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(54) Title: MONO AND BISPECIFIC ANTIBODIES FOR EPIDERMAL GROWTH FACTOR RECEPTOR VARIANT III AND CD3 AND THEIR USES

(57) Abstract: The present invention provides antibodies that specifically bind to EGFRvIII (Epidermal Growth Factor Receptor Variant III). The invention further provides bispecific antibodies that bind to EGFRvIII and another antigen (e.g., CD3) as well as antibody conjugates (e.g., antibody-drug-conjugates). The invention further relates to antibody encoding nucleic acids, and methods of obtaining such antibodies (monospecific and bispecific) and antibody conjugates. The invention further relates to therapeutic methods for use of these antibodies and antibody conjugates for the treatment of EGFRvIII-mediated pathologies, including cancer such as glioblastoma.



WO 2017/125831 A1

- 1 -

MONO AND BISPECIFIC ANTIBODIES FOR EPIDERMAL GROWTH FACTOR RECEPTOR VARIANT III AND CD3 AND THEIR USES

Field

5 The present invention relates to antibodies, e.g., full length antibodies or antigen binding fragments thereof, that specifically bind to Epidermal Growth Factor Receptor Variant III (EGFRvIII). The invention further relates to heteromultimeric antibodies (e.g., bispecific antibodies) and antibody conjugates (e.g., antibody-drug-conjugates). Compositions comprising the EGFRvIII antibodies, methods for producing and purifying
10 such antibodies, and their use in diagnostics and therapeutics are also provided.

Background

 EGFR variant III (EGFRvIII), a tumor specific mutant of EGFR, is a product of genomic rearrangement which is often associated with wild-type EGFR gene
15 amplification. EGFRvIII is formed by an in-frame deletion of exons 2-7, leading to deletion of 267 amino acids with a glycine substitution at the junction. The truncated receptor loses its ability to bind ligands but acquires constitutive kinase activity. Interestingly, EGFRvIII frequently co-expresses with full length wild-type EGFR in the same tumor cells. Moreover, EGFRvIII expressing cells exhibit increased proliferation,
20 invasion, angiogenesis and resistance to apoptosis.

 EGFRvIII is most often found in glioblastoma multiforme (GBM). It is estimated that 25-35% of GBM carries this truncated receptor. Moreover, its expression often reflects a more aggressive phenotype and poor prognosis. Besides GBM, expression of EGFRvIII has also been reported in other solid tumors such as non-small cell lung
25 cancer, head and neck cancer, breast cancer, ovarian cancer and prostate cancer. In contrast, EGFRvIII is not expressed in healthy tissues. The lack of expression in normal tissues makes EGFRvIII an ideal target for developing tumor specific targeted therapy. To date, there has not been any FDA approved monoclonal antibody (e.g., monospecific or bispecific) against EGFRvIII identified with high affinity, high specificity,
30 and high potency in treating cancers such as GBM. Accordingly, there remains a need for antibodies (e.g., monospecific or bispecific) treating cancers such as GBM with improved efficacy and safety profile, and suitable for use with human patients.

- 2 -

Summary

The invention disclosed herein is directed to antibodies (e.g., monospecific or bispecific antibodies) and antibody conjugates that specifically bind to Epidermal Growth Factor Receptor Variant III (EGFRvIII). In one aspect, the invention provides an isolated antibody which specifically binds to EGFRvIII, wherein the antibody comprises

5 (a) a heavy chain variable (VH) region comprising (i) a VH complementarity determining region one (CDR1) comprising the sequence shown in SEQ ID NO: 62, 63, 64, 74, 75, 76, 80, 81, 82, 88, 89, 90, 93, 94, 95, 99, 100, 101, 109, 110, 111, 115, 116, 117, 121, 122, 123, 132, 133, 134, 137, 138, 139, 143, 144, or 145; (ii) a VH CDR2 comprising

10 the sequence shown in SEQ ID NO: 65, 66, 68, 69, 70, 71, 77, 78, 83, 84, 86, 87, 91, 92, 96, 97, 98, 102, 103, 105, 106, 112, 113, 118, 119, 124, 125, 127, 128, 130, 131, 135, 136, 140, 141, 146, 147, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, or 237; and (iii) a VH CDR3 comprising the sequence shown in SEQ ID NO: 67, 72, 73, 79, 85, 104, 107, 108, 114, 120, 126, 129, 142, 148, 219, 220, 221, 222, 223, or

15 236; and/or a light chain variable (VL) region comprising (i) a VL CDR1 comprising the sequence shown in SEQ ID NO: 149, 154, 156, 159, 162, 165, 166, 168, 169, 170, 171, 173, 174, 176, 178, 181, 182, 185, 187, 190, 192, 195, 198, 238, or 239; (ii) a VL CDR2 comprising the sequence shown in SEQ ID NO: 150, 152, 155, 157, 160, 163, 172, 175, 179, 183, 186, 188, 191, 193, 196, or 199; and (iii) a VL CDR3 comprising the

20 sequence shown in SEQ ID NO: 151, 153, 158, 161, 164, 167, 177, 180, 184, 189, 194, 197, or 200.

In another aspect, provided is an isolated antibody which specifically binds to EGFRvIII, wherein the antibody comprises: a VH region comprising a VH CDR1, VH CDR2, and VH CDR3 of the VH sequence shown in SEQ ID NO: 1, 3, 5, 7, 9, 11, 13,

25 15, 17, 19, 21, 23, 25, 27, 30, 32, 34, 35, 37, 39, 41, 43, 44, 46, 48, 50, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 202, 203, 204, 205, 206, 207, 208, 209, 210, 214, 216, 217, or 218; and/or a VL region comprising VL CDR1, VL CDR2, and VL CDR3 of the VL sequence shown in SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 29, 31, 33, 36, 38, 40, 42, 45, 47, 49, 51, 211, 212, 213, or 215. In some embodiments, the VH

30 region as described herein comprises a variant with one or several conservative amino acid substitutions in residues that are not within a CDR and/or the VL region as described herein comprises a variant with one or several amino acid substitutions in amino acids that are not within a CDR. For example, in some embodiments, the VH or

- 3 -

VL region can comprise an amino acid sequence described above or a variant thereof with no more than 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 conservative substitutions in residues that are not within a CDR.

In some embodiments, provided is an isolated antibody which specifically binds to EGFRvIII, wherein the antibody comprises: a VH region comprising the sequence shown in SEQ ID NO: 5, 9, 11, 15, 30, 37, or 41; and/or a VL region comprising the sequence shown in SEQ ID NO: 6, 10, 12, 16, 31, 38, or 42. In some embodiments, the VH region comprises the sequence shown in SEQ ID NO: 5 and the VL region comprises the sequence shown in SEQ ID NO: 6. In some embodiments, the VH region comprises the sequence shown in SEQ ID NO: 9 and the VL region comprises the sequence shown in SEQ ID NO: 10. In some embodiments, the VH region comprises the sequence shown in SEQ ID NO: 11 and the VL region comprises the sequence shown in SEQ ID NO: 12. In some embodiments, the VH region comprises the sequence shown in SEQ ID NO: 15 and the VL region comprises the sequence shown in SEQ ID NO: 16. In some embodiments, the VH region comprises the sequence shown in SEQ ID NO: 30 and the VL region comprises the sequence shown in SEQ ID NO: 31. In some embodiments, the VH region comprises the sequence shown in SEQ ID NO: 37 and the VL region comprises the sequence shown in SEQ ID NO: 38. In some embodiments, the VH region comprises the sequence shown in SEQ ID NO: 41 and the VL region comprises the sequence shown in SEQ ID NO: 42.

In another aspect, provided is a bispecific antibody wherein the bispecific antibody is a full-length human antibody, comprising a first antibody variable domain of the bispecific antibody specifically binding to a target antigen (e.g., EGFRvIII), and comprising a second antibody variable domain of the bispecific antibody capable of recruiting the activity of a human immune effector cell by specifically binding to an effector antigen (e.g., Cluster of differentiation 3 (CD3)) located on the human immune effector cell. In some embodiments, the first antibody variable domain comprises a heavy chain variable (VH) region comprising a VH CDR1, VH CDR2, and VH CDR3 of the VH sequence shown in SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 30, 32, 34, 35, 37, 39, 41, 43, 44, 46, 48, 50, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 202, 203, 204, 205, 206, 207, 208, 209, 210, 214, 216, 217, or 218; and/or a light chain variable (VL) region comprising VL CDR1, VL CDR2, and VL CDR3 of the VL sequence shown in SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 29, 31, 33, 36,

- 4 -

38, 40, 42, 45, 47, 49, 51, 211, 212, 213, or 215. In some embodiments, the first antibody variable domain comprises (a) a heavy chain variable (VH) region comprising (i) a VH complementarity determining region one (CDR1) comprising the sequence shown in SEQ ID NO: 62, 63, 64, 74, 75, 76, 80, 81, 82, 88, 89, 90, 93, 94, 95, 99, 100, 101, 109, 110, 111, 115, 116, 117, 121, 122, 123, 132, 133, 134, 137, 138, 139, 143, 144, or 145; (ii) a VH CDR2 comprising the sequence shown in SEQ ID NO: 65, 66, 68, 69, 70, 71, 77, 78, 83, 84, 86, 87, 91, 92, 96, 97, 98, 102, 103, 105, 106, 112, 113, 118, 119, 124, 125, 127, 128, 130, 131, 135, 136, 140, 141, 146, 147, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, or 237; and (iii) a VH CDR3 comprising the sequence shown in SEQ ID NO: 67, 72, 73, 79, 85, 104, 107, 108, 114, 120, 126, 129, 142, 148, 219, 220, 221, 222, 223, or 236; and/or (b) a light chain variable (VL) region comprising (i) a VL CDR1 comprising the sequence shown in SEQ ID NO: 149, 154, 156, 159, 162, 165, 166, 168, 169, 170, 171, 173, 174, 176, 178, 181, 182, 185, 187, 190, 192, 195, 198, 238, or 239; (ii) a VL CDR2 comprising the sequence shown in SEQ ID NO: 150, 152, 155, 157, 160, 163, 172, 175, 179, 183, 186, 188, 191, 193, 196, or 199; and (iii) a VL CDR3 comprising the sequence shown in SEQ ID NO: 151, 153, 158, 161, 164, 167, 177, 180, 184, 189, 194, 197, or 200.

In some embodiments, the second antibody variable domain comprises the VH and/or VL region specific against CD3. For example, the second antibody variable domain comprises a heavy chain variable (VH) region comprising a VH CDR1, VH CDR2, and VH CDR3 of the VH sequence shown in SEQ ID NO: 240; and/or a light chain variable (VL) region comprising a VL CDR1, VL CDR2, and VL CDR3 of the VL sequence shown in SEQ ID NO: 241. In some embodiments, the second antibody variable domain comprises (a) a VH region comprising (i) a VH CDR1 comprising the sequence shown in SEQ ID NO: 244, 110, or 245; (ii) a VH CDR2 comprising the sequence shown in SEQ ID NO: 246 or 247; and (iii) a VH CDR3 comprising the sequence shown in SEQ ID NO: 248; and/or a VL region comprising (i) a VL CDR1 comprising the sequence shown in SEQ ID NO: 249; (ii) a VL CDR2 comprising the sequence shown in SEQ ID NO: 250; and (iii) a VL CDR3 comprising the sequence shown in SEQ ID NO: 251.

In some embodiments, the antibodies described herein comprise a constant region. In some embodiments, the antibodies described herein are of the human IgG1, IgG2 or IgG2Δa, IgG3, or IgG4 subclass. In some embodiments, the antibodies

- 5 -

described herein comprise a glycosylated constant region. In some embodiments, the antibodies described herein comprise a constant region having decreased binding affinity to one or more human Fc gamma receptor(s).

5 In some embodiments, both the first and the second antibody variable domains of the bispecific antibody comprise amino acid modifications at positions 223, 225, and 228 (e.g., (C223E or C223R), (E225R), and (P228E or P228R)) in the hinge region and at position 409 or 368 (e.g., K409R or L368E (EU numbering scheme)) in the CH3 region of human IgG2 (SEQ ID NO: 290).

10 In some embodiments, both the first and the second antibody variable domains of the bispecific antibody comprise amino acid modifications at position 265 (e.g., D265A) of the human IgG2.

In some embodiments, both the first and the second antibody variable domains of the bispecific antibody comprise amino acid modifications at one or more of positions 265 (e.g., D265A), 330 (e.g., A330S), and 331 (e.g., P331S) of the human IgG2. In
15 some embodiments, both the first and the second antibody variable domains of the bispecific antibody comprise amino acid modifications at each of positions 265 (e.g., D265A), 330 (e.g., A330S), and 331 (e.g., P331S) of the human IgG2.

In another aspect, the invention provides an isolated antibody comprising an acyl donor glutamine-containing tag engineered at a specific site of the EGFRvIII antibody of
20 the present invention.

In one variation, the invention provides an isolated antibody comprising an acyl donor glutamine-containing tag and an amino acid modification at position 222, 340, or 370 of the EGFRvIII antibody of the present invention. In some embodiments, the amino acid modification is a substitution from lysine to arginine.

25 In some embodiments, the EGFRvIII antibody of the present invention further comprises a linker.

In another aspect, the invention provides a conjugate of the EGFRvIII antibody as described herein, wherein the antibody is conjugated to an agent, wherein the agent is selected from the group consisting of a cytotoxic agent, an immunomodulating agent,
30 an imaging agent, a therapeutic protein, a biopolymer, and an oligonucleotide. In some embodiments, the agent is a cytotoxic agent including, but not limited to, an anthracycline, an auristatin, a camptothecin, a combretastatin, a dolastatin, a duocarmycin, an enediyne, a geldanamycin, an indolino-benzodiazepine dimer, a

- 6 -

maytansine, a puromycin, a pyrrolbenzodiazepine dimer, a taxane, a vinca alkaloid, a tubulysin, a hemiasterlin, a spliceostatin, a pladienolide, and stereoisomers, isosteres, analogs, or derivatives thereof. For example, the cytotoxic agent is MMAD (Monomethyl Auristatin D), 0101 (2-methylalanyl-N-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-
 5 [(1*R*,2*R*)-1-methoxy-2-methyl-3-oxo-3-[(1*S*)-2-phenyl-1-(1,3-thiazol-2-yl)ethyl]amino}propyl]pyrrolidin-1-yl}-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide), 3377 (N,2-dimethylalanyl-N-[(1*S*,2*R*)-4-{(2*S*)-2-[(1*R*,2*R*)-3-[(1*S*)-1-carboxyl-2-phenylethyl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-2-methoxy-1-[(1*S*)-1-methylpropyl]-4-oxobutyl]-N-methyl-L-valinamide), 0131 (2-methyl-L-
 10 prolyl-N-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-[(1*S*)-1-carboxy-2-phenylethyl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide), or 0121 (2-methyl-L-prolyl-N-[(3*R*,4*S*,5*S*)-1-{(2*S*)-2-[(1*R*,2*R*)-3-[(2*S*)-1-methoxy-1-oxo-3-phenylpropan-2-yl]amino}-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl}-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-
 15 valinamide).

In some embodiments, the present invention provides a conjugate comprising the formula: antibody-(acyl donor glutamine-containing tag)-(linker)-(cytotoxic agent).

In other embodiments, the invention provides pharmaceutical compositions comprising any of the antibodies or antibody conjugates described herein.

20 The invention also provides cell lines that recombinantly produce any of the antibodies described herein.

The invention also provides nucleic acids encoding any of the antibodies described herein. The invention also provides nucleic acids encoding a heavy chain variable region and/or a light chain variable region of any of the antibodies described
 25 herein.

The invention also provides kits comprising an effective amount of any of the antibodies or antibody conjugates described herein.

The invention also provides methods of treating subjects in need thereof comprising providing the isolated antibodies, bispecific antibodies, or antibody
 30 conjugates described herein, and administering said antibodies to said subject.

Also provided are methods of treating a condition associated with malignant cells expressing EGFRvIII in a subject comprising administering to a subject in need thereof an effective amount of a pharmaceutical composition comprising the antibodies or

- 7 -

antibody conjugates as described herein. In some embodiments, the condition is a cancer. In some embodiments, the cancer is an EGFRvIII related cancer (e.g., any cancer with EGFRvIII expression) selected from the group consisting of glioblastoma multiform, anaplastic astrocytoma, giant cell glioblastoma, gliosarcoma, anaplastic
5 oligodendroglioma, anaplastic ependymoma, anaplastic oligoastrocytoma, choroid plexus carcinoma, anaplastic ganglioglioma, pineoblastoma, pineocytoma, meningioma, medulloepithelioma, ependymblastoma, medulloblastoma, supraentorial primitive neuroectodermal tumor, atypical teratoid/rhabdoid tumor, head and neck cancer, non-small cell lung cancer, breast cancer, ovarian cancer, prostate cancer,
10 medulloblastoma, colorectal cancer, anal cancer, gastric cancer, thyroid cancer, mesothelioma, uterine cancer, and bladder cancer.

In another aspect, the invention provides a method of inhibiting tumor growth or progression in a subject who has malignant cells expressing EGFRvIII, comprising administering to the subject in need thereof an effective amount of a pharmaceutical
15 composition comprising the isolated antibodies, bispecific antibodies, or antibody conjugates, as described herein.

In another aspect, the invention provides a method inhibiting metastasis of malignant cells expressing EGFRvIII in a subject, comprising administering to the subject in need thereof an effective amount of the pharmaceutical composition
20 comprising the isolated antibodies, bispecific antibodies, or antibody conjugates, as described herein.

In another aspect, the invention provides a method inducing tumor regression in a subject who has malignant cells expressing EGFRvIII, comprising administering to the subject in need thereof an effective amount of the pharmaceutical composition of a
25 pharmaceutical composition comprising the isolated antibodies, bispecific antibodies, or antibody conjugates, as described herein.

Brief Description of the Figures/Drawings

FIGs. 1A, 1B, and 1C show examples of FACS binding histograms of three
30 EGFRvIII antibodies: mAb 42G9 (FIG. 1A), 32A10 (FIG. 1B) and 32G8 (FIG. 1C), to the three F98 cell lines: F98 (EGFR negative), F98-EGFRwt, and F98-EGFRvIII. The X-axis is fluorescence intensity; the Y-axis is percentage of maximum / normalized to mode.

- 8 -

FIGs. 2A, 2B and 2C depict histograms showing the expression of wild-type EGFR and EGFRvIII in GBM cell lines as measured by flow cytometry: LN229-EGFRvIII (FIG. 2A), LN18-EGFRvIII (FIG. 2B) and DKMG (FIG. 2C). EGFRvIII was detected with mAb 42G9 and EGFRwt was detected with an EGFR wild-type specific mAb. The X-axis is fluorescence intensity; the Y-axis is percentage of maximum / normalized to mode.

FIGs. 3A and 3B show graphs demonstrating the cytotoxicity of three EGFRvIII-CD3 bispecific antibodies in EGFRvIII transduced LN18-EGFRvIII (FIG. 3A) and parental LN18 (FIG. 3B) cells.

FIGs. 4A and 4B show graphs demonstrating the cytotoxicity of three EGFRvIII-CD3 bispecific antibodies in EGFRvIII transduced LN229-EGFRvIII (FIG. 4A) and parental LN229 (FIG. 4B) cells.

FIG. 5 shows a graph demonstrating the cytotoxicity of three EGFRvIII-CD3 bispecific antibodies in DKMG cells, which express endogenous EGFRvIII and EGFR wild-type proteins.

FIG. 6 shows a graph illustrating the *in vivo* anti-tumor activity of EGFRvIII-CD3 bispecific antibodies in a subcutaneous model of LN229-EGFRvIII GBM cell line.

Detailed Description

The invention disclosed herein provides antibodies (e.g., monospecific or bispecific) and antibody conjugates that specifically bind to EGFRvIII (e.g., human EGFRvIII). The invention also provides polynucleotides encoding these antibodies, compositions comprising these antibodies and antibody conjugates, and methods of making and using these antibodies and antibody conjugates. The invention also provides methods for treating a condition associated with EGFRvIII-mediated pathologies in a subject, such as cancer.

General Techniques

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology, biochemistry, immunology, virology, monoclonal antibody generation and engineering, which are within the skill of the art. Such techniques are explained fully in the literature, such as, Molecular Cloning: A Laboratory Manual,

second edition (Sambrook et al., 1989) Cold Spring Harbor Press; Oligonucleotide Synthesis (M.J. Gait, ed., 1984); Methods in Molecular Biology, Humana Press; Cell Biology: A Laboratory Notebook (J.E. Cellis, ed., 1998) Academic Press; Animal Cell Culture (R.I. Freshney, ed., 1987); Introduction to Cell and Tissue Culture (J.P. Mather and P.E. Roberts, 1998) Plenum Press; Cell and Tissue Culture: Laboratory Procedures (A. Doyle, J.B. Griffiths, and D.G. Newell, eds., 1993-1998) J. Wiley and Sons; Methods in Enzymology (Academic Press, Inc.); Handbook of Experimental Immunology (D.M. Weir and C.C. Blackwell, eds.); Gene Transfer Vectors for Mammalian Cells (J.M. Miller and M.P. Calos, eds., 1987); Current Protocols in Molecular Biology (F.M. Ausubel et al., eds., 1987); PCR: The Polymerase Chain Reaction, (Mullis et al., eds., 1994); Current Protocols in Immunology (J.E. Coligan et al., eds., 1991); Short Protocols in Molecular Biology (Wiley and Sons, 1999); Immunobiology (C.A. Janeway and P. Travers, 1997); Antibodies (P. Finch, 1997); Antibodies: a practical approach (D. Catty., ed., IRL Press, 1988-1989); Monoclonal antibodies: a practical approach (P. Shepherd and C. Dean, eds., Oxford University Press, 2000); Using antibodies: a laboratory manual (E. Harlow and D. Lane (Cold Spring Harbor Laboratory Press, 1999); The Antibodies (M. Zanetti and J.D. Capra, eds., Harwood Academic Publishers, 1995).

20 Definitions

An "antibody" is an immunoglobulin molecule capable of specific binding to a target, such as a carbohydrate, polynucleotide, lipid, polypeptide, etc., through at least one antigen recognition site, located in the variable region of the immunoglobulin molecule. As used herein, the term encompasses not only intact polyclonal or monoclonal antibodies, but also antigen binding fragments thereof (such as Fab, Fab', F(ab')₂, Fv), single chain (ScFv) and domain antibodies (including, for example, shark and camelid antibodies), and fusion proteins comprising an antibody, and any other modified configuration of the immunoglobulin molecule that comprises an antigen recognition site. An antibody includes an antibody of any class, such as IgG, IgA, or IgM (or sub-class thereof), and the antibody need not be of any particular class. Depending on the antibody amino acid sequence of the constant region of its heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these may be

- 10 -

further divided into subclasses (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2. The heavy-chain constant regions that correspond to the different classes of immunoglobulins are called alpha, delta, epsilon, gamma, and mu, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

The term "antigen binding fragment" or "antigen binding portion" of an antibody, as used herein, refers to one or more fragments of an intact antibody that retain the ability to specifically bind to a given antigen (e.g., EGFRvIII). Antigen binding functions of an antibody can be performed by fragments of an intact antibody. Examples of binding fragments encompassed within the term "antigen binding fragment" of an antibody include Fab; Fab'; F(ab')₂; an Fd fragment consisting of the VH and CH1 domains; an Fv fragment consisting of the VL and VH domains of a single arm of an antibody; a single domain antibody (dAb) fragment (Ward et al., Nature 341:544-546, 1989), and an isolated complementarity determining region (CDR).

An antibody, an antibody conjugate, or a polypeptide that "preferentially binds" or "specifically binds" (used interchangeably herein) to a target (e.g., EGFRvIII protein) is a term well understood in the art, and methods to determine such specific or preferential binding are also well known in the art. A molecule is said to exhibit "specific binding" or "preferential binding" if it reacts or associates more frequently, more rapidly, with greater duration and/or with greater affinity with a particular cell or substance than it does with alternative cells or substances. An antibody "specifically binds" or "preferentially binds" to a target if it binds with greater affinity, avidity, more readily, and/or with greater duration than it binds to other substances. For example, an antibody that specifically or preferentially binds to an EGFRvIII epitope is an antibody that binds this epitope with greater affinity, avidity, more readily, and/or with greater duration than it binds to other EGFRvIII epitopes or non-EGFRvIII epitopes. It is also understood that by reading this definition, for example, an antibody (or moiety or epitope) that specifically or preferentially binds to a first target may or may not specifically or preferentially bind to a second target. As such, "specific binding" or "preferential binding" does not necessarily require (although it can include) exclusive binding. Generally, but not necessarily, reference to binding means preferential binding.

- 11 -

A “variable region” of an antibody refers to the variable region of the antibody light chain or the variable region of the antibody heavy chain, either alone or in combination. As known in the art, the variable regions of the heavy and light chain each consist of four framework regions (FR) connected by three complementarity
5 determining regions (CDRs) also known as hypervariable regions. The CDRs in each chain are held together in close proximity by the FRs and, with the CDRs from the other chain, contribute to the formation of the antigen binding site of antibodies. There are at least two techniques for determining CDRs: (1) an approach based on cross-species sequence variability (i.e., Kabat et al. Sequences of Proteins of Immunological Interest,
10 (5th ed., 1991, National Institutes of Health, Bethesda MD)); and (2) an approach based on crystallographic studies of antigen-antibody complexes (Al-lazikani et al., 1997, J. Molec. Biol. 273:927-948). As used herein, a CDR may refer to CDRs defined by either approach or by a combination of both approaches.

A “CDR” of a variable domain are amino acid residues within the variable region
15 that are identified in accordance with the definitions of the Kabat, Chothia, the accumulation of both Kabat and Chothia, AbM, contact, and/or conformational definitions or any method of CDR determination well known in the art. Antibody CDRs may be identified as the hypervariable regions originally defined by Kabat et al. See, e.g., Kabat et al., 1992, Sequences of Proteins of Immunological Interest, 5th ed.,
20 Public Health Service, NIH, Washington D.C. The positions of the CDRs may also be identified as the structural loop structures originally described by Chothia and others. See, e.g., Chothia et al., Nature 342:877-883, 1989. Other approaches to CDR identification include the “AbM definition,” which is a compromise between Kabat and Chothia and is derived using Oxford Molecular's AbM antibody modeling software (now
25 Accelrys®), or the “contact definition” of CDRs based on observed antigen contacts, set forth in MacCallum et al., J. Mol. Biol., 262:732-745, 1996. In another approach, referred to herein as the “conformational definition” of CDRs, the positions of the CDRs may be identified as the residues that make enthalpic contributions to antigen binding. See, e.g., Makabe et al., Journal of Biological Chemistry, 283:1156-1166, 2008. Still
30 other CDR boundary definitions may not strictly follow one of the above approaches, but will nonetheless overlap with at least a portion of the Kabat CDRs, although they may be shortened or lengthened in light of prediction or experimental findings that particular residues or groups of residues or even entire CDRs do not significantly

- 12 -

impact antigen binding. As used herein, a CDR may refer to CDRs defined by any approach known in the art, including combinations of approaches. The methods used herein may utilize CDRs defined according to any of these approaches. For any given embodiment containing more than one CDR, the CDRs may be defined in accordance with any of Kabat, Chothia, extended, AbM, contact, and/or conformational definitions.

As used herein, "monoclonal antibody" refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally-occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to polyclonal antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler and Milstein, Nature 256:495, 1975, or may be made by recombinant DNA methods such as described in U.S. Pat. No. 4,816,567. The monoclonal antibodies may also be isolated from phage libraries generated using the techniques described in McCafferty et al., Nature 348:552-554, 1990, for example.

As used herein, "humanized" antibody refers to forms of non-human (e.g. murine) antibodies that are chimeric immunoglobulins, immunoglobulin chains, or fragments thereof (such as Fv, Fab, Fab', F(ab')₂ or other antigen binding subsequences of antibodies) that contain minimal sequence derived from non-human immunoglobulin. Preferably, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a complementary determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat, or rabbit having the desired specificity, affinity, and capacity. In some instances, Fv framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, the humanized antibody may comprise residues that are found neither in the recipient antibody nor in the imported CDR or framework sequences, but are included to further

- 13 -

refine and optimize antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region or domain (Fc), typically that of a human immunoglobulin. Preferred are antibodies having Fc regions modified as described in WO 99/58572. Other forms of humanized antibodies have one or more CDRs (CDR L1, CDR L2, CDR L3, CDR H1, CDR H2, or CDR H3) which are altered with respect to the original antibody, which are also termed one or more CDRs "derived from" one or more CDRs from the original antibody.

As used herein, "human antibody" means an antibody having an amino acid sequence corresponding to that of an antibody produced by a human and/or which has been made using any of the techniques for making human antibodies known to those skilled in the art or disclosed herein. This definition of a human antibody includes antibodies comprising at least one human heavy chain polypeptide or at least one human light chain polypeptide. One such example is an antibody comprising murine light chain and human heavy chain polypeptides. Human antibodies can be produced using various techniques known in the art. In one embodiment, the human antibody is selected from a phage library, where that phage library expresses human antibodies (Vaughan et al., Nature Biotechnology, 14:309-314, 1996; Sheets et al., Proc. Natl. Acad. Sci. (USA) 95:6157-6162, 1998; Hoogenboom and Winter, J. Mol. Biol., 227:381, 1991; Marks et al., J. Mol. Biol., 222:581, 1991). Human antibodies can also be made by immunization of animals into which human immunoglobulin loci have been transgenically introduced in place of the endogenous loci, e.g., mice in which the endogenous immunoglobulin genes have been partially or completely inactivated. This approach is described in U.S. Pat. Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; and 5,661,016. Alternatively, the human antibody may be prepared by immortalizing human B lymphocytes that produce an antibody directed against a target antigen (such B lymphocytes may be recovered from an individual or from single cell cloning of the cDNA, or may have been immunized *in vitro*). See, e.g., Cole et al. Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, p. 77, 1985; Boerner et al., J. Immunol., 147 (1):86-95, 1991; and U.S. Pat. No. 5,750,373.

- 14 -

The term "chimeric antibody" is intended to refer to antibodies in which the variable region sequences are derived from one species and the constant region sequences are derived from another species, such as an antibody in which the variable region sequences are derived from a mouse antibody and the constant region sequences are derived from a human antibody.

The terms "polypeptide", "oligopeptide", "peptide" and "protein" are used interchangeably herein to refer to chains of amino acids of any length. For example, the chain may be relatively short (e.g., 10-100 amino acids), or longer. The chain may be linear or branched, it may comprise modified amino acids, and/or may be interrupted by non-amino acids. The terms also encompass an amino acid chain that has been modified naturally or by intervention; for example, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation or modification, such as conjugation with a labeling component. Also included within the definition are, for example, polypeptides containing one or more analogs of an amino acid (including, for example, unnatural amino acids, etc.), as well as other modifications known in the art. It is understood that the polypeptides can occur as single chains or associated chains.

A "monovalent antibody" comprises one antigen binding site per molecule (e.g., IgG or Fab). In some instances, a monovalent antibody can have more than one antigen binding sites, but the binding sites are from different antigens.

A "monospecific antibody" comprises two identical antigen binding sites per molecule (e.g. IgG) such that the two binding sites bind identical epitope on the antigen. Thus, they compete with each other on binding to one antigen molecule. Most antibodies found in nature are monospecific. In some instances, a monospecific antibody can also be a monovalent antibody (e.g. Fab)

A "bivalent antibody" comprises two antigen binding sites per molecule (e.g., IgG). In some instances, the two binding sites have the same antigen specificities. However, bivalent antibodies may be bispecific.

A "bispecific" or "dual-specific" is a hybrid antibody having two different antigen binding sites. The two antigen binding sites of a bispecific antibody bind to two different epitopes, which may reside on the same or different protein targets.

- 15 -

A "bifunctional" is antibody is an antibody having identical antigen binding sites (i.e., identical amino acid sequences) in the two arms but each binding site can recognize two different antigens.

5 A "heteromultimer", "heteromultimeric complex", or "heteromultimeric polypeptide" is a molecule comprising at least a first polypeptide and a second polypeptide, wherein the second polypeptide differs in amino acid sequence from the first polypeptide by at least one amino acid residue. The heteromultimer can comprise a "heterodimer" formed by the first and second polypeptide or can form higher order tertiary structures where polypeptides in addition to the first and second polypeptide are
10 present.

A "heterodimer," "heterodimeric protein," "heterodimeric complex," or "heteromultimeric polypeptide" is a molecule comprising a first polypeptide and a second polypeptide, wherein the second polypeptide differs in amino acid sequence from the first polypeptide by at least one amino acid residue.

15 The "hinge region," "hinge sequence", and variations thereof, as used herein, includes the meaning known in the art, which is illustrated in, for example, Janeway et al., *Immunobiology: the immune system in health and disease*, (Elsevier Science Ltd., NY) (4th ed., 1999); Bloom et al., *Protein Science* (1997), 6:407-415; Humphreys et al., *J. Immunol. Methods* (1997), 209:193-202.

20 The "immunoglobulin-like hinge region," "immunoglobulin-like hinge sequence," and variations thereof, as used herein, refer to the hinge region and hinge sequence of an immunoglobulin-like or an antibody-like molecule (e.g., immunoadhesins). In some embodiments, the immunoglobulin-like hinge region can be from or derived from any IgG1, IgG2, IgG3, or IgG4 subtype, or from IgA, IgE, IgD or IgM, including chimeric
25 forms thereof, e.g., a chimeric IgG1/2 hinge region.

The term "immune effector cell" or "effector cell as used herein refers to a cell within the natural repertoire of cells in the human immune system which can be activated to affect the viability of a target cell. The viability of a target cell can include cell survival, proliferation, and/or ability to interact with other cells.

30 Antibodies of the invention can be produced using techniques well known in the art, e.g., recombinant technologies, phage display technologies, synthetic technologies or combinations of such technologies or other technologies readily known in the art

- 16 -

(see, for example, Jayasena, S.D., Clin. Chem., 45: 1628-50, 1999 and Fellouse, F.A., et al, J. Mol. Biol., 373(4):924-40, 2007).

As known in the art, "polynucleotide," or "nucleic acid," as used interchangeably herein, refer to chains of nucleotides of any length, and include DNA and RNA. The nucleotides can be deoxyribonucleotides, ribonucleotides, modified nucleotides or
5 bases, and/or their analogs, or any substrate that can be incorporated into a chain by DNA or RNA polymerase. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and their analogs. If present, modification to the nucleotide structure may be imparted before or after assembly of the chain. The sequence of
10 nucleotides may be interrupted by non-nucleotide components. A polynucleotide may be further modified after polymerization, such as by conjugation with a labeling component. Other types of modifications include, for example, "caps", substitution of one or more of the naturally occurring nucleotides with an analog, internucleotide modifications such as, for example, those with uncharged linkages (e.g., methyl
15 phosphonates, phosphotriesters, phosphoamidates, carbamates, etc.) and with charged linkages (e.g., phosphorothioates, phosphorodithioates, etc.), those containing pendant moieties, such as, for example, proteins (e.g., nucleases, toxins, antibodies, signal peptides, poly-L-lysine, etc.), those with intercalators (e.g., acridine, psoralen, etc.), those containing chelators (e.g., metals, radioactive metals, boron, oxidative metals,
20 etc.), those containing alkylators, those with modified linkages (e.g., alpha anomeric nucleic acids, etc.), as well as unmodified forms of the polynucleotide(s). Further, any of the hydroxyl groups ordinarily present in the sugars may be replaced, for example, by phosphonate groups, phosphate groups, protected by standard protecting groups, or activated to prepare additional linkages to additional nucleotides, or may be conjugated
25 to solid supports. The 5' and 3' terminal OH can be phosphorylated or substituted with amines or organic capping group moieties of from 1 to 20 carbon atoms. Other hydroxyls may also be derivatized to standard protecting groups. Polynucleotides can also contain analogous forms of ribose or deoxyribose sugars that are generally known in the art, including, for example, 2'-O-methyl-, 2'-O-allyl, 2'-fluoro- or 2'-azido-ribose,
30 carbocyclic sugar analogs, alpha- or beta-anomeric sugars, epimeric sugars such as arabinose, xyloses or lyxoses, pyranose sugars, furanose sugars, sedoheptuloses, acyclic analogs and abasic nucleoside analogs such as methyl riboside. One or more phosphodiester linkages may be replaced by alternative linking groups. These

- 17 -

alternative linking groups include, but are not limited to, embodiments wherein phosphate is replaced by P(O)S("thioate"), P(S)S ("dithioate"), (O)NR₂ ("amidate"), P(O)R, P(O)OR', CO or CH₂ ("formacetal"), in which each R or R' is independently H or substituted or unsubstituted alkyl (1-20 C) optionally containing an ether (-O-) linkage, aryl, alkenyl, cycloalkyl, cycloalkenyl or araldyl. Not all linkages in a polynucleotide need be identical. The preceding description applies to all polynucleotides referred to herein, including RNA and DNA.

As known in the art, a "constant region" of an antibody refers to the constant region of the antibody light chain or the constant region of the antibody heavy chain, either alone or in combination.

As used herein, "substantially pure" refers to material which is at least 50% pure (i.e., free from contaminants), more preferably, at least 90% pure, more preferably, at least 95% pure, yet more preferably, at least 98% pure, and most preferably, at least 99% pure.

A "host cell" includes an individual cell or cell culture that can be or has been a recipient for vector(s) for incorporation of polynucleotide inserts. Host cells include progeny of a single host cell, and the progeny may not necessarily be completely identical (in morphology or in genomic DNA complement) to the original parent cell due to natural, accidental, or deliberate mutation. A host cell includes cells transfected in vivo with a polynucleotide(s) of this invention.

As known in the art, the term "Fc region" is used to define a C-terminal region of an immunoglobulin heavy chain. The "Fc region" may be a native sequence Fc region or a variant Fc region. Although the boundaries of the Fc region of an immunoglobulin heavy chain might vary, the human IgG heavy chain Fc region is usually defined to stretch from an amino acid residue at position Cys226, or from Pro230, to the carboxyl-terminus thereof. The numbering of the residues in the Fc region is that of the EU index as in Kabat. Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md., 1991. The Fc region of an immunoglobulin generally comprises two constant regions, CH₂ and CH₃.

