPMDYHP	110	LCDR3	QQGYGRSNVDN	47
	AbM	HCDR1	GFSLSLYGVS	96	LCDR1	RASQSIGDWLA	65
		HCDR2	AVNSGGSAY	32	LCDR2	EASTLAS	44
		HCDR3	SGSGYPMDYHPI	108	LCDR3	QQGYGRSNVDNI	42
v35348	IMGT	HCDR1	GFSLSLYG	92	LCDR1	QSIGDW	40
		HCDR2	VNSGGSA	21	LCDR2	EAS	41
		HCDR3	ARSGSGYPMDYHPI	107	LCDR3	QQGYGRWHILNI	120
	Chothia	HCDR1	GFSLSLY	93	LCDR1	RASQSIGDWLA	65
		HCDR2	NSGGS	24	LCDR2	EASTLAS	44
		HCDR3	SGSGYPMDYHPI	108	LCDR3	QQGYGRWHILNI	120
	Kabat	HCDR1	LYGVS	94	LCDR1	RASQSIGDWLA	65
		HCDR2	AVNSGGSAYYADSFKG	109	LCDR2	EASTLAS	44
		HCDR3	SGSGYPMDYHPI	108	LCDR3	QQGYGRWHILNI	120
	Contact	HCDR1	SLYGVS	95	LCDR1	GDWLAWY	45
		HCDR2	WIGAVNSGGSAY	29	LCDR2	LLIYEASTLA	46
		HCDR3	ARSGSGYPMDYHP	110	LCDR3	QQGYGRWHILN	121
	AbM	HCDR1	GFSLSLYGVS	96	LCDR1	RASQSIGDWLA	65
		HCDR2	AVNSGGSAY	32	LCDR2	EASTLAS	44
		HCDR3	SGSGYPMDYHPI	108	LCDR3	QQGYGRWHILNI	120
v35350	IMGT	HCDR1	GFSLSLYG	92	LCDR1	QSIWYW	125
		HCDR2	VNSGGSA	21	LCDR2	EAS	41
		HCDR3	ARSGSGYPMDYHPI	107	LCDR3	QQGYGRWHILNI	120
	Chothia	HCDR1	GFSLSLY	93	LCDR1	RASQSIWYWLA	126
		HCDR2	NSGGS	24	LCDR2	EASTLAS	44
		HCDR3	SGSGYPMDYHPI	108	LCDR3	QQGYGRWHILNI	120
	Kabat	HCDR1	LYGVS	94	LCDR1	RASQSIWYWLA	126
		HCDR2	AVNSGGSAYYADSFKG	109	LCDR2	EASTLAS	44
		HCDR3	SGSGYPMDYHPI	108	LCDR3	QQGYGRWHILNI	120
	Contact	HCDR1	SLYGVS	95	LCDR1	WYWLAWY	127

FIG. 17 (cont.)

Variant	Numbering System	Heavy Chain CDR	Sequence	SEQ ID NO	Light Chain CDR	Sequence	SEQ ID NO
		HCDR2	WIGAVNSGGSAY	29	LCDR2	LLIYEASTLA	46
		HCDR3	ARSGSGYPMDYHP	110	LCDR3	QQGYGRWHILN	121
	AbM	HCDR1	GFSLSLYGVS	96	LCDR1	RASQSIWYWLA	126
		HCDR2	AVNSGGSAY	32	LCDR2	EASTLAS	44
		HCDR3	SGSGYPMDYHPI	108	LCDR3	QQGYGRWHILNI	120
v35354	IMGT	HCDR1	GFSLSLYG	92	LCDR1	QSIWYW	125
		HCDR2	VNSGGSA	21	LCDR2	EAS	41
		HCDR3	ARSGSGYPMDYLAI	22	LCDR3	QQGYGRWHILNI	120
	Chothia	HCDR1	GFSLSLY	93	LCDR1	RASQSIWYWLA	126
		HCDR2	NSGGS	24	LCDR2	EASTLAS	44
		HCDR3	SGSGYPMDYLAI	25	LCDR3	QQGYGRWHILNI	120
	Kabat	HCDR1	LYGVS	94	LCDR1	RASQSIWYWLA	126
		HCDR2	AVNSGGSAYYADSFKG	109	LCDR2	EASTLAS	44
		HCDR3	SGSGYPMDYLAI	25	LCDR3	QQGYGRWHILNI	120
	Contact	HCDR1	SLYGVS	95	LCDR1	WYWLAWY	127
		HCDR2	WIGAVNSGGSAY	29	LCDR2	LLIYEASTLA	46
		HCDR3	ARSGSGYPMDYLA	30	LCDR3	QQGYGRWHILN	121
	AbM	HCDR1	GFSLSLYGVS	96	LCDR1	RASQSIWYWLA	126
		HCDR2	AVNSGGSAY	32	LCDR2	EASTLAS	44
		HCDR3	SGSGYPMDYLAI	25	LCDR3	QQGYGRWHILNI	120
v35356	IMGT	HCDR1	GFSLSSYG	20	LCDR1	QSIGDW	40
		HCDR2	VNSGGSA	21	LCDR2	EAS	41
		HCDR3	ARSGSGYPMDYHPI	107	LCDR3	QQGYGRWHILNI	120
	Chothia	HCDR1	GFSLSSY	23	LCDR1	RASQSIGDWLA	65
		HCDR2	NSGGS	24	LCDR2	EASTLAS	44
		HCDR3	SGSGYPMDYHPI	108	LCDR3	QQGYGRWHILNI	120
	Kabat	HCDR1	SYGVS	26	LCDR1	RASQSIGDWLA	65
		HCDR2	AVNSGGSAYYADSFKG	109	LCDR2	EASTLAS	44
		HCDR3	SGSGYPMDYHPI	108	LCDR3	QQGYGRWHILNI	120

FIG. 17 (cont.)

