

describes the 806 antibody developed by Abbvie (ABT-806, ABT-414), which binds to EGFRvIII as well as a subset of amplified EGFR on EGFR overexpressing tumor cells (Cleary, JM et al., Invest New Drugs, 33(3), pp. 671-8, 2015; Reilly, EB., Molecular Cancer Therapeutics, Vol. 14(5), pp.1141-51, 2015). Although this antibody has been shown to bind preferentially to tumor EGFR in preclinical models, binding of this antibody to wt EGFR present in human skin has been shown to account for the cutaneous toxicity that ABT-806 exhibits in some patients (Cleary et al 2015).

Antibodies or antigen-binding fragments thereof that specifically target an epitope of EGFRvIII that is absent or not accessible in EGFR-expressing cells would be beneficial for the treatment of cancer patients.

SUMMARY

Antigen-binding agents such as antibodies or an antigen-binding fragments thereof, chimeric antigen receptors (CARs), bispecific T-cell engagers (BiTEs) and the like which specifically bind to epidermal growth factor receptor variant III (EGFRvIII) are provided.

In one aspect, the present disclosure provides an antigen-binding agent comprising an antigen-binding domain of an antibody that specifically binds to epidermal growth factor receptor variant III (EGFRvIII), wherein the antigen-binding domain comprises:

- a. a light chain variable region comprising a CDRL1 having the amino acid sequence set forth in SEQ ID NO:38, a CDRL2 having the amino acid sequence set forth in SEQ ID NO:39 and a CDRL3 having the amino acid sequence set forth in SEQ ID NO:40 and a heavy chain variable region comprising a CDRH1 having the amino acid sequence set forth in SEQ ID NO:43, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:44 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:45;
- b. a light chain variable region comprising a CDRL1 having the amino acid sequence of SEQ ID NO:8, a CDRL2 sequence of SEQ ID NO:9 and a CDRL3 sequence of SEQ ID NO:10 and a heavy chain variable region comprising a CDRH1 sequence of SEQ ID NO:13, a CDRH2 sequence of SEQ ID NO:14 and a CDRH3 sequence of SEQ ID NO:15;
- c. a light chain variable region comprising a CDRL1 having the amino acid sequence set forth in SEQ ID NO:18, a CDRL2 having the amino acid sequence set forth in SEQ ID NO:19 and a CDRL3 having the amino acid sequence set forth in SEQ ID

NO:20 and a heavy chain variable region comprising a CDRH1 having the amino acid sequence set forth in SEQ ID NO:23, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:24 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:25;

- 5 d. a light chain variable region comprising a CDRL1 having the amino acid sequence set forth in SEQ ID NO:28, a CDRL2 having the amino acid sequence set forth in SEQ ID NO:29 and a CDRL3 having the amino acid sequence set forth in SEQ ID NO:30 and a heavy chain variable region comprising a CDRH1 having the amino acid sequence set forth in SEQ ID NO:33, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:34 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:35;
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- e. a light chain variable region comprising a CDRL1 having the amino acid sequence set forth in SEQ ID NO:48, a CDRL2 having the amino acid sequence set forth in SEQ ID NO:49 and a CDRL3 having the amino acid sequence set forth in SEQ ID NO:50 and a heavy chain variable region comprising a CDRH1 having the amino acid sequence set forth in SEQ ID NO:53, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:54 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:55;
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- f. a light chain variable region comprising a CDRL1 having the amino acid sequence set forth in SEQ ID NO:58, a CDRL2 having the amino acid sequence set forth in SEQ ID NO:59 and a CDRL3 having the amino acid sequence set forth in SEQ ID NO:60 and a heavy chain variable region comprising a CDRH1 having the amino acid sequence set forth in SEQ ID NO:63, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:64 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:65;
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- g. a light chain variable region comprising a CDRL1 having the amino acid sequence set forth in SEQ ID NO:68, a CDRL2 having the amino acid sequence set forth in SEQ ID NO:69 and a CDRL3 having the amino acid sequence set forth in SEQ ID NO:70 and a heavy chain variable region comprising a CDRH1 having the amino acid sequence set forth in SEQ ID NO:78, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:79 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:80; or
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- h. a light chain variable region comprising a CDRL1 having the amino acid sequence set forth in SEQ ID NO:73, a CDRL2 having the amino acid sequence set forth in SEQ ID NO:74 and a CDRL3 having the amino acid sequence set forth in SEQ ID NO:75 and a heavy chain variable region comprising a CDRH1 having the amino acid sequence set forth in SEQ ID NO:78, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:79 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:80.

In another aspect, the present disclosure provides a pharmaceutical composition comprising the antigen-binding agent as described herein and a pharmaceutically acceptable carrier, diluent or excipient. In another aspect, the present disclosure provides a nucleic acid molecule encoding a light chain variable region and/or a heavy chain variable region of the antigen-binding agent as described herein.

In a further aspect, the present disclosure provides a use of the antigen-binding agent as described herein in the manufacture of a medicament for treating a cancer, wherein the cancer comprises cells expressing EGFRvIII. In a further aspect, the present disclosure provides a use of cells expressing the antigen-binding agent as described herein in the manufacture of a medicament for treating a subject having a cancer associated with EGFRvIII expression, wherein the antigen-binding agent is a chimeric antigen receptor, a bi-specific T-cell engager, a bispecific killer cell engager or a trispecific killer cell engager.

In a further aspect, the present disclosure provides an isolated cell population engineered to express the antigen-binding agent as described herein. In a further aspect, the present disclosure provides a pharmaceutical composition comprising the isolated cell population as described herein and a pharmaceutically acceptable buffer or excipient.

As described in more details below, some anti-EGFRvIII antibodies or antigen-binding fragments thereof may bind to EGFRvIII at the surface of cancer cells (e.g., glioblastoma cells). In some examples, the antibodies or antigen-binding fragments thereof do not significantly bind to EGFR expressed on cancer cells (e.g., U87MG-EGFR WT).

The antibodies or antigen-binding fragments thereof of the present disclosure may be internalized by cancer cells and may thus be used, in an example thereof, for delivery of cargo molecules. Particularly contemplated are anti-EGFRvIII antibodies or antigen-binding fragments thereof that are conjugated to therapeutic moieties. The anti-EGFRvIII antibodies described herein may be used for inhibiting the growth of EGFRvIII-expressing tumor cells.

In some examples of the present disclosure, the anti-EGFRvIII antibodies or antigen-binding fragments thereof may be able to bind to an epitope present in both native EGFRvIII (e.g., native recombinant EGFRvIII) and denatured EGFRvIII (e.g., denatured recombinant EGFRvIII).

Generally, the antibodies or antigen-binding fragments thereof of the present disclosure may be able to bind to a peptide comprising an EGFRvIII fragment consisting of amino acid residues 1 to 76 of EGFRvIII (SEQ ID NO:119). A subset of the antibodies or antigen-binding fragments thereof are able to bind to amino acid residues 1 to 18 of EGFRvIII (SEQ ID NO:125) and another subset of antibodies are able to bind amino acid residues 15 to 37 of EGFRvIII (SEQ ID NO:6).

More particularly, the present disclosure provides anti-EGFRvIII antibodies or antigen-binding fragments thereof that may be able to bind to one or more of the EGFRvIII fragments illustrated in Figures 4a and/or 4b.