As used in the art, "Fc receptor" and "FcR" describe a receptor that binds to the Fc region of an antibody. The preferred FcR is a native sequence human FcR. Moreover, a preferred FcR is one which binds an IgG antibody (a gamma receptor) and includes receptors of the FcγRI, FcγRII, and FcγRIII subclasses, including allelic

- 18 -

variants and alternatively spliced forms of these receptors. FcγRII receptors include FcγRIIA (an "activating receptor") and FcγRIIB (an "inhibiting receptor"), which have similar amino acid sequences that differ primarily in the cytoplasmic domains thereof. FcRs are reviewed in Ravetch and Kinet, *Ann. Rev. Immunol.*, 9:457-92, 1991; Capel et al., *Immunomethods*, 4:25-34, 1994; and de Haas et al., *J. Lab. Clin. Med.*, 126:330-41, 1995. "FcR" also includes the neonatal receptor, FcRn, which is responsible for the transfer of maternal IgGs to the fetus (Guyer et al., *J. Immunol.*, 117:587, 1976; and Kim et al., *J. Immunol.*, 24:249, 1994).

The term "compete", as used herein with regard to an antibody, means that a first antibody, or an antigen binding fragment (or portion) thereof, binds to an epitope in a manner sufficiently similar to the binding of a second antibody, or an antigen binding portion thereof, such that the result of binding of the first antibody with its cognate epitope is detectably decreased in the presence of the second antibody compared to the binding of the first antibody in the absence of the second antibody. The alternative, where the binding of the second antibody to its epitope is also detectably decreased in the presence of the first antibody, can, but need not be the case. That is, a first antibody can inhibit the binding of a second antibody to its epitope without that second antibody inhibiting the binding of the first antibody to its respective epitope. However, where each antibody detectably inhibits the binding of the other antibody with its cognate epitope or ligand, whether to the same, greater, or lesser extent, the antibodies are said to "cross-compete" with each other for binding of their respective epitope(s). Both competing and cross-competing antibodies are encompassed by the present invention. Regardless of the mechanism by which such competition or cross-competition occurs (e.g., steric hindrance, conformational change, or binding to a common epitope, or portion thereof), the skilled artisan would appreciate, based upon the teachings provided herein, that such competing and/or cross-competing antibodies are encompassed and can be useful for the methods disclosed herein.

A "functional Fc region" possesses at least one effector function of a native sequence Fc region. Exemplary "effector functions" include C1q binding; complement dependent cytotoxicity; Fc receptor binding; antibody-dependent cell-mediated cytotoxicity; phagocytosis; down-regulation of cell surface receptors (e.g. B cell receptor), etc. Such effector functions generally require the Fc region to be combined

- 19 -

with a binding domain (e.g. an antibody variable domain) and can be assessed using various assays known in the art for evaluating such antibody effector functions.

A “native sequence Fc region” comprises an amino acid sequence identical to the amino acid sequence of an Fc region found in nature. A “variant Fc region”
5 comprises an amino acid sequence which differs from that of a native sequence Fc region by virtue of at least one amino acid modification, yet retains at least one effector function of the native sequence Fc region. In some embodiments, the variant Fc region has at least one amino acid substitution compared to a native sequence Fc region or to the Fc region of a parent polypeptide, e.g. from about one to about ten amino acid
10 substitutions, and preferably, from about one to about five amino acid substitutions in a native sequence Fc region or in the Fc region of the parent polypeptide. The variant Fc region herein will preferably possess at least about 80% sequence identity with a native sequence Fc region and/or with an Fc region of a parent polypeptide, and most preferably, at least about 90% sequence identity therewith, more preferably, at least
15 about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% sequence identity therewith.

The term “effector function” refers to the biological activities attributable to the Fc region of an antibody. Examples of antibody effector functions include, but are not limited to, antibody-dependent cell-mediated cytotoxicity (ADCC), Fc receptor binding,
20 complement dependent cytotoxicity (CDC), phagocytosis, C1q binding, and down regulation of cell surface receptors (e.g., B cell receptor; BCR). See, e.g., U.S. Pat No. 6,737,056. Such effector functions generally require the Fc region to be combined with a binding domain (e.g., an antibody variable domain) and can be assessed using various assays known in the art for evaluating such antibody effector functions. An
25 exemplary measurement of effector function is through Fcγ3 and/or C1q binding.

As used herein “antibody-dependent cell-mediated cytotoxicity” or “ADCC” refers to a cell-mediated reaction in which nonspecific cytotoxic cells that express Fc receptors (FcRs) (e.g. natural killer (NK) cells, neutrophils, and macrophages) recognize bound antibody on a target cell and subsequently cause lysis of the target
30 cell. ADCC activity of a molecule of interest can be assessed using an *in vitro* ADCC assay, such as that described in U.S. Patent No. 5,500,362 or 5,821,337. Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and NK cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be

- 20 -

assessed in vivo, e.g., in an animal model such as that disclosed in Clynes et al., 1998, *PNAS (USA)*, 95:652-656.

“Complement dependent cytotoxicity” or “CDC” refers to the lysing of a target in the presence of complement. The complement activation pathway is initiated by the binding of the first component of the complement system (C1q) to a molecule (e.g. an antibody) complexed with a cognate antigen. To assess complement activation, a CDC assay, e.g. as described in Gazzano-Santoro et al., *J. Immunol. Methods*, 202: 163 (1996), may be performed.

As used herein, “treatment” is an approach for obtaining beneficial or desired clinical results. For purposes of this invention, beneficial or desired clinical results include, but are not limited to, one or more of the following: reducing the proliferation of (or destroying) neoplastic or cancerous cells, inhibiting metastasis of neoplastic cells, shrinking or decreasing the size of EGFRvIII expressing tumor, remission of an EGFRvIII associated disease (e.g., cancer), decreasing symptoms resulting from an EGFRvIII associated disease (e.g., cancer), increasing the quality of life of those suffering from an EGFRvIII associated disease (e.g., cancer), decreasing the dose of other medications required to treat an EGFRvIII associated disease (e.g., cancer), delaying the progression of an EGFRvIII associated disease (e.g., cancer), curing an EGFRvIII associated disease (e.g., cancer), and/or prolong survival of patients having an EGFRvIII associated disease (e.g., cancer).

“Ameliorating” means a lessening or improvement of one or more symptoms as compared to not administering an EGFRvIII antibody (monospecific or bispecific). “Ameliorating” also includes shortening or reduction in duration of a symptom.

As used herein, an “effective dosage” or “effective amount” of drug, compound, or pharmaceutical composition is an amount sufficient to effect any one or more beneficial or desired results. For prophylactic use, beneficial or desired results include eliminating or reducing the risk, lessening the severity, or delaying the outset of the disease, including biochemical, histological and/or behavioral symptoms of the disease, its complications and intermediate pathological phenotypes presenting during development of the disease. For therapeutic use, beneficial or desired results include clinical results such as reducing incidence or amelioration of one or more symptoms of various EGFRvIII associated diseases or conditions (such as for example multiple myeloma), decreasing the dose of other medications required to treat the disease,

- 21 -

enhancing the effect of another medication, and/or delaying the progression of the EGFRvIII associated disease of patients. An effective dosage can be administered in one or more administrations. For purposes of this invention, an effective dosage of drug, compound, or pharmaceutical composition is an amount sufficient to accomplish prophylactic or therapeutic treatment either directly or indirectly. As is understood in the clinical context, an effective dosage of a drug, compound, or pharmaceutical composition may or may not be achieved in conjunction with another drug, compound, or pharmaceutical composition. Thus, an "effective dosage" may be considered in the context of administering one or more therapeutic agents, and a single agent may be considered to be given in an effective amount if, in conjunction with one or more other agents, a desirable result may be or is achieved.

An "individual" or a "subject" is a mammal, more preferably, a human. Mammals also include, but are not limited to primates, horses, dogs, cats, mice and rats.

As used herein, "vector" means a construct, which is capable of delivering, and, preferably, expressing, one or more gene(s) or sequence(s) of interest in a host cell. Examples of vectors include, but are not limited to, viral vectors, naked DNA or RNA expression vectors, plasmid, cosmid or phage vectors, DNA or RNA expression vectors associated with cationic condensing agents, DNA or RNA expression vectors encapsulated in liposomes, and certain eukaryotic cells, such as producer cells.

As used herein, "expression control sequence" means a nucleic acid sequence that directs transcription of a nucleic acid. An expression control sequence can be a promoter, such as a constitutive or an inducible promoter, or an enhancer. The expression control sequence is operably linked to the nucleic acid sequence to be transcribed.

As used herein, "pharmaceutically acceptable carrier" or "pharmaceutical acceptable excipient" includes any material which, when combined with an active ingredient, allows the ingredient to retain biological activity and is non-reactive with the subject's immune system. Examples include, but are not limited to, any of the standard pharmaceutical carriers such as a phosphate buffered saline solution, water, emulsions such as oil/water emulsion, and various types of wetting agents. Preferred diluents for aerosol or parenteral administration are phosphate buffered saline (PBS) or normal (0.9%) saline. Compositions comprising such carriers are formulated by well known conventional methods (see, for example, Remington's Pharmaceutical Sciences, 18th

- 22 -

edition, A. Gennaro, ed., Mack Publishing Co., Easton, PA, 1990; and Remington, The Science and Practice of Pharmacy 21st Ed. Mack Publishing, 2005).

The term “acyl donor glutamine-containing tag” or “glutamine tag” as used herein refers to a polypeptide or a protein containing one or more Gln residue(s) that acts as a transglutaminase amine acceptor. See, e.g., WO2012059882 and WO2015015448.

The term “ k_{on} ” or “ k_a ”, as used herein, refers to the rate constant for association of an antibody to an antigen. Specifically, the rate constants (k_{on}/k_a and k_{off}/k_d) and equilibrium dissociation constants are measured using whole antibody (i.e. bivalent) and monomeric EGFRvIII proteins (e.g., Histidine-tagged EGFRvIII fusion protein).

The term “ k_{off} ” or “ k_d ”, as used herein, refers to the rate constant for dissociation of an antibody from the antibody/antigen complex.

The term “ K_D ”, as used herein, refers to the equilibrium dissociation constant of an antibody-antigen interaction.

Reference to “about” a value or parameter herein includes (and describes) embodiments that are directed to that value or parameter per se. For example, description referring to “about X” includes description of “X.” Numeric ranges are inclusive of the numbers defining the range. Generally speaking, the term “about” refers to the indicated value of the variable and to all values of the variable that are within the experimental error of the indicated value (e.g. within the 95% confidence interval for the mean) or within 10 percent of the indicated value, whichever is greater. Where the term “about” is used within the context of a time period (years, months, weeks, days etc.), the term “about” means that period of time plus or minus one amount of the next subordinate time period (e.g. about 1 year means 11-13 months; about 6 months means 6 months plus or minus 1 week; about 1 week means 6-8 days; etc.), or within 10 per cent of the indicated value, whichever is greater.

It is understood that wherever embodiments are described herein with the language “comprising,” otherwise analogous embodiments described in terms of “consisting of” and/or “consisting essentially of” are also provided.

Where aspects or embodiments of the invention are described in terms of a Markush group or other grouping of alternatives, the present invention encompasses not only the entire group listed as a whole, but each member of the group individually and all possible subgroups of the main group, but also the main group absent one or

- 23 -

more of the group members. The present invention also envisages the explicit exclusion of one or more of any of the group members in the claimed invention.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. In case of conflict, the present specification, including definitions, will control. Throughout this specification and claims, the word "comprise," or variations such as "comprises" or "comprising" will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers. Unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular.

Exemplary methods and materials are described herein, although methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present invention. The materials, methods, and examples are illustrative only and not intended to be limiting.

EGFRvIII Antibodies and Methods of Making Thereof

The present invention provides an antibody that binds to EGFRvIII [e.g., human EGFRvIII (e.g., accession number: P00533 Feature Identifier VAR_066493, or GenBank Acession No. AJN69267; mrpsgtagaallallaalcp asraleekkgnyvvtdhgscvracgadsyemeedgvrkckkcegpckrvncgigigefkdslsi natnikhfkntsissgdlhilpvafrgdsfthtppldpqeldilktvkeitgflliqawpenrt dlhafenleirgrtkqhggqfslavvslnitslglrslkeisdgdviisgnknlcyantinwkk lfgtsgqktkiisnrgensckatgqvchalcspegcwgpeprdcvscrnvsrgrecvdkcnlle geprefvenseciqchpeclpqamnitctgrgpdnciqcahyidgphcvktcpagvmgenntlv wkyadaghvchlchpnctygctgpglegcptngpkipsiatgmvgalllllvvalgiglfmrrr hivrkrtrlrllqerelvepltpsgeapnqallrilketefkkikvlgsagfgtvykgglwipeg ekvkipvaikelreatspkankeildeayvmasvndphvcrllogictstvtqlitqlmpfgcll dyvrehkdnigsqyllnwcqvqiakgmnyledrrlvhrdlaarnvlvktqpqhvitdfglakllg aeekeyhaeggkvpikwmalesilhrityhqsdvwsygvvtvwelmtfgskpydgipaseissil ekgerlpqppictidvymimvkcmidadsrpkfreliefskmardpqrylviqgdermhpls ptdsnfyralmdeedmddvvdadeylipqqgffsspstsrtpllsslsatsnnstvacidrngl qscpikedsflqryssdptgaltedsiddtflpvpeyinqsvpkpagsvqnpvyhngplnpap srdphyqdphstavgnpaylntvqptcvnstfdspahwaqkgshqisldnpdyqqdffpkeakp ngifkgstaenaeylrvapqssefiga(SEQ ID NO: 201))] and characterized by any one

- 24 -

or more of the following characteristics: (a) decrease or downregulate the protein expression of EGFRvIII; (b) treat, prevent, ameliorate one or more symptoms of a condition associated with malignant cells expressing EGFRvIII in a subject (e.g., cancer such as glioblastoma multiform); (c) inhibit tumor growth or progression in a subject (who has a malignant tumor expressing EGFRvIII); (d) inhibit metastasis of cancer (malignant) cells expressing EGFRvIII in a subject (who has one or more malignant cells expressing EGFRvIII); (e) induce regression (e.g., long-term regression) of a tumor expressing EGFRvIII; (f) exert cytotoxic activity in malignant cells expressing EGFRvIII; (g) block EGFRvIII interaction with other yet to be identified factors; and/or (h) induce bystander effect that kill or inhibit growth of non-EGFRvIII expressing malignant cells in the vicinity.

In one aspect, provided is an isolated antibody which specifically binds to EGFRvIII, wherein the antibody comprises (a) a heavy chain variable (VH) region comprising (i) a VH complementarity determining region one (CDR1) comprising the sequence shown in SEQ ID NO: 62, 63, 64, 74, 75, 76, 80, 81, 82, 88, 89, 90, 93, 94, 95, 99, 100, 101, 109, 110, 111, 115, 116, 117, 121, 122, 123, 132, 133, 134, 137, 138, 139, 143, 144, or 145; (ii) a VH CDR2 comprising the sequence shown in SEQ ID NO: 65, 66, 68, 69, 70, 71, 77, 78, 83, 84, 86, 87, 91, 92, 96, 97, 98, 102, 103, 105, 106, 112, 113, 118, 119, 124, 125, 127, 128, 130, 131, 135, 136, 140, 141, 146, 147, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, or 237; and iii) a VH CDR3 comprising the sequence shown in SEQ ID NO: 67, 72, 73, 79, 85, 104, 107, 108, 114, 120, 126, 129, 142, 148, 219, 220, 221, 222, 223, or 236; and/or (b) a light chain variable (VL) region comprising (i) a VL CDR1 comprising the sequence shown in SEQ ID NO: 149, 154, 156, 159, 162, 165, 166, 168, 169, 170, 171, 173, 174, 176, 178, 181, 182, 185, 187, 190, 192, 195, 198, 238, or 239; (ii) a VL CDR2 comprising the sequence shown in SEQ ID NO: 150, 152, 155, 157, 160, 163, 172, 175, 179, 183, 186, 188, 191, 193, 196, or 199; and (iii) a VL CDR3 comprising the sequence shown in SEQ ID NO: 151, 153, 158, 161, 164, 167, 177, 180, 184, 189, 194, 197, or 200.

In another aspect, provided is an isolated antibody which specifically binds to EGFRvIII, wherein the antibody comprises: a VH region comprising a VH CDR1, VH CDR2, and VH CDR3 of the VH sequence shown in SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 30, 32, 34, 35, 37, 39, 41, 43, 44, 46, 48, 50, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 202, 203, 204, 205, 206, 207, 208, 209, 210, 214, 216, 217, or

- 25 -

218; and/or a VL region comprising VL CDR1, VL CDR2, and VL CDR3 of the VL sequence shown in SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 29, 31, 33, 36, 38, 40, 42, 45, 47, 49, 51, 211, 212, 213, or 215.

In some embodiments, provided is an antibody having any one of partial light chain sequence as listed in Table 1 and/or any one of partial heavy chain sequence as listed in Table 1. In Table 1, the underlined sequences are CDR sequences according to Kabat and in bold according to Chothia.

Table 1

mAb	Light Chain	Heavy Chain
m62G7	DVVMQTQPLTSLVTIGQPASISCK <u>SSQSLLYSNGKTYLN</u> WLLQRPG QSPKRLIY <u>LVSKLDS</u> GVPDRFTG SGSGTDFTLKISRVEAEDLGFYY C <u>VQDTHFPLT</u> FGAGTKLELK (SEQ ID NO: 2)	EVQLQQSGPELVKPGASVKISCKT <u>SGYTFTD</u> YTLHWVKQSHVKSLEWI <u>GGIDPINGGTTY</u> NQKFKGKATLTV DKSSSTAYMELRSLTSEDSAVYYC AR <u>GEAMDS</u> WGQGTSVTVSS (SEQ ID NO: 1)
h62G7	DVVMTQSPLSLPVTLGQPASISC <u>KSSQSLLYSNGKTYLN</u> WFQQRPG GQSPRRLIY <u>LVSKLDS</u> GVPDRFS GSGSGTDFTLKISRVEAEDVGVY YC <u>VQDTHFPLT</u> FGGGTKVEIK (SEQ ID NO: 4)	QVQLVQSGAEVKKPGASVKVSCK A <u>SGYTFTD</u> YTLHWVRQAPGQGLE WMG <u>GINPINGGTTY</u> NQKFKGRVT MTRDTSTSTVYMELSSLRSEDVAV YYCAR <u>GEAMDS</u> WGQGTSLTVTVSS (SEQ ID NO: 3)
h62G7- EQ/L6	DVVMTQSPLSLPVTLGQPASISC <u>KSSQSLLYSNGKTYLN</u> WFQQRPG GQSPRRLIY <u>QVSKLDS</u> GVPDRFS GSGSGTDFTLKISRVEAEDVGVY YC <u>GQDTHFPLT</u> FGGGTKVEIK (SEQ ID NO: 6)	QVQLVQSGAEVKKPGASVKVSCK A <u>SGYTFTD</u> YTLHWVRQAPGQGLE WMG <u>GIWPITGGTTY</u> NQKFKGRVT MTRDTSTSTVYMELSSLRSEDVAV YYCAR <u>GEAQGS</u> WGQGTSLTVTVSS (SEQ ID NO: 5)
h62G7 H14/L1- DV	DVVMTQSPLSLPVTLGQPASISC <u>KSSQSLLYSNDKTYTN</u> WFQQRPG GQSPRRLIY <u>EVSKLDV</u> GVPDRFS GSGSGTDFTLKISRVEAEDVGVY YC <u>GQDTHFPLT</u> FGGGTKVEIK	QVQLVQSGAEVKKPGASVKVSCK A <u>SGYTFTD</u> YTLHWVRQAPGQGLE WMG <u>GIWPITGGTTY</u> NQKFKGRVT MTRDTSTSTVYMELSSLRSEDVAV YYCAR <u>GEAEGS</u> WGQGTSLTVTVSS

- 26 -

mAb	Light Chain	Heavy Chain
	(SEQ ID NO: 8)	(SEQ ID NO: 7)
42G9	EVVLTQSPATLSVSPGERATLSC <u>RASQSVRSNLA</u> WYQQKSGQAP RLLIY <u>GSTIRAT</u> GVPARFSGSGS GTEFTLTISSLQSEDFAVYYC <u>QQ</u> <u>YSDWPFT</u> FGPGTKVDIK (SEQ ID NO: 10)	QVTLKESGPVLLKPTETLTCTVS <u>GFSLSNPRMGV</u> SWIRQPPGKALE WFA <u>HIFSTDEKSL</u> KLSRLSRLTSLK DTSKSQVVLTMNMAPVDSATYY CARD <u>DSSNYEGYFDF</u> WGQGTLVTV SS (SEQ ID NO: 9)
32A10	EVVMTQSPATLSVSPGERVTLS <u>RASQSVSSNFA</u> WYQQRPQGAP RLLLY <u>GATTRAT</u> GLPGRFSGSGS GTENILTISSSLQSEDFAIYFC <u>QQY</u> <u>KDWPFT</u> FGPGSKVDIK (SEQ ID NO: 12)	QVTLKESGPVLVKPTETLTCTV <u>SGFSLSNARMGV</u> SWIRQPPGKAL EWLA <u>HIFSTDEKSIR</u> SLRSRLTSL KDTSKSQVVLTMNMDPVDTATY FCARD <u>DSSNYEGYFDY</u> WGQGTLVT VSS (SEQ ID NO: 11)
20B9	EIVMTQSPATLSVSPGERATLSC <u>RVSQSIGANLA</u> WYQQKFGQAPR LLIY <u>GASTRAT</u> GIPVRFSGGGSG TEFTLTISSLQSEDFAIYSC <u>QQYIY</u> <u>WPFT</u> FGPGTTVDIK (SEQ ID NO: 14)	QVTLKESGPVLVKPTETLTCTV <u>SGFSLSNARMGV</u> SWIRQPPGKAL EWLG <u>HIFSTDEKSYST</u> SLRGRITIS KDTSRGLVVLTLNMDPVDTATYY CARD <u>DSSNYEGYFDF</u> WGPGFLVTV SS (SEQ ID NO: 13)
14C11	EIVMTQSPATLSVSPGERATLSC <u>RASQSVSNNLA</u> WYQQKPGQAP RLLIY <u>GASTRAT</u> GVPARFSGSDS GTEFSLTISSSLQSEDFAVYFC <u>QQ</u> <u>YKDWPFT</u> FGPGTKVEIK (SEQ ID NO: 16)	QVTLKESGPVLVKPTETLTCTV <u>SGFSLNNARMGV</u> SWIRQPPGKAL EWFA <u>HIFSTDEKSFRT</u> SLRSRLTL SKDTSKSQVVLTMNMDPVDTAT YYCARD <u>DSSNYEGYFDY</u> WGQGILV TVSS (SEQ ID NO: 15)
21E11	DMVVTQSPATLSVSPGERATLSC <u>RASQSVGSDLA</u> WYQQPPGQSP RLLIY <u>GASTRAT</u> GVPARFSGSGS GTDFTLTITSLESEDFAVYYC <u>QQY</u> <u>NDWPFT</u> FGPGTKVDIK (SEQ ID NO: 18)	QVTLKESGPVLVKPTETLTCTV <u>SGFSLSNVRMGV</u> SWIRQPPGKAL EWFA <u>HIFSSDEKSIR</u> SLRSRLTSL KDTSKSQVVLTMNMDPVDTATY YCARD <u>DSSNYEGYFDF</u> WGQGTLVT VSSN (SEQ ID NO: 17)

- 27 -

mAb	Light Chain	Heavy Chain
49B11	EMEVTQSPATLSVSPGERATLSC <u>RASQNIGSDLA</u> WYQQQSGQAP RLLIS <u>GASTRAT</u> GVPTRFSGSGS GTDFTLTITSLQSEDAVYYC <u>QQ</u> <u>YNDWPFT</u> FGPGTKVDIK (SEQ ID NO: 20)	QVTLKESGPVLVKPTETLTCTV <u>SGFSLSNVR</u> MGVSWIRQPPGKAL EWFA <u>HIFSSDEKSIR</u> SLRSRLTLS KDTSKSQVVLMTNMDPVDTATY YCAR <u>DSSNYEGYFDY</u> WGQGTLVT VSS (SEQ ID NO: 19)
46E10	EVVMTQSPPNLSVSPGERATLSC <u>RASQSVTSNFA</u> WYQQRPQGQSP RLLLY <u>GASTRAT</u> GVPGRFSGSG SGTENILTISSLQSEDAVYFC <u>QQ</u> <u>YKDWPFT</u> FGPGSKVDIK (SEQ ID NO: 22)	QVTLKESGPVLVKPTETLTCTV <u>SGFSLSNARM</u> GVSWIRQPPGKAL EWLA <u>HIFSTDEKSIR</u> SLRSRLTLS KDTSKSQVVLIMTNMDPVDTATYY CARD <u>DSSNYEGYFDY</u> WGQGTLVTV SS (SEQ ID NO: 21)
12H6	EVVMTQSPATLSVSPGERATLSC <u>RASQGVSSNFA</u> WYQQRPQGQSP RLLLY <u>GASTRAT</u> GVPGRFSGSG SGTENILTISSLQSEDAIYFC <u>QQ</u> <u>YKDWPFT</u> FGPGSKVDIK (SEQ ID NO: 24)	QVTLKESGPVLVKPTETLTCTV <u>SGFSLSNARM</u> GVSWIRQPPGKAL EWLA <u>HIFSTDEKSIR</u> SLRSRLTLS KDTSKSQVVLMTNMDPVDTATY YCAR <u>DSSNYEGYFDY</u> WGQGTLVT VSS (SEQ ID NO: 23)
19A9	EVVMTQSPATLSVSPGERATLSC <u>RASQSVNRNLA</u> WYQQKPGQAP RLLIF <u>GTSTRAT</u> GIPARFSGSGSG TEFTLTIDSLQSEHSGLYYC <u>QQY</u> <u>NDWPFT</u> FGPGTKVDIK (SEQ ID NO: 26)	QVTLEESGPVLVKPTETLTCTV <u>SGFSLSNARM</u> GVSWIRQPPGKAP EWFA <u>HIFSTDEKSLR</u> LSRLSRLTL SKDTSKSQVVLMTNMDPVDTAT YYCAR <u>DSSNYEGYFDY</u> WGQGTLV TVSS (SEQ ID NO: 25)
11B11	EVLMTQSPATLSVSPGERATLSC <u>RASQSVSTNFA</u> WYQQRPQGQAP RLLLF <u>GASTRAT</u> GIPGRFSGSGS GTENILTISSLQSEDAIYFC <u>QQY</u> <u>KDWPFT</u> FGPGSKVEIK (SEQ ID NO: 28)	QVTLKESGPVLVKPTETLTCTV <u>SGFSLSNAK</u> MGVSWIRQPPGKAL EWLA <u>HIFSTDEKSIR</u> SLRSRLTM SKDTSKSQVVLMTNMDPVDTAT YYCVR <u>DSSNYEGYFDY</u> WGQGTLV TVSS (SEQ ID NO: 27)
21E7	DVVLTKSPATLSVSPGERATLSC	QVTLEESGPVLVKPTETLTCTV

- 28 -

mAb	Light Chain	Heavy Chain
	<u>RASQSVNSNLA</u> WYQQNPGQAP RLLIF <u>GSSTRAT</u> GIPASFSGSGSG TEFTLTINSLQSEHSAVYYC <u>QQY</u> <u>NDWPFT</u> FGPGTKVDIK (SEQ ID NO: 29)	<u>SGFSLSNARMGV</u> SWIRQPPGKAP EWFA <u>HIFSTDEKSLRLSLRSRLTL</u> SKDTSKSQVVLTMNMDPVDAT YYCAR <u>DSSNYEGYFDY</u> WGQGTLV TVSS (SEQ ID NO: 25)
12B2	EVVMTQSPATLSVSPGERATLSC <u>RASQSVINNLA</u> WYQQKPGQAPR LLIY <u>GTSTRAT</u> DIPARFSGSGSGT EFTLTISLQSEDFAVYYC <u>QDYN</u> <u>NWPFT</u> FGPGTKVDIK (SEQ ID NO: 31)	QVTLKESGPVLVKPTETLTCTV <u>SGFSLSNPRMGV</u> SWIRQPPGKAL EWLG <u>HIFSSDEKSYRLSLRSRLSIS</u> KDTSKSQVVLTMNMDPVDATY YCVR <u>DSSNYGGYFDY</u> WGQGTLV TVSS (SEQ ID NO: 30)
11F10	EIVMTQSPATLSVSPGERTTLSC <u>RASQSVGSNLA</u> WYQQKPGQAP RLLIY <u>GASTRASG</u> VPARFSGSGS GTEFTLTISLQSEDFAVYSC <u>QEY</u> <u>NNWPFT</u> FGQGTKVEIK (SEQ ID NO: 33)	QVTLKESGPVLVKPIETLTCTVC <u>GFSLSNPRMGV</u> SWIRQPPGKALE WLG <u>HIFSSDEKSYRLFLRSRLSISK</u> DTSKSQVVLTMNMDPVDATYY CARD <u>DSSDYEGYFDY</u> WGQGTLVTV SS (SEQ ID NO: 32)
17G11	EVVMTQSPATLSVSPGERATLSC <u>RASQSVINNLA</u> WYQQKPGQAPR LLIY <u>GTSTRAT</u> DIPARFSGSGSGT EFTLTISLQSEDFAVYYC <u>QDYN</u> <u>NWPFT</u> FGPGTKVDIK (SEQ ID NO: 31)	QVTLKESGPVLVKPTETLTCTVF <u>GFSLSNPRMGV</u> SWIRQPPGKAPE WLG <u>HIFSSDEKSYRLSLRSRLSISK</u> DTSKSQVVFXTNMDPGDPATYY CVR <u>DSSNYEEYFDY</u> WGQGTLVTV SS (SEQ ID NO: 34)
29D5	KIVMTQSPATLSVSPGERATLSC <u>RANQIVSSNLA</u> WYQQKPGQAPR LLV <u>GTSTRAT</u> GIPIRFSGSGSGT EFTLTVSSLQSEDFAVYVC <u>QQYN</u> <u>DWPFT</u> FGPGTKVDIK (SEQ ID NO: 36)	QVTLKESGPVLVKPTETLTCTV <u>SGFSLSNPRMGV</u> SWLRQPPGKAL EWFA <u>HIFSTDEKSYSPSLRGRLTV</u> SKDTSKSQVVLTLNMDPVDATY YCAR <u>DSSNYEGYFDY</u> WGQGTLVT VSS (SEQ ID NO: 35)
30D8	DIVMTQSPLSLPVTPGEPASIS <u>CR</u> <u>SSQSLLHNKRNNYLD</u> WFLQKPG	EVQLVESGGGLVKPGGSLRLSCE AS <u>GFTFSD</u> AWMSWVRQAPGKGL

- 29 -

mAb	Light Chain	Heavy Chain
	QSPQLLIY <u>LASNRAS</u> GVPDRFSG GGSGTDFTLKISRVEAEDVGVYY C <u>MQAQQTPIT</u> FGQGTRLEIK (SEQ ID NO: 38)	EWVG <u>RIKSKTDGGTTDY</u> VVPLNG RFIISRDDSRNTLYLQLNNLKTEDT AVYYCTT <u>VPGSYGY</u> WGQGTLVTV SS (SEQ ID NO: 37)
20E12	DIVLTQSPLSLSVTPGEPASISCR <u>SSQSLLYSNGKNYLD</u> WFLHKPG QSPQLLIY <u>LGSNRAS</u> GVPDRFSG SGSGIDFILKISRVEAEDVGVYYC <u>MQAQQTPIT</u> FGQGTRLEIK (SEQ ID NO: 40)	EVNLVESGGGLVKPGGSLRLSCE ASGFTFSYAWMSWVRQAPGKGL EWVG <u>RIKSIADGGATDY</u> AAPVRN RFTISRDDSRNTLYLEMHSLKTED TAVYYCTT <u>IPGNDAFDM</u> WGQGT MTVSS (SEQ ID NO: 39)
26B9	DIVLTQSPLSLPVTPGEPASISCR <u>SSQSLLHRDGFNYLD</u> WFLQKPG QSPQLLIY <u>LASSRAS</u> GVPDRFSG SDSGTDFTLKISRVEAEDVGVYY C <u>MQALQTPIT</u> FGQGTRLEIK (SEQ ID NO: 42)	EVQLVESWGVLVKPGGSLRLSCA ASGFIFNNAWMSWVRQAPGKGL WIG <u>RIKSKSDGGTTDY</u> AAPVKDRF TISRDDSKDTLYLQMNGLKTEDTA VYFCTT <u>APGGPFDY</u> WGQGTLVTV SS (SEQ ID NO: 41)
32G8	DIVLTQSPLSLSVTPGEPASISCR <u>SSQSLLYSNGKNYLD</u> WFLHKPG QSPQLLIY <u>LGSNRAS</u> GVPDRFSG SGSGIDFILKISRVEAEDVGVYYC <u>MQAQQTPIT</u> FGQGTRLEIK (SEQ ID NO: 40)	EVNLVESGGGLVKPGGSLRLSCE ASGFTFSYAWMSWVRQAPGKGL EWVG <u>RIKSITDGGVIDY</u> AAPVRNR CTISRDDSRNTLYLEMHSLKTEDT AVYYCTT <u>IPGNDDFDM</u> WGQGRM VTVSS (SEQ ID NO: 43)
34E7	DIVLTQSPLSLSVTPGEPASISCR <u>STQSLLYSNGKNYLD</u> WFLHKPG QSPQLLIF <u>LGSIRAS</u> GVPDRFSG SGSGIDFILKISRVEAEDVGVYYC <u>MQAQQTPIT</u> FGQGTRLEIK (SEQ ID NO: 45)	EVNLVESGGGLVKPGGSLRLSCE ASGFTFSYAWMSWVRQAPGKGL EWVG <u>RIKSINDGGATDY</u> ASPVRN RFTISRDDSRNMLYLEMHSLKTED TAVYYCTT <u>IPGNDAFDM</u> WGQGT LTVSS (SEQ ID NO: 44)
20G5	DIVLTQSPLSLPVTPGEPASISCR <u>SSQSLLYSDDRNYLD</u> WFLQKPG QSPHLLIY <u>LGSYRAS</u> GVPDRFSG	EVQLVESGGDLVKPGGSLRLSCA ASGFTFTNAWMSWVRQAPGKGL EWVG <u>RIKSKIDGGTTDY</u> AAPVKG

- 30 -

mAb	Light Chain	Heavy Chain
	SGSGTDFTLKISRVEAEDVGYY <u>CMQALQIPIT</u> FGQGTRLEIK (SEQ ID NO: 47)	RFIISRDDSKNTLSLQMNSLKTEDT AMYYCTT <u>APGGPFDY</u> WGQGSLV TVSS (SEQ ID NO: 46)
C6	ELQSVLTQPPSASGTPGQRTIS <u>CSGSSSNIGSNYVY</u> WYQQLPGT APKILIY <u>RNNQRPS</u> GVDPDRFSGS KSGTSASLAISGLRSEDEADYYC <u>AAWDDNLSGWV</u> FGTGTKLTVL (SEQ ID NO: 49)	QVQLVQSGAEVKKPGSSVKVSCK AS <u>GDTFSSNAIS</u> WVRQAPGGGLE WMG <u>VIIPIFGTADY</u> AQKFQGRVTIT ADESTSTAYMELSSLRSEDTAVYY CAR <u>HTYHEYAGGYGGAMPD</u> PWG QGTLVTVSS (SEQ ID NO: 48)
B5	DIQMTQSPSSLSASVGDRVITC <u>RASQSISSYLN</u> WYQQKPGKAPK LLIY <u>AASSLQS</u> GVPSRFSGSGSG TDFTLTISLQPEDFATYYC <u>QQSY</u> <u>STPLT</u> FGQGGTKVEIK (SEQ ID NO: 51)	EVQLLESGGGLVQPGGSLRLSCA AS <u>GFTFSNYAMS</u> WVRQAPGKGLE WVS <u>DISGGGGRTYY</u> ADSVKGRFTI SRDNSKNTLYLQMNSLR AEDTAV YYCAR <u>AGLLYGGGVYPMDI</u> WGQ GTLVTVSS (SEQ ID NO: 50)
42G9-1	EVVLTQSPATLSVSPGERATLSC <u>RASQSVRSNL</u> AWYQQKSGQAP RLLIY <u>GSTIRAT</u> GVPARFSGSGS GTEFTLTISLQSEDFAVYYC <u>QQ</u> <u>YSDWPFT</u> FGPGTKVDIK (SEQ ID NO: 10)	QVTLKESGPVLLKPTETLTCTVS <u>GFSLSNPRMGV</u> SWIRQPPGKALE WFA <u>HIFSTDEKSLKLSLR</u> SRLTSLK DTSKSQVVLTMNMAPVDSATYY CAR <u>X₁X₂SNYEGYFDF</u> WGQGT LVTVSS, wherein X ₁ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W, and X ₂ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W (SEQ ID NO: 52)
32A10-1	EVVMTQSPATLSVSPGERVTLS <u>RASQSVSSNF</u> AWYQQRPQAP RLLLY <u>GATTRAT</u> GLPGRFSGSGS GTENILTISSLQSEDFAIYFC <u>QQY</u> <u>KDWPFT</u> FGPGSKVDIK (SEQ ID NO: 12)	QVTLKESGPVLVKPTETLTCTV <u>SGFSLSNARMGV</u> SWIRQPPGKAL EWLA <u>HIFSTDEKSI</u> RRSLRSRLTSL KDTKSQVVLTMNMDPVDTATY FCAR <u>X₁X₂SNYEGYFDY</u> WGQGT LVTVSS, wherein X ₁ is R, H, K, D, E,

- 31 -

mAb	Light Chain	Heavy Chain
		S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W, and X ₂ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W (SEQ ID NO: 53)
20B9-1	EIVMTQSPATLSVSPGERATLSC <u>RVSQSIGANLAWYQQKFGQAPR</u> LLIY <u>GASTRAT</u> GIPVRFSGGGSG TEFTLTISLQSEDFAIYSC <u>QQYIY</u> <u>WPFT</u> FGPGTTVDIK (SEQ ID NO: 14)	QVTLKESGPVLVKPTETLTCTV <u>SGFSLSNARMGVSWIRQPPGKAL</u> EWLG <u>HIFSTDEKSYSTSLRGRITIS</u> KDTSRGLVVLTLTNMDPVDATY CAR <u>X₁X₂SNYEGYDF</u> WGPGFLVT VSS, wherein X ₁ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W, and X ₂ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W (SEQ ID NO: 54)
14C11-1	EIVMTQSPATLSVSPGERATLSC <u>RASQSVSNNLAWYQQKPGQAP</u> RLLIY <u>GASTRAT</u> GVPARFSGSDS GTEFSLTISLQSEDFAVYFC <u>QQ</u> <u>YKDWPFT</u> FGPGTKVEIK (SEQ ID NO: 16)	QVTLKESGPVLVKPTETLTCTV <u>SGFSLNNARMGVSWIRQPPGKAL</u> EWFA <u>HIFSTDEKSFRTSLRSRLTL</u> SKDTSKSQVVLTMNMDPVDAT YYCAR <u>X₁X₂SNYEGYFDY</u> WGQGIL VTVSS, wherein X ₁ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W, and X ₂ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W (SEQ ID NO: 55)
21E11-1	DMVVTQSPATLSVSPGERATLSC <u>RASQSVGSDLAWYQQPPGQSP</u> RLLIY <u>GASTRAT</u> GVPARFSGSGS GTDFTLTITSLESEDFAVYYC <u>QQY</u> <u>NDWPFT</u> FGPGTKVDIK (SEQ ID NO: 18)	QVTLKESGPVLVKPTETLTCTV <u>SGFSLSNVRMGVSWIRQPPGKAL</u> EWFA <u>HIFSSDEKSIRRSLRSRLTLS</u> KDTSKSQVVLTMNMDPVDATY YCAR <u>X₁X₂SNYEGYDF</u> WGQGTLV TVSSN, wherein X ₁ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F,