Variant	Numbering System	Heavy Chain CDR	Sequence	SEQ ID NO	Light Chain CDR	Sequence	SEQ ID NO
	Contact	HCDR1	SSYGVS	28	LCDR1	GDWLAWY	45
		HCDR2	WIGAVNSGGSAY	29	LCDR2	LLIYEASTLA	46
		HCDR3	ARSGSGYPMDYHP	110	LCDR3	QQGYGRWHILN	121
	AbM	HCDR1	GFSLSSYGVS	31	LCDR1	RASQSIGDWLA	65
		HCDR2	AVNSGGSAY	32	LCDR2	EASTLAS	44
		HCDR3	SGSGYPMDYHP1	108	LCDR3	QQGYGRWHILN1	120
v35358	IMGT	HCDR1	GFSLSSYG	20	LCDR1	QSIWYW	125
		HCDR2	VNSGGSA	21	LCDR2	EAS	41
		HCDR3	ARSGSGYPMDYHP1	107	LCDR3	QQGYGRWHILN1	120
	Chothia	HCDR1	GFSLSSY	23	LCDR1	RASQSIWYWLA	126
		HCDR2	NSGGS	24	LCDR2	EASTLAS	44
		HCDR3	SGSGYPMDYHP1	108	LCDR3	QQGYGRWHILN1	120
	Kabat	HCDR1	SYGVS	26	LCDR1	RASQSIWYWLA	126
		HCDR2	AVNSGGSAYYADSFKG	109	LCDR2	EASTLAS	44
		HCDR3	SGSGYPMDYHP1	108	LCDR3	QQGYGRWHILN1	120
	Contact	HCDR1	SSYGVS	28	LCDR1	WYWLAWY	127
		HCDR2	WIGAVNSGGSAY	29	LCDR2	LLIYEASTLA	46
		HCDR3	ARSGSGYPMDYHP	110	LCDR3	QQGYGRWHILN	121
	AbM	HCDR1	GFSLSSYGVS	31	LCDR1	RASQSIWYWLA	126
		HCDR2	AVNSGGSAY	32	LCDR2	EASTLAS	44
		HCDR3	SGSGYPMDYHP1	108	LCDR3	QQGYGRWHILN1	120
v36167	IMGT	HCDR1	GFSLSSYG	20	LCDR1	QSIWYW	125
		HCDR2	VNSGGSA	21	LCDR2	EAS	41
		HCDR3	ARSGSGYPMDYLAI	22	LCDR3	QQGYGRWHILN1	120
	Chothia	HCDR1	GFSLSSY	23	LCDR1	RASQSIWYWLA	126
		HCDR2	NSGGS	24	LCDR2	EASTLAS	44
		HCDR3	SGSGYPMDYLAI	25	LCDR3	QQGYGRWHILN1	120
	Kabat	HCDR1	SYGVS	26	LCDR1	RASQSIWYWLA	126
		HCDR2	AVNSGGSAYYADSFKG	109	LCDR2	EASTLAS	44

FIG. 17 (cont.)

Variant	Numbering System	Heavy Chain CDR	Sequence	SEQ ID NO	Light Chain CDR	Sequence	SEQ ID NO
		HCDR3	SGSGYPMDYLAI	25	LCDR3	QQGYGRWHILNI	120
	Contact	HCDR1	SSYGVS	28	LCDR1	WYWLAWY	127
		HCDR2	WIGAVNSGGSAY	29	LCDR2	LLIYEASTLA	46
		HCDR3	ARSGSGYPMDYLA	30	LCDR3	QQGYGRWHILN	121
	AbM	HCDR1	GFSLSSYGVS	31	LCDR1	RASQSIWYWLA	126
		HCDR2	AVNSGGSAY	32	LCDR2	EASTLAS	44
		HCDR3	SGSGYPMDYLAI	25	LCDR3	QQGYGRWHILNI	120
v36168	IMGT	HCDR1	GFSLSLYG	92	LCDR1	QSIWYW	125
		HCDR2	VNSGGSA	21	LCDR2	EAS	41
		HCDR3	ARSGSGYPMDYLAI	22	LCDR3	QQGYGRWHILNI	120
	Chothia	HCDR1	GFSLSLY	93	LCDR1	RASQSIWYWLA	126
		HCDR2	NSGGS	24	LCDR2	EASTLAS	44
		HCDR3	SGSGYPMDYLAI	25	LCDR3	QQGYGRWHILNI	120
	Kabat	HCDR1	LYGVS	94	LCDR1	RASQSIWYWLA	126
		HCDR2	SVNSGGSAYYADSVKG	137	LCDR2	EASTLAS	44
		HCDR3	SGSGYPMDYLAI	25	LCDR3	QQGYGRWHILNI	120
	Contact	HCDR1	SLYGVS	95	LCDR1	WYWLAWY	127
		HCDR2	WIGSVNSGGSAY	138	LCDR2	LLIYEASTLA	46
		HCDR3	ARSGSGYPMDYLA	30	LCDR3	QQGYGRWHILN	121
	AbM	HCDR1	GFSLSLYGVS	96	LCDR1	RASQSIWYWLA	126
		HCDR2	SVNSGGSAY	139	LCDR2	EASTLAS	44
		HCDR3	SGSGYPMDYLAI	25	LCDR3	QQGYGRWHILNI	120

FIG. 17 (cont.)

VH and VL Sequences of Anti-FRα Antibodies

Variant	Region	Sequence	SEQ ID NO
v23924 v30618	VH	QSVKESEGGLFKPTDTLTLTCTVSGFSLSSYGVSWVRQAPGNGLEWIGAVNSGGSAYY ANWAKSRSTITRNTNLFTVTILKMTSLAVADTATYFCARSGSGYPMDYLAIWGPGTLV TVSS	19
v30384 v36675	VH	EVQLLESGGGLVQPGGSLRLSCAVSGFSLSSYGVSWVRQAPGKGLEWIGAVNSGGSA YYADSVKGRSTISRDNSKNTVYLQMNSLRAEDTAVYYCARSGSGYPMDYLAIWGQGT LVTVSS	50
v30389	VH	EVQVLESGGGLVQPGGSLRLSCAVSGFSLSSYGVSWVRQAPGKGLEWIGAVNSGGSA YYADSVKGRSTISRDNSKNTVYLQMNSLRAEDTAVYYCARSGSGYPMDYLAIWGQGT LVTVSS	54
v30394	VH	EVQLLESGGGLVQPGGSLRLSCAVSGFSLSSYGVSWVRQAPGKGLEWIGAVNSGGSA YYADWAKGRSTISRDNSKNTVYLQMNSLRAEDTAVYYCARSGSGYPMDYLAIWGQG TLVTVSS	57
v30399	VH	EVQVLESGGGLVQPGGSLRLSCAVSGFSLSSYGVSWVRQAPGKGLEWIGAVNSGGSA YYADWAKGRSTISRDNSKNTVYLQMNSLRAEDTAVYYCARSGSGYPMDYLAIWGQG TLVTVSS	61
v31422	VH	EVQLLESGGGLFQPGGSLRLSCAVSGFSLSSYGVSWVRQAPGKGLEWIGAVNSGGSAY YADSVKGRSTISRDNSKNTVYLQMNSLRAEDTAVYYCARSGSGYPMDYLAIWGQGTL VTVSS	76
v31423	VH	EVQLLESGGGLVQPGGSLRLSCAVSGFSLSSYGVSWVRQAPGKGLEWIGAVNSGGSA YYADSVKGRSTISRDTSKNTVYLQMNSLRAEDTAVYYCARSGSGYPMDYLAIWGQGT LVTVSS	79
v31424	VH	EVQLLESGGGLVQPGGSLRLSCAVSGFSLSSYGVSWVRQAPGKGLEWIGAVNSGGSA YYADSVKGRSTISRDTSKFTVYLQMNSLRAEDTAVYYCARSGSGYPMDYLAIWGQGT LVTVSS	82
v31425	VH	EVQLLESGGGLFQPGGSLRLSCAVSGFSLSSYGVSWVRQAPGKGLEWIGAVNSGGSAY YADSVKGRSTISRDTSKFTVYLQMNSLRAEDTAVYYCARSGSGYPMDYLAIWGQGTL VTVSS	85
v31426	VH	QQLLESGGGLFQPGGSLRLSCAVSGFSLSSYGVSWVRQAPGKGLEWIGAVNSGGSAYY ADSVKGRSTISRDTSKFTVYLQMNSLRAEDTAVYYCARSGSGYPMDYLAIWGQGTLV TVSS	88
v35305	VH	EVQLLESGGGLVQPGGSLRLSCAVSGFSLSLYGVSWVRQAPGKGLEWIGAVNSGGSA YYADSVKGRSTISRDNSKNTVYLQMNSLRAEDTAVYYCARSGSGYPMDYLAIWGQGT LVTVSS	91