Examples of anti-EGFRvIII antibodies or antigen-binding fragments thereof encompassed by the present disclosure includes, for example:

- Antibodies that are able to bind to a peptide comprising an EGFRvIII fragment consisting of amino acid residues 1 to 18 of EGFRvIII (SEQ ID NO:125);
- Antibodies that are able to bind to a peptide comprising an EGFRvIII fragment consisting of amino acid residues 3 to 18 of EGFRvIII (SEQ ID NO:129);
- Antibodies that are able to bind to a peptide comprising an EGFRvIII fragment consisting of amino acid residues 15 to 37 of EGFRvIII (SEQ ID NO:6); or;
- Antibodies that are able to bind to a peptide comprising an EGFRvIII fragment consisting of amino acid residues 19 to 37 of EGFRvIII (SEQ ID NO:142)

Some particular anti-EGFRvIII antibodies or antigen-binding fragments thereof encompassed by the present disclosure include those that do not require the presence of amino acid residues 1-2 of EGFRvIII for binding. Particularly contemplated are anti-EGFRvIII antibodies or antigen-binding fragments thereof that are capable of binding to one or more EGFRvIII fragments amongst fragment 19-76 (SEQ ID NO:138), fragment 19-62 (SEQ ID NO:139), fragment 19-49 (SEQ ID NO:140), fragment 19-45 (SEQ ID NO:141), fragment 28-45 (SEQ ID NO:143), fragment 28-37 (SEQ ID NO:144), fragment 19-37 (SEQ ID NO:142), fragment 3-45 (SEQ ID NO:127), fragment 3-49 (SEQ ID NO:126), fragment 3-37 (SEQ ID NO:128), fragment 6-49 (SEQ ID NO:130), fragment 6-45 (SEQ ID NO:131), fragment 6-37 (SEQ ID NO:132), fragment 10-49 (SEQ ID NO:133), fragment 10-45 (SEQ ID NO:134), fragment 10-37 (SEQ ID

NO:135), fragment 15-49 (SEQ ID NO:136), fragment 15-45 (SEQ ID NO:137) or fragment 15-37 (SEQ ID NO:6).

The anti-EGFRvIII antibodies or antigen-binding fragments provided herein include antibodies or antigen-binding fragments that are able to bind to a peptide comprising an EGFRvIII fragment consisting of amino acid residues 3 to 37 of EGFRvIII (SEQ ID NO:128) such as for example, the F260-5G6 (referred herein also as 5G6), F263-1A8 (referred herein also as 1A8), F263-4B3 (referred herein also as 4B3), F263-4E11 (referred herein also as 4E11), F263-5D8 (referred herein also as 5D8) and F265-9C9 (referred to herein also as 9C9) antibody.

Other anti-EGFRvIII antibodies or antigen-binding fragments provided herein include antibodies or antigen-binding fragments that are able to bind to a peptide comprising an EGFRvIII fragment consisting of amino acid residues 1 to 33 of EGFRvIII (SEQ ID NO:124).

Exemplary antibodies or antigen-binding fragments thereof of the present disclosure include an antibody or antigen-binding fragment thereof that specifically binds to EGFRvIII (SEQ ID NO:5) and that is capable of binding to an EGFRvIII fragment selected from the group consisting of:

- a. a fragment consisting of amino acid residues 15 to 37 of EGFRvIII (SEQ ID NO:6);
- b. a fragment consisting of amino acid residues 1 to 76 of EGFRvIII (SEQ ID NO:119);
- c. a fragment consisting of amino acid residues 1 to 62 of EGFRvIII (SEQ ID NO: 120);
- d. a fragment consisting of amino acid residues 1 to 49 of EGFRvIII (SEQ ID NO:121);
- e. a fragment consisting of amino acid residues 1 to 45 of EGFRvIII (SEQ ID NO:122);
- f. a fragment consisting of amino acid residues 1 to 37 of EGFRvIII (SEQ ID NO:123);
- g. a fragment consisting of amino acid residues 3 to 49 of EGFRvIII (SEQ ID NO:126);
- h. a fragment consisting of amino acid residues 3 to 45 of EGFRvIII (SEQ ID NO:127);
- i. a fragment consisting of amino acid residues 3 to 37 of EGFRvIII (SEQ ID NO:128);
- j. a fragment consisting of amino acid residues 6 to 49 of EGFRvIII (SEQ ID NO:130);
- k. a fragment consisting of amino acid residues 6 to 45 of EGFRvIII (SEQ ID NO:131);
- l. a fragment consisting of amino acid residues 6 to 37 of EGFRvIII (SEQ ID NO:132);
- m. a fragment consisting of amino acid residues 10 to 49 of EGFRvIII (SEQ ID NO:133);
- n. a fragment consisting of amino acid residues 10 to 45 of EGFRvIII (SEQ ID NO:134);
- o. a fragment consisting of amino acid residues 10 to 37 of EGFRvIII (SEQ ID NO:135);
- p. a fragment consisting of amino acid residues 15 to 49 of EGFRvIII (SEQ ID NO:136);
- q. A fragment consisting of amino acid residues 15 to 45 of EGFRvIII (SEQ ID NO:137);
- r. a fragment consisting of amino acid residues 19 to 76 of EGFRvIII (SEQ ID NO:138);

- s. a fragment consisting of amino acid residues 19 to 62 of EGFRvIII (SEQ ID NO:139);
 - t. a fragment consisting of amino acid residues 19 to 49 of EGFRvIII (SEQ ID NO:140);
 - u. a fragment consisting of amino acid residues 19 to 45 of EGFRvIII (SEQ ID NO:141);
 - v. a fragment consisting of amino acid residues 19 to 37 of EGFRvIII (SEQ ID NO:142),
- 5 and;

- w. any combination of the above fragments thereof,

wherein the antibody or antigen binding fragment thereof fails to bind a peptide comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 149.

10 The antibody or antigen-binding fragment thereof of the present disclosure may be capable of binding to a peptide comprising or consisting of an amino acid sequence selected from the group consisting of SEQ ID NO:145, SEQ ID NO:146, SEQ ID NO:147, SEQ ID NO:148, SEQ ID NO:150, SEQ ID NO:151, SEQ ID NO:152, SEQ ID NO:153, SEQ ID NO:155, SEQ ID NO:156, SEQ ID NO:157, SEQ ID NO:162, SEQ ID NO:164, SEQ ID NO:165 and combination thereof.

15 The antibody or antigen-binding fragment thereof may also be capable of binding to a peptide comprising or consisting of an amino acid sequence set forth in SEQ ID NO:160.

Other exemplary examples of the disclosure include antibodies or antigen-binding fragments thereof that specifically bind to EGFRvIII (SEQ ID NO:5) and that are capable of binding to an EGFRvIII fragment selected from the group consisting of:

- a. a fragment consisting of amino acid residues 15 to 37 of EGFRvIII (SEQ ID NO:6);
 - 20 b. a fragment consisting of amino acid residues 1 to 76 of EGFRvIII (SEQ ID NO:119);
 - c. a fragment consisting of amino acid residues 1 to 49 of EGFRvIII (SEQ ID NO:121);
 - d. a fragment consisting of amino acid residues 1 to 37 of EGFRvIII (SEQ ID NO:123);
 - e. a fragment consisting of amino acid residues 3 to 37 of EGFRvIII (SEQ ID NO:128);
- and;

- 25 f. any combination of the above fragments thereof,

wherein the antibodies or antigen binding fragments thereof fail to bind a peptide comprising or consisting of the amino acid sequence set forth in SEQ ID NO: 149.