- 32 -

mAb	Light Chain	Heavy Chain
		Y, or W, and X ₂ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W (SEQ ID NO: 56)
49B11-1	EMEVNQSPATLSVSPGERATLSC <u>RASQNI</u>GSDLA WYQQQSGQAP RLLIS <u>G</u>ASTRAT GVPTRFSGSGS GTDFTLTITSLQSEDFAVYYC <u>QQ</u> <u>YNDWPFT</u> FGPGTKVDIK (SEQ ID NO: 20)	QVTLKESGPVLVKPTETLTCTV <u>SGFSL</u>SNVRMGV SWIRQPPGKAL EWFA <u>HIFSSDEKSIR</u> SLRSRLTSL KDTSKSQVVLMTNMDPVDATY YCAR <u>X₁X₂SNYEGYFDY</u> WGQGTLV TVSS, wherein X ₁ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W, and X ₂ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W (SEQ ID NO: 57)
46E10-1	EVVMTQSPPNLSVSPGERATLSC <u>RASQSV</u>TSNFA WYQQRPGQSP RLLLY <u>G</u>ASTRAT GVPGRFSGSG SGTENILTISSLQSEDFAVYFC <u>QQ</u> <u>YKDWPFT</u> FGPGSKVDIK (SEQ ID NO: 22)	QVTLKESGPVLVKPTETLTCTV <u>SGFSL</u>SNARMGV SWIRQPPGKAL EWLA <u>HIFSTDEKSIR</u> SLRSRLTSL KDTSKSQVVLIMTNMDPVDATYY CAR <u>X₁X₂SNYEGYFDY</u> WGQGTLVT VSS, wherein X ₁ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W, and X ₂ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W (SEQ ID NO: 58)
12H6-1	EVVMTQSPATLSVSPGERATLSC <u>RASQGV</u>SSNFA WYQQRPGQSP RLLLY <u>G</u>ASTRAT GVPGRFSGSG SGTENILTISSLQSEDFAIYFC <u>QQ</u> <u>YKDWPFT</u> FGPGSKVDIK (SEQ ID NO: 24)	QVTLKESGPVLVKPTETLTCTV <u>SGFSL</u>SNARMGV SWIRQPPGKAL EWLA <u>HIFSTDEKSIR</u> SLRSRLTSL KDTSKSQVVLMTNMDPVDATY YCAR <u>X₁X₂SNYEGYFDY</u> WGQGTLV TVSS, wherein X ₁ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W, and X ₂ is R, H, K, D, E, S,

- 33 -

mAb	Light Chain	Heavy Chain
		T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W (SEQ ID NO: 59)
19A9-1	EVVMTQSPATLSVSPGERATLSC <u>RASQSVNRNLA</u> WYQQKPGQAP RLLIF <u>GTSTRAT</u> GIPARFSGSGSG TEFTLTIDSLQSEHSGLYYC <u>QQY</u> <u>NDWPFT</u> FGPGTKVDIK (SEQ ID NO: 26)	QVTLEESGPVLVKPTETLTCTV <u>SGFSLSNARM</u> GVSWIRQPPGKAP EWFA <u>HIFSTDEKSLRLSLRSRLTL</u> SKDTSKSQVVLTMNMDPVDAT YYCAR <u>X₁X₂SNYEGYFDY</u> WGQGTL VTVSS, wherein X ₁ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W, and X ₂ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W (SEQ ID NO: 60)
11B11-1	EVLMTQSPATLSVSPGERATLSC <u>RASQSVSTNFA</u> WYQQRPQAP RLLLF <u>GASTRAT</u> GIPGRFSGSGS GTENILTISSLQSEDFAIYFC <u>QQY</u> <u>KDWPFT</u> FGPGSKVEIK (SEQ ID NO: 28)	QVTLKESGPVLVKPTETLTCTV <u>SGFSLSNAKMGV</u> SWIRQPPGKAL EWLA <u>HIFSTDEKSIRRSRLSRLTM</u> SKDTSKSQVVLTMNMDPVDAT YYCVR <u>X₁X₂SNYEGYFDY</u> WGQGTL VTVSS, wherein X ₁ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W, and X ₂ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W (SEQ ID NO: 61)
21E7-1	DVVLTKSPATLSVSPGERATLSC <u>RASQSVNSNLA</u> WYQQNPGQAP RLLIF <u>GSSTRAT</u> GIPASFSGSGSG TEFTLTINSLQSEHSAVYYC <u>QQY</u> <u>NDWPFT</u> FGPGTKVDIK (SEQ ID NO: 29)	QVTLEESGPVLVKPTETLTCTV <u>SGFSLSNARM</u> GVSWIRQPPGKAP EWFA <u>HIFSTDEKSLRLSLRSRLTL</u> SKDTSKSQVVLTMNMDPVDAT YYCAR <u>X₁X₂SNYEGYFDY</u> WGQGTL VTVSS, wherein X ₁ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W, and X ₂ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y,

- 34 -

mAb	Light Chain	Heavy Chain
		or W (SEQ ID NO: 202)
12B2-1	EVVMTQSPATLSVSPGERATLSC <u>RASQSVINNLA</u> WYQQKPGQAPR LLIY <u>GTSTRAT</u> DIPARFSGSGSGT EFTLTISLQSEDFAVYYC <u>QDYN</u> <u>NWPFT</u> FGPGTKVDIK (SEQ ID NO: 31)	QVTLKESGPVLVKPTETLTCTV <u>SGFSLSNPRMGV</u> SWIRQPPGKAL EWLG <u>HIFSSDEKSYRLSLRS</u> LSIS KDTSKSQVVLTMNMDPVDATY YCVR <u>X₁X₂SNYGGYFDY</u> WGQGTL VTVSS, wherein X ₁ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W, and X ₂ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W (SEQ ID NO: 203)
11F10-1	EIVMTQSPATLSVSPGERTTLSC <u>RASQSVGSNLA</u> WYQQKPGQAP RLLIY <u>GASTRASG</u> VPARFSGSGS GTEFTLTISLQSEDFAVYSC <u>QEY</u> <u>NNWPFT</u> FGQGTKVEIK (SEQ ID NO: 33)	QVTLKESGPVLVKPIETLTCTVC <u>GFSLSNPRMGV</u> SWIRQPPGKALE WLG <u>HIFSSDEKSYRLFLRS</u> RLSISK DTSKSKVVLTMNMDPVDATYY CAR <u>X₁X₂SDYEGYFDY</u> WGQGTLVT VSS, wherein X ₁ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W, and X ₂ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W (SEQ ID NO: 204)
17G11- 1	EVVMTQSPATLSVSPGERATLSC <u>RASQSVINNLA</u> WYQQKPGQAPR LLIY <u>GTSTRAT</u> DIPARFSGSGSGT EFTLTISLQSEDFAVYYC <u>QDYN</u> <u>NWPFT</u> FGPGTKVDIK (SEQ ID NO: 31)	QVTLKESGPVLVKPTETLTCTVF <u>GFSLSNPRMGV</u> SWIRQPPGKAPE WLG <u>HIFSSDEKSYRLSLRS</u> LSISK DTSKSKVVFXTNMDPGDPATYY CVR <u>X₁X₂SNYEEYFDY</u> WGQGTLVT VSS, wherein X ₁ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W, and X ₂ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W (SEQ ID NO: 205)

- 35 -

mAb	Light Chain	Heavy Chain
29D5-1	KIVMTQSPATLSVSPGERATLSC <u>RANQIVSSNL</u> AWYQQKPGQAPR LLVF <u>GTSTRAT</u> GIPIRFSGSGSGT EFTLTVSSLQSEDAVYVC <u>QQYN</u> <u>DWPFT</u> FGPGTKVDIK (SEQ ID NO: 36)	QVTLKESGPVLVKPTETLTCTV <u>SGFSLSNPRM</u> GVSWLRQPPGKAL EWFA <u>HIFSTDEKSY</u> SPSLRGRLTV SKDTSKQVVLTLTNMDPVDATY YCAR <u>X₁X₂SNYEGYFDY</u> WGQGTLV TVSS, wherein X ₁ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W, and X ₂ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W (SEQ ID NO: 206)
30D8-1	DIVMTQSPLSLPVTPGEPASISCR <u>SSQSLLHNKRNNYLD</u> WFLQKPG QSPQLLIY <u>LASNRAS</u> GVPDRFSG GGSGTDFTLKISRVEAEDVGVYY <u>CMQAQQTPIT</u> FGQGTRLEIK (SEQ ID NO: 38)	EVQLVESGGGLVKKPGGSLRLSCE <u>ASGFTFSD</u> AWMSWVRQAPGKGL EWWG <u>RIKSKTX₁X₂GTTDY</u> VVPLN GRFIISRDDSRNTLYLQLNNLKTED TAVYYCTT <u>VPGSYGY</u> WGQGTLVT VSS, wherein X ₁ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W, and X ₂ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W (SEQ ID NO: 207)
20E12-1	DIVLTQSPLSLSVTPGEPASISCR <u>SSQSLLYSX₁X₂KNYLD</u> WFLHKP GQSPQLLIY <u>LGSNRAS</u> GVPDRFS GSGSGIDFILKISRVEAEDVGVYY <u>CMQAQQTPIT</u> FGQGTRLEIK, , wherein X ₁ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W, and X ₂ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W (SEQ ID NO: 211)	EVNLVESGGGLVKKPGGSLRLSCE <u>ASGFTFSY</u> AWMSWVRQAPGKGL EWWG <u>RIKSIAX₁X₂GATDY</u> AAPVRN RFTISRDDSRNTLYLEMHSLKTED TAVYYCTT <u>IPGNDAFDM</u> WGQGT MTVSS, wherein X ₁ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W, and X ₂ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W (SEQ ID NO: 208)
26B9-1	DIVLTQSPLSLPVTPGEPASISCR	EVQLVESWGVLVKKPGGSLRLSCA

mAb	Light Chain	Heavy Chain
	<p><u>SSQSLLHRX₁X₂FNYLD</u>WFLQKP GQSPQLLIY<u>LASSRAS</u>GVPDRFS GSDSGTDFTLKISRVEAEDVGVY YC<u>MQALQTPIT</u>FGQGTRLEIK, wherein X₁ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W, and X₂ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W (SEQ ID NO: 212)</p>	<p><u>ASGFIFNNAWMSWVRQAPGKGLE</u> WIG<u>RIKSKSX₁X₂GTTDY</u>AAPVKDR FTISRDDSKDTLYLQMNGLKTEDT AVYFCTT<u>APGGPFDY</u>WGQGTLT VSS, wherein X₁ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W, and X₂ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W (SEQ ID NO: 209)</p>
32G8-1	<p>DIVLTQSPLSLSVTPGEPASISCR<u>R</u> <u>SSQSLLYSX₁X₂KNYLD</u>WFLHKP GQSPQLLIY<u>LGSNRAS</u>GVPDRFS GSGSGIDFILKISRVEAEDVGVYY C<u>MQAQQTPI</u>TFGQGTRLEIK, wherein X₁ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W, and X₂ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W (SEQ ID NO: 213)</p>	<p>EVNLVESGGGLVKPGGSLRLSCE <u>ASGFTFSYAWMSWVRQAPGKGL</u> EWVG<u>RIKSITX₁X₂GVIDY</u>AAPVRN RCTISRDDSRNTLYLEMHSLKTED TAVYYCTT<u>IPGNDDFDM</u>WGQGRM VTVSS, wherein X₁ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W, and X₂ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W (SEQ ID NO: 210)</p>
34E7-1	<p>DIVLTQSPLSLSVTPGEPASISCR<u>R</u> <u>STQSLLYSX₁X₂KNYLD</u>WFLHKP GQSPQLLIF<u>LGSIRAS</u>GVPDRFS GSGSGIDFILKISRVEAEDVGVYY C<u>MQAQQTPI</u>TFGQGTRLEIK, , wherein X₁ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W, and X₂ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W (SEQ ID NO: 215)</p>	<p>EVNLVESGGGLVKPGGSLRLSCE <u>ASGFTFSYAWMSWVRQAPGKGL</u> EWVG<u>RIKSINX₁X₂GATDY</u>ASPVRN RFTISRDDSRNMLYLEMHSLKTED TAVYYCTT<u>IPGNDAFDM</u>WGQGTL VTVSS, wherein X₁ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W, and X₂ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W (SEQ ID NO: 214)</p>
20G5-1	<p>DIVLTQSPLSLPVTGEPASISCR<u>R</u> <u>SSQSLLYSDDRNYLD</u>WFLQKPG</p>	<p>EVQLVESGGDLVKPGGSLRLSCA <u>ASGFTFTNAWMSWVRQAPGKGL</u></p>

- 37 -

mAb	Light Chain	Heavy Chain
	QSPHLLIY <u>LGSYRAS</u> GVPDRFSG SGSGTDFTLKISRVEAEDVGVYY <u>CMQALQIPIT</u> FGQGTRLEIK (SEQ ID NO: 47)	EWVG <u>RIKSKIX₁X₂GTTDY</u> AAPVKG RFIISRDDSKNTLSLQMNSLKTEDT AMYYCTT <u>APGGPFDY</u> WGQGSLV TVSS, wherein X ₁ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W, and X ₂ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W (SEQ ID NO: 216)
C6-1	ELQSVLTQPPSASGTPGQRTVIS <u>CSGSSSNIGSNYVY</u> WYQQLPGT APKILIY <u>RNNQRPS</u> GVPDRFSGS KSGTSASLAISGLRSEDEADYYC <u>AAWDDNLSGWV</u> FGTGTKLTVL (SEQ ID NO: 49)	QVQLVQSGAEVKKPGSSVKVSCK <u>ASGDTFSSNAIS</u> WVRQAPGGGLE WMG <u>VIIPIFGTADY</u> AQKFQGRVTIT ADESTSTAYMELSSLRSEDYAVYY CAR <u>HTYHEYAGGYGGAMX₁X₂</u> W GGGTLVTVSS, wherein X ₁ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W, and X ₂ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W (SEQ ID NO: 217)
B5-1	DIQMTQSPSSLSASVGDRVTITC <u>RASQSISSYLN</u> WYQQKPGKAPK LLIY <u>AASSLQS</u> GVPSRFSGSGSG TDFTLTISLQPEDFATYYC <u>QQSY</u> <u>STPLT</u> FGQGTKVEIK (SEQ ID NO: 51)	EVQLLESGGGLVQPGGSLRLSCA <u>ASGFTFSNYAMS</u> WVRQAPGKGLE WVS <u>DISGGGGRTYYAX₁X₂</u> VKGRF TISRDNKNTLYLQMNSLRAEDTA VYYCAR <u>AGLLYGGGVYPMDI</u> WG GGTLVTVS, wherein X ₁ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W, and X ₂ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W (SEQ ID NO: 218)

Also provided herein are CDR portions of antigen binding domains of antibodies to EGFRvIII (including Chothia, Kabat CDRs, and CDR contact regions). Determination of CDR regions is well within the skill of the art. It is understood that in some

- 38 -

embodiments, CDRs can be a combination of the Kabat and Chothia CDR (also termed "combined CRs" or "extended CDRs"). In some embodiments, the CDRs are the Kabat CDRs. In other embodiments, the CDRs are the Chothia CDRs. In other words, in 5 combination CDRs, or combinations thereof. Table 2 provides examples of CDR sequences provided herein.

Table 2

Heavy Chain			
mAb	CDRH1	CDRH2	CDRH3
m62G7	TDYTLH (SEQ ID NO: 62) (Kabat); GYTFTD (SEQ ID NO: 63) (Chothia); GYTFTDYTLH (SEQ ID NO: 64) (extended)	GIDPINGGTTYNQKFK G (SEQ ID NO: 65) (Kabat) GIDPINGGTTY (SEQ ID NO: 66) (Chothia)	GEAMDS (SEQ ID NO: 67)
h62G7	TDYTLH (SEQ ID NO: 62) (Kabat); GYTFTD (SEQ ID NO: 63) (Chothia); GYTFTDYTLH (SEQ ID NO: 64) (extended)	GINPINGGTTYNQKFK G (SEQ ID NO: 68) (Kabat) GINPINGGTTY (SEQ ID NO: 69) (Chothia)	GEAMDS (SEQ ID NO: 67)
h62G7-H14	TDYTLH (SEQ ID NO: 62) (Kabat); GYTFTD (SEQ ID NO: 63) (Chothia); GYTFTDYTLH (SEQ ID NO: 64) (extended)	GIWPITGGTTYNQKFK G (SEQ ID NO: 70) (Kabat) GIWPITGGTTY (SEQ ID NO: 71) (Chothia)	GAEAGS (SEQ ID NO: 72)
h62G7-EQ	TDYTLH (SEQ ID NO: 62) (Kabat); GYTFTD (SEQ ID NO: 63) (Chothia);	GIWPITGGTTYNQKFK G (SEQ ID NO: 70) (Kabat) GIWPITGGTTY (SEQ	GEAQGS (SEQ ID NO: 73)

- 39 -

	GYTFTDYTLH (SEQ ID NO: 64) (extended)	ID NO: 71) (Chothia)	
42G9	SNPRMGVS (SEQ ID NO: 74) (Kabat); GFSLSNPR (SEQ ID NO: 75) (Chothia); GFSLSNPRMGVS (SEQ ID NO: 76) (extended)	HIFSTDEKSLKLSLRS(S EQ ID NO: 77) (Kabat) HIFSTDEKSL (SEQ ID NO: 78) (Chothia)	DSSNYEGYFDF (SEQ ID NO: 79)
32A10	SNARMGV (SEQ ID NO: 80) (Kabat); GFSLSNAR (SEQ ID NO: 81) (Chothia); GFSLSNARMGV (SEQ ID NO: 82) (extended)	HIFSTDEKSIRRLRS (SEQ ID NO: 83) (Kabat) HIFSTDEKSI (SEQ ID NO: 84) (Chothia)	DSSNYEGYFDY (SEQ ID NO: 85)
20B9	SNARMGV (SEQ ID NO: 80) (Kabat); GFSLSNAR (SEQ ID NO: 81) (Chothia); GFSLSNARMGV (SEQ ID NO: 82) (extended)	HIFSTDEKSYSTSLRG(S EQ ID NO: 86) (Kabat) HIFSTDEKSY (SEQ ID NO: 87) (Chothia)	DSSNYEGYFDF (SEQ ID NO: 79)
14C11	NNARMGV (SEQ ID NO: 88) (Kabat); GFSLNNAR (SEQ ID NO: 89) (Chothia); GFSLNNARMGV (SEQ ID NO: 90) (extended)	HIFSTDEKSFRTSLRS(S EQ ID NO: 91) (Kabat) HIFSTDEKSF (SEQ ID NO: 92) (Chothia)	DSSNYEGYFDY (SEQ ID NO: 85)
21E11	SNVRMGVS (SEQ ID NO: 93) (Kabat); GFSLSNVR (SEQ ID NO: 94) (Chothia); GFSLSNVRMGVS (SEQ ID NO: 95) (extended)	HIFSSDEKSIRRLRS(SE Q ID NO: 96) (Kabat) HIFSSDEKSI (SEQ ID NO: 97) (Chothia)	DSSNYEGYFDF (SEQ ID NO: 79)

- 40 -

	95) (extended)		
49B11	SNVRMGVS (SEQ ID NO: 93) (Kabat); GFSLSNVR (SEQ ID NO: 94) (Chothia); GFSLSNVRMGVS (SEQ ID NO: 95) (extended)	HIFSSDEKSIRRLRS(SEQ ID NO: 96) (Kabat) HIFSSDEKSI (SEQ ID NO: 97) (Chothia)	DSSNYEGYFDY (SEQ ID NO: 85)
46E10 12H6	SNARMGV (SEQ ID NO: 80) (Kabat); GFSLSNAR (SEQ ID NO: 81) (Chothia); GFSLSNARMGV (SEQ ID NO: 82) (extended)	HIFSTDEKSIRRLRS (SEQ ID NO: 83) (Kabat) HIFSTDEKSI (SEQ ID NO: 84) (Chothia)	DSSNYEGYFDY (SEQ ID NO: 85)
19A9 21E7	SNARMGV (SEQ ID NO: 80) (Kabat); GFSLSNAR (SEQ ID NO: 81) (Chothia); GFSLSNARMGV (SEQ ID NO: 82) (extended)	HIFSTDEKSLRLRS (SEQ ID NO: 98) (Kabat) HIFSTDEKSL (SEQ ID NO: 78) (Chothia)	DSSNYEGYFDY (SEQ ID NO: 85)
11B11	SNAKMGVS (SEQ ID NO: 99) (Kabat); GFSLSNAK (SEQ ID NO: 100) (Chothia); GFSLSNAKMGVS (SEQ ID NO: 101) (extended)	HIFSTDEKSIRRLRS (SEQ ID NO: 83) (Kabat) HIFSTDEKSI (SEQ ID NO: 84) (Chothia)	DSSNYEGYFDY (SEQ ID NO: 85)
12B2	SNPRMGVS (SEQ ID NO: 74) (Kabat); GFSLSNPR (SEQ ID NO: 75) (Chothia); GFSLSNPRMGVS (SEQ ID NO: 76) (extended)	HIFSSDEKSYRLRS (SEQ ID NO: 102) (Kabat) HIFSSDEKSY (SEQ ID NO: 103) (Chothia)	DSSNYGGYFDY (SEQ ID NO: 104)

- 41 -

11F10	SNPRMGVS (SEQ ID NO: 74) (Kabat); GFSLSNPR (SEQ ID NO: 75) (Chothia); GFSLSNPRMGVS (SEQ ID NO: 76) (extended)	HIFSSDEKSYRLFLRS (SEQ ID NO: 105) (Kabat) HIFSSDEKSY (SEQ ID NO: 103) (Chothia)	DSSDYEGYFDY (SEQ ID NO: 107)
17G11	SNPRMGVS (SEQ ID NO: 74) (Kabat); GFSLSNPR (SEQ ID NO: 75) (Chothia); GFSLSNPRMGVS (SEQ ID NO: 76) (extended)	HIFSSDEKSYRLSLRS (SEQ ID NO: 102) (Kabat) HIFSSDEKSY (SEQ ID NO: 103) (Chothia)	DSSNYEEYFDY (SEQ ID NO: 108)
29D5	SNPRMGVS (SEQ ID NO: 74) (Kabat); GFSLSNPR (SEQ ID NO: 75) (Chothia); GFSLSNPRMGVS (SEQ ID NO: 76) (extended)	HIFSTDEKSYSPSLRG (SEQ ID NO: 106) (Kabat) HIFSTDEKSY (SEQ ID NO: 87) (Chothia)	DSSNYEGYFDY (SEQ ID NO: 85)
30D8	SDAWMS (SEQ ID NO: 109) (Kabat); GFTFSD (SEQ ID NO: 110) (Chothia); GFTFSDAWMS (SEQ ID NO: 111) (extended)	RIKSKTDGGTTDYVPL NG (SEQ ID NO: 112) (Kabat) RIKSKTDGGTTDY (SEQ ID NO: 113) (Chothia)	VPGSYGY (SEQ ID NO: 114)
20E12	SYAWMS (SEQ ID NO: 115) (Kabat); GFTFSY (SEQ ID NO: 116) (Chothia); GFTFSYAWMS (SEQ ID NO: 117) (extended)	RIKSIADGGATDYAAP VRN (SEQ ID NO: 118) (Kabat) RIKSIADGGATDY (SEQ ID NO: 119) (Chothia)	IPGNDAFDM (SEQ ID NO: 120)

- 42 -

26B9	NNAWMS (SEQ ID NO: 121) (Kabat); GFIFNN (SEQ ID NO: 122) (Chothia); GFIFNNAWMS (SEQ ID NO: 123) (extended)	RIKSKSDGGTTDYAAP VKD (SEQ ID NO: 124) (Kabat) RIKSKSDGGTTDY (SEQ ID NO: 125) (Chothia)	APGGPFDY (SEQ ID NO: 126)
32G8	SYAWMS (SEQ ID NO: 115) (Kabat); GFTFSY (SEQ ID NO: 116) (Chothia); GFTFSYAWMS (SEQ ID NO: 117) (extended)	RIKSITDGGVIDYAAPV RN (SEQ ID NO: 127) (Kabat) RIKSITDGGVIDY (SEQ ID NO: 128) (Chothia)	IPGNDDFDM (SEQ ID NO: 129)
34E7	SYAWMS (SEQ ID NO: 115) (Kabat); GFTFSY (SEQ ID NO: 116) (Chothia); GFTFSYAWMS (SEQ ID NO: 117) (extended)	RIKSINDGGATDYASPV RN (SEQ ID NO: 130) (Kabat) RIKSINDGGATDY (SEQ ID NO: 131) (Chothia)	IPGNDAFDM (SEQ ID NO: 120)
20G5	TNAWMS (SEQ ID NO: 132) (Kabat); GFTFTN (SEQ ID NO: 133) (Chothia); GFTFTNAWMS (SEQ ID NO: 134) (extended)	RIKSKIDGGTTDYAAPV KG (SEQ ID NO: 135) (Kabat) RIKSKIDGGTTDY (SEQ ID NO: 136) (Chothia)	APGGPFDY (SEQ ID NO: 126)
C6	SSNAIS (SEQ ID NO: 137) (Kabat); GDTFSS (SEQ ID NO: 138) (Chothia); GDTFSSNAIS (SEQ ID NO: 139) (extended)	VIIPIFGTADYAQKFQG (SEQ ID NO: 140) (Kabat) VIIPIFGTADY (SEQ ID NO: 141) (Chothia)	HTYHEYAGGYGG AMDP (SEQ ID NO: 142)
B5	SNYAMS (SEQ ID NO: 143)	DISGGGGRTYYADSVK	AGLLYGGGVYPM

	(Kabat); GFTFSN (SEQ ID NO: 144) (Chothia); GFTFSNYAMS (SEQ ID NO: 145) (extended)	G (SEQ ID NO: 146) (Kabat) DISGGGGRTYY (SEQ ID NO: 147) (Chothia)	DI (SEQ ID NO: 148)
42G9-1	SNPRMGVS (SEQ ID NO: 74) (Kabat); GFSLSNPR (SEQ ID NO: 75) (Chothia); GFSLSNPRMGVS (SEQ ID NO: 76) (extended)	HIFSTDEKSLKLSLRS (SEQ ID NO: 77) (Kabat) HIFSTDEKSL (SEQ ID NO: 78) (Chothia)	X ₁ X ₂ SNYEGYFDF, wherein X ₁ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W, and X ₂ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W (SEQ ID NO: 219)
32A10-1	SNARMGVVS (SEQ ID NO: 80) (Kabat); GFSLSNAR (SEQ ID NO: 81) (Chothia); GFSLSNARMGVVS (SEQ ID NO: 82) (extended)	HIFSTDEKSIRRLRS (SEQ ID NO: 83) (Kabat) HIFSTDEKSI (SEQ ID NO: 84) (Chothia)	X ₁ X ₂ SNYEGYFDY, wherein X ₁ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W, and X ₂ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W (SEQ ID NO: 220)
20B9-1	SNARMGVVS (SEQ ID NO: 80) (Kabat); GFSLSNAR (SEQ ID NO: 81) (Chothia); GFSLSNARMGVVS (SEQ ID NO: 82) (extended)	HIFSTDEKSYSTSLRG(S EQ ID NO: 86) (Kabat) HIFSTDEKSY (SEQ ID NO: 87) (Chothia)	X ₁ X ₂ SNYEGYFDF, wherein X ₁ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W, and X ₂ is R, H, K,

			D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W (SEQ ID NO: 219)
14C11-1	NNARMGVS (SEQ ID NO: 88) (Kabat); GFSLNNAR (SEQ ID NO: 89) (Chothia); GFSLNNARMGVS (SEQ ID NO: 90) (extended)	HIFSTDEKSFRTSLRS (SEQ ID NO: 91) (Kabat) HIFSTDEKSF (SEQ ID NO: 92) (Chothia)	X ₁ X ₂ SNYEGYFDY, wherein X ₁ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W, and X ₂ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W (SEQ ID NO: 220)
21E11-1	SNVRMGVS (SEQ ID NO: 93) (Kabat); GFSLSNVR (SEQ ID NO: 94) (Chothia); GFSLSNVRMGVS (SEQ ID NO: 95) (extended)	HIFSSDEKSIRRLRS (SEQ ID NO: 96) (Kabat) HIFSSDEKSI (SEQ ID NO: 97) (Chothia)	X ₁ X ₂ SNYEGYFDF, wherein X ₁ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W, and X ₂ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W (SEQ ID NO: 219)
49B11-1	SNVRMGVS (SEQ ID NO: 93) (Kabat); GFSLSNVR (SEQ ID NO: 94) (Chothia); GFSLSNVRMGVS (SEQ ID NO: 95) (extended)	HIFSSDEKSIRRLRS(SEQ ID NO: 96) (Kabat) HIFSSDEKSI (SEQ ID NO: 97) (Chothia)	X ₁ X ₂ SNYEGYFDY, wherein X ₁ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W, and X ₂ is R, H, K, D, E, S, T, N, Q, C,

			G, P, A, V, I, L, M, F, Y, or W (SEQ ID NO: 220)
46E10-1 12H6-1	SNARMGVS (SEQ ID NO: 80) (Kabat); GFSLSNAR (SEQ ID NO: 81) (Chothia); GFSLSNARMGVS (SEQ ID NO: 82) (extended)	HIFSTDEKSIRRLRS (SEQ ID NO: 83) (Kabat) HIFSTDEKSI (SEQ ID NO: 84) (Chothia)	X ₁ X ₂ SNYEGYFDY, wherein X ₁ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W, and X ₂ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W (SEQ ID NO: 220)
19A9-1 21E7-1	SNARMGVS (SEQ ID NO: 80) (Kabat); GFSLSNAR (SEQ ID NO: 81) (Chothia); GFSLSNARMGVS (SEQ ID NO: 82) (extended)	HIFSTDEKSLRLRS (SEQ ID NO: 98) (Kabat) HIFSTDEKSL (SEQ ID NO: 78) (Chothia)	X ₁ X ₂ SNYEGYFDY, wherein X ₁ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W, and X ₂ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W (SEQ ID NO: 220)
11B11-1	SNAKMGVS (SEQ ID NO: 99) (Kabat); GFSLSNAK (SEQ ID NO: 100) (Chothia); GFSLSNAKMGVS (SEQ ID NO: 101) (extended)	HIFSTDEKSIRRLRS (SEQ ID NO: 83) (Kabat) HIFSTDEKSI (SEQ ID NO: 84) (Chothia)	X ₁ X ₂ SNYEGYFDY, wherein X ₁ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W, and X ₂ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M,

- 46 -

			F, Y, or W (SEQ ID NO: 220)
12B2-1	SNPRMGVS (SEQ ID NO: 74) (Kabat); GFSLSNPR (SEQ ID NO: 75) (Chothia); GFSLSNPRMGVS (SEQ ID NO: 76) (extended)	HIFSSDEKSYRLSLRS (SEQ ID NO: 102) (Kabat) HIFSSDEKSY (SEQ ID NO: 103) (Chothia)	X ₁ X ₂ SNYGGYFDY, wherein X ₁ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W, and X ₂ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W (SEQ ID NO: 221)
11F10-1	SNPRMGVS (SEQ ID NO: 74) (Kabat); GFSLSNPR (SEQ ID NO: 75) (Chothia); GFSLSNPRMGVS (SEQ ID NO: 76) (extended)	HIFSSDEKSYRLFLRS (SEQ ID NO: 105) (Kabat) HIFSSDEKSY (SEQ ID NO: 103) (Chothia)	X ₁ X ₂ SDYEGYFDY, wherein X ₁ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W, and X ₂ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W (SEQ ID NO: 222)
17G11-1	SNPRMGVS (SEQ ID NO: 74) (Kabat); GFSLSNPR (SEQ ID NO: 75) (Chothia); GFSLSNPRMGVS (SEQ ID NO: 76) (extended)	HIFSSDEKSYRLSLRS (SEQ ID NO: 102) (Kabat) HIFSSDEKSY (SEQ ID NO: 103) (Chothia)	X ₁ X ₂ SNYEEYFDY, wherein X ₁ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W, and X ₂ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W (SEQ ID

- 47 -

			NO: 223)
29D5-1	SNPRMGVS (SEQ ID NO: 74) (Kabat); GFSLSNPR (SEQ ID NO: 75) (Chothia); GFSLSNPRMGVS (SEQ ID NO: 76) (extended)	HIFSTDEKSYSPSLRG(S EQ ID NO: 106) (Kabat) HIFSTDEKSY (SEQ ID NO: 87) (Chothia)	X ₁ X ₂ SNYEGYFDY, wherein X ₁ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W, and X ₂ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W (SEQ ID NO: 220)
30D8-1	SDAWMS (SEQ ID NO: 109) (Kabat); GFTFSD (SEQ ID NO: 110) (Chothia); GFTFSDAWMS (SEQ ID NO: 111) (extended)	RIKSKTX ₁ X ₂ GTTDYVW PLNG, wherein X ₁ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W, and X ₂ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W (SEQ ID NO: 224) (Kabat) RIKSKTX ₁ X ₂ GTTDY, wherein X ₁ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W, and X ₂ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W (SEQ ID NO: 225) (Chothia)	VPGSYGY (SEQ ID NO: 114)
20E12-1	SYAWMS (SEQ ID NO: 115) (Kabat);	RIKSIAX ₁ X ₂ GATDYAAP VRN, wherein X ₁ is R,	IPGNDAFDM (SEQ ID NO: 120)

- 48 -

	GFTFSY (SEQ ID NO: 116) (Chothia); GFTFSYAWMS (SEQ ID NO: 117) (extended)	H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W, and X ₂ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W (SEQ ID NO: 226) (Kabat) RIKSIAX ₁ X ₂ GATDY, wherein X ₁ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W, and X ₂ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W (SEQ ID NO: 227) (Chothia)	
26B9-1	NNAWMS (SEQ ID NO: 121) (Kabat); GFIFNN (SEQ ID NO: 122) (Chothia); GFIFNNAWMS (SEQ ID NO: 123) (extended)	RIKSKSX ₁ X ₂ GTDDYAAP VKD, wherein X ₁ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W, and X ₂ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W (SEQ ID NO: 228) (Kabat) RIKSKSX ₁ X ₂ GTDDY, wherein X ₁ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W, and X ₂ is R, H, K, D, E, S, T, N, Q, C, G,	APGGPFDY (SEQ ID NO: 126)

- 49 -

		P, A, V, I, L, M, F, Y, or W (SEQ ID NO: 229) (Chothia)	
32G8-1	SYAWMS (SEQ ID NO: 115) (Kabat); GFTFSY (SEQ ID NO: 116) (Chothia); GFTFSYAWMS (SEQ ID NO: 117) (extended)	RIKSITX ₁ X ₂ GVIDYAAP VRN, wherein X ₁ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W, and X ₂ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W (SEQ ID NO: 230) (Kabat) RIKSITX ₁ X ₂ GVIDY, wherein X ₁ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W, and X ₂ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W (SEQ ID NO: 231) (Chothia)	IPGNDDFDM (SEQ ID NO: 129)
34E7-1	SYAWMS (SEQ ID NO: 115) (Kabat); GFTFSY (SEQ ID NO: 116) (Chothia); GFTFSYAWMS (SEQ ID NO: 117) (extended)	RIKSINX ₁ X ₂ GATDYASP VRN, wherein X ₁ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W, and X ₂ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W (SEQ ID NO: 232) (Kabat) RIKSINX ₁ X ₂ GATDY,	IPGNDAFDM (SEQ ID NO: 120)

- 50 -

		wherein X_1 is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W, and X_2 is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W (SEQ ID NO: 233) (Chothia)	
20G5-1	TNAWMS (SEQ ID NO: 132) (Kabat); GFTFTN (SEQ ID NO: 133) (Chothia); GFTFTNAWMS (SEQ ID NO: 134) (extended)	RIKSKIX ₁ X ₂ GTDDYAAP VKG, wherein X_1 is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W, and X_2 is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W (SEQ ID NO: 234) (Kabat) RIKSKIX ₁ X ₂ GTDDY, wherein X_1 is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W, and X_2 is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W (SEQ ID NO: 235) (Chothia)	APGGPFDY (SEQ ID NO: 126)
C6-1	SSNAIS (SEQ ID NO: 137) (Kabat); GDTFSS (SEQ ID NO: 138) (Chothia); GDTFSSNAIS (SEQ ID NO: 139) (Chothia)	VIIPIFGTADYAQKFQG (SEQ ID NO: 140) (Kabat) VIIPIFGTADY (SEQ ID NO: 141) (Chothia)	HTYHEYAGGYGG AMX ₁ X ₂ , wherein X_1 is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y,

- 51 -

	139) (extended)		or W, and X ₂ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W (SEQ ID NO: 236)
B5-1	SNYAMS (SEQ ID NO: 143) (Kabat); GFTFSN (SEQ ID NO: 144) (Chothia); GFTFSNYAMS (SEQ ID NO: 145) (extended)	DISGGGGRTYYAX ₁ X ₂ V KG, wherein X ₁ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W, and X ₂ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W (SEQ ID NO: 237) (Kabat) DISGGGGRTYY (SEQ ID NO: 147) (Chothia)	AGLLYGGGVYPM DI (SEQ ID NO: 148)
Light Chain			
mAb	CDRL1	CDRL2	CDRL3
m62G7 h62G7	KSSQSLLYSNGKTYLN (SEQ ID NO: 149)	LVSKLDS (SEQ ID NO: 150)	VQDTHFPLT (SEQ ID NO: 151)
h62G7-L6	KSSQSLLYSNGKTYLN (SEQ ID NO: 149)	QVSKLDS (SEQ ID NO: 152)	GQDTHFPLT (SEQ ID NO: 153)
h62G7-L1-DV	KSSQSLLYSNDKTYTN (SEQ ID NO: 154)	EVSCLDV (SEQ ID NO: 155)	GQDTHFPLT (SEQ ID NO: 153)
42G9	RASQSVRSNLA (SEQ ID NO: 156)	GSTIRAT (SEQ ID NO: 157)	QQYSDWPFT (SEQ ID NO: 158)
32A10	RASQSVSSNFA (SEQ ID NO: 159)	GATTRAT (SEQ ID NO: 160)	QQYKDWPF (SEQ ID NO: 161)
20B9	RVSQSIGANLA (SEQ ID NO: 162)	GASTRAT (SEQ ID NO: 163)	QQYIYWPFT (SEQ ID NO: 164)

- 52 -

14C11	RASQSVSNLA (SEQ ID NO: 165)	GASTRAT (SEQ ID NO: 163)	QQYKDWPF (SEQ ID NO: 161)
21E11	RASQSVGSDLA (SEQ ID NO: 166)	GASTRAT (SEQ ID NO: 163)	QQYNDWPF (SEQ ID NO: 167)
49B11	RASQNIGSDLA (SEQ ID NO: 168)	GASTRAT (SEQ ID NO: 163)	QQYNDWPF (SEQ ID NO: 167)
46E10	RASQSVTSNFA (SEQ ID NO: 169)	GASTRAT (SEQ ID NO: 163)	QQYKDWPF (SEQ ID NO: 161)
12H6	RASQGVSSNFA (SEQ ID NO: 170)	GASTRAT (SEQ ID NO: 163)	QQYKDWPF (SEQ ID NO: 161)
19A9	RASQSVNRNLA (SEQ ID NO: 171)	GTSTRAT (SEQ ID NO: 172)	QQYNDWPF (SEQ ID NO: 167)
11B11	RASQSVSTNFA (SEQ ID NO: 173)	GASTRAT (SEQ ID NO: 163)	QQYKDWPF (SEQ ID NO: 161)
21E7	RASQSVNSNLA (SEQ ID NO: 174)	GSSTRAT (SEQ ID NO: 175)	QQYNDWPF (SEQ ID NO: 167)
12B2 17G11	RASQSVINNLA (SEQ ID NO: 176)	GTSTRAT (SEQ ID NO: 172)	QDYNNWPF (SEQ ID NO: 177)
11F10	RASQSVGSNLA (SEQ ID NO: 178)	GASTRASG (SEQ ID NO: 179)	QEYNNWPF (SEQ ID NO: 180)
29D5	RANQIVSSNLA (SEQ ID NO: 181)	GTSTRAT (SEQ ID NO: 172)	QQYNDWPF (SEQ ID NO: 167)
30D8	RSSQSLLHNKRNNYLD (SEQ ID NO: 182)	LASNRA (SEQ ID NO: 183)	MQAQQTPI (SEQ ID NO: 184)
20E12 32G8	RSSQSLLYSNGKNYLD (SEQ ID NO: 185)	LGSNRA (SEQ ID NO: 186)	MQAQQTPI (SEQ ID NO: 184)
26B9	RSSQSLLHRDGFNYLD (SEQ ID NO: 187)	LASSRA (SEQ ID NO: 188)	MQALQTP (SEQ ID NO: 189)
34E7	RSTQSLLYSNGKNYLD (SEQ ID NO: 190)	LGSIRA (SEQ ID NO: 191)	MQAQQTPI (SEQ ID NO: 184)

- 53 -

20G5	RSSQSLLYSDRRNYLD (SEQ ID NO: 192)	LGSYRAS (SEQ ID NO: 193)	MQALQIPIT (SEQ ID NO: 194)
C6	SGSSSNIGSNYVY (SEQ ID NO: 195)	RNNQRPS (SEQ ID NO: 196)	AAWDDNLSGWV (SEQ ID NO: 197)
B5	RASQSISSYLN (SEQ ID NO: 198)	AASSLQS (SEQ ID NO: 199)	QQSYSTPLT (SEQ ID NO: 200)
20E12-1 32G8-1	RSSQSLLYSX ₁ X ₂ KNYLD, wherein X ₁ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W, and X ₂ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W (SEQ ID NO: 238)	LGSNRAS (SEQ ID NO: 186)	MQAQQTPT (SEQ ID NO: 184)
26B9-1	RSSQSLHRX ₁ X ₂ FNYLD, wherein X ₁ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W, and X ₂ is R, H, K, D, E, S, T, N, Q, C, G, P, A, V, I, L, M, F, Y, or W (SEQ ID NO: 239)	LASSRAS (SEQ ID NO: 188)	MQALQTPIT (SEQ ID NO: 189)

In some embodiments, the present invention provides an antibody that binds to and competes with the antibody as described herein, including m62G7, h62G7, h62G7-H14/L1-DV, h62G7-EQ/L6, 42G9, 32A10, 20B9, 14C11, 21E11, 49B11, 46E10, 12H6, 19A9, 21E7, 11B11, 12B2, 11F10, 17G11, 29D5, 30D8, 20E12, 26B9, 32G8, 34E7, 20G5, C6, B5, 42G9-1, 32A10-1, 20B9-1, 14C11-1, 21E11-1, 49B11-1, 46E10-1, 12H6-1, 19A9-1, 21E7-1, 11B11-1, 12B2-1, 11F10-1, 17G11-1, 29D5-1, 30D8-1, 20E12-1, 26B9-1, 32G8-1, 34E7-1, 20G5-1, C6-1, and B5-1.