FIG. 18

Variant	Region	Sequence	SEQ ID NO
v35342	VH	EVQLLESGGGLVQPGGSLRLSCAVSGFSLSLYGVSWVRQAPGKGLEWIGSLNSGGSAY YADSVKGRSTISRDNSKNTVYLQMNSLRAEDTAVYYCARSGSGYPMDYLAIWGQGTL VTVSS	99
v35347 v35348 v35350	VH	EVQLLESGGGLVQPGGSLRLSCAVSGFSLSLYGVSWVRQAPGKGLEWIGAVNSGGSA YYADSFKGRSTISRDNSKNTVYLQMNSLRAEDTAVYYCARSGSGYPMDYHPIWGQGT LVTVSS	106
v35354	VH	EVQLLESGGGLVQPGGSLRLSCAVSGFSLSLYGVSWVRQAPGKGLEWIGAVNSGGSA YYADSFKGRSTISRDNSKNTVYLQMNSLRAEDTAVYYCARSGSGYPMDYLAIWGQGT LVTVSS	113
v35356 v35358	VH	EVQLLESGGGLVQPGGSLRLSCAVSGFSLSSYGVSWVRQAPGKGLEWIGAVNSGGSA YYADSFKGRSTISRDNSKNTVYLQMNSLRAEDTAVYYCARSGSGYPMDYHPIWGQGT LVTVSS	116
v36167	VH	EVQLLESGGGLVQPGGSLRLSCAVSGFSLSSYGVSWVRQAPGKGLEWIGAVNSGGSA YYADSFKGRSTISRDNSKNTVYLQMNSLRAEDTAVYYCARSGSGYPMDYLAIWGQGT LVTVSS	133
v36168	VH	EVQLLESGGGLVQPGGSLRLSCAVSGFSLSLYGVSWVRQAPGKGLEWIGSVNSGGSAY YADSVKGRSTISRDNSKNTVYLQMNSLRAEDTAVYYCARSGSGYPMDYLAIWGQGTL VTVSS	136
v23924 v30618	VL	AYDMTQTPASVEVAVGGTVTIKCQASQSIGDWLAWYQQKPGQPPRLLIYEASTLASG VPSRFSGSGSGTQFTLTISGVECADAATYYCQQGYGRSNVDNIFGGGTEVVVK	39
v30384 v30389 v30394 v30399 v31422 v31423 v31424 v31425 v31426 v35347 v36675	VL	DYQMTQSPSSVSASVGDRVTIITCRASQSIGDWLAWYQQKPGKAPKLLIYEASTLASGV PSRFSGSGSGTDFTLTISSVQPEDAATYYCQQGYGRSNVDNIFGGGTKVEVK	64
v35348 v35356	VL	DYQMTQSPSSVSASVGDRVTIITCRASQSIGDWLAWYQQKPGKAPKLLIYEASTLASGV PSRFSGSGSGTDFTLTISSVQPEDAATYYCQQGYGRWHILNIFGGGTKVEVK	119

FIG. 18 (cont.)

Variant	Region	Sequence	SEQ ID NO
v35305	VL	DYQMTQSPSSVSASVGDRVTITCRASQSIWYWLAWYQQKPGKAPKLLIYEASTLASGV PSRFSGSGSGTDFTLTISSVQPEDAATYYCQQGYGRSNVDNIFGGGTKVEVK	124
v35342 v35350 v35354 v35358 v36167 v36168	VL	DYQMTQSPSSVSASVGDRVTITCRASQSIWYWLAWYQQKPGKAPKLLIYEASTLASGV PSRFSGSGSGTDFTLTISSVQPEDAATYYCQQGYGRWHILNIFGGGTKVEVK	130

FIG. 18 (cont.)