30 The antibody or antigen-binding fragment thereof of the present disclosure may be capable of binding to a peptide comprising or consisting of an amino acid sequence selected from the group consisting of SEQ ID NO:145, SEQ ID NO:146, SEQ ID NO:147, SEQ ID NO:151, SEQ ID NO:152, SEQ ID NO:153, SEQ ID NO:155, SEQ ID NO:156, SEQ ID NO:157, SEQ ID NO:158, SEQ ID NO:160, SEQ ID NO:161, SEQ ID NO:162, SEQ ID NO:164, SEQ ID NO:165 and combination thereof. The antibody or antigen-binding fragment thereof may also be capable of

binding to a peptide comprising or consisting of an amino acid sequence set forth in SEQ ID NO:154. The antibody or antigen-binding fragment thereof may also be capable of binding to a peptide comprising or consisting of an amino acid sequence set forth in SEQ ID NO:159.

Yet other exemplary examples of the disclosure include antibodies or antigen-binding fragments thereof that specifically bind to EGFRvIII (SEQ ID NO:5) and that are capable of binding to an EGFRvIII fragment selected from the group consisting of:

- a. a fragment consisting of amino acid residues 15 to 37 of EGFRvIII (SEQ ID NO:6);
- b. a fragment consisting of amino acid residues 1 to 76 of EGFRvIII (SEQ ID NO:119);
- c. a fragment consisting of amino acid residues 1 to 62 of EGFRvIII (SEQ ID NO: 120);
- d. a fragment consisting of amino acid residues 1 to 49 of EGFRvIII (SEQ ID NO:121);
- e. a fragment consisting of amino acid residues 1 to 45 of EGFRvIII (SEQ ID NO:122);
- f. a fragment consisting of amino acid residues 1 to 37 of EGFRvIII (SEQ ID NO:123);
- g. a fragment consisting of amino acid residues 3 to 49 of EGFRvIII (SEQ ID NO:126);
- h. a fragment consisting of amino acid residues 3 to 45 of EGFRvIII (SEQ ID NO:127);
- i. a fragment consisting of amino acid residues 3 to 37 of EGFRvIII (SEQ ID NO:128);
- j. a fragment consisting of amino acid residues 6 to 49 of EGFRvIII (SEQ ID NO:130);
- k. a fragment consisting of amino acid residues 6 to 45 of EGFRvIII (SEQ ID NO:131);
- l. a fragment consisting of amino acid residues 6 to 37 of EGFRvIII (SEQ ID NO:132);
- m. a fragment consisting of amino acid residues 10 to 49 of EGFRvIII (SEQ ID NO:133);
- n. a fragment consisting of amino acid residues 10 to 45 of EGFRvIII (SEQ ID NO:134);
- o. a fragment consisting of amino acid residues 10 to 37 of EGFRvIII (SEQ ID NO:135);
- p. a fragment consisting of amino acid residues 15 to 49 of EGFRvIII (SEQ ID NO:136);
- q. a fragment consisting of amino acid residues 15 to 45 of EGFRvIII (SEQ ID NO:137);
- and;
- r. any combination of the above fragments thereof.

The antibodies or antigen-binding fragments thereof may also bind to:

- a. a fragment consisting of amino acid residues 19 to 49 of EGFRvIII (SEQ ID NO:140);
 - b. a fragment consisting of amino acid residues 19 to 37 of EGFRvIII (SEQ ID NO:142);
 - c. a fragment consisting of amino acid residues 28 to 45 of EGFRvIII (SEQ ID NO:143);
 - d. a fragment consisting of amino acid residues 28 to 37 of EGFRvIII (SEQ ID NO:144),
- or;
- any combination of the above fragments thereof.

The antibody or antigen-binding fragment thereof may also be capable of binding to a peptide comprising or consisting of an amino acid sequence selected from the group consisting SEQ ID NO:145, SEQ ID NO:147, SEQ ID NO:148, SEQ ID NO:150, SEQ ID NO:151, SEQ ID NO:152, SEQ ID NO:153, SEQ ID NO:155, SEQ ID NO:156, SEQ ID NO:157, SEQ ID NO:165 and combination thereof. The antibody or antigen-binding fragment thereof may also be capable of binding to a peptide comprising or consisting of an amino acid sequence set forth in SEQ ID NO:149.

Also provided are anti-EGFRvIII antibodies or antigen-binding fragments thereof that are able to bind an epitope comprising or involving amino acid residue Cys20 in said peptide. These include for example, anti-EGFRvIII antibodies or antigen-binding fragments that bind EGFRvIII and/or a peptide comprising an EGFRvIII fragment consisting of the amino acid sequence set forth in SEQ ID NO:6 but that are not able to bind a peptide comprising or consisting of the amino acid sequence SCVRAAGADSYEMEEDGVRKCKK (SEQ ID NO:149). Such antibodies or antigen binding fragments encompass, for example, the 4B3, 5D8 and 4E11 antibodies.

Also specifically encompassed by the present disclosure are anti-EGFRvIII antibodies or antigen-binding fragments thereof that are able to bind an epitope comprising or involving amino acid residue Cys35 in said peptide. These include for example, anti-EGFRvIII antibodies or antigen-binding fragments that bind EGFRvIII and/or a peptide comprising an EGFRvIII fragment consisting of the amino acid sequence set forth in SEQ ID NO:6 but that are not able to bind a peptide comprising or consisting of the amino acid sequence SCVRACGADSYEMEEDGVRKAKK (SEQ ID NO:163). Such antibodies or antigen binding fragments encompass, for example, the 4B3, 5D8, 9C9 and 4E11 antibodies.

Further encompassed by the present disclosure are anti-EGFRvIII antibodies or antigen-binding fragments thereof that are able to bind an epitope in a peptide comprising or involving amino acid residue Cys20 and Cys35 in said peptide. These include for example, anti-EGFRvIII antibodies or antigen-binding fragments that bind EGFRvIII and/or a peptide comprising or consisting of an EGFRvIII fragment set forth in SEQ ID NO:6 but that are not able to bind a peptide comprising or consisting of the amino acid sequence selected from SCVRAAGADSYEMEEDGVRKCKK (SEQ ID NO:149) or SCVRACGADSYEMEEDGVRKAKK (SEQ ID NO:163). Such antibodies or antigen binding fragments encompass, for example, the 4B3, 5D8 and 4E11 antibodies.

In addition to amino acid residues Cys20 and/or Cys35, the epitope to which the anti-EGFRvIII antibodies or antigen-binding fragments thereof bind or which are involved in its binding

may further include Glu26, Asp30, Gly31 and/or Arg33. The epitope may also further include Asp23 and/or Val32.

For example, anti-EGFRvIII antibodies or antigen-binding fragments thereof encompassed by the present disclosure include those that bind to an epitope comprising or
5 involving:

- Cys20, Glu26, Asp30, Gly31, Arg33 and Cys35, or;
- Cys20, Asp23, Glu26, Asp30, Gly31, Val32, Arg33 and Cys35.

In addition to amino acid residues Cys20 and/or Cys35, the epitope to which the anti-EGFRvIII antibodies or antigen-binding fragments thereof bind or involved in its binding may
10 further include Arg18 and/or Gly21. The epitope may also further include Glu26 and/or Gly31.