In some embodiments, the invention also provides CDR portions of antibodies to EGFRvIII antibodies based on CDR contact regions. CDR contact regions are regions of an antibody that imbue specificity to the antibody for an antigen. In general, CDR contact regions include the residue positions in the CDRs and Vernier zones which are

- 54 -

constrained in order to maintain proper loop structure for the antibody to bind a specific antigen. See, e.g., Makabe et al., J. Biol. Chem., 283:1156-1166, 2007. Determination of CDR contact regions is well within the skill of the art.

The binding affinity (K_D) of the EGFRvIII antibody as described herein to
5 EGFRvIII (such as human EGFRvIII (e.g., (SEQ ID NO: 201)) can be about 0.001 to about 5000 nM. In some embodiments, the binding affinity is about any of 5000 nM, 4500 nM, 4000 nM, 3500 nM, 3000 nM, 2500 nM, 2000 nM, 1789 nM, 1583 nM, 1540 nM, 1500 nM, 1490 nM, 1064 nM, 1000 nM, 933 nM, 894 nM, 750 nM, 705 nM, 678 nM, 532 nM, 500 nM, 494 nM, 400 nM, 349 nM, 340 nM, 353 nM, 300 nM, 250 nM, 244
10 nM, 231 nM, 225 nM, 207 nM, 200 nM, 186 nM, 172 nM, 136 nM, 113 nM, 104 nM, 101 nM, 100 nM, 90 nM, 83 nM, 79 nM, 74 nM, 54 nM, 50 nM, 45 nM, 42 nM, 40 nM, 35 nM, 32 nM, 30 nM, 25 nM, 24 nM, 22 nM, 20 nM, 19 nM, 18 nM, 17 nM, 16 nM, 15 nM, 12 nM, 10 nM, 9 nM, 8 nM, 7.5 nM, 7 nM, 6.5 nM, 6 nM, 5.5 nM, 5 nM, 4 nM, 3 nM, 2 nM, 1 nM, 0.5 nM, 0.3 nM, 0.1 nM, 0.01 nM, or 0.001 nM. In some embodiments, the
15 binding affinity is less than about any of 5000 nM, 4000 nM, 3000 nM, 2000 nM, 1000 nM, 900 nM, 800 nM, 250 nM, 200 nM, 100 nM, 50 nM, 30 nM, 20 nM, 10 nM, 7.5 nM, 7 nM, 6.5 nM, 6 nM, 5 nM, 4.5 nM, 4 nM, 3.5 nM, 3 nM, 2.5 nM, 2 nM, 1.5 nM, 1 nM, or 0.5 nM.

Bispecific antibodies, monoclonal antibodies that have binding specificities for at
20 least two different antigens, can be prepared using the antibodies disclosed herein. Methods for making bispecific antibodies are known in the art (see, e.g., Suresh et al., Methods in Enzymology 121:210, 1986). Traditionally, the recombinant production of bispecific antibodies was based on the coexpression of two immunoglobulin heavy chain-light chain pairs, with the two heavy chains having different specificities (Millstein
25 and Cuellar, Nature 305, 537-539, 1983). Accordingly, in one aspect, provided is a bispecific antibody wherein the bispecific antibody is a full-length human antibody, comprising a first antibody variable domain of the bispecific antibody specifically binding to a target antigen (e.g., EGFRvIII), and comprising a second antibody variable domain of the bispecific antibody capable of recruiting the activity of a human immune effector
30 cell by specifically binding to an effector antigen located on the human immune effector cell.

The human immune effector cell can be any of a variety of immune effector cells known in the art. For example, the immune effector cell can be a member of the human

- 55 -

lymphoid cell lineage, including, but not limited to, a T cell (e.g., a cytotoxic T cell), a B cell, and a natural killer (NK) cell. The immune effector cell can also be, for example without limitation, a member of the human myeloid lineage, including, but not limited to, a monocyte, a neutrophilic granulocyte, and a dendritic cell. Such immune effector cells may have either a cytotoxic or an apoptotic effect on a target cell or other desired effect upon activation by binding of an effector antigen.

The effector antigen is an antigen (e.g., a protein or a polypeptide) that is expressed on the human immune effector cell. Examples of effector antigens that can be bound by the heterodimeric protein (e.g., a heterodimeric antibody or a bispecific antibody) include, but are not limited to, human CD3 (or CD3 (Cluster of Differentiation) complex), CD16, NKG2D, NKp46, CD2, CD28, CD25, CD64, and CD89.

The target cell can be a cell that is native or foreign to humans. In a native target cell, the cell may have been transformed to be a malignant cell or pathologically modified (e.g., a native target cell infected with a virus, a plasmodium, or a bacterium). In a foreign target cell, the cell is an invading pathogen, such as a bacterium, a plasmodium, or a virus.

The target antigen is expressed on a target cell in a diseased condition (e.g., an inflammatory disease, a proliferative disease (e.g., cancer), an immunological disorder, a neurological disease, a neurodegenerative disease, an autoimmune disease, an infectious disease (e.g., a viral infection or a parasitic infection), an allergic reaction, a graft-versus-host disease or a host-versus-graft disease). A target antigen is not effector antigen. In some embodiments, the target antigen is EGFRvIII.

In some embodiments, provided is a bispecific antibody wherein the bispecific antibody is a full-length human antibody, comprising a first antibody variable domain of the bispecific antibody specifically binding to a target antigen, and comprising a second antibody variable domain of the bispecific antibody capable of recruiting the activity of a human immune effector cell by specifically binding to an effector antigen located on the human immune effector cell, wherein the first antibody variable domain comprises a heavy chain variable (VH) region comprising a VH CDR1, VH CDR2, and VH CDR3 of the VH sequence shown in SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 30, 32, 34, 35, 37, 39, 41, 43, 44, 46, 48, 50, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 202, 203, 204, 205, 206, 207, 208, 209, 210, 214, 216, 217, or 218; and/or a light chain variable (VL) region comprising a VL CDR1, VL CDR2, and VL CDR3 of the VL

- 56 -

sequence shown in SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 29, 31, 33, 36, 38, 40, 42, 45, 47, 49, 51, 211, 212, 213, or 215.

In some embodiments, provided is a bispecific antibody wherein the bispecific antibody is a full-length human antibody, comprising a first antibody variable domain of the bispecific antibody specifically binding to a target antigen, and comprising a second antibody variable domain of the bispecific antibody capable of recruiting the activity of a human immune effector cell by specifically binding to an effector antigen located on the human immune effector cell, wherein the first antibody variable domain comprises (a) a heavy chain variable (VH) region comprising (i) a VH complementarity determining region one (CDR1) comprising the sequence shown in SEQ ID NO: 62, 63, 64, 74, 75, 76, 80, 81, 82, 88, 89, 90, 93, 94, 95, 99, 100, 101, 109, 110, 111, 115, 116, 117, 121, 122, 123, 132, 133, 134, 137, 138, 139, 143, 144, or 145; (ii) a VH CDR2 comprising the sequence shown in SEQ ID NO: 65, 66, 68, 69, 70, 71, 77, 78, 83, 84, 86, 87, 91, 92, 96, 97, 98, 102, 103, 105, 106, 112, 113, 118, 119, 124, 125, 127, 128, 130, 131, 135, 136, 140, 141, 146, 147, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, or 237; and (iii) a VH CDR3 comprising the sequence shown in SEQ ID NO: 67, 72, 73, 79, 85, 104, 107, 108, 114, 120, 126, 129, 142, 148, 219, 220, 221, 222, 223, or 236; and/or (b) a light chain variable (VL) region comprising (i) a VL CDR1 comprising the sequence shown in SEQ ID NO: 149, 154, 156, 159, 162, 165, 166, 168, 169, 170, 171, 173, 174, 176, 178, 181, 182, 185, 187, 190, 192, 195, 198, 238, or 239; (ii) a VL CDR2 comprising the sequence shown in SEQ ID NO: 150, 152, 155, 157, 160, 163, 172, 175, 179, 183, 186, 188, 191, 193, 196, or 199; and (iii) a VL CDR3 comprising the sequence shown in SEQ ID NO: 151, 153, 158, 161, 164, 167, 177, 180, 184, 189, 194, 197, or 200.

In some embodiments, the second antibody variable domain comprises a heavy chain variable (VH) region comprising a VH CDR1, VH CDR2, and VH CDR3 of the VH sequence shown in SEQ ID NO: 240; and/or a light chain variable (VL) region comprising VL CDR1, VL CDR2, and VL CDR3 of the VL sequence shown in SEQ ID NO: 241.

In some embodiments, the second antibody variable domain comprises (a) a heavy chain variable (VH) region comprising (i) a VH complementary determining region one (CDR1) comprising the sequence shown in SEQ ID NO: 244, 110, or 245; (ii) a VH CDR2 comprising the sequence shown in SEQ ID NO: 246 or 247; and (iii) a

- 57 -

VH CDR3 comprising the sequence shown in SEQ ID NO: 248; and/or (b) a light chain variable (VL) region comprising (i) a VL CDR1 comprising the sequence shown in SEQ ID NO: 249; (ii) a VL CDR2 comprising the sequence shown in SEQ ID NO: 250; and (iii) a VL CDR3 comprising the sequence shown in SEQ ID NO: 251.

- 5 Table 3 shows the specific amino acid and nucleic acid sequences of the second antibody variable domain, which is specific to CD3. In Table 3, the underlined sequences are CDR sequences according to Kabat and in bold according to Chothia.

Table 3

mAb	Light Chain	Heavy Chain
h2B4_ HNPS _VH 1d_T2 4K_VL	DIVMTQSPDSLAVSLGERATINC KSSQSLFNVR SRKNYL AWYQQK PGQPPKLLIS WASTRES GVPDF SGSGSGTDFLTISLQAEDVAV YYC KQSYDLFT FGSGTKLEIK (SEQ ID NO: 241)	EVQLVESGGGLVQPGGSLRLSCA AS GFTFSD YYMTWVRQAPGKGLE WVA FIRNRARGYTSD HNPSVKGR FTISRDNAKNSLYLQMNSLRAEDT AVYYCARD DRPSYYVLDY WGQGT VTVSS (SEQ ID NO: 240)
h2B4_ HNPS _VH 1d_T2 4K_VL	GACATTGTGATGACTCAATCCC CCGACTCCCTGGCTGTGTCCCT CGGCGAACGCGCAACTATCAAC TGTAAGCAGCCAGTCCCTGT TCAACGTCGGTCGAGGAAGAA CTACCTGGCCTGGTATCAGCAG AAACCTGGGCAGCCGCCGAAG CTTCTGATCTCATGGGCCTCAA CTCGGGAAAGCGGAGTGCCAG ATAGATTCTCCGGATCTGGCTC CGGAACCGACTTCACCCTGACG ATTCGAGCTTGCAAGCGGAGG ATGTGGCCGTGTACTACTGCAA GCAGTCCTACGACCTCTTCACC TTTGGTTCCGGCACCAAGCTGG AGATCAAA (SEQ ID NO: 243)	GAAGTCCAACCTTGTCGAATCGGG AGGAGGCCTTGTCGAACCCGGT GGATCCCTGAGGCTGTCTATGCG CGGCCTCGGGCTTCACCTTTTCC GATTACTACATGACCTGGGTCAG ACAGGCCCTGGAAAGGGGTTG GAATGGGTGGCATTCTCCGGA ATAGAGCCCGCGGATACACTTCC GACCACAACCCAGCGTGAAGG GGCGGTTACCATAGCCGCGA CAACGCCAAGAACTCCCTCTACC TCCAAATGAACAGCCTGCGGGC GGAGGATACCGCTGTGTACTACT GCGCCCGCGACCGGCCGTCTTA CTATGTGCTGGACTACTGGGGC CAGGGTACTACGGTCACCGTCT CCTCA (SEQ ID NO: 242)

10

Table 4 shows the examples of CDR sequences of the second antibody variable domain, which is specific to CD3.

Table 4

- 58 -

Heavy Chain			
mAb	CDRH1	CDRH2	CDRH3
h2B4_H NPS	SDYYMT (SEQ ID NO: 244) (Kabat); GFTFSD (SEQ ID NO: 110) (Chothia); GFTFSDYYMT (SEQ ID NO: 245) (Extended)	FIRNRARGYTSDH (SEQ ID NO: 246) (Kabat) FIRNRARGYTSDHNPSVKG (SEQ ID NO: 247) (Extended)	DRPSYYVLDY (SEQ ID NO: 248)
Light Chain			
mAb	CDRH1	CDRH2	CDRH3
h2B4- 1d_T24 K	KSSQSLFNVRSRKN YLA (SEQ ID NO: 249)	WASTRES (SEQ ID NO: 250)	KQSYDLFT (SEQ ID NO: 251)

In some embodiments, a bispecific antibody provided herein which contains a CD3-specific variable domain contains an anti-CD3 sequence as provided in U.S. Publication No. 20160297885, which is hereby incorporated by reference for all purposes.

According to one approach to making bispecific antibodies, antibody variable domains with the desired binding specificities (antibody-antigen combining sites) are fused to immunoglobulin constant region sequences. The fusion preferably is with an immunoglobulin heavy chain constant region, comprising at least part of the hinge, CH2 and CH3 regions. It is preferred to have the first heavy chain constant region (CH1), containing the site necessary for light chain binding, present in at least one of the fusions. DNAs encoding the immunoglobulin heavy chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are cotransfected into a suitable host organism. This provides for great flexibility in adjusting the mutual proportions of the three polypeptide fragments in embodiments when unequal ratios of the three polypeptide chains used in the construction provide the optimum yields. It is, however, possible to insert the coding sequences for two or all three polypeptide chains in one expression vector when the expression of at least two

- 59 -

polypeptide chains in equal ratios results in high yields or when the ratios are of no particular significance.

In another approach, the bispecific antibodies are composed of a hybrid immunoglobulin heavy chain with a first binding specificity in one arm, and a hybrid
5 immunoglobulin heavy chain-light chain pair (providing a second binding specificity) in the other arm. This asymmetric structure, with an immunoglobulin light chain in only one half of the bispecific molecule, facilitates the separation of the desired bispecific compound from unwanted immunoglobulin chain combinations. This approach is described in PCT Publication No. WO 94/04690.

10 In another approach, the bispecific antibodies are composed of amino acid modification in the first hinge region in one arm, and the substituted/replaced amino acid in the first hinge region has an opposite charge to the corresponding amino acid in the second hinge region in another arm. This approach is described in International Patent Application No. PCT/US2011/036419 (WO2011/143545).

15 In another approach, the formation of a desired heteromultimeric or heterodimeric protein (e.g., bispecific antibody) is enhanced by altering or engineering an interface between a first and a second immunoglobulin-like Fc region (e.g., a hinge region and/or a CH3 region). In this approach, the bispecific antibodies may be composed of a CH3 region, wherein the CH3 region comprises a first CH3 polypeptide
20 and a second CH3 polypeptide which interact together to form a CH3 interface, wherein one or more amino acids within the CH3 interface destabilize homodimer formation and are not electrostatically unfavorable to homodimer formation. This approach is described in International Patent Application No. PCT/US2011/036419 (WO2011/143545).

25 In another approach, the bispecific antibodies can be generated using a glutamine-containing peptide tag engineered to the antibody directed to an epitope (e.g., EGFRvIII) in one arm and another peptide tag (e.g., a Lys-containing peptide tag or a reactive endogenous Lys) engineered to a second antibody directed to a second epitope in another arm in the presence of transglutaminase. This approach is
30 described in International Patent Application No. PCT/IB2011/054899 (WO2012/059882).

In some embodiments, the heterodimeric protein (e.g., bispecific antibody) as described herein comprises a full-length human antibody, wherein a first antibody

- 60 -

variable domain of the bispecific antibody specifically binding to a target antigen (e.g., EGFRvIII), and comprising a second antibody variable domain of the bispecific antibody capable of recruiting the activity of a human immune effector cell by specifically binding to an effector antigen (e.g., CD3) located on the human immune effector cell, wherein the first and second antibody variable domain of the heterodimeric protein comprise amino acid modifications at positions 223, 225, and 228 (e.g., (C223E or C223R), (E225R), and (P228E or P228R)) in the hinge region and at position 409 or 368 (e.g., K409R or L368E (EU numbering scheme)) in the CH3 region of human IgG2 (SEQ ID NO: 290).

In some embodiments, the first and second antibody variable domains of the heterodimeric protein comprise amino acid modifications at positions 221 and 228 (e.g., (D221R or D221E) and (P228R or P228E)) in the hinge region and at position 409 or 368 (e.g., K409R or L368E (EU numbering scheme)) in the CH3 region of human IgG1 (SEQ ID NO: 291).

In some embodiments, the first and second antibody variable domains of the heterodimeric protein comprise amino acid modifications at positions 228 (e.g., (P228E or P228R)) in the hinge region and at position 409 or 368 (e.g., R409 or L368E (EU numbering scheme)) in the CH3 region of human IgG4 (SEQ ID NO: 292).

The amino acid sequence of the wild type Fc regions of human IgG1, IgG2, and IgG4 are listed below:

IgG2 (SEQ ID NO: 290)

ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ
SSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTKVERKCCVECPPCPAPPVA
GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPRE
EQFNSTFRVSVSLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTL
PPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPMLDSDGSFFLYS
KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

IgG1 (SEQ ID NO: 291)

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
QSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAP
ELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK
PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV
YTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFL
YSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

IgG4 (SEQ ID NO: 292)

- 61 -

ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ
 SSGLYSLSSVVTVPSSSLGKTYTCNVDPKPSNTKVDKRVESKYGPPCPPCPAPEFLG
 GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPRE
 EQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTL
 5 PPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYS
 RLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLGLGK

The antibodies useful in the present invention can encompass monoclonal
 antibodies, polyclonal antibodies, antibody fragments (e.g., Fab, Fab', F(ab')₂, Fv, Fc,
 10 etc.), chimeric antibodies, bispecific antibodies, heteroconjugate antibodies, single
 chain (ScFv), mutants thereof, fusion proteins comprising an antibody portion (e.g., a
 domain antibody), humanized antibodies, and any other modified configuration of the
 immunoglobulin molecule that comprises an antigen recognition site of the required
 specificity, including glycosylation variants of antibodies, amino acid sequence variants
 15 of antibodies, and covalently modified antibodies. The antibodies may be murine, rat,
 human, or any other origin (including chimeric or humanized antibodies).

In some embodiments, the EGFRvIII antibody as described herein is a
 monoclonal antibody. For example, the EGFRvIII antibody is a humanized monoclonal
 antibody or a chimeric monoclonal antibody.

20 In some embodiments, the antibody comprises a modified constant region, such
 as, for example without limitation, a constant region that has increased potential for
 provoking an immune response. For example, the constant region may be modified to
 have increased affinity to an Fc gamma receptor such as, e.g., FcγRI, FcγRIIA, or
 FcγRIII.

25 In some embodiments, the antibody comprises a modified constant region, such
 as a constant region that is immunologically inert, that is, having a reduced potential for
 provoking an immune response. In some embodiments, the constant region is modified
 as described in Eur. J. Immunol., 29:2613-2624, 1999; PCT Application No.
 PCT/GB99/01441; and/or UK Patent Application No. 98099518. The Fc can be human
 30 IgG1, human IgG2, human IgG3, or human IgG4. The Fc can be human IgG2
 containing the mutation A330P331 to S330S331 (IgG2Δa), in which the amino acid
 residues are numbered with reference to the wild type IgG2 sequence. Eur. J.
 Immunol., 29:2613-2624, 1999. In some embodiments, the antibody comprises a
 constant region of IgG₄ comprising the following mutations (Armour et al., Molecular
 35 Immunology 40 585-593, 2003): E233F234L235 to P233V234A235 (IgG4Δc), in which

- 62 -

the numbering is with reference to wild type IgG4. In yet another embodiment, the Fc is human IgG4 E233F234L235 to P233V234A235 with deletion G236 (IgG4Δb). In another embodiment, the Fc is any human IgG4 Fc (IgG4, IgG4Δb or IgG4Δc) containing hinge stabilizing mutation S228 to P228 (Aalberse et al., Immunology 105, 9-19, 2002). In another embodiment, the Fc can be aglycosylated Fc.

In some embodiments, the constant region is aglycosylated by mutating the oligosaccharide attachment residue (such as Asn297) and/or flanking residues that are part of the glycosylation recognition sequence in the constant region. In some embodiments, the constant region is aglycosylated for N-linked glycosylation enzymatically. The constant region may be aglycosylated for N-linked glycosylation enzymatically or by expression in a glycosylation deficient host cell.

In some embodiments, the constant region has a modified constant region that removes or reduces Fc gamma receptor binding. For example, the Fc can be human IgG2 containing the mutation D265, in which the amino acid residues are numbered with reference to the wild type IgG2 sequence (SEQ ID NO: 290). Accordingly, in some embodiments, the constant region has a modified constant region having the sequence shown in SEQ ID NO: 252:

ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ
SSGLYSLSVVTVPSNFGTQTYTCNVDHKPSNTKVDKTVRKCRVRCPRCPAPPVA
GPSVFLFPPKPKDTLMISRTPEVTCVVAVSHEDPEVQFNWYVDGVEVHNAKTKPRE
EQFNSTFRVSVLTVVHQDWLNGKEYKCKVSNKGLPSSIEKTISKTKGQPREPQVYTL
PPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPMLDSDGSFFFLYS
RLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK.

The nucleic acid encoding the sequence shown in SEQ ID NO: 252 is shown in SEQ ID NO: 253:

GCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCGCCCTGCTCCAGGAGCACC
TCCGAGAGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCG
GTGACGGTGTCTGTGGAACCTCAGGCGCTCTGACCAGCGGCGTGACACCTTCCCG
GCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTAGTGACCGTGCCCT
CCAGCAACTTCGGCACCCAGACCTACACCTGCAACGTAGATCACAAGCCCAGCAA
CACCAAGGTGGACAAGACAGTTGAGCGCAAATGTCGTGTCAGGTGCCCAAGGTG
CCCAGCACCACTGTGGCAGGACCGTCAGTCTTCTTCCCCCCAAAACCCAAG

- 63 -

GACACCCTCATGATCTCCCGGACCCCTGAGGTCACGTGCGTGGTGGTGGCCGTG
 AGCCACGAAGACCCCGAGGTCCAGTTCAACTGGTACGTGGACGGCGTGGAGGTG
 CATAATGCCAAGACAAAGCCACGGGAGGAGCAGTTCAACAGCACGTTCCGTGTGG
 TCAGCGTCCTCACCGTCGTGCACCAGGACTGGCTGAACGGCAAGGAGTACAAGT
 5 GCAAGGTCTCCAACAAAGGCCTCCCATCCTCCATCGAGAAAACCATCTCCAAAAC
 CAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGA
 GATGACCAAGAACCAGGTGAGCCTGACCTGCCTGGTCAAAGGCTTCTACCCAGC
 GACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACACTACAAGACC
 ACACCTCCCATGCTGGACTCCGACGGCTCCTTCTTCTCTACAGCAGGCTCACCG
 10 TGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGA
 GGCTCTGCACAACCACTACACACAGAAGAGCCTCTCCCTGTCTCCGGGTAAA.

In some embodiments, the constant region has a modified constant region
 having the sequence shown in SEQ ID NO: 254:

15 ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ
 SSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVRKCEVECPECPAPPVA
 GPSVFLFPPKPKDTLMISRTPEVTCVAVSHEDPEVQFNWYVDGVEVHNAKTKPRE
 EQFNSTFRVSVLTVVHQDWLNGKEYKCKVSNKGLPSSIEKTKGQPREPQVYTL
 PPSREEMTKNQVSLTCEVKGFYPSDIAVEWESNGQPENNYKTPPMLDSDGSFFLYS
 20 KLTVDKSRWQQGNVFCSSVMHEALHNHYTQKSLSLSPGK.

The nucleic acid encoding the sequence shown in SEQ ID NO: 254 is shown in
 SEQ ID NO: 255:

GCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCGCCCTGCTCCAGGAGCACC
 TCCGAGAGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCG
 25 GTGACGGTGTCTGTGAACTCAGGCGCTCTGACCAGCGGCGTGCACACCTTCCCG
 GCTGTCTTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTAGTGACCGTGCCCT
 CCAGCAACTTCGGCACCCAGACCTACACCTGCAACGTAGATCACAAGCCCAGCAA
 CACCAAGGTGGACAAGACAGTTGAGCGCAAATGTGAGGTCGAGTGCCCAGAGTG
 CCCAGCACCACTGTGGCAGGACCGTCAGTCTTCTCTTCCCCCAAAACCCAAG
 30 GACACCCTCATGATCTCCCGGACCCCTGAGGTCACGTGCGTGGTGGTGGCCGTG
 AGCCACGAAGACCCCGAGGTCCAGTTCAACTGGTACGTGGACGGCGTGGAGGTG
 CATAATGCCAAGACAAAGCCACGGGAGGAGCAGTTCAACAGCACGTTCCGTGTGG
 TCAGCGTCCTCACCGTCGTGCACCAGGACTGGCTGAACGGCAAGGAGTACAAGT

- 64 -

GCAAGGTCTCCAACAAAGGCCTCCCATCCTCCATCGAGAAAACCATCTCCAAAAC
 CAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGA
 GATGACCAAGAACCAGGTCAGCCTGACCTGCGAGGTCAAAGGCTTCTACCCCAGC
 GACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACACTACAAGACC
 5 ACACCTCCCATGCTGGACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTCACCG
 TGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGA
 GGCTCTGCACAACCACTACACACAGAAGAGCCTCTCCCTGTCTCCGGGTAAA.

The amino acid of the human Kappa constant region is shown in SEQ ID NO:

256:

10 GTVAAPSVFIFPPSDEQLKSGTASVVCLLNFPYAPREKVQWKVDNALQSGNSQESVT
 EQDSKDSTYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC. And the
 nucleic acid encoding the sequence of SEQ ID NO: 256 is shown in SEQ ID NO: 257:
 GGAAGTGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAA
 ATCTGGAAGTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCA
 15 AAGTACAGTGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGT
 CACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCTGACGCT
 GAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAG
 GGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTAG.

One way of determining binding affinity of antibodies to EGFRvIII is by
 20 measuring binding affinity of the bivalent antibody to monomeric EGFRvIII protein. The
 affinity of an EGFRvIII antibody can be determined by surface plasmon resonance
 (Biacore™ 3000™ surface plasmon resonance (SPR) system, Biacore™, INC,
 Piscataway NJ) equipped with pre-immobilized anti-mouse Fc or anti-human Fc using
 HBS-EP running buffer (0.01M HEPES, pH 7.4, 0.15 NaCl, 3 mM EDTA, 0.005% v/v
 25 Surfactant P20). Monomeric 8-histidine tagged human EGFRvIII extracellular domain
 can be diluted into HBS-EP buffer to a concentration of less than 0.5 µg/mL and
 injected across the individual chip channels using variable contact times, to achieve two
 ranges of antigen density, either 50-200 response units (RU) for detailed kinetic studies
 or 800-1,000 RU for screening assays. Regeneration studies have shown that 25 mM
 30 NaOH in 25% v/v ethanol effectively removes the bound EGFRvIII protein while keeping
 the activity of EGFRvIII antibodies on the chip for over 200 injections. Typically, serial
 dilutions (spanning concentrations of 0.1-10x estimated K_D) of purified 8-histidine
 tagged EGFRvIII samples are injected for 1 min at 100 µL/minute and dissociation

- 65 -

times of up to 2 hours are allowed. The concentrations of the EGFRvIII proteins are determined by absorbance at 280nm based on sequence specific extinction coefficient of the 8-histidine tagged EGFRvIII protein. Kinetic association rates (k_{on} or k_a) and dissociation rates (k_{off} or k_d) are obtained simultaneously by fitting the data globally to a 1:1 Langmuir binding model (Karlsson, R. Roos, H. Fagerstam, L. Petersson, B. (1994). Methods Enzymology 6. 99-110) using the BIAevaluation program. Equilibrium dissociation constant (K_D) values are calculated as k_{off}/k_{on} . This protocol is suitable for use in determining binding affinity of an antibody to any monomeric EGFRvIII, including human EGFRvIII, EGFRvIII of another mammal (such as mouse EGFRvIII, rat EGFRvIII, or primate EGFRvIII), as well as different forms of EGFRvIII (e.g., glycosylated EGFRvIII). Binding affinity of an antibody is generally measured at 25°C, but can also be measured at 37°C.

The antibodies as described herein may be made by any method known in the art. For the production of hybridoma cell lines, the route and schedule of immunization of the host animal are generally in keeping with established and conventional techniques for antibody stimulation and production, as further described herein. General techniques for production of human and mouse antibodies are known in the art and/or are described herein.

It is contemplated that any mammalian subject including humans or antibody producing cells therefrom can be manipulated to serve as the basis for production of mammalian, including human and hybridoma cell lines. Typically, the host animal is inoculated intraperitoneally, intramuscularly, orally, subcutaneously, intraplantar, and/or intradermally with an amount of immunogen, including as described herein.

Hybridomas can be prepared from the lymphocytes and immortalized myeloma cells using the general somatic cell hybridization technique of Kohler, B. and Milstein, C., Nature 256:495-497, 1975 or as modified by Buck, D. W., et al., In Vitro, 18:377-381, 1982. Available myeloma lines, including but not limited to X63-Ag8.653 and those from the Salk Institute, Cell Distribution Center, San Diego, Calif., USA, may be used in the hybridization. Generally, the technique involves fusing myeloma cells and lymphoid cells using a fusogen such as polyethylene glycol, or by electrical means well known to those skilled in the art. After the fusion, the cells are separated from the fusion medium and grown in a selective growth medium, such as hypoxanthine-aminopterin-thymidine (HAT) medium, to eliminate unhybridized parent cells. Any of

- 66 -

the media described herein, supplemented with or without serum, can be used for culturing hybridomas that secrete monoclonal antibodies. As another alternative to the cell fusion technique, EBV immortalized B cells may be used to produce the monoclonal antibodies of the subject invention. The hybridomas are expanded and subcloned, if
5 desired, and supernatants are assayed for anti-immunogen activity by conventional immunoassay procedures (e.g., radioimmunoassay, enzyme immunoassay, or fluorescence immunoassay).

Hybridomas that may be used as source of antibodies encompass all derivatives, progeny cells of the parent hybridomas that produce monoclonal antibodies specific for
10 EGFRvIII, or portions thereof.

Hybridomas that produce such antibodies may be grown *in vitro* or *in vivo* using known procedures. The monoclonal antibodies may be isolated from the culture media or body fluids, by conventional immunoglobulin purification procedures such as ammonium sulfate precipitation, gel electrophoresis, dialysis, chromatography, and
15 ultrafiltration, if desired. Undesired activity, if present, can be removed, for example, by running the preparation over adsorbents made of the immunogen attached to a solid phase and eluting or releasing the desired antibodies off the immunogen. Immunization of a host animal with cells expressing human EGFRvIII, a human EGFRvIII protein, or a fragment containing the target amino acid sequence conjugated to a protein that is
20 immunogenic in the species to be immunized, e.g., keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, or soybean trypsin inhibitor using a bifunctional or derivatizing agent, for example, maleimidobenzoyl sulfosuccinimide ester (conjugation through cysteine residues), N-hydroxysuccinimide (through lysine residues), glutaraldehyde, succinic anhydride, SOCl_2 , or $\text{R}^1\text{N}=\text{C}=\text{NR}$, where R and R^1 are different
25 alkyl groups, can yield a population of antibodies (e.g., monoclonal antibodies).

If desired, the antibody (monoclonal or polyclonal) of interest may be sequenced and the polynucleotide sequence may then be cloned into a vector for expression or propagation. The sequence encoding the antibody of interest may be maintained in vector in a host cell and the host cell can then be expanded and frozen for future use.
30 Production of recombinant monoclonal antibodies in cell culture can be carried out through cloning of antibody genes from B cells by means known in the art. See, e.g. Tiller et al., J. Immunol. Methods 329, 112, 2008; U.S. Pat. No. 7,314,622.

- 67 -

In an alternative, the polynucleotide sequence may be used for genetic manipulation to "humanize" the antibody or to improve the affinity, or other characteristics of the antibody. For example, the constant region may be engineered to more nearly resemble human constant regions to avoid immune response if the antibody is used in clinical trials and treatments in humans. It may be desirable to genetically manipulate the antibody sequence to obtain greater affinity to EGFRvIII and greater efficacy in inhibiting EGFRvIII.

There are four general steps to humanize a monoclonal antibody. These are: (1) determining the nucleotide and predicted amino acid sequence of the starting antibody light and heavy variable domains (2) designing the humanized antibody, i.e., deciding which antibody framework region to use during the humanizing process (3) the actual humanizing methodologies/techniques and (4) the transfection and expression of the humanized antibody. See, for example, U.S. Pat. Nos. 4,816,567; 5,807,715; 5,866,692; 6,331,415; 5,530,101; 5,693,761; 5,693,762; 5,585,089; and 6,180,370.