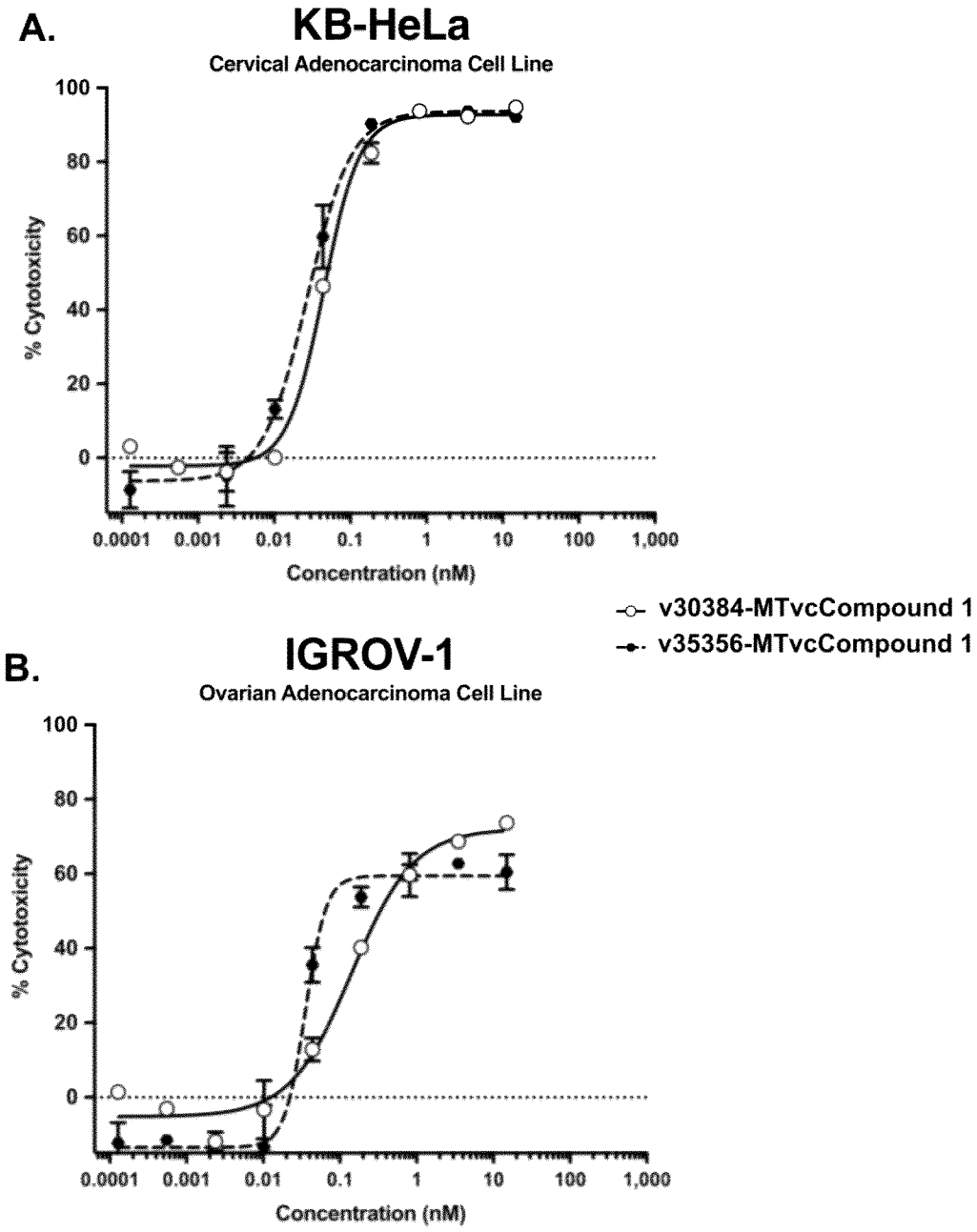

A.
KB-HeLa
Cervical Adenocarcinoma Cell Line
% Cytotoxicity
100
80
60
40
20
0
0.0001
0.001
0.01
0.1
1
10
100
1,000
Concentration (nM)
v30384-MTvcCompound 1
v35356-MTvcCompound 1
B.
IGROV-1
Ovarian Adenocarcinoma Cell Line
% Cytotoxicity
100
80
60
40
20
0
0.0001
0.001
0.01
0.1
1
10
100
1,000
Concentration (nM)

FIG. 19

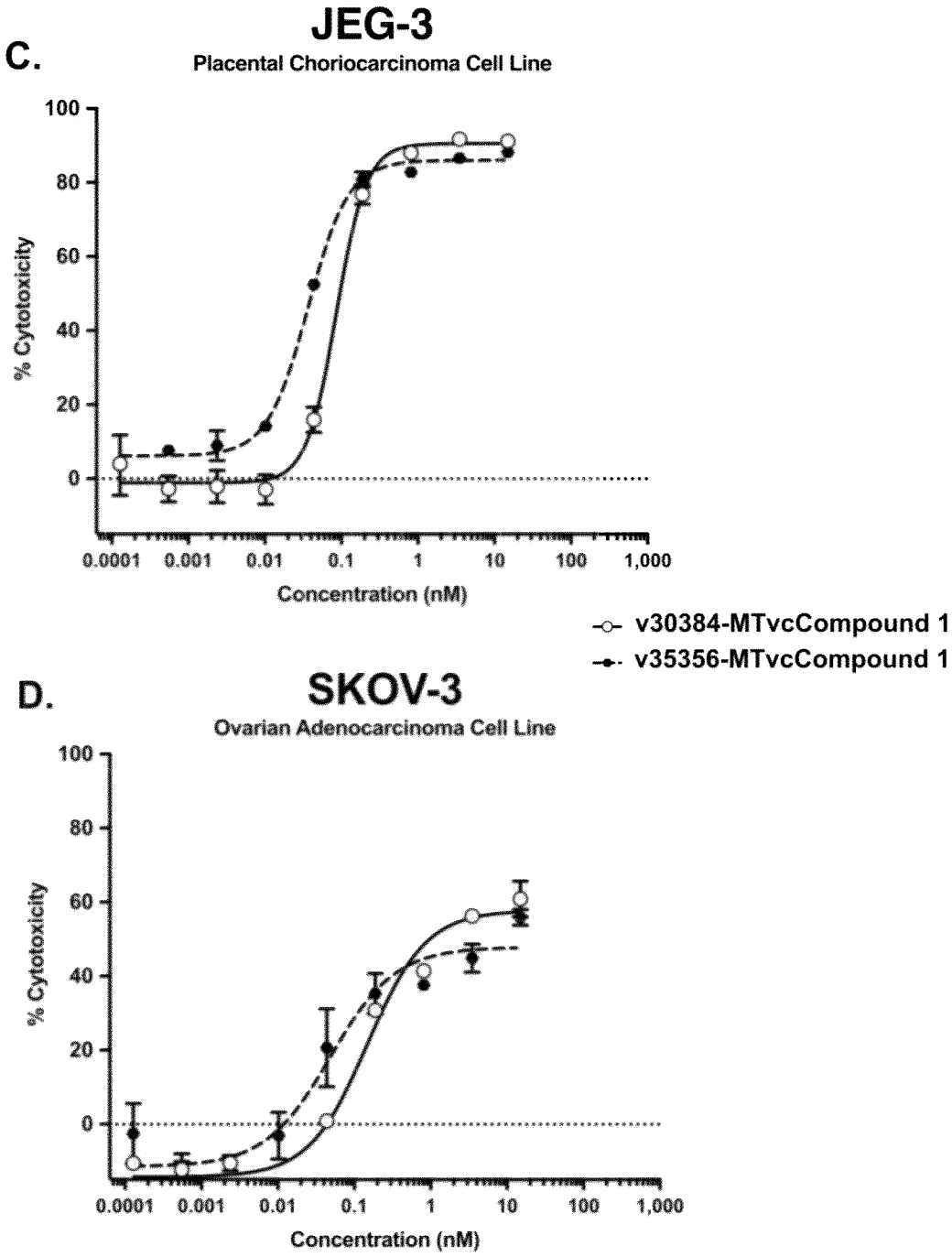

C.
JEG-3
Placental Choriocarcinoma Cell Line
% Cytotoxicity
100
80
60
40
20
0
0.0001
0.001
0.01
0.1
1
10
100
1,000
Concentration (nM)
v30384-MTvcCompound 1
v35356-MTvcCompound 1
D.
SKOV-3
Ovarian Adenocarcinoma Cell Line
% Cytotoxicity
100
80
60
40
20
0
0.0001
0.001
0.01
0.1
1
10
100
1,000
Concentration (nM)

FIG. 19 (cont.)