For example, the anti-EGFRvIII antibodies or antigen-binding fragments thereof encompassed by the present disclosure include those that bind to an epitope comprising or involving:

- Arg18, Cys20, Gly21 and Cys35, or;
- Arg18, Cys20, Gly21, Glu26, Gly31 and Cys35.

Additional anti-EGFRvIII antibodies or antigen-binding fragments thereof encompassed by the present disclosure include those that bind to an epitope comprising or involving:

- Cys16, Glu26, Gly31, Val32, Arg33, Lys34, Cys35 and Lys36, or;
- Cys16, Cys20, Glu26, Asp30, Gly31, Val32, Arg33, Lys34, Cys35 and Lys36.

Examples of the disclosure particularly include anti-EGFRvIII antibodies or antigen-binding fragments thereof that are able to compete with the 4E11 antibody or antigen-binding fragment thereof or that are able to compete with an antibody or antigen-binding fragment thereof that comprises the CDRs of the 4E11 antibody.

Other examples of the present disclosure include anti-EGFRvIII antibodies or antigen-binding fragments thereof that are able to compete with the 5G6 antibody or antigen-binding fragment thereof or that are able to compete with an antibody or antigen-binding fragment thereof that comprises the CDRs of the 5G6 antibody.

Further examples of the present disclosure include anti-EGFRvIII antibodies or antigen-binding fragments thereof that are able to compete with the 9C9 antibody or antigen-binding fragment thereof or that are able to compete with an antibody or antigen-binding fragment thereof that comprises the CDRs of the 9C9 antibody.

A particular example encompassed by the present disclosure relates to an anti-EGFRvIII antibody or antigen-binding fragment thereof comprising the CDRs of the 4E11 antibody.

The present disclosure provides anti-EGFRvIII antibodies or antigen binding fragments comprising a sequence selected from the group consisting of:

- 5 - an antibody or fragment thereof comprising CDRL1 (SEQ ID NO:38), CDRL2 (SEQ ID NO:39), CDRL3 (SEQ ID NO:40), CDRH1 (SEQ ID NO:43), CDRH2 (SEQ ID NO:44) and CDRH3 (SEQ ID NO:45) of the 4E11 antibody;
- an antibody or fragment thereof comprising CDRL1 (SEQ ID NO:8), CDRL2 (SEQ ID NO:9), CDR L3 (SEQ ID NO:10), CDRH1 (SEQ ID NO:13), CDRH2 (SEQ ID NO:14) and CDRH3
10 (SSEQ ID NO:15) of the 5G6 antibody;
- an antibody or fragment thereof comprising CDRL1 (SEQ ID NO:18), CDRL2 (SEQ ID NO:19), CDRL3 (SEQ ID NO:20), CDRH1 (SEQ ID NO:23), CDRH2 (SEQ ID NO:24) and CDRH3 (SEQ ID NO:25) of the 1A8 antibody;
- an antibody or fragment thereof comprising CDRL1 (SEQ ID NO:28), CDRL2 (SEQ ID NO:29), CDRL3 (SEQ ID NO:30), CDRH1 (SEQ ID NO:33), CDRH2 (SEQ ID NO: 34) and CDRH3
15 (SSEQ ID NO:35) of the 4B3 antibody;
- an antibody or fragment thereof comprising CDRL1 (SEQ ID NO:48), CDRL2 (SEQ ID NO:49), CDRL3 (SEQ ID NO:50), CDRH1 (SEQ ID NO:53), CDRH2 (SEQ ID NO:54) and CDRH3 (SEQ ID NO:55) of the 5D8 antibody;
- 20 - an antibody or fragment thereof comprising CDRL1 (SEQ ID NO:58), CDRL2 (SEQ ID NO:59), CDRL3 (SEQ ID NO:60), CDRH1 (SEQ ID NO:63), CDRH2 (SEQ ID NO:64), CDRH3 (SEQ ID NO:65) of the 9C9 antibody; and
- an antibody or fragment thereof comprising CDRL1 (SEQ ID NO: 68 or 73), CDRL2 (SEQ ID NO:69 or 74), CDRL3 (SEQ ID NO:70 or 75), CDRH1 (SEQ ID NO:78), CDRH2 (SEQ ID NO:79) and CDRH3 (SEQ ID NO:80) of the F266-11B1 (referred to herein as 11B1), F266-11C8 (referred to herein as 11C8), F266-11H5 (referred to herein as 11H5) and/or F266-11H3 (referred to herein as 11H3) antibodies.
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Examples encompassed by the present disclosure relates to an anti-EGFRvIII antibody or antigen-binding fragment thereof comprising the CDRs of the 5G6 antibody,

- 30 Another example encompassed by the present disclosure relates to an anti-EGFRvIII antibody or antigen-binding fragment thereof comprising the CDRs of the 1A8 antibody.

A further example encompassed by the present disclosure relates to an anti-EGFRvIII antibody or antigen-binding fragment thereof comprising the CDRs of the 4B3 antibody.

Another example encompassed by the present disclosure relates to an anti-EGFRvIII antibody or antigen-binding fragment thereof comprising the CDRs of the 5D8 antibody.

5 A further example encompassed by the present disclosure relates to an anti-EGFRvIII antibody or antigen-binding fragment thereof comprising the CDRs of the 9C9 antibody.

Another example encompassed by the present disclosure relates to an anti-EGFRvIII antibody or antigen-binding fragment thereof comprising the CDRs of the 11B1 or of the 11C8 antibody.

10 The present disclosure provides anti-EGFRvIII antibodies or antigen-binding fragments selected from the group consisting of:

- an antibody or antigen-binding fragment thereof comprising CDR sequences consisting of CDRL1 (SEQ ID NO:8), CDRL2 (SEQ ID NO:9), CDRL3 (SEQ ID NO:10), CDRH1 (SEQ ID NO:13), CDRH2 (SEQ ID NO:14), CDRH3 (SEQ ID NO:15);
- 15 - an antibody or antigen-binding fragment thereof comprising CDR sequences consisting of CDRL1 (SEQ ID NO:18), CDRL2 (SEQ ID NO:19), CDRL3 (SEQ ID NO:20), CDRH1 (SEQ ID NO:23), CDRH2 (SEQ ID NO:24), CDRH3 (SEQ ID NO:25);
- an antibody or antigen-binding fragment thereof comprising CDR sequences consisting of CDRL1 (SEQ ID NO:28), CDRL2 (SEQ ID NO:29), CDRL3 (SEQ ID NO:30), CDRH1 (SEQ ID NO:33), CDRH2 (SEQ ID NO:34), CDRH3 (SEQ ID NO:35);
- 20 - an antibody or antigen-binding fragment thereof comprising CDR sequences consisting of CDRL1 (SEQ ID NO:38), CDRL2 (SEQ ID NO:39), CDRL3 (SEQ ID NO:40), CDRH1 (SEQ ID NO:43), CDRH2 (SEQ ID NO:44), CDRH3 (SEQ ID NO:45);
- an antibody or antigen-binding fragment thereof comprising CDR sequences consisting of CDRL1 (SEQ ID NO:48), CDRL2 (SEQ ID NO:49), CDRL3 (SEQ ID NO:50), CDRH1 (SEQ ID NO:53), CDRH2 (SEQ ID NO:54), CDRH3 (SEQ ID NO:55);
- 25 - an antibody or antigen-binding fragment thereof comprising CDR sequences consisting of CDRL1 (SEQ ID NO:58), CDRL2 (SEQ ID NO:59), CDRL3 (SEQ ID NO:60), CDRH1 (SEQ ID NO:63), CDRH2 (SEQ ID NO:64), CDRH3 (SEQ ID NO:65);

- an antibody or fragment thereof comprising CDR sequences consisting of CDRL1 (SEQ ID NO:68), CDRL2 (SEQ ID NO:69), CDRL3 (SEQ ID NO:70), CDRH1 (SEQ ID NO:78), CDRH2 (SEQ ID NO:79), CDRH3 (SEQ ID NO:80), and;
- an antibody or antigen-binding fragment thereof comprising CDR sequences consisting of CDRL1 (SEQ ID NO:73), CDRL2 (SEQ ID NO:74), CDRL3 (SEQ ID NO:75), CDRH1 (SEQ ID NO:78), CDRH2 (SEQ ID NO:79), CDRH3 (SEQ ID NO:80).