A number of "humanized" antibody molecules comprising an antigen binding site derived from a non-human immunoglobulin have been described, including chimeric antibodies having rodent or modified rodent V regions and their associated CDRs fused to human constant regions. See, for example, Winter et al. Nature 349:293-299, 1991, Lobuglio et al. Proc. Nat. Acad. Sci. USA 86:4220-4224, 1989, Shaw et al. J Immunol. 138:4534-4538, 1987, and Brown et al. Cancer Res. 47:3577-3583, 1987. Other references describe rodent CDRs grafted into a human supporting framework region (FR) prior to fusion with an appropriate human antibody constant region. See, for example, Riechmann et al. Nature 332:323-327, 1988, Verhoeven et al. Science 239:1534-1536, 1988, and Jones et al. Nature 321:522-525, 1986. Another reference describes rodent CDRs supported by recombinantly engineered rodent framework regions. See, for example, European Patent Publication No. 0519596. These "humanized" molecules are designed to minimize unwanted immunological response toward rodent anti-human antibody molecules which limits the duration and effectiveness of therapeutic applications of those moieties in human recipients. For example, the antibody constant region can be engineered such that it is immunologically inert (e.g., does not trigger complement lysis). See, e.g. PCT Publication No. PCT/GB99/01441; UK Patent Application No. 9809951.8. Other methods of humanizing antibodies that may also be utilized are disclosed by Daugherty

- 68 -

et al., Nucl. Acids Res. 19:2471-2476, 1991, and in U.S. Pat. Nos. 6,180,377; 6,054,297; 5,997,867; 5,866,692; 6,210,671; and 6,350,861; and in PCT Publication No. WO 01/27160.

5 The general principles related to humanized antibodies discussed above are also applicable to customizing antibodies for use, for example, in dogs, cats, primate, equines and bovines. Further, one or more aspects of humanizing an antibody described herein may be combined, e.g., CDR grafting, framework mutation and CDR mutation.

10 In one variation, fully human antibodies may be obtained by using commercially available mice that have been engineered to express specific human immunoglobulin proteins. Transgenic animals that are designed to produce a more desirable (e.g., fully human antibodies) or more robust immune response may also be used for generation of humanized or human antibodies. Examples of such technology are Xenomouse™ from Abgenix, Inc. (Fremont, CA) and HuMAb-Mouse® and TC Mouse™ from Medarex, 15 Inc. (Princeton, NJ).

In an alternative, antibodies may be made recombinantly and expressed using any method known in the art. In another alternative, antibodies may be made recombinantly by phage display technology. See, for example, U.S. Pat. Nos. 5,565,332; 5,580,717; 5,733,743; and 6,265,150; and Winter et al., Annu. Rev. 20 Immunol. 12:433-455, 1994. Alternatively, the phage display technology (McCafferty et al., Nature 348:552-553, 1990) can be used to produce human antibodies and antibody fragments *in vitro*, from immunoglobulin variable (V) domain gene repertoires from unimmunized donors. According to this technique, antibody V domain genes are cloned in-frame into either a major or minor coat protein gene of a filamentous 25 bacteriophage, such as M13 or fd, and displayed as functional antibody fragments on the surface of the phage particle. Because the filamentous particle contains a single-stranded DNA copy of the phage genome, selections based on the functional properties of the antibody also result in selection of the gene encoding the antibody exhibiting those properties. Thus, the phage mimics some of the properties of the B cell. Phage 30 display can be performed in a variety of formats; for review see, e.g., Johnson, Kevin S. and Chiswell, David J., Current Opinion in Structural Biology 3:564-571, 1993. Several sources of V-gene segments can be used for phage display. Clackson et al., Nature 352:624-628, 1991, isolated a diverse array of anti-oxazolone antibodies from a small

- 69 -

random combinatorial library of V genes derived from the spleens of immunized mice. A repertoire of V genes from unimmunized human donors can be constructed and antibodies to a diverse array of antigens (including self-antigens) can be isolated essentially following the techniques described by Mark et al., J. Mol. Biol. 222:581-597, 1991, or Griffith et al., EMBO J. 12:725-734, 1993. In a natural immune response, antibody genes accumulate mutations at a high rate (somatic hypermutation). Some of the changes introduced will confer higher affinity, and B cells displaying high-affinity surface immunoglobulin are preferentially replicated and differentiated during subsequent antigen challenge. This natural process can be mimicked by employing the technique known as "chain shuffling." (Marks et al., Bio/Technol. 10:779-783, 1992). In this method, the affinity of "primary" human antibodies obtained by phage display can be improved by sequentially replacing the heavy and light chain V region genes with repertoires of naturally occurring variants (repertoires) of V domain genes obtained from unimmunized donors. This technique allows the production of antibodies and antibody fragments with affinities in the pM-nM range. A strategy for making very large phage antibody repertoires (also known as "the mother-of-all libraries") has been described by Waterhouse et al., Nucl. Acids Res. 21:2265-2266, 1993. Gene shuffling can also be used to derive human antibodies from rodent antibodies, where the human antibody has similar affinities and specificities to the starting rodent antibody. According to this method, which is also referred to as "epitope imprinting", the heavy or light chain V domain gene of rodent antibodies obtained by phage display technique is replaced with a repertoire of human V domain genes, creating rodent-human chimeras. Selection on antigen results in isolation of human variable regions capable of restoring a functional antigen binding site, i.e., the epitope governs (imprints) the choice of partner. When the process is repeated in order to replace the remaining rodent V domain, a human antibody is obtained (see PCT Publication No. WO 93/06213). Unlike traditional humanization of rodent antibodies by CDR grafting, this technique provides completely human antibodies, which have no framework or CDR residues of rodent origin.

Antibodies may be made recombinantly by first isolating the antibodies and antibody producing cells from host animals, obtaining the gene sequence, and using the gene sequence to express the antibody recombinantly in host cells (e.g., CHO cells). Another method which may be employed is to express the antibody sequence in plants (e.g., tobacco) or transgenic milk. Methods for expressing antibodies recombinantly in

- 70 -

plants or milk have been disclosed. See, for example, Peeters, et al. Vaccine 19:2756, 2001; Lonberg, N. and D. Huszar Int. Rev. Immunol 13:65, 1995; and Pollock, et al., J Immunol Methods 231:147, 1999. Methods for making derivatives of antibodies, e.g., humanized, single chain, etc. are known in the art.

5 Immunoassays and flow cytometry sorting techniques such as fluorescence activated cell sorting (FACS) can also be employed to isolate antibodies that are specific for EGFRvIII, or tumor antigens of interest.

 The antibodies as described herein can be bound to many different carriers. Carriers can be active and/or inert. Examples of well-known carriers include
10 polypropylene, polystyrene, polyethylene, dextran, nylon, amylases, glass, natural and modified celluloses, polyacrylamides, agaroses, and magnetite. The nature of the carrier can be either soluble or insoluble for purposes of the invention. Those skilled in the art will know of other suitable carriers for binding antibodies, or will be able to ascertain such, using routine experimentation. In some embodiments, the carrier
15 comprises a moiety that targets the myocardium.

 DNA encoding the monoclonal antibodies is readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the monoclonal antibodies). The hybridoma cells serve as a preferred source of such DNA. Once
20 isolated, the DNA may be placed into expression vectors (such as expression vectors disclosed in PCT Publication No. WO 87/04462), which are then transfected into host cells such as E. coli cells, simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. See, e.g., PCT
25 Publication No. WO 87/04462. The DNA also may be modified, for example, by substituting the coding sequence for human heavy and light chain constant regions in place of the homologous murine sequences, Morrison et al., Proc. Nat. Acad. Sci. 81:6851, 1984, or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide. In that manner,
30 “chimeric” or “hybrid” antibodies are prepared that have the binding specificity of a monoclonal antibody herein.

 The EGFRvIII antibodies as described herein can be identified or characterized using methods known in the art, whereby reduction of EGFRvIII expression levels are

- 71 -

detected and/or measured. In some embodiments, an EGFRvIII antibody is identified by incubating a candidate agent with EGFRvIII and monitoring binding and/or attendant reduction of EGFRvIII expression levels. The binding assay may be performed with purified EGFRvIII polypeptide(s), or with cells naturally expressing, or transfected to
5 express, EGFRvIII polypeptide(s). In one embodiment, the binding assay is a competitive binding assay, where the ability of a candidate antibody to compete with a known EGFRvIII antibody for EGFRvIII binding is evaluated. The assay may be performed in various formats, including the ELISA format.

Following initial identification, the activity of a candidate EGFRvIII antibody can
10 be further confirmed and refined by bioassays, known to test the targeted biological activities. Alternatively, bioassays can be used to screen candidates directly. Some of the methods for identifying and characterizing antibodies are described in detail in the Examples.

EGFRvIII antibodies may be characterized using methods well known in the art.
15 For example, one method is to identify the epitope to which it binds, or "epitope mapping." There are many methods known in the art for mapping and characterizing the location of epitopes on proteins, including solving the crystal structure of an antibody-antigen complex, competition assays, gene fragment expression assays, and synthetic peptide-based assays, as described, for example, in Chapter 11 of Harlow
20 and Lane, Using Antibodies, a Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1999. In an additional example, epitope mapping can be used to determine the sequence to which an antibody binds. Epitope mapping is commercially available from various sources, for example, Pepscan Systems (Edelhertweg 15, 8219 PH Lelystad, The Netherlands). The epitope can be a
25 linear epitope, i.e., contained in a single stretch of amino acids, or a conformational epitope formed by a three-dimensional interaction of amino acids that may not necessarily be contained in a single stretch. Peptides of varying lengths (e.g., at least 4-6 amino acids long) can be isolated or synthesized (e.g., recombinantly) and used for binding assays with an EGFRvIII or other tumor antigen antibody. In another example,
30 the epitope to which the EGFRvIII antibody binds can be determined in a systematic screening by using overlapping peptides derived from the EGFRvIII sequence and determining binding by the EGFRvIII antibody. According to the gene fragment expression assays, the open reading frame encoding EGFRvIII is fragmented either

- 72 -

randomly or by specific genetic constructions and the reactivity of the expressed fragments of EGFRvIII with the antibody to be tested is determined. The gene fragments may, for example, be produced by PCR and then transcribed and translated into protein in vitro, in the presence of radioactive amino acids. The binding of the antibody to the radioactively labeled EGFRvIII is then determined by immunoprecipitation and gel electrophoresis. Certain epitopes can also be identified by using large libraries of random peptide sequences displayed on the surface of phage particles (phage libraries). Alternatively, a defined library of overlapping peptide fragments can be tested for binding to the test antibody in simple binding assays. In an additional example, mutagenesis of an antigen binding domain, domain swapping experiments and alanine scanning mutagenesis can be performed to identify residues required, sufficient, and/or necessary for epitope binding. For example, domain swapping experiments can be performed using a mutant EGFRvIII in which various fragments of the EGFRvIII protein have been replaced (swapped) with sequences from EGFRvIII from another species (e.g., mouse), or a closely related, but antigenically distinct protein (e.g., Trop-1). By assessing binding of the antibody to the mutant EGFRvIII, the importance of the particular EGFRvIII fragment to antibody binding can be assessed. In the case of EGFRvIII specific antibody (i.e. antibody that does not bind EGFRwt (wild type) or any other proteins), epitope can be deduced from the sequence alignment of EGFRvIII to EGFRwt.

Yet another method which can be used to characterize an EGFRvIII antibody is to use competition assays with other antibodies known to bind to the same antigen, i.e., various fragments on EGFRvIII, to determine if the EGFRvIII antibody binds to the same epitope as other antibodies. Competition assays are well known to those of skill in the art.

An expression vector can be used to direct expression of an EGFRvIII antibody. One skilled in the art is familiar with administration of expression vectors to obtain expression of an exogenous protein in vivo. See, e.g., U.S. Pat. Nos. 6,436,908; 6,413,942; and 6,376,471. Administration of expression vectors includes local or systemic administration, including injection, oral administration, particle gun or catheterized administration, and topical administration. In another embodiment, the expression vector is administered directly to the sympathetic trunk or ganglion, or into a coronary artery, atrium, ventricle, or pericardium.

- 73 -

Targeted delivery of therapeutic compositions containing an expression vector, or subgenomic polynucleotides can also be used. Receptor-mediated DNA delivery techniques are described in, for example, Findeis et al., Trends Biotechnol., 1993, 11:202; Chiou et al., Gene Therapeutics: Methods And Applications Of Direct Gene Transfer, J.A. Wolff, ed., 1994; Wu et al., J. Biol. Chem., 263:621, 1988; Wu et al., J. Biol. Chem., 269:542, 1994; Zenke et al., Proc. Natl. Acad. Sci. USA, 87:3655, 1990; and Wu et al., J. Biol. Chem., 266:338, 1991. Therapeutic compositions containing a polynucleotide are administered in a range of about 100 ng to about 200 mg of DNA for local administration in a gene therapy protocol. Concentration ranges of about 500 ng to about 50 mg, about 1 µg to about 2 mg, about 5 µg to about 500 µg, and about 20 µg to about 100 µg of DNA can also be used during a gene therapy protocol. The therapeutic polynucleotides and polypeptides can be delivered using gene delivery vehicles. The gene delivery vehicle can be of viral or non-viral origin (see generally, Jolly, Cancer Gene Therapy, 1:51, 1994; Kimura, Human Gene Therapy, 5:845, 1994; Connelly, Human Gene Therapy, 1995, 1:185; and Kaplitt, Nature Genetics, 6:148, 1994). Expression of such coding sequences can be induced using endogenous mammalian or heterologous promoters. Expression of the coding sequence can be either constitutive or regulated.

Viral-based vectors for delivery of a desired polynucleotide and expression in a desired cell are well known in the art. Exemplary viral-based vehicles include, but are not limited to, recombinant retroviruses (see, e.g., PCT Publication Nos. WO 90/07936; WO 94/03622; WO 93/25698; WO 93/25234; WO 93/11230; WO 93/10218; WO 91/02805; U.S. Pat. Nos. 5, 219,740 and 4,777,127; GB Pat. No. 2,200,651; and EP Pat. No. 0 345 242), alphavirus-based vectors (e.g., Sindbis virus vectors, Semliki forest virus (ATCC VR-67; ATCC VR-1247), Ross River virus (ATCC VR-373; ATCC VR-1246) and Venezuelan equine encephalitis virus (ATCC VR-923; ATCC VR-1250; ATCC VR 1249; ATCC VR-532)), and adeno-associated virus (AAV) vectors (see, e.g., PCT Publication Nos. WO 94/12649, WO 93/03769; WO 93/19191; WO 94/28938; WO 95/11984 and WO 95/00655). Administration of DNA linked to killed adenovirus as described in Curiel, Hum. Gene Ther., 1992, 3:147 can also be employed.

Non-viral delivery vehicles and methods can also be employed, including, but not limited to, polycationic condensed DNA linked or unlinked to killed adenovirus alone (see, e.g., Curiel, Hum. Gene Ther., 3:147, 1992); ligand-linked DNA (see, e.g., Wu, J.

- 74 -

Biol. Chem., 264:16985, 1989); eukaryotic cell delivery vehicles cells (see, e.g., U.S. Pat. No. 5,814,482; PCT Publication Nos. WO 95/07994; WO 96/17072; WO 95/30763; and WO 97/42338) and nucleic charge neutralization or fusion with cell membranes. Naked DNA can also be employed. Exemplary naked DNA introduction methods are described in PCT Publication No. WO 90/11092 and U.S. Pat. No. 5,580,859. Liposomes that can act as gene delivery vehicles are described in U.S. Pat. No. 5,422,120; PCT Publication Nos. WO 95/13796; WO 94/23697; WO 91/14445; and EP 0524968. Additional approaches are described in Philip, Mol. Cell Biol., 14:2411, 1994 and in Woffendin, Proc. Natl. Acad. Sci., 91:1581, 1994.

In some embodiments, the invention encompasses compositions, including pharmaceutical compositions, comprising antibodies described herein or made by the methods and having the characteristics described herein. As used herein, compositions comprise one or more antibodies that bind to EGFRvIII, and/or one or more polynucleotides comprising sequences encoding one or more these antibodies. These compositions may further comprise suitable excipients, such as pharmaceutically acceptable excipients including buffers, which are well known in the art.

The invention also provides methods of making any of these antibodies. The antibodies of this invention can be made by procedures known in the art. The polypeptides can be produced by proteolytic or other degradation of the antibodies, by recombinant methods (i.e., single or fusion polypeptides) as described above or by chemical synthesis. Polypeptides of the antibodies, especially shorter polypeptides up to about 50 amino acids, are conveniently made by chemical synthesis. Methods of chemical synthesis are known in the art and are commercially available. For example, an antibody could be produced by an automated polypeptide synthesizer employing the solid phase method. See also, U.S. Pat. Nos. 5,807,715; 4,816,567; and 6,331,415.

In another alternative, the antibodies can be made recombinantly using procedures that are well known in the art. In one embodiment, a polynucleotide comprises a sequence encoding the heavy chain and/or the light chain variable regions of antibody m62G7, h62G7, h62G7-H14/L1-DV, h62G7-EQ/L6, 42G9, 32A10, 20B9, 14C11, 21E11, 49B11, 46E10, 12H6, 19A9, 21E7, 11B11, 12B2, 11F10, 17G11, 29D5, 30D8, 20E12, 26B9, 32G8, 34E7, 20G5, C6, B5, 42G9-1, 32A10-1, 20B9-1, 14C11-1, 21E11-1, 49B11-1, 46E10-1, 12H6-1, 19A9-1, 21E7-1, 11B11-1, 12B2-1, 11F10-1, 17G11-1, 29D5-1, 30D8-1, 20E12-1, 26B9-1, 32G8-1, 34E7-1, 20G5-1, C6-1, or B5-1.

- 75 -

The sequence encoding the antibody of interest may be maintained in a vector in a host cell and the host cell can then be expanded and frozen for future use. Vectors (including expression vectors) and host cells are further described herein.

Heteroconjugate antibodies, comprising two covalently joined antibodies, are also within the scope of the invention. Such antibodies have been used to target immune system cells to unwanted cells (U.S. Pat. No. 4,676,980), and for treatment of HIV infection (PCT Publication Nos. WO 91/00360 and WO 92/200373; EP 03089). Heteroconjugate antibodies may be made using any convenient cross-linking methods. Suitable cross-linking agents and techniques are well known in the art, and are described in U.S. Pat. No. 4,676,980.

Chimeric or hybrid antibodies also may be prepared *in vitro* using known methods of synthetic protein chemistry, including those involving cross-linking agents. For example, immunotoxins may be constructed using a disulfide exchange reaction or by forming a thioether bond. Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptobutyrimidate.

In the recombinant humanized antibodies, the Fc γ portion can be modified to avoid interaction with Fc γ receptor and the complement and immune systems. The techniques for preparation of such antibodies are described in WO 99/58572. For example, the constant region may be engineered to more resemble human constant regions to avoid immune response if the antibody is used in clinical trials and treatments in humans. See, for example, U.S. Pat. Nos. 5,997,867 and 5,866,692.

The invention encompasses modifications to the antibodies and polypeptides of the invention including variants shown in Table 5, including functionally equivalent antibodies which do not significantly affect their properties and variants which have enhanced or decreased activity and/or affinity. For example, the amino acid sequence may be mutated to obtain an antibody with the desired binding affinity to EGFRvIII. Modification of polypeptides is routine practice in the art and need not be described in detail herein. Examples of modified polypeptides include polypeptides with conservative substitutions of amino acid residues, one or more deletions or additions of amino acids which do not significantly deleteriously change the functional activity, or which mature (enhance) the affinity of the polypeptide for its ligand, or use of chemical analogs.

- 76 -

Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl
 5 residue or the antibody fused to an epitope tag. Other insertional variants of the antibody molecule include the fusion to the N- or C-terminus of the antibody of an enzyme or a polypeptide which increases the half-life of the antibody in the blood circulation.

Substitution variants have at least one amino acid residue in the antibody
 10 molecule removed and a different residue inserted in its place. The sites of greatest interest for substitutional mutagenesis include the hypervariable regions, but FR alterations are also contemplated. Conservative substitutions are shown in Table 5 under the heading of "conservative substitutions." If such substitutions result in a change in biological activity, then more substantial changes, denominated "exemplary
 15 substitutions" in Table 5, or as further described below in reference to amino acid classes, may be introduced and the products screened. In some embodiments, substitution variants of antibodies provided herein have no more than 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 conservative substitution in the VH or VL region as compared to the reference parent antibody. In some embodiments, the substitutions
 20 are not within a CDR of the VH or VL region.

Table 5: Amino Acid Substitutions

Original Residue (naturally occurring amino acid)	Conservative Substitutions	Exemplary Substitutions
Ala (A)	Val	Val; Leu; Ile
Arg (R)	Lys	Lys; Gln; Asn
Asn (N)	Gln	Gln; His; Asp, Lys; Arg
Asp (D)	Glu	Glu; Asn
Cys (C)	Ser	Ser; Ala
Gln (Q)	Asn	Asn; Glu
Glu (E)	Asp	Asp; Gln
Gly (G)	Ala	Ala
His (H)	Arg	Asn; Gln; Lys; Arg

- 77 -

Original Residue (naturally occurring amino acid)	Conservative Substitutions	Exemplary Substitutions
Ile (I)	Leu	Leu; Val; Met; Ala; Phe; Norleucine
Leu (L)	Ile	Norleucine; Ile; Val; Met; Ala; Phe
Lys (K)	Arg	Arg; Gln; Asn
Met (M)	Leu	Leu; Phe; Ile
Phe (F)	Tyr	Leu; Val; Ile; Ala; Tyr
Pro (P)	Ala	Ala
Ser (S)	Thr	Thr
Thr (T)	Ser	Ser
Trp (W)	Tyr	Tyr; Phe
Tyr (Y)	Phe	Trp; Phe; Thr; Ser
Val (V)	Leu	Ile; Leu; Met; Phe; Ala; Norleucine

Substantial modifications in the biological properties of the antibody are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Naturally occurring amino acid residues are divided into groups based on common side-chain properties:

- (1) Non-polar: Norleucine, Met, Ala, Val, Leu, Ile;
- (2) Polar without charge: Cys, Ser, Thr, Asn, Gln;
- (3) Acidic (negatively charged): Asp, Glu;
- (4) Basic (positively charged): Lys, Arg;
- (5) Residues that influence chain orientation: Gly, Pro; and
- (6) Aromatic: Trp, Tyr, Phe, His.

Non-conservative substitutions are made by exchanging a member of one of these classes for another class.

Any cysteine residue not involved in maintaining the proper conformation of the antibody also may be substituted, generally with serine, to improve the oxidative stability of the molecule and prevent aberrant cross-linking. Conversely, cysteine

- 78 -

bond(s) may be added to the antibody to improve its stability, particularly where the antibody is an antibody fragment such as an Fv fragment.

Amino acid modifications can range from changing or modifying one or more amino acids to complete redesign of a region, such as the variable region. Changes in the variable region can alter binding affinity and/or specificity. In some embodiments, no more than one to five conservative amino acid substitutions are made within a CDR domain. In other embodiments, no more than one to three conservative amino acid substitutions are made within a CDR domain. In still other embodiments, the CDR domain is CDR H3 and/or CDR L3.

Modifications also include glycosylated and nonglycosylated polypeptides, as well as polypeptides with other post-translational modifications, such as, for example, glycosylation with different sugars, acetylation, and phosphorylation. Antibodies are glycosylated at conserved positions in their constant regions (Jefferis and Lund, Chem. Immunol. 65:111-128, 1997; Wright and Morrison, TibTECH 15:26-32, 1997). The oligosaccharide side chains of the immunoglobulins affect the protein's function (Boyd et al., Mol. Immunol. 32:1311-1318, 1996; Wittwe and Howard, Biochem. 29:4175-4180, 1990) and the intramolecular interaction between portions of the glycoprotein, which can affect the conformation and presented three-dimensional surface of the glycoprotein (Jefferis and Lund, supra; Wyss and Wagner, Current Opin. Biotech. 7:409-416, 1996). Oligosaccharides may also serve to target a given glycoprotein to certain molecules based upon specific recognition structures. Glycosylation of antibodies has also been reported to affect antibody-dependent cellular cytotoxicity (ADCC). In particular, CHO cells with tetracycline-regulated expression of $\beta(1,4)$ -N-acetylglucosaminyltransferase III (GnTIII), a glycosyltransferase catalyzing formation of bisecting GlcNAc, was reported to have improved ADCC activity (Umana et al., Mature Biotech. 17:176-180, 1999).

Glycosylation of antibodies is typically either N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. The tripeptide sequences asparagine-X-serine, asparagine-X-threonine, and asparagine-X-cysteine, where X is any amino acid except proline, are the recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain. Thus, the presence of either of these tripeptide sequences in a polypeptide creates a potential glycosylation site. O-linked glycosylation refers to the attachment of

- 79 -

one of the sugars N-acetylgalactosamine, galactose, or xylose to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used.

5 Addition of glycosylation sites to the antibody is conveniently accomplished by altering the amino acid sequence such that it contains one or more of the above-described tripeptide sequences (for N-linked glycosylation sites). The alteration may also be made by the addition of, or substitution by, one or more serine or threonine residues to the sequence of the original antibody (for O-linked glycosylation sites).

10 The glycosylation pattern of antibodies may also be altered without altering the underlying nucleotide sequence. Glycosylation largely depends on the host cell used to express the antibody. Since the cell type used for expression of recombinant glycoproteins, e.g. antibodies, as potential therapeutics is rarely the native cell, variations in the glycosylation pattern of the antibodies can be expected (see, e.g. Hse et al., J. Biol. Chem. 272:9062-9070, 1997).

15 In addition to the choice of host cells, factors that affect glycosylation during recombinant production of antibodies include growth mode, media formulation, culture density, oxygenation, pH, purification schemes and the like. Various methods have been proposed to alter the glycosylation pattern achieved in a particular host organism including introducing or overexpressing certain enzymes involved in oligosaccharide production (U.S. Pat. Nos. 5,047,335; 5,510,261 and 5,278,299). Glycosylation, or
20 certain types of glycosylation, can be enzymatically removed from the glycoprotein, for example, using endoglycosidase H (Endo H), N-glycosidase F, endoglycosidase F1, endoglycosidase F2, endoglycosidase F3. In addition, the recombinant host cell can be genetically engineered to be defective in processing certain types of polysaccharides.
25 These and similar techniques are well known in the art.

Other methods of modification include using coupling techniques known in the art, including, but not limited to, enzymatic means, oxidative substitution and chelation. Modifications can be used, for example, for attachment of labels for immunoassay. Modified polypeptides are made using established procedures in the art and can be
30 screened using standard assays known in the art, some of which are described below and in the Examples.

Other antibody modifications include antibodies that have been modified as described in PCT Publication No. WO 99/58572. These antibodies comprise, in

- 80 -

addition to a binding domain directed at the target molecule, an effector domain having an amino acid sequence substantially homologous to all or part of a constant region of a human immunoglobulin heavy chain. These antibodies are capable of binding the target molecule without triggering significant complement dependent lysis, or cell-mediated destruction of the target. In some embodiments, the effector domain is capable of specifically binding FcRn and/or FcγRIIb. These are typically based on chimeric domains derived from two or more human immunoglobulin heavy chain C_H2 domains. Antibodies modified in this manner are particularly suitable for use in chronic antibody therapy, to avoid inflammatory and other adverse reactions to conventional antibody therapy.

The invention includes affinity matured embodiments. For example, affinity matured antibodies can be produced by procedures known in the art (Marks et al., *Bio/Technology*, 10:779-783, 1992; Barbas et al., *Proc Nat. Acad. Sci, USA* 91:3809-3813, 1994; Schier et al., *Gene*, 169:147-155, 1995; Yelton et al., *J. Immunol.*, 155:1994-2004, 1995; Jackson et al., *J. Immunol.*, 154(7):3310-9, 1995, Hawkins et al., *J. Mol. Biol.*, 226:889-896, 1992; and PCT Publication No. WO2004/058184).

The following methods may be used for adjusting the affinity of an antibody and for characterizing a CDR. One way of characterizing a CDR of an antibody and/or altering (such as improving) the binding affinity of a polypeptide, such as an antibody, termed "library scanning mutagenesis". Generally, library scanning mutagenesis works as follows. One or more amino acid positions in the CDR are replaced with two or more (such as 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20) amino acids using art recognized methods. This generates small libraries of clones (in some embodiments, one for every amino acid position that is analyzed), each with a complexity of two or more members (if two or more amino acids are substituted at every position). Generally, the library also includes a clone comprising the native (unsubstituted) amino acid. A small number of clones, e.g., about 20-80 clones (depending on the complexity of the library), from each library are screened for binding affinity to the target polypeptide (or other binding target), and candidates with increased, the same, decreased, or no binding are identified. Methods for determining binding affinity are well-known in the art. Binding affinity may be determined using Biacore™ surface plasmon resonance analysis, which detects differences in binding affinity of about 2-fold or greater. Biacore™ is particularly useful when the starting

- 81 -

antibody already binds with a relatively high affinity, for example a K_D of about 10 nM or lower. Screening using Biacore™ surface plasmon resonance is described in the Examples, herein.

Binding affinity may be determined using Kinexa Biocensor, scintillation proximity
5 assays, ELISA, ORIGEN immunoassay (IGEN), fluorescence quenching, fluorescence transfer, and/or yeast display. Binding affinity may also be screened using a suitable bioassay.

In some embodiments, every amino acid position in a CDR is replaced (in some
embodiments, one at a time) with all 20 natural amino acids using art recognized
10 mutagenesis methods (some of which are described herein). This generates small libraries of clones (in some embodiments, one for every amino acid position that is analyzed), each with a complexity of 20 members (if all 20 amino acids are substituted at every position).

In some embodiments, the library to be screened comprises substitutions in two
15 or more positions, which may be in the same CDR or in two or more CDRs. Thus, the library may comprise substitutions in two or more positions in one CDR. The library may comprise substitution in two or more positions in two or more CDRs. The library may comprise substitution in 3, 4, 5, or more positions, said positions found in two, three, four, five or six CDRs. The substitution may be prepared using low redundancy
20 codons. See, e.g., Table 2 of Balint et al., Gene 137(1):109-18, 1993.

The CDR may be CDRH3 and/or CDRL3. The CDR may be one or more of CDRL1, CDRL2, CDRL3, CDRH1, CDRH2, and/or CDRH3. The CDR may be a Kabat CDR, a Chothia CDR, or an extended CDR.

Candidates with improved binding may be sequenced, thereby identifying a CDR
25 substitution mutant which results in improved affinity (also termed an "improved" substitution). Candidates that bind may also be sequenced, thereby identifying a CDR substitution which retains binding.

Multiple rounds of screening may be conducted. For example, candidates (each
comprising an amino acid substitution at one or more position of one or more CDR) with
30 improved binding are also useful for the design of a second library containing at least the original and substituted amino acid at each improved CDR position (i.e., amino acid position in the CDR at which a substitution mutant showed improved binding). Preparation, and screening or selection of this library is discussed further below.

- 82 -

Library scanning mutagenesis also provides a means for characterizing a CDR, in so far as the frequency of clones with improved binding, the same binding, decreased binding or no binding also provide information relating to the importance of each amino acid position for the stability of the antibody-antigen complex. For example, if a position of the CDR retains binding when changed to all 20 amino acids, that position is identified as a position that is unlikely to be required for antigen binding. Conversely, if a position of CDR retains binding in only a small percentage of substitutions, that position is identified as a position that is important to CDR function. Thus, the library scanning mutagenesis methods generate information regarding positions in the CDRs that can be changed to many different amino acids (including all 20 amino acids), and positions in the CDRs which cannot be changed or which can only be changed to a few amino acids.

Candidates with improved affinity may be combined in a second library, which includes the improved amino acid, the original amino acid at that position, and may further include additional substitutions at that position, depending on the complexity of the library that is desired, or permitted using the desired screening or selection method. In addition, if desired, adjacent amino acid position can be randomized to at least two or more amino acids. Randomization of adjacent amino acids may permit additional conformational flexibility in the mutant CDR, which may in turn, permit or facilitate the introduction of a larger number of improving mutations. The library may also comprise substitution at positions that did not show improved affinity in the first round of screening.

The second library is screened or selected for library members with improved and/or altered binding affinity using any method known in the art, including screening using Biacore™ surface plasmon resonance analysis, and selection using any method known in the art for selection, including phage display, yeast display, and ribosome display.

The invention also encompasses fusion proteins comprising one or more fragments or regions from the antibodies of this invention. In one embodiment, a fusion polypeptide is provided that comprises at least 10 contiguous amino acids of the variable light chain region shown in SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 29, 31, 33, 36, 38, 40, 42, 45, 47, 49, 51, 211, 212, 213, or 215, and/or at least 10 amino acids of the variable heavy chain region shown in SEQ ID NOs: 1, 3, 5,

- 83 -

7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 30, 32, 34, 35, 37, 39, 41, 43, 44, 46, 48, 50, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 202, 203, 204, 205, 206, 207, 208, 209, 210, 214, 216, 217, or 218. In other embodiments, a fusion polypeptide is provided that comprises at least about 10, at least about 15, at least about 20, at least about 25, or at least about 30 contiguous amino acids of the variable light chain region and/or at least about 10, at least about 15, at least about 20, at least about 25, or at least about 30 contiguous amino acids of the variable heavy chain region. In another embodiment, the fusion polypeptide comprises one or more CDR(s). In still other embodiments, the fusion polypeptide comprises CDR H3 (VH CDR3) and/or CDR L3 (VL CDR3). For purposes of this invention, a fusion protein contains one or more antibodies and another amino acid sequence to which it is not attached in the native molecule, for example, a heterologous sequence or a homologous sequence from another region. Exemplary heterologous sequences include, but are not limited to a "tag" such as a FLAG tag or a 6His tag. Tags are well known in the art.

A fusion polypeptide can be created by methods known in the art, for example, synthetically or recombinantly. Typically, the fusion proteins of this invention are made by preparing an expressing a polynucleotide encoding them using recombinant methods described herein, although they may also be prepared by other means known in the art, including, for example, chemical synthesis.

This invention also provides compositions comprising antibodies conjugated (for example, linked) to an agent that facilitate coupling to a solid support (such as biotin or avidin). For simplicity, reference will be made generally to antibodies with the understanding that these methods apply to any of the EGFRvIII antibody embodiments described herein. Conjugation generally refers to linking these components as described herein. The linking (which is generally fixing these components in proximate association at least for administration) can be achieved in any number of ways. For example, a direct reaction between an agent and an antibody is possible when each possesses a substituent capable of reacting with the other. For example, a nucleophilic group, such as an amino or sulfhydryl group, on one may be capable of reacting with a carbonyl-containing group, such as an anhydride or an acid halide, or with an alkyl group containing a good leaving group (e.g., a halide) on the other.

The invention also provides isolated polynucleotides encoding the antibodies of the invention, and vectors and host cells comprising the polynucleotide.

- 84 -

Accordingly, the invention provides polynucleotides (or compositions, including pharmaceutical compositions), comprising polynucleotides encoding any of the following: m62G7, h62G7, h62G7-H14/L1-DV, h62G7-EQ/L6, 42G9, 32A10, 20B9, 14C11, 21E11, 49B11, 46E10, 12H6, 19A9, 21E7, 11B11, 12B2, 11F10, 17G11, 29D5, 30D8, 20E12, 26B9, 32G8, 34E7, 20G5, C6, B5, 42G9-1, 32A10-1, 20B9-1, 14C11-1, 21E11-1, 49B11-1, 46E10-1, 12H6-1, 19A9-1, 21E7-1, 11B11-1, 12B2-1, 11F10-1, 17G11-1, 29D5-1, 30D8-1, 20E12-1, 26B9-1, 32G8-1, 34E7-1, 20G5-1, C6-1, and B5-1, or any fragment or part thereof having the ability to bind EGFRvIII.

In another aspect, the invention provides polynucleotides encoding any of the antibodies (including antibody fragments) and polypeptides described herein, such as antibodies and polypeptides having impaired effector function. Polynucleotides can be made and expressed by procedures known in the art.

In another aspect, the invention provides compositions (such as a pharmaceutical compositions) comprising any of the polynucleotides of the invention. In some embodiments, the composition comprises an expression vector comprising a polynucleotide encoding any of the antibodies described herein.

Expression vectors, and administration of polynucleotide compositions are further described herein.

In another aspect, the invention provides a method of making any of the polynucleotides described herein.

Polynucleotides complementary to any such sequences are also encompassed by the present invention. Polynucleotides may be single-stranded (coding or antisense) or double-stranded, and may be DNA (genomic, cDNA or synthetic) or RNA molecules. RNA molecules include HnRNA molecules, which contain introns and correspond to a DNA molecule in a one-to-one manner, and mRNA molecules, which do not contain introns. Additional coding or non-coding sequences may, but need not, be present within a polynucleotide of the present invention, and a polynucleotide may, but need not, be linked to other molecules and/or support materials.

Polynucleotides may comprise a native sequence (i.e., an endogenous sequence that encodes an antibody or a portion thereof) or may comprise a variant of such a sequence. Polynucleotide variants contain one or more substitutions, additions, deletions and/or insertions such that the immunoreactivity of the encoded polypeptide is not diminished, relative to a native immunoreactive molecule. The effect on the

- 85 -

immunoreactivity of the encoded polypeptide may generally be assessed as described herein. Variants preferably exhibit at least about 70% identity, more preferably, at least about 80% identity, yet more preferably, at least about 90% identity, and most preferably, at least about 95% identity to a polynucleotide sequence that encodes a native antibody or a portion thereof.

Two polynucleotide or polypeptide sequences are said to be "identical" if the sequence of nucleotides or amino acids in the two sequences is the same when aligned for maximum correspondence as described below. Comparisons between two sequences are typically performed by comparing the sequences over a comparison window to identify and compare local regions of sequence similarity. A "comparison window" as used herein, refers to a segment of at least about 20 contiguous positions, usually 30 to about 75, or 40 to about 50, in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned.

Optimal alignment of sequences for comparison may be conducted using the Megalign program in the Lasergene suite of bioinformatics software (DNASTAR, Inc., Madison, WI), using default parameters. This program embodies several alignment schemes described in the following references: Dayhoff, M.O., 1978, A model of evolutionary change in proteins - Matrices for detecting distant relationships. In Dayhoff, M.O. (ed.) Atlas of Protein Sequence and Structure, National Biomedical Research Foundation, Washington DC Vol. 5, Suppl. 3, pp. 345-358; Hein J., 1990, Unified Approach to Alignment and Phylogenesis pp. 626-645 Methods in Enzymology vol. 183, Academic Press, Inc., San Diego, CA; Higgins, D.G. and Sharp, P.M., 1989, CABIOS 5:151-153; Myers, E.W. and Muller W., 1988, CABIOS 4:11-17; Robinson, E.D., 1971, Comb. Theor. 11:105; Santou, N., Nes, M., 1987, Mol. Biol. Evol. 4:406-425; Sneath, P.H.A. and Sokal, R.R., 1973, Numerical Taxonomy the Principles and Practice of Numerical Taxonomy, Freeman Press, San Francisco, CA; Wilbur, W.J. and Lipman, D.J., 1983, Proc. Natl. Acad. Sci. USA 80:726-730.