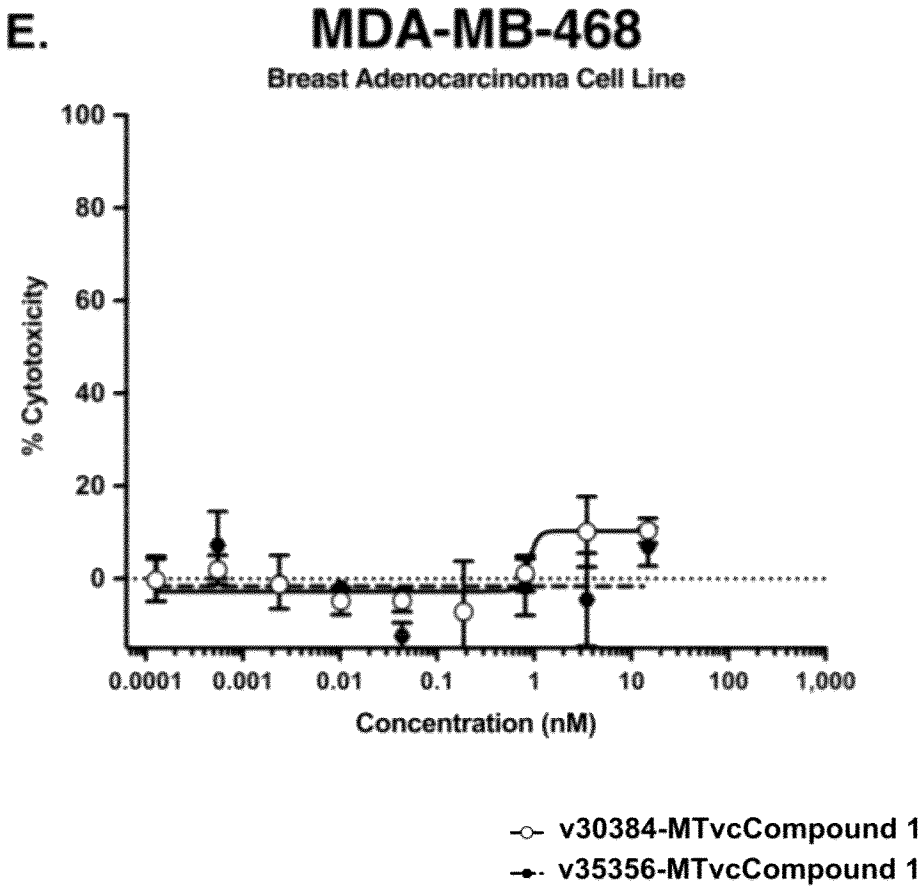

E.
MDA-MB-468
Breast Adenocarcinoma Cell Line
% Cytotoxicity
100
80
60
40
20
0
0.0001
0.001
0.01
0.1
1
10
100
1,000
Concentration (nM)
v30384-MTvcCompound 1
v35356-MTvcCompound 1

FIG. 19 (cont.)

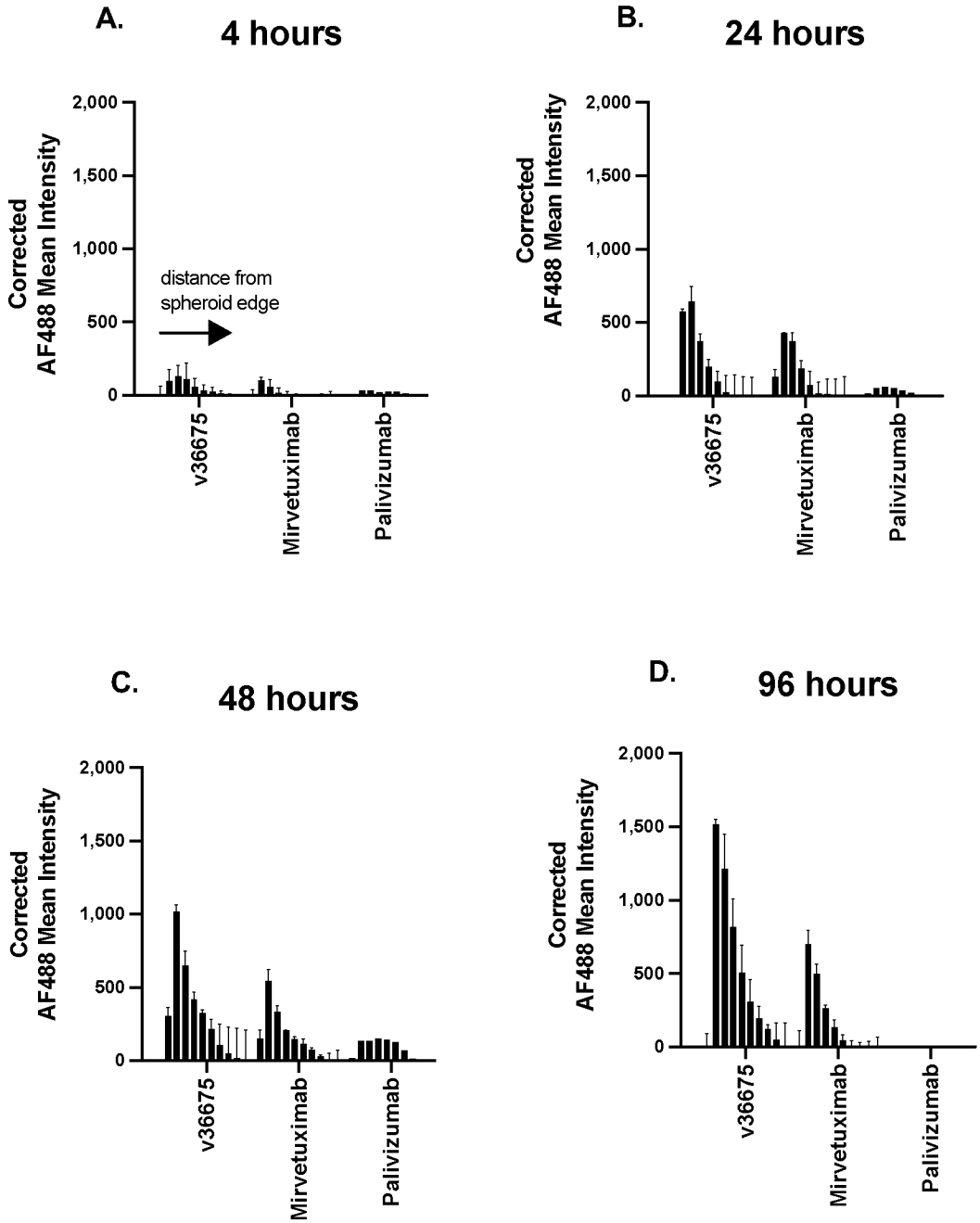

A.
4 hours
Corrected AF488 Mean Intensity
2,000
1,500
1,000
500
0
distance from spheroid edge
v36675
Mirvetuximab
Palivizumab
B.
24 hours
C.
48 hours
D.
96 hours