The present disclosure provides in some examples, an antigen-binding agent such as an antibody or an antigen-binding fragment thereof, CAR, BiTE and the like which specifically binds to EGFRvIII and which may comprise for example:

- a. a light chain variable region which may comprise a CDRL1 having the amino acid sequence set forth in SEQ ID NO:8, a CDRL2 having the amino acid sequence set forth in SEQ ID NO:9 and a CDRL3 having the amino acid sequence set forth in SEQ ID NO:10 and a heavy chain variable region which may comprise a CDRH1 having the amino acid sequence set forth in SEQ ID NO:13, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:14 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:15;
- b. a light chain variable region which may comprise a CDRL1 having the amino acid sequence set forth in SEQ ID NO:18, a CDRL2 having the amino acid sequence set forth in SEQ ID NO:19 and a CDRL3 having the amino acid sequence set forth in SEQ ID NO:20 and a heavy chain variable region which may comprise a CDRH1 having the amino acid sequence set forth in SEQ ID NO:23, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:24 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:25;
- c. a light chain variable region which may comprise a CDRL1 having the amino acid sequence set forth in SEQ ID NO:28, a CDRL2 having the amino acid sequence set forth in SEQ ID NO:29 and a CDRL3 having the amino acid sequence set forth in SEQ ID NO:30 and a heavy chain variable region which may comprise a CDRH1 having the amino acid sequence set forth in SEQ ID NO:33, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:34 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:35;
- d. a light chain variable region which may comprise a CDRL1 having the amino acid sequence set forth in SEQ ID NO:38, a CDRL2 having the amino acid sequence

- 5 set forth in SEQ ID NO:39 and a CDRL3 having the amino acid sequence set forth in SEQ ID NO:40 and a heavy chain variable region which may comprise a CDRH1 having the amino acid sequence set forth in SEQ ID NO:43, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:44 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:45;
- 10 e. a light chain variable region which may comprise a CDRL1 having the amino acid sequence set forth in SEQ ID NO:48, a CDRL2 having the amino acid sequence set forth in SEQ ID NO:49 and a CDRL3 having the amino acid sequence set forth in SEQ ID NO:50 and a heavy chain variable region which may comprise a CDRH1 having the amino acid sequence set forth in SEQ ID NO:53, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:54 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:55;
- 15 f. a light chain variable region which may comprise a CDRL1 having the amino acid sequence set forth in SEQ ID NO:58, a CDRL2 having the amino acid sequence set forth in SEQ ID NO:59 and a CDRL3 having the amino acid sequence set forth in SEQ ID NO:60 and a heavy chain variable region which may comprise a CDRH1 having the amino acid sequence set forth in SEQ ID NO:63, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:64 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:65;
- 20 g. a light chain variable region which may comprise a CDRL1 having the amino acid sequence set forth in SEQ ID NO:68, a CDRL2 having the amino acid sequence set forth in SEQ ID NO:69 and a CDRL3 having the amino acid sequence set forth in SEQ ID NO:70 and a heavy chain variable region which may comprise a CDRH1 having the amino acid sequence set forth in SEQ ID NO:78, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:79 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:80, or;
- 25 h. a light chain variable region which may comprise a CDRL1 having the amino acid sequence set forth in SEQ ID NO:73, a CDRL2 having the amino acid sequence set forth in SEQ ID NO:74 and a CDRL3 having the amino acid sequence set forth in SEQ ID NO:75 and a heavy chain variable region which may comprise a CDRH1 having the amino acid sequence set forth in SEQ ID NO:78, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:79 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:80.
- 30

The present disclosure provides in additional examples, an antigen-binding agent such as an antibody or an antigen-binding fragment thereof, CAR, BiTE and the like which specifically binds to EGFRvIII which may comprise:

- 5 a. A light chain variable region which may comprise an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO: 118 or substantially identical to SEQ ID NO:118 and/or a heavy chain variable region which may comprise an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO:116 or substantially identical to SEQ ID NO:116;
- 10 b. A light chain variable region which may comprise an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO: 115 or substantially identical to SEQ ID NO:115 and a heavy chain variable region which may comprise an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO:116 or substantially identical to SEQ ID NO:116,
- 15 or;
- 20 c. A light chain variable region which may comprise an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO: 118 or substantially identical to SEQ ID NO:118 and a heavy chain variable region which may comprise an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO:62 or substantially identical to SEQ ID NO:62.

In an example thereof, antigen-binding agents having light chain at least 80% identical or substantially identical to the amino acid sequence set forth in SEQ ID NO: 115 or SEQ ID NO: 118 may have CDRs identical to those of SEQ ID NO: 115 or SEQ ID NO: 118 respectively.

25 In an example thereof, antigen-binding agents having heavy chain at least 80% identical or substantially identical to the amino acid sequence set forth in SEQ ID NO: 62 or SEQ ID NO: 116 may have CDRs identical to those of SEQ ID NO: 62 or SEQ ID NO: 116 respectively.

The present disclosure provides in further examples, an antigen-binding agent such as an antibody or an antigen-binding fragment thereof, CAR, BiTE and the like which specifically binds to EGFRvIII which may comprise:

- 30 a. A light chain variable region which may comprise an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO:7 or substantially identical to SEQ ID NO:7 and a heavy chain variable region which may comprise

an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO:12 or substantially identical to SEQ ID NO:12;

- 5 b. A light chain variable region which may comprise an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO: 17 or substantially identical to SEQ ID NO:17 and a heavy chain variable region which may comprise an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO:22 or substantially identical to SEQ ID NO:22;
- 10 c. A light chain variable region which may comprise an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO:27 or substantially identical to SEQ ID NO:27 and a heavy chain variable region which may comprise an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO:32 or substantially identical to SEQ ID NO:32;
- 15 d. A light chain variable region which may comprise an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO: 37 or substantially identical to SEQ ID NO:37 and a heavy chain variable region which may comprise an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO:42 or substantially identical to SEQ ID NO:42;
- 20 e. A light chain variable region which may comprise an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO:47 or substantially identical to SEQ ID NO:47 and a heavy chain variable region which may comprise an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO:52 or substantially identical to SEQ ID NO:52;
- 25 f. A light chain variable region which may comprise an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO:57 or substantially identical to SEQ ID NO:57 and a heavy chain variable region which may comprise an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO:62 or substantially identical to SEQ ID NO:62;
- 30 g. A light chain variable region which may comprise an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO:67 or substantially identical to SEQ ID NO:67 and a heavy chain variable region which may comprise an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO:77 or substantially identical to SEQ ID NO: 77,

at least 80% identical to the amino acid set forth in SEQ ID NO:92 or substantially identical to SEQ ID NO:92 or at least 80% identical to the amino acid sequence set forth in SEQ ID NO:102 or substantially identical to SEQ ID NO:102, or;

- 5 h. A light chain variable region which may comprise an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO:72 or substantially identical to SEQ ID NO: 72 and a heavy chain variable region which may comprise an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO:77 or substantially identical to SEQ ID NO: 77 or at least 80% identical to the amino acid set forth in SEQ ID NO:92 or substantially identical to SEQ ID NO:92.