Preferably, the "percentage of sequence identity" is determined by comparing two optimally aligned sequences over a window of comparison of at least 20 positions, wherein the portion of the polynucleotide or polypeptide sequence in the comparison window may comprise additions or deletions (i.e., gaps) of 20 percent or less, usually 5 to 15 percent, or 10 to 12 percent, as compared to the reference sequences (which

- 86 -

does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid bases or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the reference sequence (i.e. the window size) and multiplying the results by 100 to yield the percentage of sequence identity.

Variants may also, or alternatively, be substantially homologous to a native gene, or a portion or complement thereof. Such polynucleotide variants are capable of hybridizing under moderately stringent conditions to a naturally occurring DNA sequence encoding a native antibody (or a complementary sequence).

Suitable "moderately stringent conditions" include prewashing in a solution of 5 X SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0); hybridizing at 50°C-65°C, 5 X SSC, overnight; followed by washing twice at 65°C for 20 minutes with each of 2X, 0.5X and 0.2X SSC containing 0.1 % SDS.

As used herein, "highly stringent conditions" or "high stringency conditions" are those that: (1) employ low ionic strength and high temperature for washing, for example 0.015 M sodium chloride/0.0015 M sodium citrate/0.1% sodium dodecyl sulfate at 50°C; (2) employ during hybridization a denaturing agent, such as formamide, for example, 50% (v/v) formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50 mM sodium phosphate buffer at pH 6.5 with 750 mM sodium chloride, 75 mM sodium citrate at 42°C; or (3) employ 50% formamide, 5 x SSC (0.75 M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50 µg/ml), 0.1% SDS, and 10% dextran sulfate at 42°C, with washes at 42°C in 0.2 x SSC (sodium chloride/sodium citrate) and 50% formamide at 55°C, followed by a high-stringency wash consisting of 0.1 x SSC containing EDTA at 55°C. The skilled artisan will recognize how to adjust the temperature, ionic strength, etc. as necessary to accommodate factors such as probe length and the like.

It will be appreciated by those of ordinary skill in the art that, as a result of the degeneracy of the genetic code, there are many nucleotide sequences that encode a polypeptide as described herein. Some of these polynucleotides bear minimal homology to the nucleotide sequence of any native gene. Nonetheless, polynucleotides that vary due to differences in codon usage are specifically contemplated by the present

- 87 -

invention. Further, alleles of the genes comprising the polynucleotide sequences provided herein are within the scope of the present invention. Alleles are endogenous genes that are altered as a result of one or more mutations, such as deletions, additions and/or substitutions of nucleotides. The resulting mRNA and protein may, but need not, have an altered structure or function. Alleles may be identified using standard techniques (such as hybridization, amplification and/or database sequence comparison).

The polynucleotides of this invention can be obtained using chemical synthesis, recombinant methods, or PCR. Methods of chemical polynucleotide synthesis are well known in the art and need not be described in detail herein. One of skill in the art can use the sequences provided herein and a commercial DNA synthesizer to produce a desired DNA sequence.

For preparing polynucleotides using recombinant methods, a polynucleotide comprising a desired sequence can be inserted into a suitable vector, and the vector in turn can be introduced into a suitable host cell for replication and amplification, as further discussed herein. Polynucleotides may be inserted into host cells by any means known in the art. Cells are transformed by introducing an exogenous polynucleotide by direct uptake, endocytosis, transfection, F-mating or electroporation. Once introduced, the exogenous polynucleotide can be maintained within the cell as a non-integrated vector (such as a plasmid) or integrated into the host cell genome. The polynucleotide so amplified can be isolated from the host cell by methods well known within the art. See, e.g., Sambrook et al., 1989.

Alternatively, PCR allows reproduction of DNA sequences. PCR technology is well known in the art and is described in U.S. Patent Nos. 4,683,195, 4,800,159, 4,754,065 and 4,683,202, as well as PCR: The Polymerase Chain Reaction, Mullis et al. eds., Birkhäuser Press, Boston, 1994.

RNA can be obtained by using the isolated DNA in an appropriate vector and inserting it into a suitable host cell. When the cell replicates and the DNA is transcribed into RNA, the RNA can then be isolated using methods well known to those of skill in the art, as set forth in Sambrook et al., 1989, supra, for example.

Suitable cloning vectors may be constructed according to standard techniques, or may be selected from a large number of cloning vectors available in the art. While the cloning vector selected may vary according to the host cell intended to be used,

- 88 -

useful cloning vectors will generally have the ability to self-replicate, may possess a single target for a particular restriction endonuclease, and/or may carry genes for a marker that can be used in selecting clones containing the vector. Suitable examples include plasmids and bacterial viruses, e.g., pUC18, pUC19, Bluescript (e.g., pBS SK+) and its derivatives, mp18, mp19, pBR322, pMB9, ColE1, pCR1, RP4, phage DNAs, and shuttle vectors such as pSA3 and pAT28. These and many other cloning vectors are available from commercial vendors such as BioRad, Strategene, and Invitrogen.

Expression vectors generally are replicable polynucleotide constructs that contain a polynucleotide according to the invention. It is implied that an expression vector must be replicable in the host cells either as episomes or as an integral part of the chromosomal DNA. Suitable expression vectors include but are not limited to plasmids, viral vectors, including adenoviruses, adeno-associated viruses, retroviruses, cosmids, and expression vector(s) disclosed in PCT Publication No. WO 87/04462. Vector components may generally include, but are not limited to, one or more of the following: a signal sequence; an origin of replication; one or more marker genes; suitable transcriptional controlling elements (such as promoters, enhancers and terminator). For expression (i.e., translation), one or more translational controlling elements are also usually required, such as ribosome binding sites, translation initiation sites, and stop codons.

The vectors containing the polynucleotides of interest can be introduced into the host cell by any of a number of appropriate means, including electroporation, transfection employing calcium chloride, rubidium chloride, calcium phosphate, DEAE-dextran, or other substances; microprojectile bombardment; lipofection; and infection (e.g., where the vector is an infectious agent such as vaccinia virus). The choice of introducing vectors or polynucleotides will often depend on features of the host cell.

The invention also provides host cells comprising any of the polynucleotides described herein. Any host cells capable of over-expressing heterologous DNAs can be used for the purpose of isolating the genes encoding the antibody, polypeptide or protein of interest. Non-limiting examples of mammalian host cells include but not limited to COS, HeLa, and CHO cells. See also PCT Publication No. WO 87/04462. Suitable non-mammalian host cells include prokaryotes (such as *E. coli* or *B. subtilis*) and yeast (such as *S. cerevisiae*, *S. pombe*; or *K. lactis*). Preferably, the host cells express the cDNAs at a level of about 5 fold higher, more preferably, 10 fold higher,

- 89 -

even more preferably, 20 fold higher than that of the corresponding endogenous antibody or protein of interest, if present, in the host cells. Screening the host cells for a specific binding to EGFRvIII is effected by an immunoassay or FACS. A cell overexpressing the antibody or protein of interest can be identified.

5

EGFRvIII Antibody Conjugates

The present invention also provides a conjugate (or immunoconjugate) of the EGFRvIII antibody as described herein, wherein the antibody is conjugated to an agent (e.g., a cytotoxic agent) for targeted immunotherapy (e.g., antibody-drug conjugates) either directly or indirectly via a linker. For example, a cytotoxic agent can be linked or
10 conjugated to the EGFRvIII antibody as described herein for targeted local delivery of the cytotoxic agent moiety to tumors (e.g., EGFRvIII expressing tumor).

Methods for conjugating cytotoxic agent or other therapeutic agents to antibodies have been described in various publications. For example, chemical modification can
15 be made in the antibodies either through lysine side chain amines or through cysteine sulfhydryl groups activated by reducing interchain disulfide bonds for the conjugation reaction to occur. See, e.g., Tanaka et al., FEBS Letters 579:2092-2096, 2005, and Gentle et al., Bioconjugate Chem. 15:658-663, 2004. Reactive cysteine residues engineered at specific sites of antibodies for specific drug conjugation with defined
20 stoichiometry have also been described. See, e.g., Junutula et al., Nature Biotechnology, 26:925-932, 2008. Conjugation using an acyl donor glutamine-containing tag or an endogenous glutamine made reactive (i.e., the ability to form a covalent bond as an acyl donor) by polypeptide engineering in the presence of transglutaminase and an amine (e.g., a cytotoxic agent comprising or attached to a
25 reactive amine) is also described in international applications WO2012/059882 and WO2015015448 .

In some embodiments, the EGFRvIII antibody or the conjugate as described herein comprises an acyl donor glutamine-containing tag engineered at a specific site of the antibody (e.g., a carboxyl terminus, an amino terminus, or at another site in the
30 EGFRvIII antibody). In some embodiments, the tag comprises an amino acid glutamine (Q) or an amino acid sequence LQG, LLQGG (SEQ ID NO: 258), LLQG (SEQ ID NO: 259), LSLSQG (SEQ ID NO: 260), GGGLLQGG (SEQ ID NO: 261), GLLQG (SEQ ID NO: 262), LLQ, GSPLAQSHGG (SEQ ID NO: 263), GLLQGGG (SEQ ID NO: 264),

- 90 -

GLLQGG (SEQ ID NO: 265), GLLQ (SEQ ID NO: 266), LLQLLQGA (SEQ ID NO: 267), LLQGA (SEQ ID NO: 268), LLQYQGA (SEQ ID NO: 269), LLQGSG (SEQ ID NO: 270), LLQYQG (SEQ ID NO: 271), LLQLLQG (SEQ ID NO: 272), SLLQG (SEQ ID NO: 273), LLQLQ (SEQ ID NO: 274), LLQLLQ (SEQ ID NO: 275), LLQGR (SEQ ID NO: 276), LLQGPP (SEQ ID NO: 277), LLQGPA (SEQ ID NO: 278), GLLQGPP (SEQ ID NO: 279), GLLQGA (SEQ ID NO: 280), LLQGPGK (SEQ ID NO: 281), LLQGPG (SEQ ID NO: 282), LLQGP (SEQ ID NO: 283), LLQP (SEQ ID NO: 284), LLQPGK (SEQ ID NO: 285), LLQAPGK (SEQ ID NO: 286), LLQGAPG (SEQ ID NO: 287), LLQGAP (SEQ ID NO: 288), and LLQLQG (SEQ ID NO: 289).

Also provided is an isolated antibody comprising an acyl donor glutamine-containing tag and an amino acid modification at position 222, 340, or 370 of the antibody (EU numbering scheme) wherein the modification is an amino acid deletion, insertion, substitution, mutation, or any combination thereof. In some embodiments, the amino acid modification is a substitution from lysine to arginine (e.g., K222R, K340R, or K370R).

The agents that can be conjugated to the EGFRvIII antibodies of the present invention include, but are not limited to, cytotoxic agents, immunomodulating agents, imaging agents, therapeutic proteins, biopolymers, or oligonucleotides.

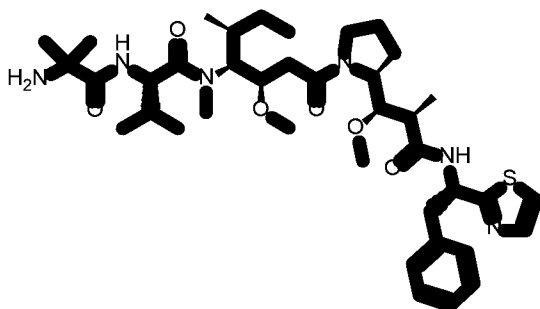
Examples of a cytotoxic agent include, but are not limited to, anthracycline, an auristatin, a dolastatin, a combretastatin, a duocarmycin, a pyrrolobenzodiazepine dimer, an indolino-benzodiazepine dimer, an enediyne, a geldanamycin, a maytansine, a puromycin, a taxane, a vinca alkaloid, a camptothecin, a tubulysin, a hemiasterlin, a spliceostatin, a pladienolide, and stereoisomers, isosteres, analogs, or derivatives thereof.

The anthracyclines are derived from bacteria *Streptomyces* and have been used to treat a wide range of cancers, such as leukemias, lymphomas, breast, uterine, ovarian, and lung cancers. Exemplary anthracyclines include, but are not limited to, daunorubicin, doxorubicin (i.e., adriamycin), epirubicin, idarubicin, valrubicin, and mitoxantrone.

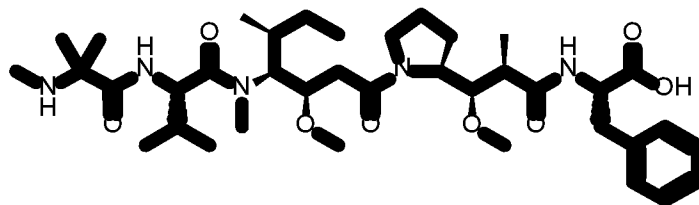
Dolastatins and their peptidic analogs and derivatives, auristatins, are highly potent antimitotic agents that have been shown to have anticancer and antifungal activity. See, e.g., U.S. Pat. No. 5,663,149 and Pettit et al., *Antimicrob. Agents Chemother.* 42:2961-2965, 1998. Exemplary dolastatins and auristatins include, but

- 91 -

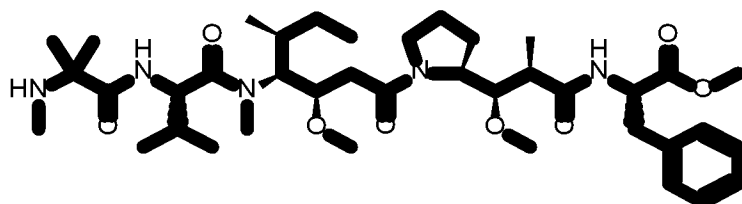
are not limited to, dolastatin 10, auristatin E, auristatin EB (AEB), auristatin EFP (AEFP), MMAD (Monomethyl Auristatin D or monomethyl dolastatin 10), MMAF (Monomethyl Auristatin F or N-methylvaline-valine-dolaisoleuine-dolaproine-phenylalanine), MMAE (Monomethyl Auristatin E or N-methylvaline-valine-dolaisoleuine-dolaproine-norephedrine), 5-benzoylvaleric acid-AE ester (AEVB), and other novel auristatins (such as the ones described in U.S. Publication No. 2013/0129753). In some embodiments, the auristatin is 0101 (2-methylalanyl-N-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-2-methyl-3-oxo-3-[(1*S*)-2-phenyl-1-(1,3-thiazol-2-yl)ethyl]amino}propyl]pyrrolidin-1-yl]-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide) having the following structure:



In some embodiments, the auristatin is 3377 (N,2-dimethylalanyl-N-[(1*S*,2*R*)-4-[(2*S*)-2-[(1*R*,2*R*)-3-[(1*S*)-1-carboxyl-2-phenylethyl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-2-methoxy-1-[(1*S*)-1-methylpropyl]-4-oxobutyl]-N-methyl-L-valinamide) having the following structure:

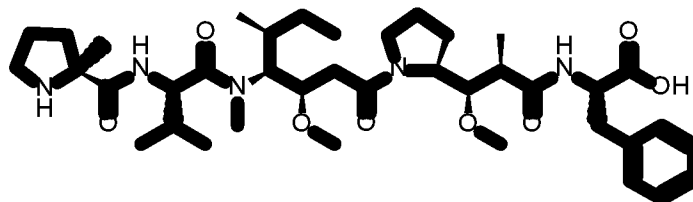


In some embodiments, the auristatin is 0131-OMe (N,2-dimethylalanyl-N-[(3*R*,4*S*,5*S*)-3-methoxy-1-{(2*S*)-2-[(1*R*,2*R*)-1-methoxy-3-[(2*S*)-1-methoxy-1-oxo-3-phenylpropan-2-yl]amino}-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide) having the following structure:

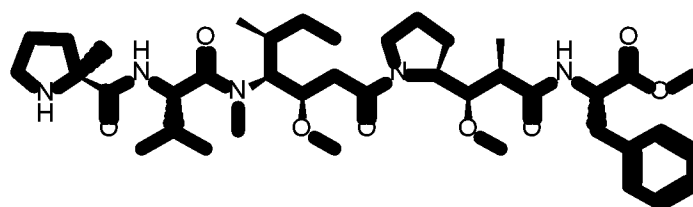


- 92 -

In other embodiments, the auristatin is 0131 (2-methyl-L-prolyl-N-[(3R,4S,5S)-1-[(2S)-2-[(1R,2R)-3-[(1S)-1-carboxy-2-phenylethyl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide) having the following structure:



In other embodiments, the auristatin is 0121 (2-methyl-L-prolyl-N-[(3R,4S,5S)-1-[(2S)-2-[(1R,2R)-3-[(2S)-1-methoxy-1-oxo-3-phenylpropan-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide) having the following structure:



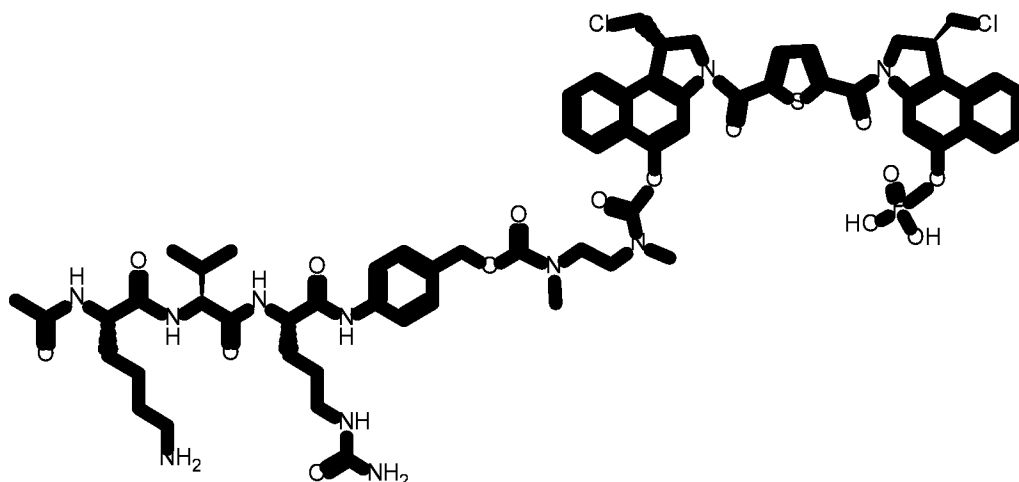
Camptothecin is a cytotoxic quinoline alkaloid which inhibits the enzyme topoisomerase I. Examples of camptothecin and its derivatives include, but are not limited to, topotecan and irinotecan, and their metabolites, such as SN-38.

Combretastatins are natural phenols with vascular disruption properties in tumors. Exemplary combretastatins and their derivatives include, but are not limited to, combretastatin A-4 (CA-4) and ombrabulin.

Duocarmycin and CC-1065 are DNA alkylating agents with cytotoxic potency. See Boger and Johnson, *PNAS* 92:3642-3649 (1995). Exemplary duocarmycin and CC-1065 include, but are not limited to, (+)-duocarmycin A and (+)-duocarmycin SA, (+)-CC-1065, and the compounds as disclosed in the international application PCT/IB2015/050280 including, but not limited to, N~2~-acetyl-L-lysyl-L-valyl-N~5~-carbamoyl-N-[4-([(2-([(1S)-1-(chloromethyl)-3-[(5-[(1S)-1-(chloromethyl)-5-(phosphonooxy)-1,2-dihydro-3H-benzo[e]indol-3-yl]carbonyl]thiophen-2-yl)carbonyl]-2,3-di

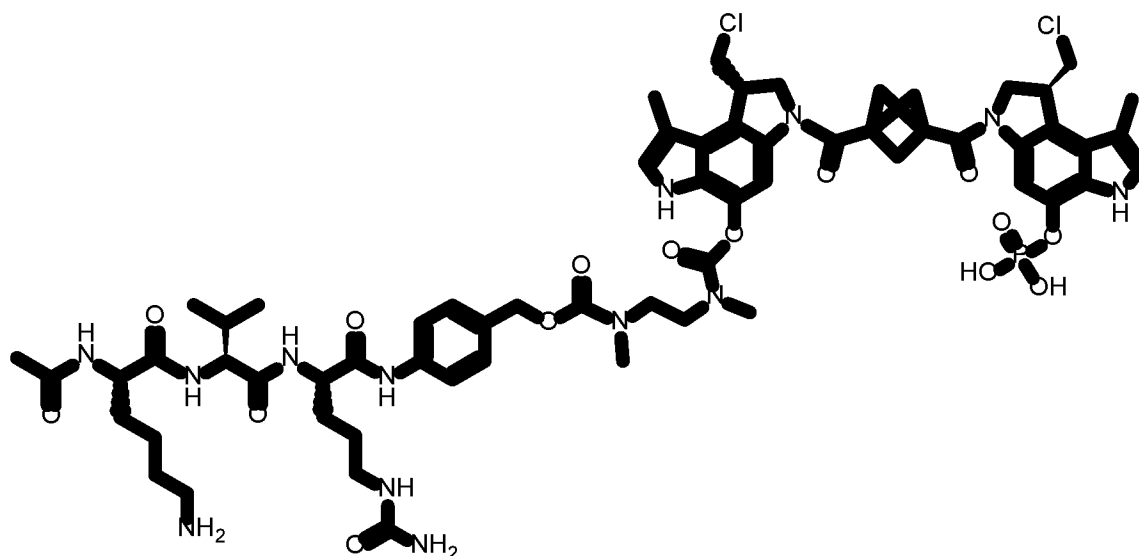
hydro-1H-benzo[e]indol-5-yl]oxy)carbonyl](methyl)amino)ethyl)(methyl)carbamoyl]oxy}methyl)phenyl]-L-ornithinamide having the structure:

- 93 -



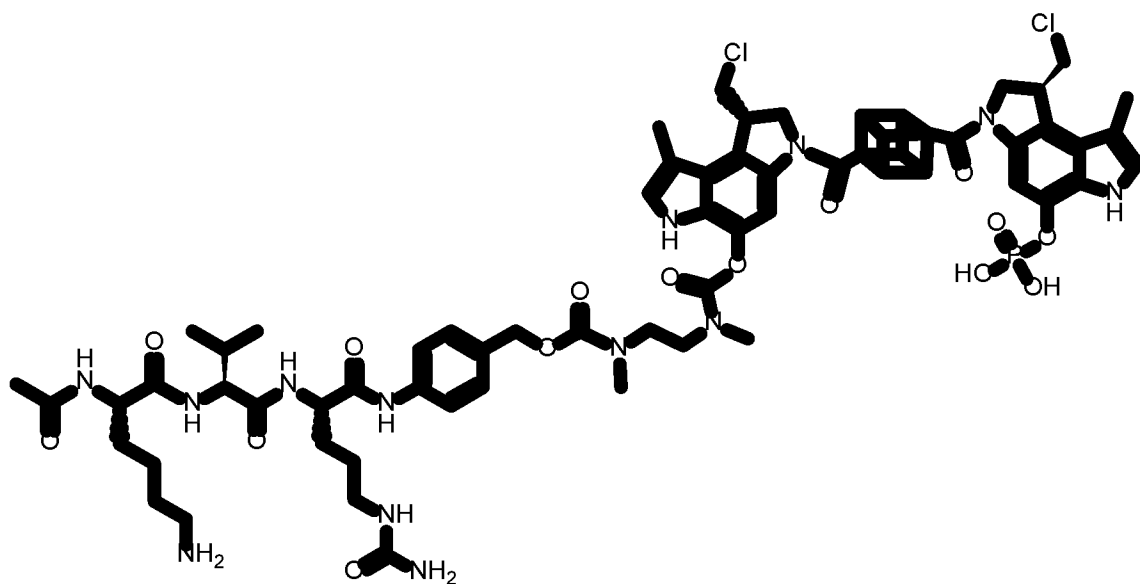
N₂-acetyl-L-lysyl-L-valyl-N₅-carbamoyl-N-[4-({[(2-({[(8S)-8-(chloromethyl)-6-[(3-
 {[(1S)-1-(chloromethyl)-8-methyl-5-(phosphonooxy)-1,6-dihydropyrrolo[3,2-e]indol-
 3(2H)-yl]carbonyl}bicyclo[1.1.1]p ent-1-yl)carbonyl]-1-methyl-3,6,7,8-

5 tetrahydropyrrolo[3,2-e]indol-4-
 yl}oxy)carbonyl](methyl)amino}ethyl)(methyl)carbamoyl]oxy}methyl)phenyl]-L-
 ornithinamide having the structure:



10 N₂-acetyl-L-lysyl-L-valyl-N₅-carbamoyl-N-[4-({[(2-({[(8S)-8-(chloromethyl)-6-[(4-
 {[(1S)-1-(chloromethyl)-8-methyl-5-(phosphonooxy)-1,6-dihydropyrrolo[3,2-e]indol-
 3(2H)-yl]carbonyl}pentacyclo[4.2.0.0~2,5~.0~3,8~.0~4,7~]oct-1-yl)carbonyl]-1-methyl-
 3,6,7,8-tetrahydropyrrolo[3,2-e]indol-4-
 yl}oxy)carbonyl](methyl)amino}ethyl)(methyl)carbamoyl]oxy}methyl)phenyl]-L-
 ornithinamide having the structure:

- 94 -



Enediynes are a class of anti-tumor bacterial products characterized by either nine- and ten-membered rings or the presence of a cyclic system of conjugated triple-
 5 double-triple bonds. Exemplary enediynes include, but are not limited to, calicheamicin, esperamicin, uncialamicin, dynemicin, and their derivatives.

Geldanamycins are benzoquinone ansamycin antibiotic that bind to Hsp90 (Heat Shock Protein 90) and have been used antitumor drugs. Exemplary geldanamycins include, but are not limited to, 17-AAG (17-N-Allylamino-17-Demethoxygeldanamycin)
 10 and 17-DMAG (17-Dimethylaminoethylamino-17-demethoxygeldanamycin).

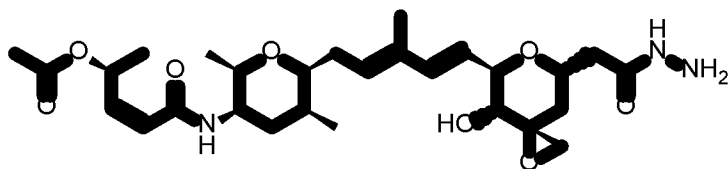
Hemiasterlin and its analogues (e.g., HTI-286) bind to the tubulin, disrupt normal microtubule dynamics, and, at stoichiometric amounts, depolymerize microtubules.

Maytansines or their derivatives maytansinoids inhibit cell proliferation by inhibiting the microtubules formation during mitosis through inhibition of polymerization
 15 of tubulin. See Remillard et al., Science 189:1002-1005, 1975. Exemplary maytansines and maytansinoids include, but are not limited to, mertansine (DM1) and its derivatives as well as ansamitocin.

Pyrrolobenzodiazepine dimers (PBDs) and indolino-benzodiazepine dimers (IGNs) are anti-tumor agents that contain one or more imine functional groups, or
 20 their equivalents, that bind to duplex DNA. PBD and IGN molecules are based on the natural product athramycin, and interact with DNA in a sequence-selective manner, with a preference for purine-guanine-purine sequences. Exemplary PBDs and their analogs include, but are not limited to, SJG-136.

- 95 -

Spliceostatins and pladienolides are anti-tumor compounds which inhibit splicing and interacts with spliceosome, SF3b. Examples of spliceostatins include, but are not limited to, spliceostatin A, FR901464, and (2S,3Z)-5-[[[(2R,3R,5S,6S)-6-[(2E,4E)-5-[(3R,4R,5R,7S)-7-(2-hydrazinyl-2-oxoethyl)-4-hydroxy-1,6-dioxaspiro[2.5]oct-5-yl]-3-methylpenta-2,4-dien-1-yl]-2,5-dimethyltetrahydro-2H-pyran-3-yl]amino}-5-oxopent-3-en-2-yl acetate having the structure of



. Examples of pladienolides

include, but are not limited to, Pladienolide B, Pladienolide D, or E7107.

Taxanes are diterpenes that act as anti-tubulin agents or mitotic inhibitors. Exemplary taxanes include, but are not limited to, paclitaxel (e.g., TAXOL®) and docetaxel (TAXOTERE®).

Tubulysins are natural products isolated from a strain of myxobacteria that has been shown to depolymerize microtubules and induce mitotic arrest. Exemplary tubulysins include, but are not limited to, tubulysin A, tubulysin B, and tubulysin D.

Vinca alkylolids are also anti-tubulin agents. Exemplary vinca alkylolids include, but are not limited to, vincristine, vinblastine, vindesine, and vinorelbine.

Accordingly, in some embodiments, the cytotoxic agent is selected from the group consisting of MMAD (Monomethyl Auristatin D), 0101 (2-methylalanyl-N-[(3R,4S,5S)-3-methoxy-1-[(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-oxo-3-[(1S)-2-phenyl-1-(1,3-thiazol-2-yl)ethyl]amino}propyl]pyrrolidin-1-yl]-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide), 3377 (N,2-dimethylalanyl-N-[(1S,2R)-4-[(2S)-2-[(1R,2R)-3-[(1S)-1-carboxyl-2-phenylethyl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-2-methoxy-1-[(1S)-1-methylpropyl]-4-oxobutyl]-N-methyl-L-valinamide), 0131 (2-methyl-L-prolyl-N-[(3R,4S,5S)-1-[(2S)-2-[(1R,2R)-3-[(1S)-1-carboxyl-2-phenylethyl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide), 0131-OMe (N,2-dimethylalanyl-N-[(3R,4S,5S)-3-methoxy-1-[(2S)-2-[(1R,2R)-1-methoxy-3-[(2S)-1-methoxy-1-oxo-3-phenylpropan-2-yl]amino}-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide), 0121 (2-methyl-L-prolyl-N-[(3R,4S,5S)-1-[(2S)-2-[(1R,2R)-3-[(2S)-1-methoxy-1-oxo-3-phenylpropan-2-yl]amino]-1-methoxy-2-methyl-3-oxopropyl]pyrrolidin-1-yl]-3-methoxy-

- 96 -

5-methyl-1-oxoheptan-4-yl]-N-methyl-L-valinamide), and (2S,3Z)-5-[(2R,3R,5S,6S)-6-[(2E,4E)-5-[(3R,4R,5R,7S)-7-(2-hydrazinyl-2-oxoethyl)-4-hydroxy-1,6-dioxaspiro[2.5]oct-5-yl]-3-methylpenta-2,4-dien-1-yl]-2,5-dimethyltetrahydro-2H-pyran-3-yl]amino}-5-oxopent-3-en-2-yl acetate.

5 In some embodiments, the agent is an immunomodulating agent. Examples of an immunomodulating agent include, but are not limited to, gancyclovir, etanercept, tacrolimus, sirolimus, voclosporin, cyclosporine, rapamycin, cyclophosphamide, azathioprine, mycophenolate mofetil, methotrexate, glucocorticoid and its analogs, cytokines, stem cell growth factors, lymphotoxins, tumor necrosis factor (TNF),
 10 hematopoietic factors, interleukins (e.g., interleukin-1 (IL-1), IL-2, IL-3, IL-6, IL-10, IL-12, IL-18, and IL-21), colony stimulating factors (e.g., granulocyte-colony stimulating factor (G-CSF) and granulocyte macrophage-colony stimulating factor (GM-CSF)), interferons (e.g., interferons- α , - β and - γ), the stem cell growth factor designated "S 1 factor," erythropoietin and thrombopoietin, or a combination thereof.

15 In some embodiments, the agent moiety is an imaging agent (e.g., a fluorophore or a chelator), such as fluorescein, rhodamine, lanthanide phosphors, and their derivatives thereof, or a radioisotope bound to a chelator. Examples of fluorophores include, but are not limited to, fluorescein isothiocyanate (FITC) (e.g., 5-FITC), fluorescein amidite (FAM) (e.g., 5-FAM), eosin, carboxyfluorescein, erythrosine, Alexa
 20 Fluor[®] (e.g., Alexa 350, 405, 430, 488, 500, 514, 532, 546, 555, 568, 594, 610, 633, 647, 660, 680, 700, or 750), carboxytetramethylrhodamine (TAMRA) (e.g., 5-TAMRA), tetramethylrhodamine (TMR), and sulforhodamine (SR) (e.g., SR101). Examples of chelators include, but are not limited to, 1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid (DOTA), 1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA), 1,4,7-
 25 triazacyclononane-1-glutaric acid-4,7-acetic acid (deferioxamine), diethylenetriaminepentaacetic acid (DTPA), and 1,2-bis(o-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid (BAPTA).

In some embodiments, therapeutic or diagnostic radioisotopes or other labels (e.g., PET or SPECT labels) can be incorporated in the agent for conjugation to the
 30 EGFRvIII antibodies as described herein. Examples of a radioisotope or other labels include, but are not limited to, ³H, ¹¹C, ¹³N, ¹⁴C, ¹⁵N, ¹⁵O, ³⁵S, ¹⁸F, ³²P, ³³P, ⁴⁷Sc, ⁵¹Cr, ⁵⁷Co, ⁵⁸Co, ⁵⁹Fe, ⁶²Cu, ⁶⁴Cu, ⁶⁷Cu, ⁶⁷Ga, ⁶⁸Ga, ⁷⁵Se, ⁷⁶Br, ⁷⁷Br, ⁸⁶Y, ⁸⁹Zr, ⁹⁰Y, ⁹⁴Tc, ⁹⁵Ru, ⁹⁷Ru, ⁹⁹Tc, ¹⁰³Ru, ¹⁰⁵Rh, ¹⁰⁵Ru, ¹⁰⁷Hg, ¹⁰⁹Pd, ¹¹¹Ag, ¹¹¹In, ¹¹³In, ¹²¹Te, ¹²²Te, ¹²³I,

- 97 -

¹²⁴I, ¹²⁵I, ¹²⁵Te, ¹²⁶I, ¹³¹I, ¹³¹In, ¹³³I, ¹⁴²Pr, ¹⁴³Pr, ¹⁵³Pb, ¹⁵³Sm, ¹⁶¹Tb, ¹⁶⁵Tm, ¹⁶⁶Dy, ¹⁶⁶H, ¹⁶⁷Tm, ¹⁶⁸Tm, ¹⁶⁹Yb, ¹⁷⁷Lu, ¹⁸⁶Re, ¹⁸⁸Re, ¹⁸⁹Re, ¹⁹⁷Pt, ¹⁹⁸Au, ¹⁹⁹Au, ²⁰¹Tl, ²⁰³Hg, ²¹¹At, ²¹²Bi, ²¹²Pb, ²¹³Bi, ²²³Ra, ²²⁴Ac, or ²²⁵Ac.

In some embodiments, the agent is a therapeutic protein including, but is not limited to, a toxin, a hormone, an enzyme, and a growth factor.

Examples of a toxin protein (or polypeptide) include, but are not limited to, diphtheria (e.g., diphtheria A chain), *Pseudomonas* exotoxin and endotoxin, ricin (e.g., ricin A chain), abrin (e.g., abrin A chain), modeccin (e.g., modeccin A chain), alpha-sarcin, Aleurites fordii proteins, dianthin proteins, ribonuclease (RNase), DNase I, *Staphylococcal* enterotoxin-A, pokeweed antiviral protein, gelonin, diphtherin toxin, *Phytolaca americana* proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, mitogellin, restrictocin, phenomycin, enomycin, tricothecenes, inhibitor cystine knot (ICK) peptides (e.g., ceratotoxins), and conotoxin (e.g., KIIIA or SmIIIA).

In some embodiments, the agent is a biocompatible polymer. The EGFRvIII antibodies as described herein can be conjugated to the biocompatible polymer to increase serum half-life and bioactivity, and/or to extend *in vivo* half-lives. Examples of biocompatible polymers include water-soluble polymer, such as polyethylene glycol (PEG) or its derivatives thereof and zwitterion-containing biocompatible polymers (e.g., a phosphorylcholine containing polymer).

In some embodiments, the agent is an oligonucleotide, such as anti-sense oligonucleotides.

In another aspect, the invention provides a conjugate of the antibody as described herein, wherein the conjugate comprises the formula: antibody-(acyl donor glutamine-containing tag)-(linker)-(cytotoxic agent).

Examples of a linker containing one or more reactive amines include, but are not limited to, Ac-Lys-Gly (acetyl-lysine-glycine), aminocaproic acid, Ac-Lys-β-Ala (acetyl-lysine-β-alanine), amino-PEG2 (polyethylene glycol)-C2, amino-PEG3-C2, amino-PEG6-C2 (or amino PEG6-propionyl), Ac-Lys-Val-Cit-PABC (acetyl-lysine-valine-citrulline-*p*-aminobenzyloxycarbonyl), amino-PEG6-C2-Val-Cit-PABC, aminocaproyl-Val-Cit-PABC, [(3R,5R)-1-{3-[2-(2-aminoethoxy)ethoxy]propanoyl}]piperidine-3,5-diyl]bis-Val-Cit-PABC, [(3S,5S)-1-{3-[2-(2-aminoethoxy)ethoxy]propanoyl}]piperidine-3,5-diyl]bis-Val-Cit-PABC, putrescine, or Ac-Lys-putrescine.

Methods of Using the EGFRvIII Antibodies and the Antibody Conjugates Thereof

The antibodies or the antibody conjugates of the present invention are useful in various applications including, but are not limited to, therapeutic treatment methods and
5 diagnostic treatment methods.

The antibodies (e.g., monospecific and bispecific) and the antibody conjugates obtained by the methods described above can be used as a medicament. In some embodiments, such a medicament can be used for treating cancer, including solid tumors and liquid tumors. In some embodiments, the cancer is EGFRvIII related cancer
10 including, but not limited to, glioblastoma (e.g., glioblastoma multiform), anaplastic astrocytoma, giant cell glioblastoma, gliosarcoma, anaplastic oligodendroglioma, anaplastic ependymoma, anaplastic oligoastrocytoma, choroid plexus carcinoma, anaplastic ganglioglioma, pineoblastoma, pineocytoma, meningioma, medulloepithelioma, ependymblastoma, medulloblastoma, supraentorial primitive
15 neuroectodermal tumor, atypical teratoid/rhabdoid tumor, mixed glioma, head and neck cancer, non-small cell lung cancer, breast cancer, ovarian cancer, prostate cancer, medulloblastoma, colorectal cancer, anal cancer, cervical cancer, renal cancer, skin cancer, pancreatic cancer, liver cancer, bladder cancer, gastric cancer, thyroid cancer, mesothelioma, uterine cancer, lymphoma, or leukemia.