FIG. 20

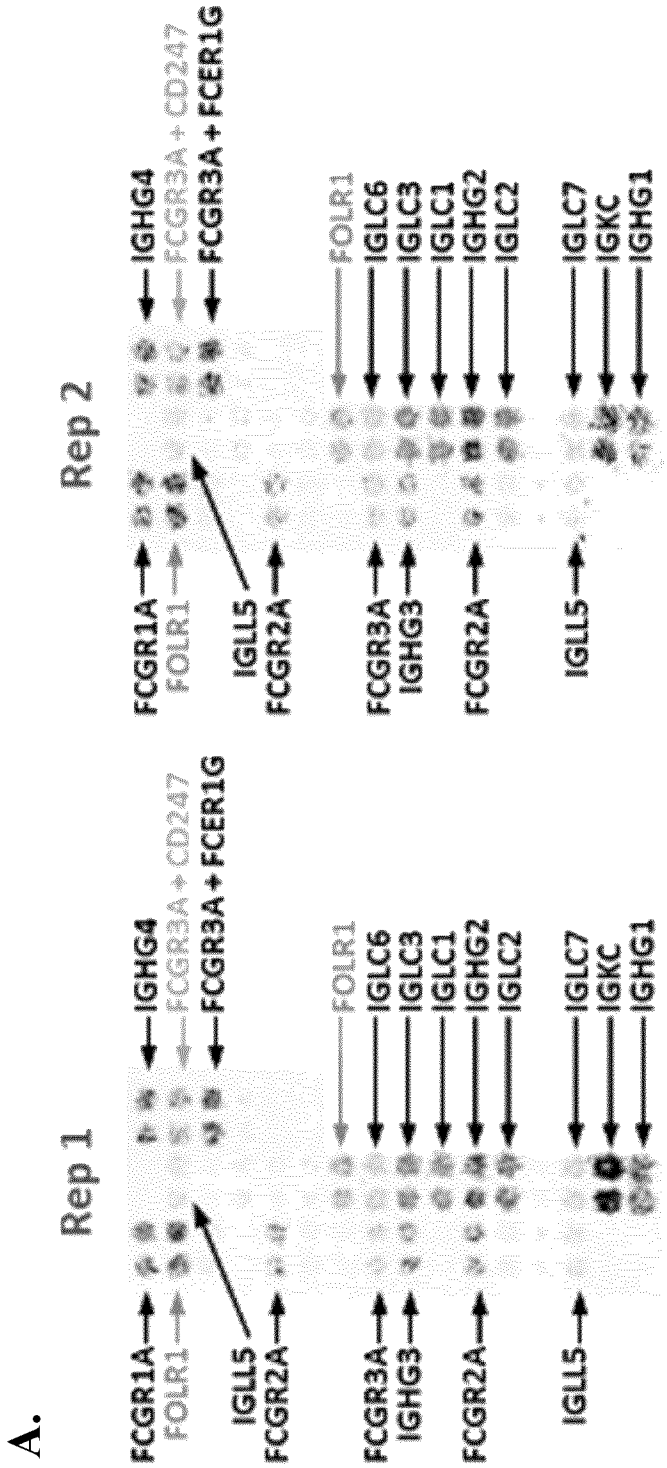

A.
Rep 1
FCGR1A
FOLR1
IGLL5
FCGR2A
IGHG4
FCGR3A + CD247
FCGR3A + FCER1G
FOLR1
FCGR3A
IGHG3
FCGR2A
IGLL5
IGLC6
IGLC3
IGLC1
IGHG2
IGLC2
IGLC7
IGKC
IGHG1
Rep 2
FCGR1A
FOLR1
IGLL5
FCGR2A
IGHG4
FCGR3A + CD247
FCGR3A + FCER1G
FOLR1
FCGR3A
IGHG3
FCGR2A
IGLL5
IGLC6
IGLC3
IGLC1
IGHG2
IGLC2
IGLC7
IGKC
IGHG1

FIG. 21

B.

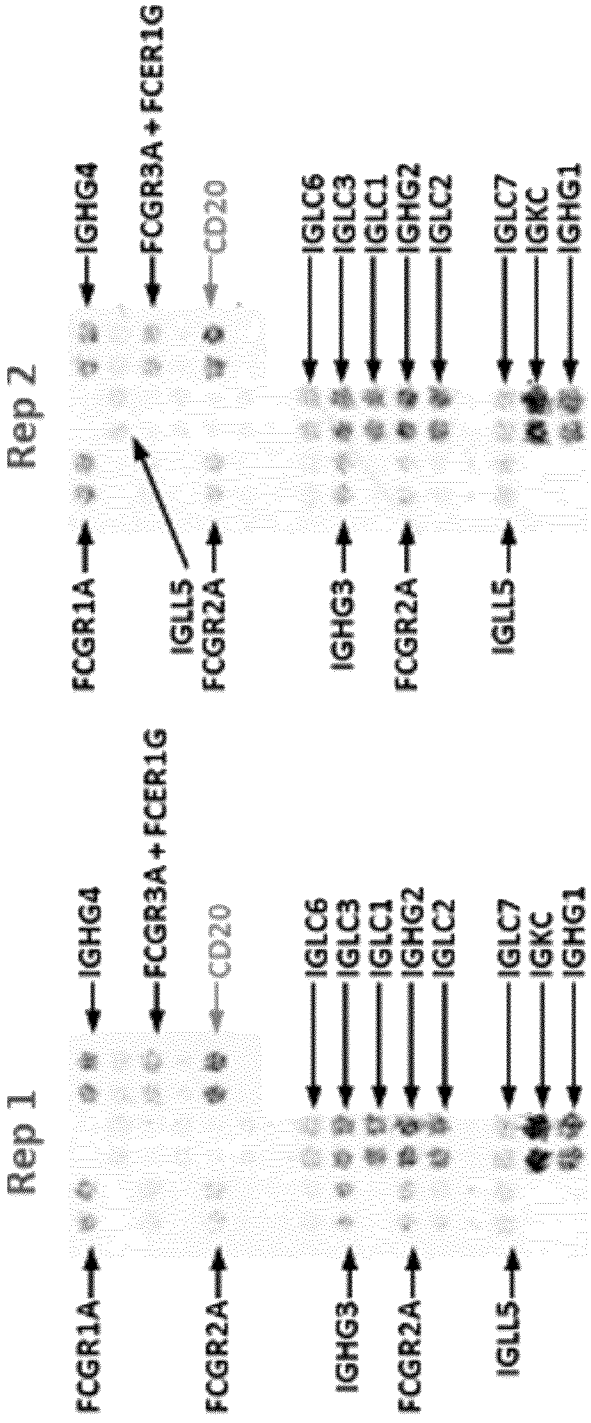

Rep 1
Rep 2
FCGR1A
IGHG4
FCGR3A + FCER1G
CD20
IGLL5
FCGR2A
IGHG3
IGLC6
IGLC3
IGLC1
IGHG2
IGLC2
IGLC7
IGKC
IGHG1

FIG. 21 (cont.)