10 The light chain variable regions, light chains, heavy chain variable regions or heavy chains which may comprise an amino acid sequence at least 80% identical to that of given antibody may have CDRs that are identical to that antibody. In an example of the present disclosure, the VL and VH sequences of the antibodies and antigen-binding fragments provided in the present disclosure may comprise a sequence substantially identical to the VL and VH sequences provided herein, or may comprise a sequence having at least 80%, at least 90%, or at least 95% sequence identity, wherein sequence variation is preferably outside the CDRs of the VL and VH sequences provided.

15 Moreover, the present disclosure specifically provides antigen-binding agent such as an antibody or an antigen-binding fragment thereof, CAR, BiTE and the like which specifically binds to EGFRvIII and which may comprise:

- 20 a. A light chain which may comprise an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO:108 or substantially identical to SEQ ID NO:108 and a heavy chain which may comprise an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO:107 or substantially identical to SEQ ID NO:107, or;
- 25 b. A light chain which may comprise an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO:110 or substantially identical to SEQ ID NO:110 and a heavy chain which may comprise an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO:109 or substantially identical to SEQ ID NO:109.
- 30

The present disclosure particularly provides antigen-binding agent such as anti-EGFRvIII antibodies or an antigen-binding fragments thereof, CARs, BiTEs and the like which may comprise:

- 5 a. a CDRL1 having the amino acid sequence set forth in SEQ ID NO:38, a CDRL2 having the amino acid sequence set forth in SEQ ID NO:39 and a CDRL3 having the amino acid sequence set forth in SEQ ID NO:40, a CDRH1 having the amino acid sequence set forth in SEQ ID NO:43, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:44 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:45;
- 10 b. A light chain variable region which may comprise an amino acid sequence at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical or identical to the amino acid sequence set forth in SEQ ID NO: 37 and a heavy chain variable region which may comprise an amino acid sequence at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical or
15 identical to the amino acid sequence set forth in SEQ ID NO:42 or;
- 20 c. A light chain which may comprise an amino acid sequence at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical or identical to the amino acid sequence set forth in SEQ ID NO:108 and a heavy chain which may comprise an amino acid sequence at least 80% identical, at least 85% identical, at least 90% identical, at least 95% identical or identical to the amino acid
25 sequence set forth in SEQ ID NO:107.

In accordance with the present disclosure, the antibody or antigen-binding fragment thereof of item b. or c. may have CDRs identical or substantially identical to those set forth in SEQ ID NOs:38, 39, 40, 43, 44 and 45.

25 In accordance with the present disclosure, the antibody or antigen-binding fragment thereof may have, for example, an affinity of less than 100 nM such as for example, an affinity of 50 nM or less, 20 nM or less, 10 nM or less, 5 nM or less, etc.

30 Exemplary examples of the present disclosure include antibodies or antigen-binding fragments thereof which may comprise a human IgG constant region. Antibodies or antigen-binding fragments of the present disclosure may comprise, for example and without limitation, a human IgG1 constant region or a human IgG2 constant region.

In an exemplary example, the antigen-binding agents disclosed herein may comprise humanized framework regions.

In accordance with the present disclosure, the antibody or antigen-binding fragment thereof may be monoclonal antibody, a polyclonal antibody, a humanized antibody, a chimeric antibody, a human antibody, a single chain antibody, or a multispecific antibody (e.g., a bispecific antibody).

Bispecific antibodies or antigen-binding fragments thereof of the present disclosure includes those that may comprise a first arm that specifically binds to a first human EGFRvIII epitope and a second arm that specifically binds to a second (non-overlapping) human EGFRvIII epitope (e.g. a biparatopic antibody).

Additional examples of bispecific antibodies or antigen-binding fragments thereof of the present disclosure includes those that may comprise a first arm that specifically binds to a first human EGFRvIII epitope and a second arm that specifically binds to another antigen.

The bispecific antibody or antigen-binding fragment thereof of the present disclosure include bispecific immune cell engagers such as those comprising a first arm that specifically binds to human EGFRvIII and a second arm that specifically binds to CD3.

In accordance with the present disclosure, the antigen-binding fragment comprises, for example, a scFv, a Fab, a Fab' or a (Fab')₂.

In a further example, the present disclosure provides anti-EGFRvIII antibodies or antigen-binding fragments thereof which may be linked to a cargo molecule.

In accordance with the present disclosure, the cargo molecule may comprise a therapeutic moiety, such as for example, a cytotoxic agent, a cytostatic agent, an anti-cancer agent or a radiotherapeutic. In particular examples of the disclosure, the antibody drug conjugates may comprise a cytotoxic agent. Another particular example of the disclosure relates to antibody drug conjugates comprising a radiotherapeutic.

In accordance with the present disclosure, the cargo molecule may comprise a detectable moiety.

The antibodies or antigen-binding fragments thereof of the present disclosure may be provided in the form of pharmaceutical compositions. The pharmaceutical composition may comprise, for example, a pharmaceutically acceptable carrier, diluent or excipient.

The present disclosure additionally provides nucleic acid molecules encoding a light chain variable region and/or a heavy chain variable region of the antibody or antigen-binding fragment thereof disclosed herein.

Exemplary examples of nucleic acid molecules of the present disclosure include:

- 5 a. a nucleic acid molecule which may comprise a sequence at least 80% identical to the nucleotide sequence set forth in SEQ ID NO: 11 and/or a nucleic acid molecule which may comprise a sequence at least 80% identical to the nucleotide sequence set forth in SEQ ID NO:16 or that encodes an amino acid sequence substantially identical to the amino acid sequence of SEQ ID NO: 7 and/or SEQ ID NO: 12;
- 10 b. a nucleic acid molecule which may comprise a sequence at least 80% identical to the nucleotide sequence set forth in SEQ ID NO:21 and/or a nucleic acid molecule which may comprise a sequence at least 80% identical to the nucleotide sequence set forth in SEQ ID NO:26 or that encodes an amino acid sequence substantially identical to the amino acid sequence of SEQ ID NO:17 and/or SEQ ID NO:22;
- 15 c. a nucleic acid molecule which may comprise a sequence at least 80% identical to the nucleotide sequence set forth in SEQ ID NO:31 and/or a nucleic acid molecule which may comprise a sequence at least 80% identical to the nucleotide sequence set forth in SEQ ID NO:36 or that encodes an amino acid sequence substantially identical to the amino acid sequence of SEQ ID NO:27 and/or SEQ ID NO:32
- 20 d. a nucleic acid molecule which may comprise a sequence at least 80% identical to the nucleotide sequence set forth in SEQ ID NO:41 and/or a nucleic acid molecule which may comprise a sequence at least 80% identical to the nucleotide sequence set forth in SEQ ID NO:46 or that encodes an amino acid sequence substantially identical to the sequence of SEQ ID NO:37 and/or SEQ ID NO:42;
- 25 e. a nucleic acid molecule which may comprise a sequence at least 80% identical to the nucleotide sequence set forth in SEQ ID NO:51 and/or a nucleic acid molecule which may comprise a sequence at least 80% identical to the nucleotide sequence set forth in SEQ ID NO:56 or that encodes an amino acid sequence substantially identical to the sequence of SEQ ID NO:47 and/or SEQ ID NO:52;
- 30 f. a nucleic acid molecule which may comprise a sequence at least 80% identical to the nucleotide sequence set forth in SEQ ID NO:61 and/or a nucleic acid molecule which may comprise a sequence at least 80% identical to the nucleotide sequence

set forth in SEQ ID NO:66 or that encodes an amino acid sequence substantially identical to the sequence of SEQ ID NO:57 and/or SEQ ID NO:62;