In some embodiments, provided is a method of inhibiting tumor growth or progression in a subject who has malignant cells expressing EGFRvIII, comprising administering to the subject in need thereof an effective amount of a composition comprising the EGFRvIII antibodies or the EGFRvIII antibody conjugates as described
20 herein. In other embodiments, provided is a method of inhibiting metastasis of cells expressing EGFRvIII in a subject, comprising administering to the subject in need thereof an effective amount of a composition comprising the EGFRvIII antibodies or the EGFRvIII antibody conjugates as described herein. In other embodiments, provided is a method of inducing tumor regression in malignant cells in a subject, comprising administering to the subject in need thereof an effective amount of a composition
25 comprising the EGFRvIII antibodies or the EGFRvIII antibody conjugates as described herein.
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- 99 -

In some embodiments, the antibody (e.g., monospecific or bispecific) or the antibody conjugate according to the invention can be used in the manufacture of a medicament for treatment of a cancer in a patient in need thereof.

In some embodiments, the treatment can be in combination with one or more therapies against cancer selected from the group of antibodies therapy, chemotherapy, cytokines therapy, targeted therapy, vaccine therapy, dendritic cell therapy, gene therapy, hormone therapy, surgical resection, laser light therapy and radiation therapy. For example, the antibody (monospecific or bispecific) or the antibody conjugate of the invention can be administered to a patient in conjunction with (e.g., before, simultaneously, or following) 1) standard of care, including radiation, surgical resection, chemotherapy (e.g., temozolomide, procarbazine, carmustine, lomustine, vincristine etc.), antibody therapy such as bevacizumab, anti-angiogenic therapy, and/or tumor treating fields; 2) vaccine, including EGFRvIII vaccine; 3) targeted therapy, such as kinase inhibitors (e.g. everolimus); and 4) immunotherapies, including but not limited to anti-PD-1, anti-PD-L1, anti-PD-L2, anti-41BB, anti-TIM3, anti-LAG3, anti-TIGIT, anti-OX40, anti-HVEM, anti-BTLA, anti-CD40, anti-CD47, anti-CSF1R, anti-CSF1, anti-MARCO, anti-IL8, anti-CXCR4, and anti-CTLA4 antibodies.

The administration of the antibodies (e.g., monospecific or bispecific) or the antibody conjugates according to the invention may be carried out in any convenient manner, including by aerosol inhalation, injection, ingestion, transfusion, implantation or transplantation. The compositions described herein may be administered to a patient subcutaneously, intradermally, intratumorally, intracranially, intranodally, intramedullary, intramuscularly, by intravenous or intralymphatic injection, or intraperitoneally. In one embodiment, the heteromultimeric antibody compositions of the invention are preferably administered by intravenous injection.

In some embodiments, the administration of the antibodies (e.g., monospecific or bispecific) or the antibody conjugates can comprise administration of, for example, about 0.01 to about 20 mg per kg body weight including all integer values of mg per kg within those ranges. In some embodiments, the administration of the antibodies or the antibody conjugates can comprise administration of about 0.1 to 10 mg per kg body weight including all integer values of mg per kg within those ranges. The heteromultimeric antibody can be administered in one or more doses. In some embodiments, said effective amount of the antibody or the antibody conjugate can be

- 100 -

administered as a single dose. In some embodiments, said effective amount of antibodies can be administered as more than one dose over a period time. Timing of administration is within the judgment of managing physician and depends on the clinical condition of the patient. While individual needs vary, determination of optimal ranges of effective amounts of a given antibody (e.g., monospecific or bispecific) or antibody conjugate for a particular disease or conditions within the skill of the art. An effective amount means an amount which provides a therapeutic or prophylactic benefit. The dosage administered will be dependent upon the age, health and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment and the nature of the effect desired. In some embodiments, an effective amount of heteromultimeric antibody or composition comprising those antibodies are administered parenterally. In some embodiments, administration can be an intravenous administration. In some embodiments, administration can be directly done by injection within a tumor.

In some embodiments, anti-EGFRvIII antibodies provided herein may be used for diagnostic purposes, such in assays to identify EGFRvIII protein in samples (e.g. in immunohistochemistry assays) or in patients.

Compositions

In one aspect, the present invention provides a pharmaceutical composition comprising an antibody (e.g., monospecific or bispecific) or an antibody conjugate, of the invention or portion thereof as described above in a pharmaceutically acceptable carrier. In certain embodiments, the polypeptides of the invention may be present in a neutral form (including zwitter ionic forms) or as a positively or negatively-charged species. In some embodiments, the polypeptides may be complexed with a counterion to form a "pharmaceutically acceptable salt," which refers to a complex comprising one or more polypeptides and one or more counterions, where the counterions are derived from pharmaceutically acceptable inorganic and organic acids and bases.

The antibody (e.g., monospecific or bispecific) or the antibody conjugate, or portions thereof, may be administered alone or in combination with one or more other polypeptides of the invention or in combination with one or more other drugs (or as any combination thereof). The pharmaceutical compositions, methods and uses of the invention thus also encompass embodiments of combinations (co-administration) with other active agents, as detailed below.

- 101 -

As used herein, the terms “co-administration,” “co-administered” and “in combination with,” referring to the antibodies of the invention and one or more other therapeutic agents, is intended to mean, and does refer to and include the following: (i) simultaneous administration of such combination of a heterodimer disclosed herein and therapeutic agent(s) to a patient in need of treatment, when such components are formulated together into a single dosage form which releases said components at substantially the same time to said patient; (ii) substantially simultaneous administration of such combination of a heterodimer disclosed herein and therapeutic agent(s) to a patient in need of treatment, when such components are formulated apart from each other into separate dosage forms which are taken at substantially the same time by said patient, whereupon said components are released at substantially the same time to said patient; (iii) sequential administration of such combination of a heterodimer disclosed herein and therapeutic agent(s) to a patient in need of treatment, when such components are formulated apart from each other into separate dosage forms which are taken at consecutive times by said patient with a significant time interval between each administration, whereupon said components are released at substantially different times to said patient; and (iv) sequential administration of such combination of a heterodimer disclosed herein and therapeutic agent(s) to a patient in need of treatment, when such components are formulated together into a single dosage form which releases said components in a controlled manner whereupon they are concurrently, consecutively, and/or overlappingly released at the same and/or different times to said patient, where each part may be administered by either the same or a different route.

Generally, the antibody (e.g., monospecific or bispecific) or the antibody conjugate disclosed herein or portions thereof are suitable to be administered as a formulation in association with one or more pharmaceutically acceptable excipient(s). The term 'excipient' is used herein to describe any ingredient other than the compound(s) of the invention. The choice of excipient(s) will to a large extent depend on factors such as the particular mode of administration, the effect of the excipient on solubility and stability, and the nature of the dosage form. As used herein, “pharmaceutically acceptable excipient” includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. Some examples of pharmaceutically acceptable excipients are water, saline, phosphate buffered saline,

- 102 -

dextrose, glycerol, ethanol and the like, as well as combinations thereof. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Additional examples of pharmaceutically acceptable substances are wetting agents or minor amounts of auxiliary substances such as wetting or emulsifying agents, preservatives or buffers, which enhance the shelf life or effectiveness of the antibody.

Pharmaceutical compositions of the present invention and methods for their preparation will be readily apparent to those skilled in the art. Such compositions and methods for their preparation may be found, for example, in Remington's Pharmaceutical Sciences, 19th Edition (Mack Publishing Company, 1995). Pharmaceutical compositions are preferably manufactured under GMP conditions.

A pharmaceutical composition of the invention may be prepared, packaged, or sold in bulk, as a single unit dose, or as a plurality of single unit doses. As used herein, a "unit dose" is discrete amount of the pharmaceutical composition comprising a predetermined amount of the active ingredient. The amount of the active ingredient is generally equal to the dosage of the active ingredient which would be administered to a subject or a convenient fraction of such a dosage such as, for example, one-half or one-third of such a dosage. Any method for administering peptides, proteins or antibodies accepted in the art may suitably be employed for the heterodimeric proteins and portions thereof disclosed herein.

The pharmaceutical compositions of the invention are typically suitable for parenteral administration. As used herein, "parenteral administration" of a pharmaceutical composition includes any route of administration characterized by physical breaching of a tissue of a subject and administration of the pharmaceutical composition through the breach in the tissue, thus generally resulting in the direct administration into the blood stream, into muscle, or into an internal organ. Parenteral administration thus includes, but is not limited to, administration of a pharmaceutical composition by injection of the composition, by application of the composition through a surgical incision, by application of the composition through a tissue-penetrating non-surgical wound, and the like. In particular, parenteral administration is contemplated to include, but is not limited to, subcutaneous, intraperitoneal, intramuscular, intrasternal, intravenous, intraarterial, intrathecal, intraventricular, intraurethral, intracranial,

- 103 -

intrasynovial injection or infusions; and kidney dialytic infusion techniques. Preferred embodiments include the intravenous and the subcutaneous routes.

Formulations of a pharmaceutical composition suitable for parenteral administration typically generally comprise the active ingredient combined with a pharmaceutically acceptable carrier, such as sterile water or sterile isotonic saline. Such formulations may be prepared, packaged, or sold in a form suitable for bolus administration or for continuous administration. Injectable formulations may be prepared, packaged, or sold in unit dosage form, such as in ampoules or in multi dose containers containing a preservative. Formulations for parenteral administration include, but are not limited to, suspensions, solutions, emulsions in oily or aqueous vehicles, pastes, and the like. Such formulations may further comprise one or more additional ingredients including, but not limited to, suspending, stabilizing, or dispersing agents. In one embodiment of a formulation for parenteral administration, the active ingredient is provided in dry (i.e. powder or granular) form for reconstitution with a suitable vehicle (e.g. sterile pyrogen free water) prior to parenteral administration of the reconstituted composition. Parenteral formulations also include aqueous solutions which may contain excipients such as salts, carbohydrates and buffering agents (preferably to a pH of from 3 to 9), but, for some applications, they may be more suitably formulated as a sterile non-aqueous solution or as a dried form to be used in conjunction with a suitable vehicle such as sterile, pyrogen-free water. Exemplary parenteral administration forms include solutions or suspensions in sterile aqueous solutions, for example, aqueous propylene glycol or dextrose solutions. Such dosage forms can be suitably buffered, if desired. Other parentally-administrable formulations which are useful include those which comprise the active ingredient in microcrystalline form, or in a liposomal preparation. Formulations for parenteral administration may be formulated to be immediate and/or modified release. Modified release formulations include controlled, delayed, sustained, pulsed, targeted and programmed release formulations. For example, in one aspect, sterile injectable solutions can be prepared by incorporating the heterodimeric protein, e.g., bispecific antibody, in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated

- 104 -

above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile filtered solution thereof. The proper fluidity of a solution can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prolonged absorption of injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin.

An exemplary, non-limiting pharmaceutical composition of the invention is a formulation as a sterile aqueous solution having a pH that ranges from about 5.0 to about 6.5 and comprising from about 1 mg/mL to about 200 mg/mL of an antibody (e.g., monospecific or bispecific) or an antibody conjugate disclosed herein, from about 1 millimolar to about 100 millimolar of histidine buffer, from about 0.01 mg/mL to about 10 mg/mL of polysorbate 80, from about 100 millimolar to about 400 millimolar of trehalose, and from about 0.01 millimolar to about 1.0 millimolar of disodium EDTA dihydrate.

Dosage regimens may be adjusted to provide the optimum desired response. For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form, as used herein, refers to physically discrete units suited as unitary dosages for the patients/subjects to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are generally dictated by and directly dependent on (a) the unique characteristics of the chemotherapeutic agent and the particular therapeutic or prophylactic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

Thus, the skilled artisan would appreciate, based upon the disclosure provided herein, that the dose and dosing regimen is adjusted in accordance with methods well-

- 105 -

known in the therapeutic arts. That is, the maximum tolerable dose can be readily established, and the effective amount providing a detectable therapeutic benefit to a patient may also be determined, as can the temporal requirements for administering each agent to provide a detectable therapeutic benefit to the patient. Accordingly, while
5 certain dose and administration regimens are exemplified herein, these examples in no way limit the dose and administration regimen that may be provided to a patient in practicing the present invention.

It is to be noted that dosage values may vary with the type and severity of the condition to be alleviated, and may include single or multiple doses. It is to be further
10 understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that dosage ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition. Further, the dosage regimen with the
15 compositions of this invention may be based on a variety of factors, including the type of disease, the age, weight, sex, medical condition of the patient, the severity of the condition, the route of administration, and the particular antibody employed. Thus, the dosage regimen can vary widely, but can be determined routinely using standard methods. For example, doses may be adjusted based on pharmacokinetic or
20 pharmacodynamic parameters, which may include clinical effects such as toxic effects and/or laboratory values. Thus, the present invention encompasses intra-patient dose-escalation as determined by the skilled artisan. Determining appropriate dosages and regimens are well-known in the relevant art and would be understood to be encompassed by the skilled artisan once provided the teachings disclosed herein.

25 For administration to human subjects, the total monthly dose of an antibody (e.g., monospecific or bispecific) or an antibody conjugate disclosed herein is typically in the range of about 0.5 to about 1200 mg per patient, depending, of course, on the mode of administration, mechanism of action, and target biology. For example, an intravenous monthly dose may require about 1 to about 1000 mg/patient. The total monthly dose
30 may be administered in single or divided doses and may, at the physician's discretion, fall outside of the typical range given herein.

An exemplary, non-limiting range for a therapeutically or prophylactically effective amount of an antibody (e.g., monospecific or bispecific) or an antibody

- 106 -

conjugate, disclosed herein is about 1 to about 1000 mg/patient/month. In certain embodiments, the heterodimeric protein may be administered at about 1 to about 200 or about 1 to about 150 mg/patient/month.

5 Kits

The invention also provides kits for use in the instant methods. Kits of the invention include one or more containers comprising the antibody (e.g., monospecific or bispecific) or the antibody conjugate as described herein and instructions for use in accordance with any of the methods of the invention described herein. Generally, these
10 instructions comprise a description of administration of the heterodimeric protein for the above described therapeutic treatments.

The instructions relating to the use of the antibody (e.g., monospecific or bispecific) or the antibody conjugate as described herein generally include information as to dosage, dosing schedule, and route of administration for the intended treatment.
15 The containers may be unit doses, bulk packages (e.g., multi-dose packages) or sub-unit doses. Instructions supplied in the kits of the invention are typically written instructions on a label or package insert (e.g., a paper sheet included in the kit), but machine-readable instructions (e.g., instructions carried on a magnetic or optical storage disk) are also acceptable.

20 The kits of this invention are in suitable packaging. Suitable packaging includes, but is not limited to, vials, bottles, jars, flexible packaging (e.g., sealed Mylar or plastic bags), and the like. Also contemplated are packages for use in combination with a specific device, such as an inhaler, nasal administration device (e.g., an atomizer) or an infusion device such as a minipump. A kit may have a sterile access port (for example
25 the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). The container may also have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is a bispecific antibody. The container may further comprise a second
30 pharmaceutically active agent.

Kits may optionally provide additional components such as buffers and interpretive information. Normally, the kit comprises a container and a label or package insert(s) on or associated with the container.

- 107 -

Incorporated by reference herein for all purposes is the content of U.S. Provisional Patent Application Nos. 62/281,543 (filed January 21, 2016) and 62/431,766 (Filed December 8, 2016).

5 Biological Deposit

Representative materials of the present invention were deposited in the American Type Culture Collection, 10801 University Boulevard, Manassas, Va. 20110-2209, USA, on _____. Vector _____ having ATCC Accession No. _____ is a polynucleotide encoding the _____ light chain variable region, and vector _____ having
10 ATCC Accession No. _____ is a polynucleotide encoding the _____ heavy chain variable region. The deposits were made under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure and Regulations thereunder (Budapest Treaty). This assures maintenance of a viable culture of the deposit for 30 years from the date of deposit. The deposit will be
15 made available by ATCC under the terms of the Budapest Treaty, and subject to an agreement between Pfizer, Inc. and ATCC, which assures permanent and unrestricted availability of the progeny of the culture of the deposit to the public upon issuance of the pertinent U.S. patent or upon laying open to the public of any U.S. or foreign patent application, whichever comes first, and assures availability of the progeny to one
20 determined by the U.S. Commissioner of Patents and Trademarks to be entitled thereto according to 35 U.S.C. § 122 and the Commissioner's rules pursuant thereto (including 37 C.F.R. § 1.14 with particular reference to 886 OG 638).

The assignee of the present application has agreed that if a culture of the materials on deposit should die or be lost or destroyed when cultivated under suitable
25 conditions, the materials will be promptly replaced on notification with another of the same. Availability of the deposited material is not to be construed as a license to practice the invention in contravention of the rights granted under the authority of any government in accordance with its patent laws.

30 The following examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way. Indeed, various modifications of the invention in addition to those shown and described herein will

- 108 -

become apparent to those skilled in the art from the foregoing description and fall within the scope of the appended claims.

Examples

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Example 1: Affinity Determination for Recombinant Anti-EGFRvIII Murine-Human Chimeric Antibody and Humanized Antibodies

This example determines the affinity of chimeric and humanized anti-EGFRvIII antibodies at 25°C and 37°C.

10 Anti-EGFRvIII mouse (m) antibody, m62G7, generated from hybridomas was sequenced and subcloned into suitable vectors for expression as murine-human chimeric antibodies. The CDRs of mouse antibody m62G7 were grafted onto human framework and expressed as human IgG1 recombinant antibody, h62G7. Affinity variants of h62G7 were made by introducing mutations in the CDRs of the heavy and
15 light chains. The affinities of recombinant anti-EGFRvIII chimeric antibody m62G7 and humanized h62G7 antibodies were measured on a surface plasmon resonance Biacore™ T200 biosensor equipped with a research-grade anti-human Fc coupled CM4 sensor chip (GE Healthcare Inc., Piscataway, NJ). Anti-EGFRvIII antibodies were then captured by anti-human Fc. Monomeric 8-histidine tagged human EGFRvIII
20 extracellular domain was then injected as the analyte at 10-fold dilution series with top concentration at 1000 nM. Affinity of anti-EGFRvIII antibodies towards human EGFRvIII was measured at both 25°C and 37°C (Table 6). None of these antibodies showed detectable binding to 1000 nM 8-histidine tagged recombinant wild-type protein EGFRwt under the same assay condition.

25 In Table 6, variants of h62G7 are described with reference to the heavy chain variation then the light chain variation. For example, antibody clone “h62G7-EQ/L6” refers to the h62G7 clone containing the “EQ” variation in the heavy chain (also referred to herein as “h62G7-EQ”) and the “L6” variation in the light chain (also referred to herein as “h62G7-L6”). These heavy chain and light chain amino acid sequences are
30 provided in Table 2. Also, in the present application, a h62G7 variant may be referred to with either the heavy chain or the light chain variant written first – so, for example, “h62G7-EQ/L6” and “h62G7-L6/EQ” both refer to an antibody which contains a h62G7-EQ heavy chain and a h62G7-L6 light chain.

Table 6

Antibody	25°C			37°C		
	k_a (1/Ms)	k_d (1/s)	K_D (nM)	k_a (1/Ms)	k_d (1/s)	K_D (nM)
m62G7	7.30E+05	6.40E-02	88.7	8.00E+05	1.70E-01	207.0
h62G7-EQ/L6	2.40E+05	1.00E-02	43.8	6.60E+05	7.40E-02	112.8
h62G7-EQ/L1-DV	2.00E+05	1.20E-05	59.9	3.70E+05	6.90E-02	185.8
h62G7-H14/L1-DV	1.80E+04	2.00E-02	1087.9	6.60E+04	1.00E-01	1539.6
h62G7-H14/L6	1.30E+04	1.30E-02	992.2	4.30E+04	6.80E-02	1583.3

Example 2: Affinity Determination for Human Anti-EGFRvIII Antibodies

This example determines the affinity of various human anti-EGFRvIII antibodies at 37°C.

To generate human antibodies against EGFRvIII, transgenic AlivaMab mice (Ablexis LLC, San Francisco, CA) were immunized with alternating schedule of rat glioblastoma cell line expressing EGFRvIII, F98-npEGFRvIII (American Type Culture Collection, Manassas, VA) and peptides (SEQ ID NO: 227:

CGSGSGLEEKKGNYVVTDH) directed to the junction region in EGFRvIII. Hybridomas were generated using standard techniques. To determine the binding affinity and specificity of these hybridomas to EGFRvIII, antibodies in culture supernatants were captured by anti-mouse Fc using Biacore™ T200 biosensor equipped with anti-mouse Fc coupled CM4 sensor chips (Biacore™ AB, Uppsala, Sweden – now GE Healthcare). Monomeric 8-histidine tagged human EGFRvIII extracellular domain was then injected as the analyte at 10-fold dilution series starting with top concentration 1000 nM. Affinity of anti-EGFRvIII antibodies towards human EGFRvIII was measured at 37°C (Table 7). None of these hybridoma antibodies showed detectable binding to 1000 nM 8-histidine tagged recombinant wild-type protein EGFRwt under the same assay condition.

Table 7

Antibody	EGFRvIII binding at 37°C		
	k_a (1/Ms)	k_d (1/s)	K_D (nM)
42G9	6.88E+04	5.63E-04	8.2
32A10	6.54E+04	6.26E-04	9.6
21E11	6.66E+04	6.32E-04	9.5
49B11	7.64E+04	6.95E-04	9.1
46E10	5.97E+04	7.16E-04	12.0
12H6	5.93E+04	7.33E-04	12.4
19A9	5.58E+04	1.04E-03	18.6
11B11	5.21E+04	1.13E-03	21.7

- 110 -

21E7	6.52E+04	1.30E-03	19.9
20B9	4.67E+04	1.50E-03	32.1
12B2	7.38E+04	1.79E-03	24.3
11F10	6.63E+04	2.81E-03	42.4
17G11	5.61E+04	3.00E-03	53.5
29D5	1.02E+05	4.24E-03	41.6
14C11	7.55E+04	5.93E-03	78.5
20E12	3.99E+04	1.41E-02	353.4
20G5	1.25E+05	2.89E-02	231.2
26B9	1.31E+05	3.20E-02	244.3
30D8	1.61E+05	2.77E-02	172.0
32G8	6.82E+03	1.22E-02	1788.9
34E7	3.77E+04	1.28E-02	339.5

Example 3: Binding Specificity of Anti-EGFRvIII Antibodies to EGFRvIII expressing Cell Lines by Flow Cytometry

5 This example demonstrates the cell binding specificity of anti-EGFRvIII antibodies to EGFRvIII expressing cells.

To assess the cell binding specificity of anti-EGFRvIII antibodies generated from the AlivaMab mice, three isogenic rat glioblastoma cell lines and a human cancer cell line were used: F98 (does not express any form of human EGFR), F98-EGFRwt (expresses wild-type EGFR), F98-npEGFRvIII (expresses EGFRvIII) and A431 (an epidermoid carcinoma cell line with wild-type EGFR over-expression), all obtained from American Type Culture Collection (Manassas, VA). For cell staining, 500,000 cells were incubated with 50 μ l hybridoma supernatants for 45 min at 4°C, washed with binding buffer (PBS (Phosphate Buffered Saline) + 0.5% BSA (Bovine Serum Albumin)), followed by incubation with FITC-conjugated goat anti-mouse Fc specific secondary antibody from Jackson ImmunoResearch Laboratories (West Grove, PA). Tables 8A and 8B show mean fluorescent intensities (MFI) of EGFRvIII antibodies (except clone 20G5) on EGFRvIII expressing cell line were at least 10-fold higher than on non-expressing cell lines. FIG. 1A, FIG. 1B, and FIG. 1C show examples of the FACS binding histograms of three EGFRvIII specific clones which had been cloned and expressed as recombinant human IgG1 antibodies, 42G9 (FIG. 1A), 32A10 (FIG. 1B) and 32G8 (FIG. 1C), to the three F98 cell lines.

Table 8A

	F98	F98-EGFRwt	F98-EGFRvIII	A431
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- 111 -

Antibody	MFI	% positive	MFI	% positive	MFI	% positive	MFI	% positive
2nd Ab only	170	0.6	202	1.7	258	2.3	592	0.4
anti-EGFR(wt and vIII)	163	0.5	9608	98.3	5329	99.4	55240	100.0
42G9	159	0.4	185	1.6	3247	98.5	538	0.3
32A10	159	0.5	185	1.4	3349	98.3	531	0.2
21E11	159	0.3	184	1.3	3105	98.5	555	0.5
49B11	156	0.6	185	1.3	2980	98.5	599	0.8
46E10	158	0.4	187	1.6	2986	98.7	560	0.5
12H6	157	0.5	188	1.9	3445	98.3	569	0.8
19A9	158	0.5	168	1.6	3100	98.1	578	1.0
11B11	161	0.6	187	1.7	3391	98.2	589	1.2
21E7	159	0.3	184	1.3	3105	98.5	603	1.1
20B9	157	0.3	189	1.8	3418	98.3	558	0.7
12B2	156	0.4	185	1.5	2749	97.9	571	0.8
11F10	155	0.5	187	1.6	3283	98.0	582	1.1
17G11	157	0.6	184	1.5	3357	98.1	556	0.7
29D5	155	0.3	185	1.3	2829	97.9	531	0.4
14C11	157	0.4	185	1.3	3213	98.2	580	0.8

Table 8B

	F98		F98-EGFRwt		F98-EGFRvIII		A431	
Antibody	MFI	% positive	MFI	% positive	MFI	% positive	MFI	% positive
2nd Ab only	235	0.2	252	0.2	322	1.3	185	0.7
anti-EGFR(wt and vIII)	245	0.3	6857	97.2	5827	99.4	44493	100.0
20E12	381	6.0	348	3.4	3976	97.9	302	2.6
20G5	1248	16.8	1070	12.6	4639	98.5	391	2.0
26B9	310	4.1	298	2.3	5405	98.6	276	1.7
30D8	296	4.0	280	1.7	5165	98.6	269	1.3
32G8	329	4.9	301	1.6	3734	98.6	271	1.2
34E7	485	6.9	371	4.0	4128	98.5	294	1.1

Example 4: Affinity Determination for Fully Human Anti-EGFRvIII Antibodies from phage library

This example determines the affinity of various human anti-EGFRvIII antibodies at 25°C.

Human anti-EGFRvIII antibodies obtained from phage library screen were sequenced and subcloned into suitable vectors for expression as recombinant human

- 112 -

IgG1 antibodies. The affinities of antibodies were measured at 25°C (Table 9) on a surface plasmon resonance Biacore™ T200 biosensor equipped with an anti-human Fc coupled CM4 sensor chip (GE Healthcare Inc., Piscataway, NJ). Anti-EGFRvIII antibodies were captured by anti-human Fc. Monomeric 8-histidine tagged human EGFRvIII extracellular domain was then injected as the analyte at 10-fold dilution series starting at 1000 nM. Among the two antibodies, only C6 showed very weak but detectable binding to 1000 nM 8-histidine tagged recombinant wild-type protein EGFRwt at 25°C.

10 Table 9

Antibody	EGFRvIII binding at 25°C		
	k_a (1/Ms)	k_d (1/s)	K_D (nM)
B5	2.08E+04	1.41E-02	677.9
C6	1.68E+04	8.94E-03	532.1

Example 5: Generation and Characterization of GBM Cell Lines Expression EGFRvIII

This example demonstrates the expression of wild-type EGFR and EGFRvIII in GBM cell lines.

Five GFP (green fluorescent protein) and luciferase transduced human glioblastoma cell lines, DKMG, LN18, LN18-EGFRvIII, LN229 and LN229-EGFRvIII were used for functional characterization. DKMG, which expresses both endogenous wild-type EGFR and EGFRvIII, was obtained from DSMZ (Braunschweig, Germany). LN18 and LN229, which express only wild-type EGFR, were obtained from American Type Culture Collection (Manassas, VA). To generate GFP-luciferase labeled cell lines, DKMG, LN18 and LN229 were transduced with lentivirus particles (Amsbio, Cambridge, MA) encoding both GFP (green fluorescent protein) and luciferase in a bicistronic system. LN18-EGFRvIII and LN229-EGFRvIII were then generated by transduction of the parental cell lines, with a lentivirus vector encoding the full length EGFRvIII gene (SEQ ID NO: 201). Wild-type EGFR and EGFRvIII expression in each cell line was then analysed using flow cytometer. For cell staining, 300,000 cells were incubated with 3 µg EGFR wild-type specific or EGFRvIII specific antibody in 100 µl binding buffer (PBS (Phosphate Buffered Saline) + 2% FBS) for 45 min at 4°C, washed with binding buffer, followed by incubation with Alexa Fluor 647-conjugated goat anti-human Fc specific secondary antibody from Jackson ImmunoResearch Laboratories (West Grove,

- 113 -

PA). FIGs. 2A-C show the expression profiles of wild-type EGFR and EGFRvIII in LN229-EGFRvIII, LN18-EGFRvIII and DKMG, respectively.

Example 6: In vitro cytotoxicity assays with EGFRvIII-CD3 bispecific antibodies

5 This example demonstrates the cytotoxicity of EGFRvIII-CD3 bispecific antibodies towards EGFRvIII expressing GBM cell lines.

To generate EGFRvIII-CD3 bispecific antibodies, the heavy-chain variable domains of anti-EGFRvIII and anti-CD3 antibodies were subcloned into the appropriate human IgG2 based bispecific vectors and expressed with their corresponding light-
10 chain in HEK293 cells. Purification of the EGFRvIII-CD3 bispecific antibodies was done according to published methods (J Mol Biol, 2012, 3, pp204-219; US patent publication 2013/0115208). In these assays, the EGFRvIII-CD3 bispecific antibodies contain the anti-EGFRvIII sequence of anti-EGFRvIII clones h62G7-EQ/L6, 30D8, or 42G9.

Target cells in this Example were: EGFRvIII transduced LN18-EGFRvIII cells
15 (FIG. 3A) and parental LN18 (FIG. 3B) cells; EGFRvIII transduced LN229-EGFRvIII (FIG. 4A) and parental LN229 (FIG. 4B) cells; and DKMG cells (which express endogenous EGFRvIII and EGFR wild-type proteins) (FIG. 5).

For the cytotoxicity assays, luciferase transduced target cells were plated in white 96-well plates at 10,000 cells/well in PBMC media (RPMI, 10% FBS, 2 mM L-
20 glutamine, 1% Pen/Strep, 20 μ M β -mercaptoethanol, 10 mM HEPES, 1% non-essential amino acids, 1 mM sodium pyruvate) and incubated at 37 °C. Twenty-four hours later, activated T cells at the desired T:E (target:effector) ratio (10,000 T cells for 1:1, for LN18 and LN229 cells; 50,000 T cells for 1:5, for DKMG cells) were added to target cells along with the EGFRvIII-CD3 bispecific antibodies, negative control human IgG,
25 negative control CD3 monovalent antibody in bispecific Fc backbone, or negative control bivalent anti-EGFRvIII mAb 42G9 in wild-type human IgG. Cells were incubated for another 24 h at 37 °C. To detect the amount of viable target cells at the end of assay, the media was discarded and 100 μ l of 150 μ g/ml luciferin was added to each well. Luminescence signal was acquired on SpectraMax M5 Plate Reader (Molecular
30 Devices, Sunnyvale, CA). Percentage of live target cells was determined by normalizing the luminescence reading for each sample to that of control well containing only target cells.

- 114 -

The results are summarized in FIGs. 3A, 3B, 4A, 4B, and 5. In the graphs, the EGFRvIII-CD3 bispecific antibody data are represented by open symbols, and the negative control antibody data are represented by solid symbols

Target cells that expressed EGFRvIII showed a dose-dependent response to treatment with EGFRvIII-CD3 bispecific antibodies h62G7-EQ/L6/CD3 biFc, 30D8/CD3 biFc, and 42G9/CD3 biFc. In contrast, target cells that expressed EGFR wild-type protein only were not killed, thus indicating the specificity of the EGFRvIII-CD3 bispecific antibodies for cells expressing EGFRvIII. In addition, target cells that expressed EGFRvIII did not show a response to treatment with negative control antibodies human IgG, CD3 monovalent biFc, or 42G1 hIgG1 (anti-EGFRvIII antibody).

For example, LN18-EGFRvIII target cells treated with 0.01 nM h62G7-EQ/L6/CD3 biFc were only about 20% viable at the end of the assay. In contrast, LN18-EGFRvIII target cells treated with 0.1 nM control IgG, CD3 mono biFc, or 42G9 hIgG1 were about 100% viable at the end of the assay (FIG. 3A). In addition, parental cell line LN18 target cells treated with 0.01 nM h62G7-EQ/L6/CD3 biFc were about 100% viable at the end of the assay (FIG 3B).

In another example, LN229-EGFRvIII target cells treated with 0.01 nM 42G9/CD3 biFc were only about 35% viable at the end of the assay. In contrast, LN229-EGFRvIII target cells treated with 0.1 nM control IgG, CD3 mono biFc, or 42G9 hIgG1 were about 90-100% viable at the end of the assay (FIG. 4A). In addition, parental cell line LN229 target cells treated with 0.01 nM 42G9/CD3 biFc were about 100% viable at the end of the assay (FIG 4B).

In another example, DKMG target cells treated with 1 nM h62G7-EQ/L6/CD3 biFc were only about 35% viable at the end of the assay (FIG. 5). In contrast, DKMG target cells treated with 1 nM control IgG, CD3 mono biFc, or 42G9 hIgG1 were about 100% viable at the end of the assay (FIG. 5).

These data demonstrate that EGFRvIII-CD3 bispecific antibodies effectively mediate killing by T cells of EGFRvIII expressing cells.

Example 7: In Vivo Study of Anti-EGFRvIII-CD3 Bispecific Antibodies in a GBM model LN229-EGFRvIII

This example determines the *in vivo* anti-tumor activity of anti-EGFRvIII bispecific antibodies in a subcutaneous LN229-EGFRvIII GBM cell line model.

- 115 -

Three million LN229-EGFRvIII cells were implanted subcutaneously into 5-6 weeks old NSG mice (Jackson Laboratory, Sacramento, CA). Tumor volume was measured once a week by a caliper device and calculated with the following formula: Tumor volume = (length x width²) / 2. On day 18 post tumor implantation, animals were
5 randomized by tumor sizes into five animals per group. A single dose of 20 million fresh pan T cells was administered intraperitoneally, followed by bolus tail vein injection of 0.5 mg/kg of EGFRvIII-CD3 bispecific antibodies (the antibodies contained the anti-EGFRvIII sequence of anti-EGFRvIII clones h62G7-EQ/L6, 30D8, or 42G9), or CD3 monovalent control in bispecific Fc backbone.

10 The results are summarized in FIG. 6. In the graph, the EGFRvIII-CD3 bispecific antibody data are represented by open symbols, and the negative control antibody data are represented by solid symbols.

The EGFRvIII-CD3 bispecific antibodies h62G7-EQ/L6/CD3 biFc, 30D8/CD3 biFc, and 42G9/CD3 biFc inhibited the *in vivo* growth of the EGFRvIII-expressing
15 LN229-EGFRvIII GBM cells. In contrast, the negative control antibody CD3 monovalent biFc and a no treatment control (i.e. the mouse was not dosed with T cells or antibody) did not inhibit the *in vivo* growth of the LN229-EGFRvIII GBM cells. For example, at day 37 post tumor implantation, the mean tumor volume for mice treated with the EGFRvIII-CD3 bispecific antibodies 30D8/CD3 biFc and 42G9/CD3 biFc was less than
20 100 mm³, whereas the mean tumor volume for mice without treatment or treated with the CD3 monovalent biFc was greater than 1200 mm³.

These data demonstrate the *in vivo* anti-tumor activities of EGFRvIII-CD3 bispecific antibodies against EGFRvIII expressing tumor cells.

25 Although the disclosed teachings have been described with reference to various applications, methods, kits, and compositions, it will be appreciated that various changes and modifications can be made without departing from the teachings herein and the claimed invention below. The foregoing examples are provided to better illustrate the disclosed teachings and are not intended to limit the scope of the
30 teachings presented herein. While the present teachings have been described in terms of these exemplary embodiments, the skilled artisan will readily understand that numerous variations and modifications of these exemplary embodiments are possible without undue experimentation. All such variations and modifications are within the

- 116 -

scope of the current teachings.

All references cited herein, including patents, patent applications, papers, text books, and the like, and the references cited therein, to the extent that they are not already, are hereby incorporated by reference in their entirety. In the event that one or
5 more of the incorporated literature and similar materials differs from or contradicts this application, including but not limited to defined terms, term usage, described techniques, or the like, this application controls.

The foregoing description and Examples detail certain specific embodiments of the invention and describes the best mode contemplated by the inventors. It will be
10 appreciated, however, that no matter how detailed the foregoing may appear in text, the invention may be practiced in many ways and the invention should be construed in accordance with the appended claims and any equivalents thereof.

- 117 -

Claims

It is claimed:

1. An isolated antibody, which specifically binds to Epidermal Growth Factor Receptor Variant III (EGFRvIII), wherein the antibody comprises

- 5 (a) a heavy chain variable (VH) region comprising (i) a VH complementarity determining region one (CDR1) comprising the sequence shown in SEQ ID NO: 62, 63, 64, 74, 75, 76, 80, 81, 82, 88, 89, 90, 93, 94, 95, 99, 100, 101, 109, 110, 111, 115, 116, 117, 121, 122, 123, 132, 133, 134, 137, 138, 139, 143, 144, or 145; (ii) a VH CDR2 comprising the sequence shown in SEQ ID NO: 65, 66, 68, 10 69, 70, 71, 77, 78, 83, 84, 86, 87, 91, 92, 96, 97, 98, 102, 103, 105, 106, 112, 113, 118, 119, 124, 125, 127, 128, 130, 131, 135, 136, 140, 141, 146, 147, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, or 237; and (iii) a VH CDR3 comprising the sequence shown in SEQ ID NO: 67, 72, 73, 79, 85, 104, 107, 108, 114, 120, 126, 129, 142, 148, 219, 220, 221, 222, 223, or 236; and/or
- 15 (b) a light chain variable (VL) region comprising (i) a VL CDR1 comprising the sequence shown in SEQ ID NO: 149, 154, 156, 159, 162, 165, 166, 168, 169, 170, 171, 173, 174, 176, 178, 181, 182, 185, 187, 190, 192, 195, 198, 238, or 239; (ii) a VL CDR2 comprising the sequence shown in SEQ ID NO: 150, 152, 155, 157, 160, 163, 172, 175, 179, 183, 186, 188, 191, 193, 196, or 199; and (iii)
- 20 a VL CDR3 comprising the sequence shown in SEQ ID NO: 151, 153, 158, 161, 164, 167, 177, 180, 184, 189, 194, 197, or 200.