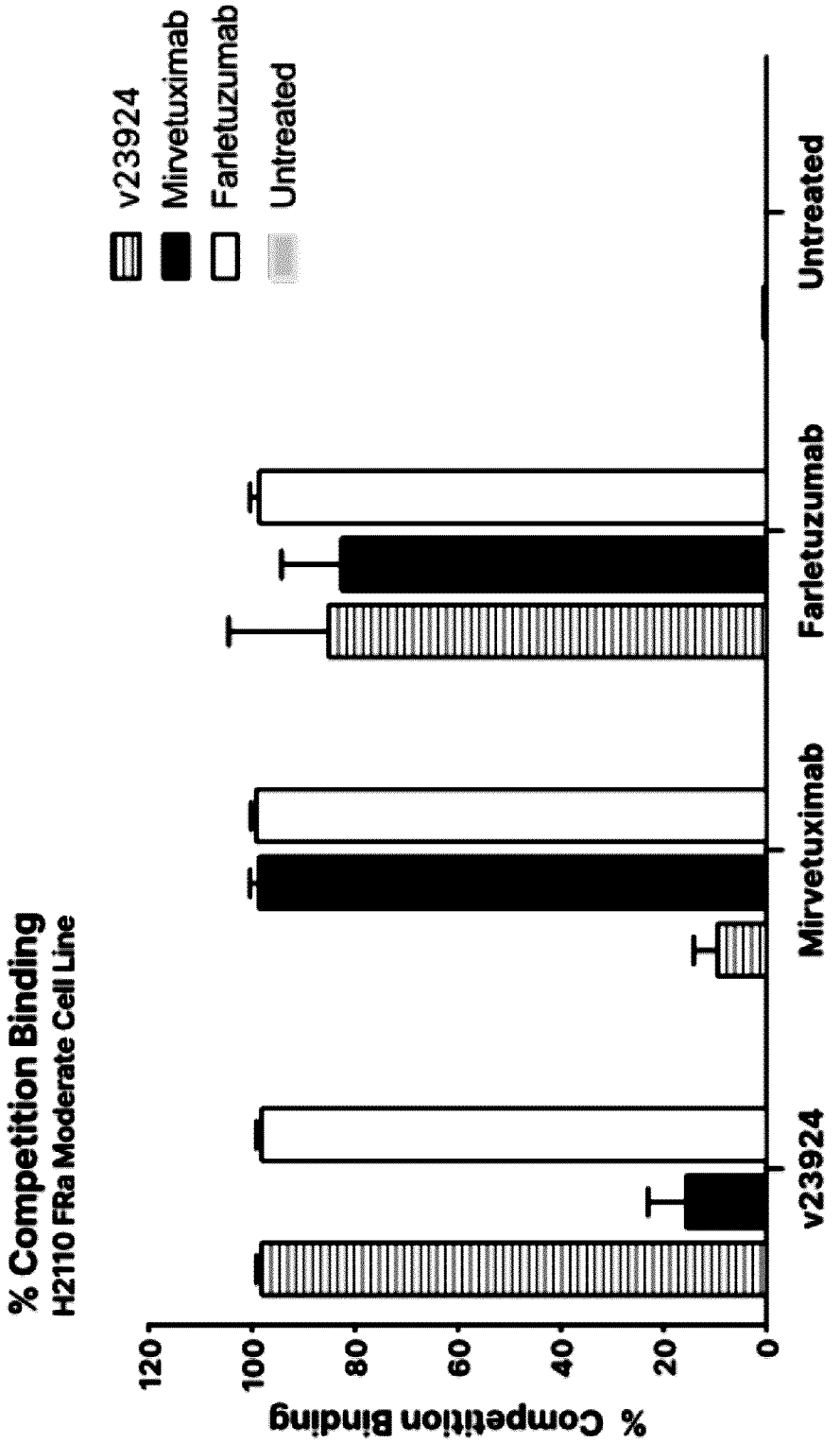

% Competition Binding
H2110 FRa Moderate Cell Line
v23924
Mirvetuximab
Farletuzumab
Untreated
% Competition Binding
120
100
80
60
40
20
0
v23924
Mirvetuximab
Farletuzumab
Untreated