- 5 g. a nucleic acid molecule which may comprise the nucleotide sequence a sequence at least 80% identical to set forth in SEQ ID NO:71 and/or a nucleic acid molecule which may comprise a sequence at least 80% identical to the nucleotide sequence set forth in SEQ ID NO:81 or that encodes an amino acid sequence substantially identical to the sequence of SEQ ID NO:67 and/or SEQ ID NO:77;
- 10 h. a nucleic acid molecule which may comprise the nucleotide sequence a sequence at least 80% identical to set forth in SEQ ID NO:76 and/or a nucleic acid molecule which may comprise a sequence at least 80% identical to the nucleotide sequence set forth in SEQ ID NO:81 or that encodes an amino acid sequence substantially identical to the sequence of SEQ ID NO:72 and/or SEQ ID NO:77;
- 15 i. a nucleic acid molecule which may comprise a sequence at least 80% identical to the nucleotide sequence set forth in SEQ ID NO:86 and/or a nucleic acid molecule which may comprise a sequence at least 80% identical to the nucleotide sequence set forth in SEQ ID NO:96 or that encodes an amino acid sequence substantially identical to the sequence of SEQ ID NO:82 and/or SEQ ID NO:92;
- 20 j. a nucleic acid molecule which may comprise a sequence at least 80% identical to the nucleotide sequence set forth in SEQ ID NO:91 and/or a nucleic acid molecule which may comprise a sequence at least 80% identical to the nucleotide sequence set forth in SEQ ID NO:96 or that encodes an amino acid sequence substantially identical to the sequence of SEQ ID NO:87 and/or SEQ ID NO:92, or;
- 25 k. a nucleic acid molecule which may comprise a sequence at least 80% identical to the nucleotide sequence set forth in SEQ ID NO:101 and/or a nucleic acid molecule which may comprise a sequence at least 80% identical to the nucleotide sequence set forth in SEQ ID NO:106 or that encodes an amino acid sequence substantially identical to the sequence of SEQ ID NO:97 and/or SEQ ID NO:102.

In a further example, the present disclosure provides a kit comprising at least one of the antibody or antigen-binding fragments thereof disclosed herein.

- 30 Additional examples of the present disclosure relate to a vector or set of vectors which may comprise a nucleic acid encoding a light chain variable region and a heavy chain variable region of the antibody or antigen-binding fragment disclosed herein. The nucleic acids encoding

the light chain variable region or the light chain and the heavy chain variable region or the heavy chain may be provided on the same vector or on separate vectors.

Further examples of the present disclosure relate to isolated cells comprising the vector or set of vectors described herein. The isolated cells may be capable of expressing, assembling
5 and/or secreting the antibody or antigen-binding fragment thereof.

Other examples of the present disclosure relate to a kit comprising a first vial comprising a nucleic acid or vector encoding the light chain of the antibody or antigen-binding fragment thereof of the present disclosure and a second vial comprising a nucleic acid or vector encoding the heavy chain of the antibody or antigen-binding fragment thereof.

10 Additional examples of the present disclosure relate to a method of treating cancer which comprises cells (e.g., tumor cells) expressing EGFRvIII. The method may comprise administering the antibody or antigen-binding fragment thereof described herein to subject in need. Antibody or antigen-binding fragments that are conjugated to a therapeutic moiety (ADCs) are particularly contemplated in methods of treatments.

15 The present disclosure additionally relates to the use of the antibody or antigen-binding fragment thereof described herein in the treatment of cancer.

The present disclosure further relates to the use of the antibody or antigen-binding fragment thereof described herein in the manufacture of a medicament for the treatment of cancer.

20 In accordance with the present disclosure, the antibody or antigen-binding fragment thereof may be used in combination with a chemotherapeutic.

In accordance with the present disclosure, the subject in need has or is suspected of having glioblastoma multiforme.

25 Further in accordance with the present disclosure, the subject in need has or is suspected of having a carcinoma.

Further examples of the present disclosure relate to a method of detecting EGFRvIII. The method may comprise contacting a sample comprising or suspected of comprising EGFRvIII with the antibody or antigen-binding fragment described herein.

30 Additional examples of the present disclosure relate to a method of making the antibody or antigen-binding fragment thereof of the present disclosure by culturing a cell comprising nucleic acids or vectors encoding the antibody or antigen-binding fragment so that the antibody or

antigen-binding fragment thereof is produced. The antibody or antigen-binding fragment thereof may thus be isolated and/or purified.

The method may further comprise conjugating the antibody or antigen-binding fragment thereof with a cargo molecule, such as for example, a therapeutic moiety.

5 Further scope, applicability and advantages of the present disclosure will become apparent from the non-restrictive detailed description given hereinafter. It should be understood, however, that this detailed description, while indicating exemplary examples of the disclosure, is given by way of example only, with reference to the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

10 **Figures 1A-1K** represent histograms obtained using flow cytometry on supernatants of selected hybridomas on U87MG cell lines overexpressing wild type human EGFR (U87WT) or EGFRvIII (U87vIII) as indicated.

Figures 2A-2D represent dose-response binding curves obtained from flow cytometry data of purified monoclonal antibodies on U87MG glioblastoma cell lines overexpressing EGFR
15 vIII (U87MG EGFR vIII) or wild type EGFR (U87MG EGFR wt) as indicated.

Figure 3 is an alignment between the amino acid sequence of the extracellular domains of wild type human EGFR and EGFRvIII. Identical amino acids are underlined.

Figures 4A and 4B show the results of anti-hEGFRvIII mAbs binding properties to various fragments of the EGFRvIII displayed on yeast cells (+++ represents 95% +/- 5% positive yeast
20 cells which is characterized by positive antibody binding with high affinity; ++ represents 70% +/- 20% positive yeast cells which is characterized by positive antibody binding with medium affinity; + represents 30% +/- 20% positive yeast cells which is characterized by positive antibody binding with low affinity; (+) represents 5-9% positive yeast cells which is characterized by positive antibody binding with very low affinity; -/+ represents less than 5% positive yeast cells which is
25 characterized by ambiguous antibody binding, - represents 0% positive yeast cells which is characterized by no binding; nt = not tested.

Figure 5A is an alignment between the amino acid sequence of 11B1 (SEQ ID NO: 77), 11C8 (SEQ ID NO:92) and 11H3 (SEQ ID NO:102) heavy chain variable regions where " * " means that the residues in that column are identical in all sequences in the alignment, " : " means
30 that conserved substitutions have been observed (Sievers F. et al., Molecular Systems Biology, 11 Oct 2011, 7:539).