2. An isolated antibody which specifically binds to Epidermal Growth Factor Receptor Variant III (EGFRvIII), wherein the antibody comprises:

- 25 a VH region comprising a VH CDR1, VH CDR2, and VH CDR3 of the VH sequence shown in SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 30, 32, 34, 35, 37, 39, 41, 43, 44, 46, 48, 50, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 202, 203, 204, 205, 206, 207, 208, 209, 210, 214, 216, 217, or 218; and/or

- 30 a VL region comprising VL CDR1, VL CDR2, and VL CDR3 of the VL sequence shown in SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 29, 31, 33, 36, 38, 40, 42, 45, 47, 49, 51, 211, 212, 213, or 215.

- 118 -

3. An isolated antibody which specifically binds to EGFRvIII and competes with the antibody of claim 1 or 2.

4. A bispecific antibody wherein the bispecific antibody is a full-length human antibody, comprising a first antibody variable domain of the bispecific antibody specifically binding to a target antigen, and comprising a second antibody variable domain of the bispecific antibody capable of recruiting the activity of a human immune effector cell by specifically binding to an effector antigen located on the human immune effector cell, wherein the first antibody variable domain comprises a heavy chain variable (VH) region comprising a VH CDR1, VH CDR2, and VH CDR3 of the VH sequence shown in SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 30, 32, 34, 35, 37, 39, 41, 43, 44, 46, 48, 50, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 202, 203, 204, 205, 206, 207, 208, 209, 210, 214, 216, 217, or 218; and/or a light chain variable (VL) region comprising VL CDR1, VL CDR2, and VL CDR3 of the VL sequence shown in SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 29, 31, 33, 36, 38, 40, 42, 45, 47, 49, 51, 211, 212, 213, or 215.

5. The bispecific antibody of claim 4, wherein the second antibody variable domain comprises a heavy chain variable (VH) region comprising a VH CDR1, VH CDR2, and VH CDR3 of the VH sequence shown in SEQ ID NO: 240; and/or a light chain variable (VL) region comprising VL CDR1, VL CDR2, and VL CDR3 of the VL sequence shown in SEQ ID NO: 241.

6. A bispecific antibody wherein the bispecific antibody is a full-length human antibody, comprising a first antibody variable domain of the bispecific antibody specifically binding to a target antigen, and comprising a second antibody variable domain of the bispecific antibody capable of recruiting the activity of a human immune effector cell by specifically binding to an effector antigen located on the human immune effector cell, wherein the first antibody variable domain comprises

a. a heavy chain variable (VH) region comprising (i) a VH complementarity determining region one (CDR1) comprising the sequence shown in SEQ ID NO: 62, 63, 64, 74, 75, 76, 80, 81, 82, 88, 89, 90, 93, 94, 95, 99, 100, 101, 109, 110, 111, 115, 116, 117, 121, 122, 123, 132, 133, 134, 137, 138, 139, 143, 144, or 145; (ii) a VH CDR2 comprising the sequence shown in SEQ ID NO: 65, 66, 68, 69, 70, 71, 77, 78, 83, 84, 86, 87, 91,

- 119 -

92, 96, 97, 98, 102, 103, 105, 106, 112, 113, 118, 119, 124, 125, 127, 128, 130, 131, 135, 136, 140, 141, 146, 147, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, or 237; and iii) a VH CDR3 comprising the sequence shown in SEQ ID NO: 67, 72, 73, 79, 85, 104, 107, 108, 114, 120, 126, 129, 142, 148, 219, 220, 221, 222, 223, or 236; and/or

b. a light chain variable (VL) region comprising (i) a VL CDR1 comprising the sequence shown in SEQ ID NO: 149, 154, 156, 159, 162, 165, 166, 168, 169, 170, 171, 173, 174, 176, 178, 181, 182, 185, 187, 190, 192, 195, 198, 238, or 239; (ii) a VL CDR2 comprising the sequence shown in SEQ ID NO: 150, 152, 155, 157, 160, 163, 172, 175, 179, 183, 186, 188, 191, 193, 196, or 199; and (iii) a VL CDR3 comprising the sequence shown in SEQ ID NO: 151, 153, 158, 161, 164, 167, 177, 180, 184, 189, 194, 197, or 200.

7. The bispecific antibody of claim 6, wherein the second antibody variable domain specifically binds to the effector antigen CD3.

8. The bispecific antibody of claim 7, wherein the second antibody variable domain comprises

a. a heavy chain variable (VH) region comprising (i) a VH complementary determining region one (CDR1) comprising the sequence shown in SEQ ID NO: 244, 110, or 245; (ii) a VH CDR2 comprising the sequence shown in SEQ ID NO: 246 or 247; and iii) a VH CDR3 comprising the sequence shown in SEQ ID NO: 248; and/or

b. a light chain variable (VL) region comprising (i) a VL CDR1 comprising the sequence shown in SEQ ID NO: 249; (ii) a VL CDR2 comprising the sequence shown in SEQ ID NO: 250; and (iii) a VL CDR3 comprising the sequence shown in SEQ ID NO: 251.

9. The bispecific antibody of any one of claims 4-8, wherein both the first and the second antibody variable domains of the heterodimeric protein comprise amino acid modifications at positions 223, 225, and 228 in the hinge region and at position 409 or 368 (EU numbering scheme) in the CH3 region of a human IgG2 (SEQ ID NO: 290).

- 120 -

10. The bispecific antibody of claim 9, further comprising an amino acid modification at one or more of positions 265, 330 and 331 of the human IgG2.

11. The antibody of any one of claims 1-3, wherein the antibody comprises an acyl donor glutamine-containing tag engineered at a specific site.

5 12. The antibody of claim 11, wherein the antibody comprises a linker.

13. A nucleic acid encoding the antibody of any one of claims 1-12.

14. A vector comprising the nucleic acid of claim 13.

15. A host cell comprising the nucleic acid of claim 13 or the vector of claim 14.

10 16. A conjugate of the antibody of any one of claims 1-3 and 11-12, wherein the antibody is conjugated to an agent, wherein the agent is selected from the group consisting of a cytotoxic agent, an immunomodulating agent, an imaging agent, a therapeutic protein, a biopolymer, and an oligonucleotide.

15 17. The antibody of any one of claims 1-3 and 11-12, the bispecific antibody of any one of claims 4-10, or the conjugate of claim 16 for use as a medicament.

18. The antibody, bispecific antibody, or the conjugate of claim 17, wherein the medicament is for use in treatment of an EGFRvIII related cancer selecting from the group consisting of glioblastoma multiform, anaplastic astrocytoma, giant cell glioblastoma, gliosarcoma, anaplastic oligodendroglioma, anaplastic ependymoma, 20 anaplastic oligoastrocytoma, choroid plexus carcinoma, anaplastic ganglioglioma, pineoblastoma, pineocytoma, meningioma, medulloepithelioma, ependymoblastoma, medulloblastoma, supraentorial primitive neuroectodermal tumor, atypical teratoid/rhabdoid tumor, mixed glioma, head and neck cancer, non-small cell lung cancer, breast cancer, ovarian cancer, prostate cancer, medulloblastoma, colorectal 25 cancer, anal cancer, cervical cancer, renal cancer, skin cancer, pancreatic cancer, liver cancer, bladder cancer, gastric cancer, thyroid cancer, mesothelioma, uterine cancer, lymphoma, and leukemia.

19. The antibody, bispecific antibody, or the conjugate of claim 18, wherein the cancer is glioblastoma multiforme, head and neck cancer, non-small cell lung 30 cancer, breast cancer, ovarian cancer, or prostate cancer.

- 121 -

20. A method of treating a subject in need thereof comprising:

a. providing the antibody according to any one of claims 1-3, the bispecific antibody according to any one of claims 4-10, or the conjugate of claim 16; and

5 b. administering said antibody, bispecific antibody, or conjugate to said patient.

21. A pharmaceutical composition comprising the antibody of any one of claims 1-3, the bispecific antibody of any one of claims 4-10, or the conjugate of claim 16.

10 22. A method of treating a condition associated with malignant cells expressing EGFRvIII in a subject comprising administering to a subject in need thereof an effective amount of the pharmaceutical composition of claim 21.

23. The method of claim 22, wherein the condition is a cancer.

15 24. The method of claim 23, wherein the cancer is an EGFRvIII related cancer selecting from the group consisting of glioblastoma multiforme, anaplastic astrocytoma, giant cell glioblastoma, gliosarcoma, anaplastic oligodendroglioma, anaplastic ependymoma, anaplastic oligoastrocytoma, choroid plexus carcinoma, anaplastic ganglioglioma, pineoblastoma, pineocytoma, meningioma, medulloepithelioma, ependymblastoma, medulloblastoma, supraentorial primitive neuroectodermal tumor, 20 atypical teratoid/rhabdoid tumor, mixed glioma, head and neck cancer, non-small cell lung cancer, breast cancer, ovarian cancer, prostate cancer, medulloblastoma, colorectal cancer, anal cancer, cervical cancer, renal cancer, skin cancer, pancreatic cancer, liver cancer, bladder cancer, gastric cancer, thyroid cancer, mesothelioma, uterine cancer, lymphoma, and leukemia.

25 25. The method of claim 24, wherein the cancer is glioblastoma multiforme, head and neck cancer, non-small cell lung cancer, breast cancer, ovarian cancer, or prostate cancer.

26. A method of inhibiting tumor growth or progression in a subject who has malignant cells expressing EGFRvIII, comprising administering to the subject in need

- 122 -

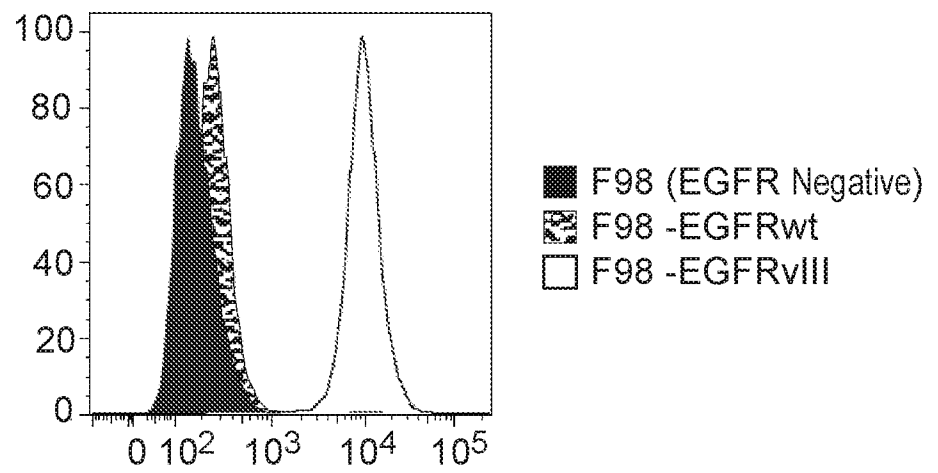
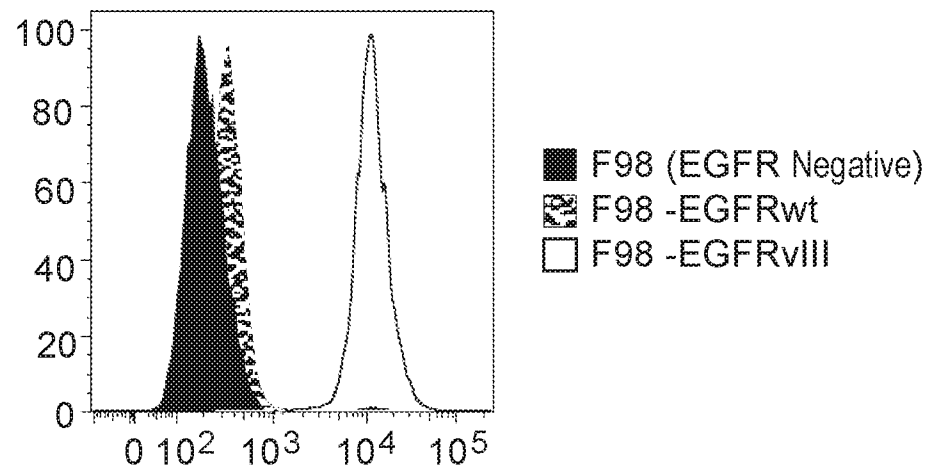
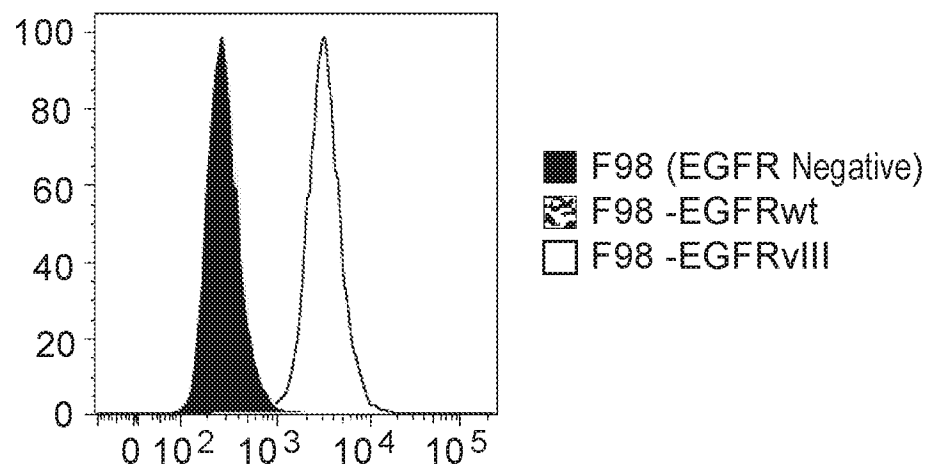
thereof an effective amount of the pharmaceutical composition of claim 21 to the subject.

27. A method of inhibiting metastasis of malignant cells expressing EGFRvIII in a subject, comprising administering to the subject in need thereof an effective amount
5 of the pharmaceutical composition of claim 21 to the subject.

28. A method of inducing tumor regression in a subject who has malignant cells expressing EGFRvIII, comprising administering to the subject in need thereof an effective amount of the pharmaceutical composition of claim 21 to the subject.

29. A method of producing an antibody, comprising culturing the host cell of
10 claim 15 under conditions that result in production of the antibody or bispecific antibody, and isolating the antibody or bispecific antibody from the host cell or culture.

1/6

FIG. 1A**FIG. 1B****FIG. 1C**

2/6

FIG. 2A

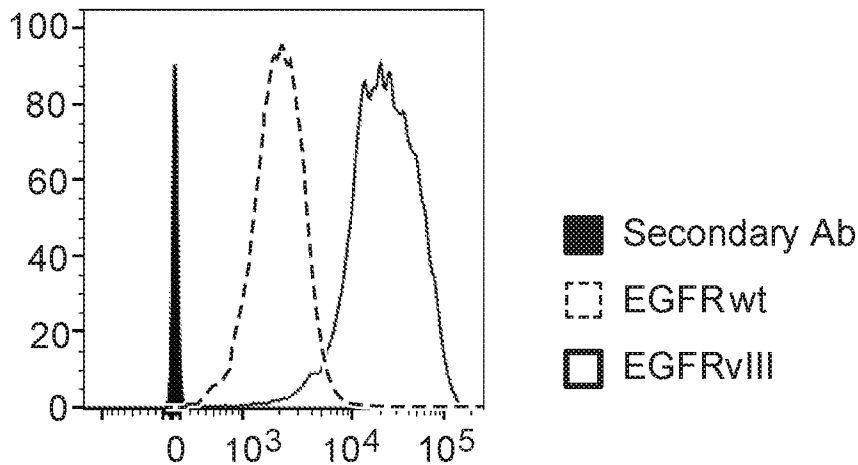


FIG. 2B

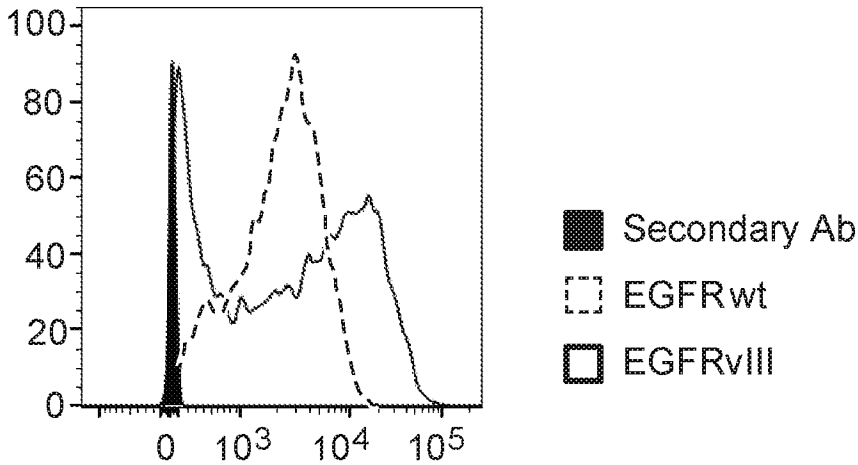
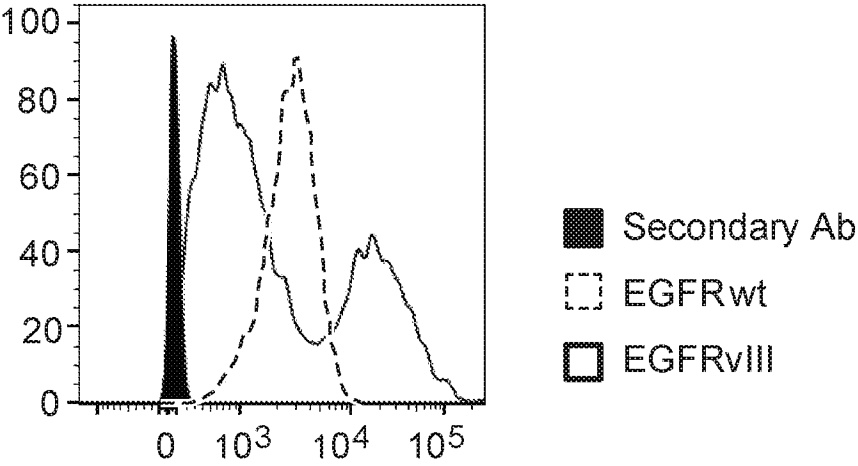


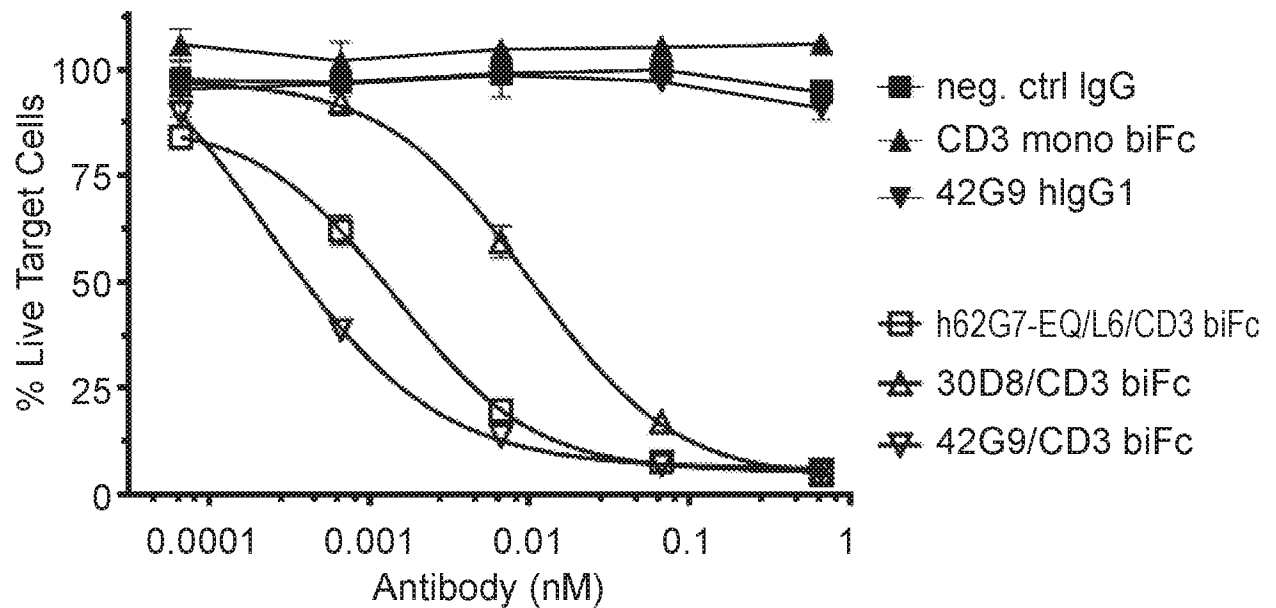
FIG. 2C



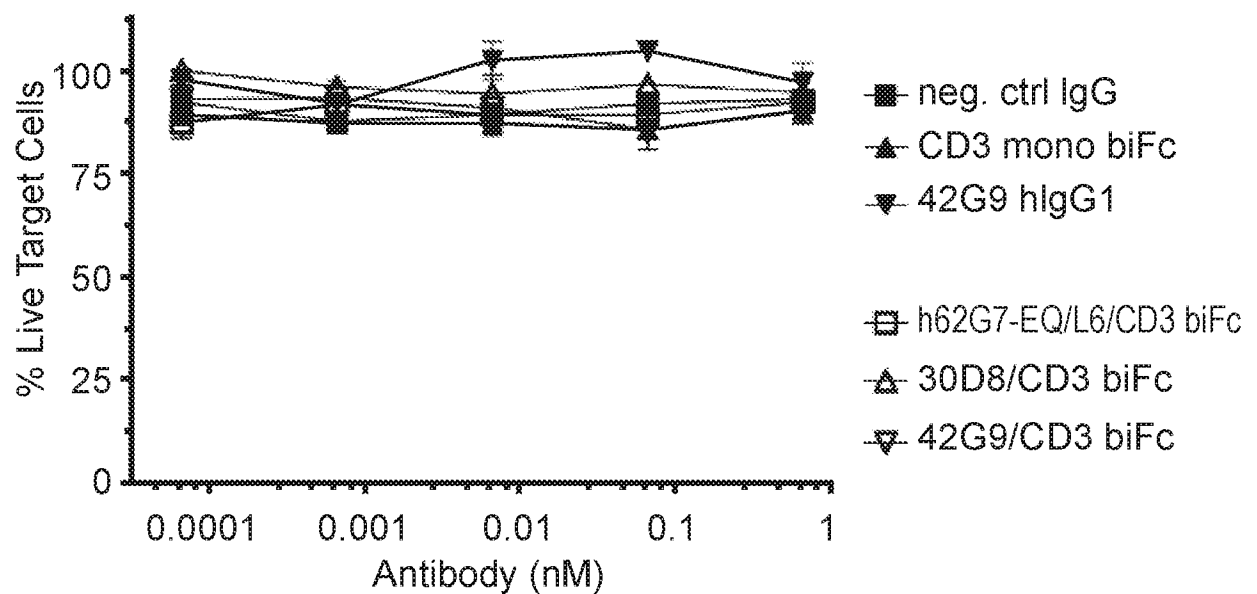
3/6

FIG. 3A

LN18-EGFRvIII

**FIG. 3B**

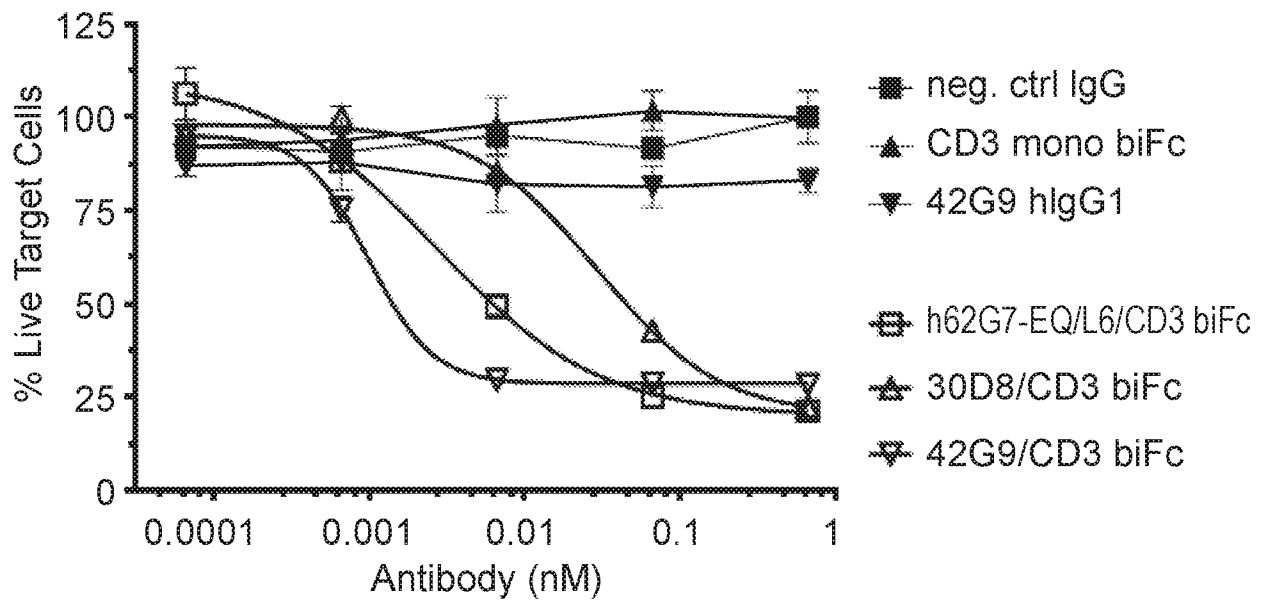
LN18



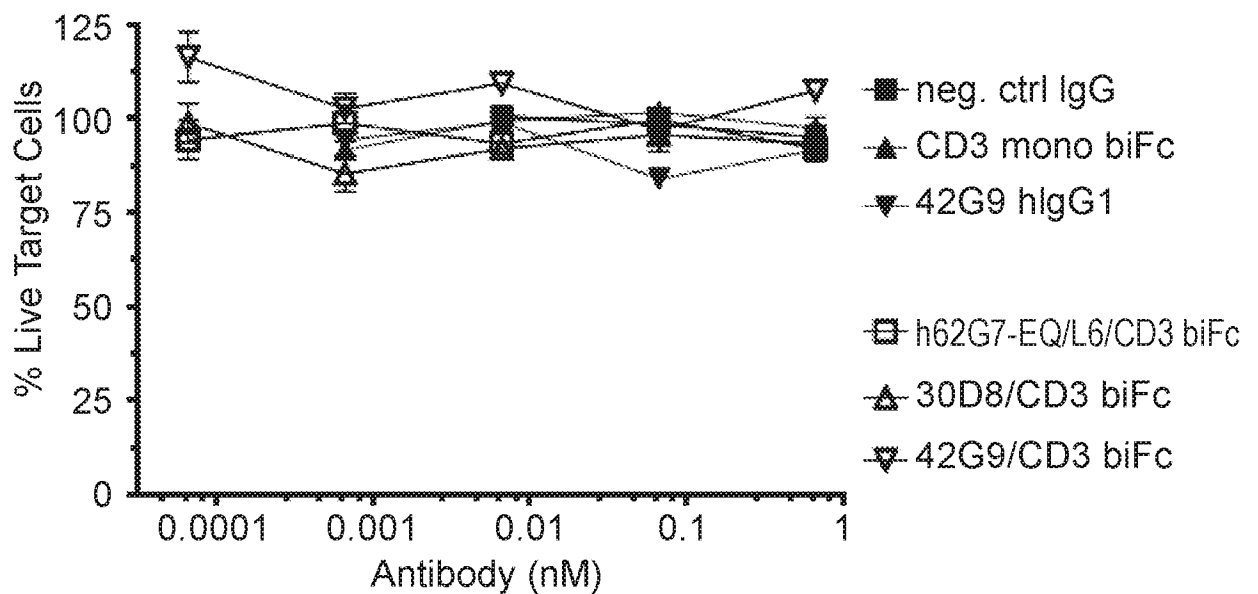
4/6

FIG. 4A

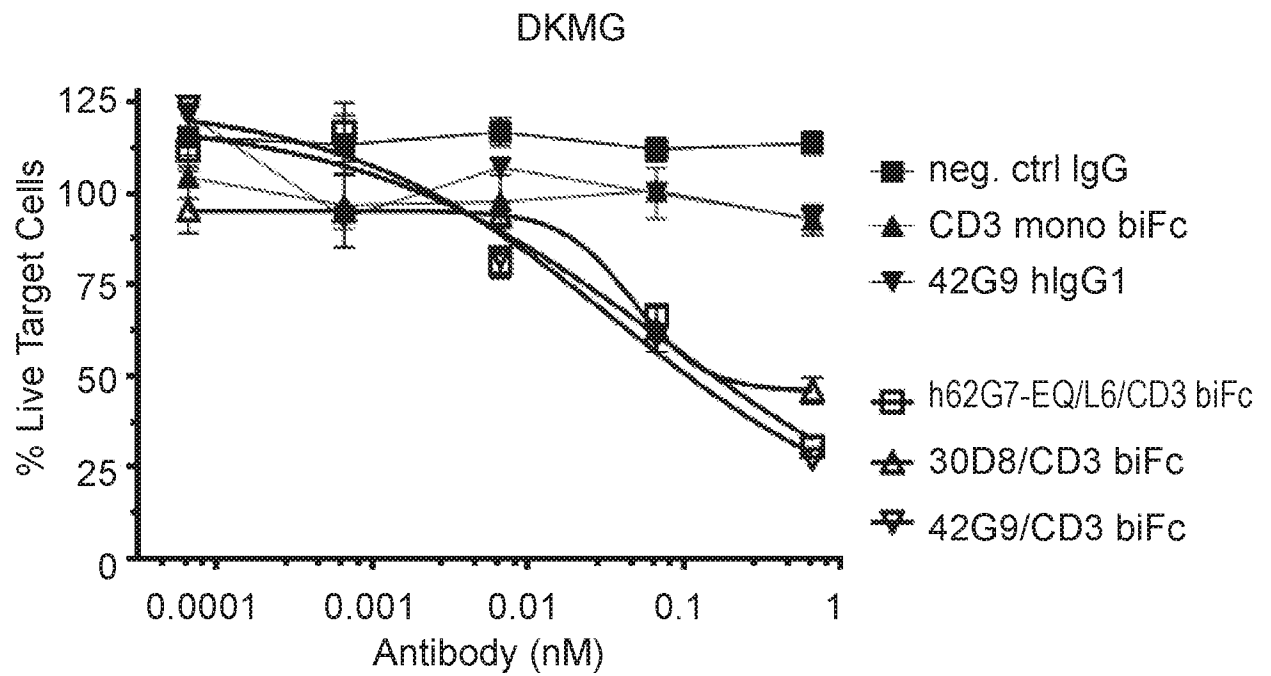
LN229-EGFRvIII

**FIG. 4B**

LN229



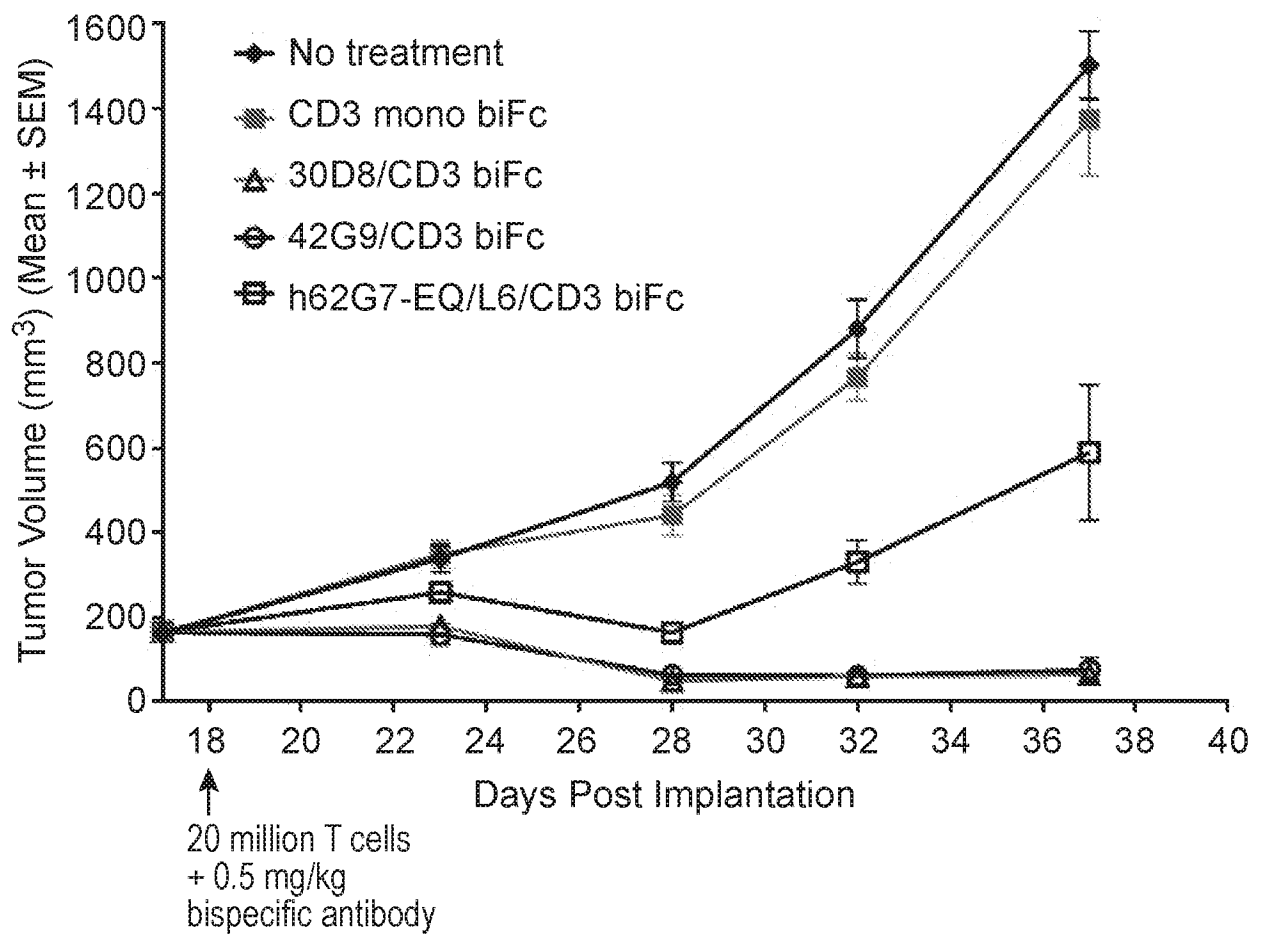
5/6

FIG. 5

6/6

FIG. 6

LN229-EGFRvIII



INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2017/050109

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/28 C07K16/30 A61P35/00
ADD.

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)
C07K

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

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

EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2015/006482 A1 (UNIV DUKE [US]; US GOVERNMENT [US]) 15 January 2015 (2015-01-15) examples	1-16
X	WO 2013/185010 A1 (UNIV DUKE [US]; US HEALTH [US]) 12 December 2013 (2013-12-12) examples figure 8	1-29
X	US 2012/189630 A1 (BIGNER DARELL D [US] ET AL) 26 July 2012 (2012-07-26) figure 8 examples	1-29
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Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

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"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

15 March 2017

Date of mailing of the international search report

02/05/2017

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Fax: (+31-70) 340-3016

Authorized officer

Covone-van Hees, M

INTERNATIONAL SEARCH REPORT

International application No

PCT/IB2017/050109

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2014/153002 A1 (CALIFORNIA INST BIOMEDICAL RES [US]; SCRIPPS RESEARCH INST [US]) 25 September 2014 (2014-09-25) paragraph [0222] examples	1-29
X	----- BRYAN D. CHOI ET AL: "A novel bispecific antibody recruits T cells to eradicate tumors in the "immunologically privileged" central nervous system", ONCOIMMUNOLOGY, vol. 2, no. 4, 27 April 2013 (2013-04-27), page e23639, XP055306563, ISSN: 2162-4011, DOI: 10.4161/onci.23639 the whole document	1-29
X	----- B. D. CHOI ET AL: "Systemic administration of a bispecific antibody targeting EGFRvIII successfully treats intracerebral glioma", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, vol. 110, no. 1, 17 December 2012 (2012-12-17), pages 270-275, XP055156305, ISSN: 0027-8424, DOI: 10.1073/pnas.1219817110 the whole document	1-29
A	----- CHAN-JUAN SHEN ET AL: "Chimeric antigen receptor containing ICOS signaling domain mediates specific and efficient antitumor effect of T cells against EGFRvIII expressing glioma", JOURNAL OF HEMATOLOGY & ONCOLOGY, BIOMED CENTRAL LTD, LONDON UK, vol. 6, no. 1, 9 May 2013 (2013-05-09), page 33, XP021151586, ISSN: 1756-8722, DOI: 10.1186/1756-8722-6-33 the whole document	1-29
A	----- IAN M ZITRON ET AL: "Targeting and killing of glioblastoma with activated T cells armed with bispecific antibodies", BMC CANCER, BIOMED CENTRAL, LONDON, GB, vol. 13, no. 1, 22 February 2013 (2013-02-22), page 83, XP021141258, ISSN: 1471-2407, DOI: 10.1186/1471-2407-13-83 the whole document	1-29
3 2	E ----- WO 2017/021370 A1 (AMGEN RES GMBH [DE]) 9 February 2017 (2017-02-09) examples	1-29

INTERNATIONAL SEARCH REPORT

International application No.

PCT/IB2017/050109

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:
- ☒ in the form of an Annex C/ST.25 text file.
- ☐ on paper or in the form of an image file.
- b. ☐ furnished together with the international application under PCT Rule 13~~ter~~.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
- c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
- ☐ in the form of an Annex C/ST.25 text file (Rule 13~~ter~~.1(a)).
- ☐ on paper or in the form of an image file (Rule 13~~ter~~.1(b) and Administrative Instructions, Section 713).
2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/IB2017/050109

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2015006482 A1	15-01-2015	AU 2014287244 A1 CA 2917919 A1 EP 3019532 A1 JP 2016523562 A US 2016168263 A1 WO 2015006482 A1	04-02-2016 15-01-2015 18-05-2016 12-08-2016 16-06-2016 15-01-2015
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WO 2017021370 A1	09-02-2017	US 2017029512 A1 WO 2017021370 A1	02-02-2017 09-02-2017