FIG. 22

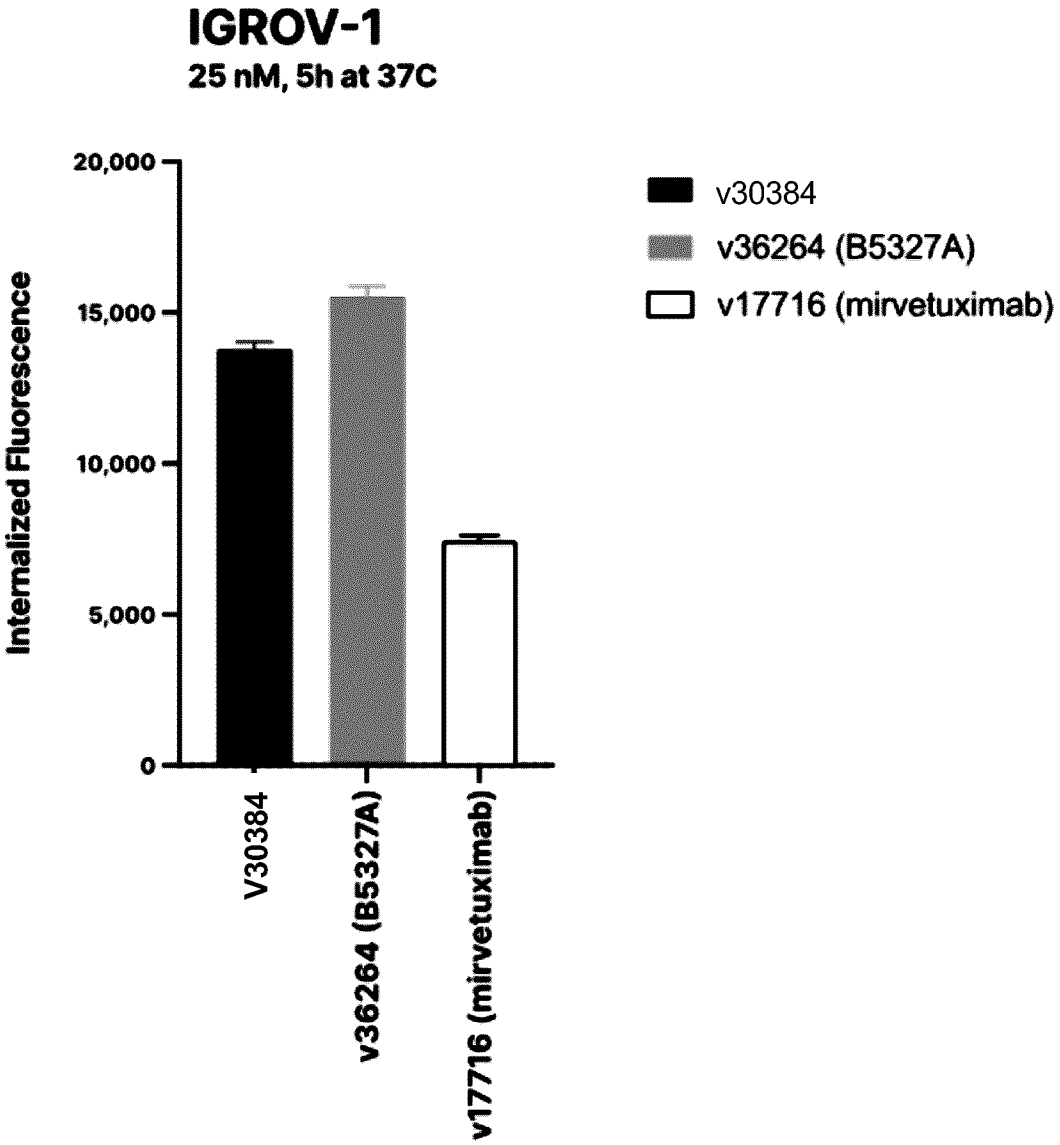

IGROV-1
25 nM, 5h at 37C
Internalized Fluorescence
20,000
15,000
10,000
5,000
0
V30384
v36264 (B5327A)
v17716 (mirvetuximab)
v30384
v36264 (B5327A)
v17716 (mirvetuximab)

FIG. 23

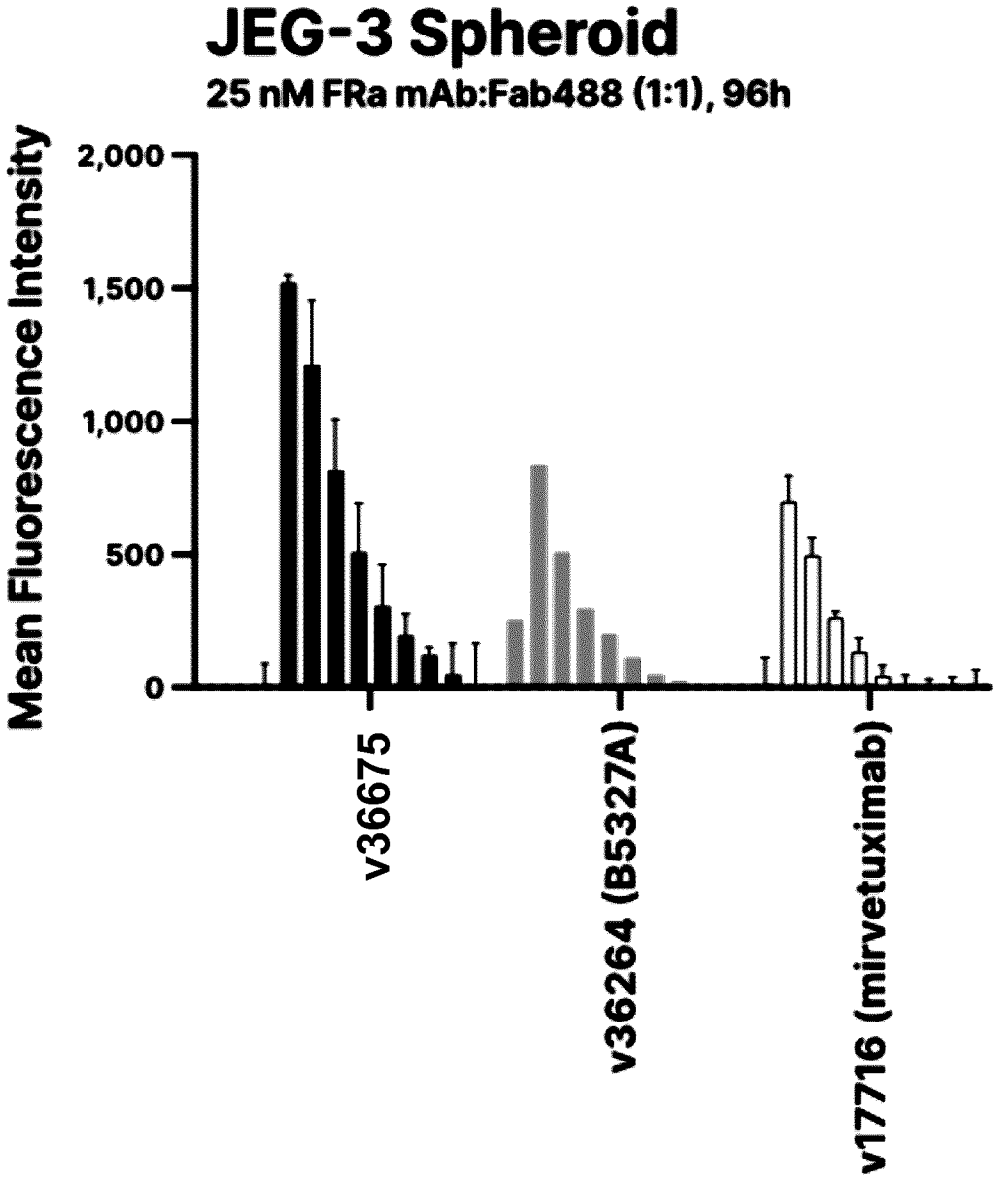

JEG-3 Spheroid
25 nM FRa mAb:Fab488 (1:1), 96h
Mean Fluorescence Intensity
2,000
1,500
1,000
500
0
v36675
v36264 (B5327A)
v17716 (mirvetuximab)

FIG. 24

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2023/050405

A. CLASSIFICATION OF SUBJECT MATTER

IPC: ***C07K 16/28*** (2006.01), ***A61K 39/395*** (2006.01), ***A61K 47/68*** (2017.01), ***A61P 35/00*** (2006.01), ***C12N 15/13*** (2006.01)

CPC: , A61K 39/3955 (2020.01), A61K 47/6849 (2020.01), A61K 47/6851 (2020.01), A61P 35/00 (2020.01), C07K 16/28 (2020.01), C12N 15/11 (2020.01), C07K 2317/35 (2020.01), C07K 2317/50 (2020.01)

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)
Keywords used 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
Keywords: anti-FRA, anti-FRalpha, anti-FRα, anti-FOLR1, anti-folate receptor, folate, epitope*, ADC, antibody-drug conjugate, cancer,
Search of SEQ ID NOs: 3-8, 39, 64, 119, 124 and 130, and the epitope defined in claims 1 and 4 (EDXRTSY).

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	FURUUCHI, K. et al. Antibody-drug conjugate MORAb-202 exhibits long-lasting antitumor efficacy in TNBC PDx models. Cancer Science. June 2021, Vol. 112, No. 6, pages 2467-2480. *see whole document*	1-3, 14-21 and 23-44

☐ 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 29 June 2023 (29-06-2023)	Date of mailing of the international search report 29 June 2023 (29-06-2023)
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 Alexandre Martel (819) 230-4870

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2023/050405

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:

It is noted that the sequence listing furnished by the Applicant does not have a proper sequence associated with SEQ ID NO : 7. Consequently, for searching purposes, the sequence of "EAS" was used for SEQ ID NO: 7, as is defined in Table 3 of the description (pages 18-19).