Figure 5B is an alignment between the amino acid sequence of 4B3 (SEQ ID NO:27) and 5D8 (SE ID NO:47) light chain variable regions, where " * " means that the residues in that column are identical in all sequences in the alignment, " : " means that conserved substitutions have been observed and " . " means that semi-conserved substitutions are observed.

5 **Figure 5C** is an alignment between the amino acid sequence of 4B3 (SEQ ID NO:32) and 5D8 (SEQ ID NO:52) heavy chain variable regions.

CLAIMS:

1. An antigen-binding agent comprising an antigen-binding domain of an antibody that specifically binds to epidermal growth factor receptor variant III (EGFRvIII), wherein the antigen-binding domain comprises:

- 5 a. a light chain variable region comprising a CDRL1 having the amino acid sequence set forth in SEQ ID NO:38, a CDRL2 having the amino acid sequence set forth in SEQ ID NO:39 and a CDRL3 having the amino acid sequence set forth in SEQ ID NO:40 and a heavy chain variable region comprising a CDRH1 having the amino acid sequence set forth in SEQ ID NO:43, a CDRH2 having the amino acid
10 sequence set forth in SEQ ID NO:44 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:45;
- b. a light chain variable region comprising a CDRL1 having the amino acid sequence of SEQ ID NO:8, a CDRL2 sequence of SEQ ID NO:9 and a CDRL3 sequence of
15 SEQ ID NO:10 and a heavy chain variable region comprising a CDRH1 sequence of SEQ ID NO:13, a CDRH2 sequence of SEQ ID NO:14 and a CDRH3 sequence of SEQ ID NO:15;
- c. a light chain variable region comprising a CDRL1 having the amino acid sequence set forth in SEQ ID NO:18, a CDRL2 having the amino acid sequence set forth in
20 SEQ ID NO:19 and a CDRL3 having the amino acid sequence set forth in SEQ ID NO:20 and a heavy chain variable region comprising a CDRH1 having the amino acid sequence set forth in SEQ ID NO:23, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:24 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:25;
- d. a light chain variable region comprising a CDRL1 having the amino acid sequence set forth in SEQ ID NO:28, a CDRL2 having the amino acid sequence set forth in
25 SEQ ID NO:29 and a CDRL3 having the amino acid sequence set forth in SEQ ID NO:30 and a heavy chain variable region comprising a CDRH1 having the amino acid sequence set forth in SEQ ID NO:33, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:34 and a CDRH3 having the amino acid
30 sequence set forth in SEQ ID NO:35;

- 5 e. a light chain variable region comprising a CDRL1 having the amino acid sequence set forth in SEQ ID NO:48, a CDRL2 having the amino acid sequence set forth in SEQ ID NO:49 and a CDRL3 having the amino acid sequence set forth in SEQ ID NO:50 and a heavy chain variable region comprising a CDRH1 having the amino acid sequence set forth in SEQ ID NO:53, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:54 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:55;
- 10 f. a light chain variable region comprising a CDRL1 having the amino acid sequence set forth in SEQ ID NO:58, a CDRL2 having the amino acid sequence set forth in SEQ ID NO:59 and a CDRL3 having the amino acid sequence set forth in SEQ ID NO:60 and a heavy chain variable region comprising a CDRH1 having the amino acid sequence set forth in SEQ ID NO:63, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:64 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:65;
- 15 g. a light chain variable region comprising a CDRL1 having the amino acid sequence set forth in SEQ ID NO:68, a CDRL2 having the amino acid sequence set forth in SEQ ID NO:69 and a CDRL3 having the amino acid sequence set forth in SEQ ID NO:70 and a heavy chain variable region comprising a CDRH1 having the amino acid sequence set forth in SEQ ID NO:78, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:79 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:80; or
- 20
- 25 h. a light chain variable region comprising a CDRL1 having the amino acid sequence set forth in SEQ ID NO:73, a CDRL2 having the amino acid sequence set forth in SEQ ID NO:74 and a CDRL3 having the amino acid sequence set forth in SEQ ID NO:75 and a heavy chain variable region comprising a CDRH1 having the amino acid sequence set forth in SEQ ID NO:78, a CDRH2 having the amino acid sequence set forth in SEQ ID NO:79 and a CDRH3 having the amino acid sequence set forth in SEQ ID NO:80.
2. The antigen-binding agent of claim 1, wherein the antigen binding agent comprises;
- 30 a. a light chain variable region comprising an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO: 37 or substantially identical to SEQ ID NO:37 and a heavy chain variable region comprising an amino

acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO:42 or substantially identical to SEQ ID NO:42;

- 5 b. a light chain variable region comprising a sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO:7 or substantially identical to SEQ ID NO:7 and a heavy chain variable region comprising a sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO:12 or substantially identical to SEQ ID NO:12;
- 10 c. a light chain variable region comprising an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO: 17 or substantially identical to SEQ ID NO:17 and a heavy chain variable region comprising an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO:22 or substantially identical to SEQ ID NO:22;
- 15 d. a light chain variable region comprising an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO:27 or substantially identical to SEQ ID NO:27 and a heavy chain variable region comprising an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO:32 or substantially identical to SEQ ID NO:32;
- 20 e. a light chain variable region comprising an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO:47 or substantially identical to SEQ ID NO:47 and a heavy chain variable region comprising an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO:52 or substantially identical to SEQ ID NO:52;
- 25 f. a light chain variable region comprising an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO:57 or substantially identical to SEQ ID NO:57 and a heavy chain variable region comprising an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO:62 or substantially identical to SEQ ID NO:62;
- 30 g. a light chain variable region comprising an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO:67 or substantially identical to SEQ ID NO:67 and a heavy chain variable region comprising an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO:77 or substantially identical to SEQ ID NO:77, the amino acid set forth in

SEQ ID NO:92 or substantially identical to SEQ ID NO:92 or the amino acid sequence set forth in SEQ ID NO:102 or substantially identical to SEQ ID NO:102; or

- 5 h. a light chain variable region comprising an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO:72 or substantially identical to SEQ ID NO: 72 and a heavy chain variable region comprising an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO:77 or substantially identical to SEQ ID NO:77 or the amino acid set forth in SEQ ID NO:92 or substantially identical to SEQ ID NO:92.
- 10 3. The antigen-binding agent of claim 1 or 2, wherein the antigen-binding agent is an antibody or an antigen-binding fragment thereof.
4. The antigen-binding agent of claim 3, wherein the antibody is a monoclonal antibody, a polyclonal antibody, a humanized antibody, a chimeric antibody, a human antibody, a single chain antibody, or a multispecific antibody.
- 15 5. The antigen-binding agent of claim 3, wherein the antibody or antigen-binding fragment thereof comprises:
- 20 a. a light chain variable region comprising an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO: 118 or substantially identical to SEQ ID NO:118 and a heavy chain variable region comprising an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO:116 or substantially identical to SEQ ID NO:116;
- 25 b. a light chain variable region comprising an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO: 115 or substantially identical to SEQ ID NO:115 and a heavy chain variable region comprising an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO:116 or substantially identical to SEQ ID NO:116; or
- 30 c. a light chain variable region comprising an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO: 118 or substantially identical to SEQ ID NO:118 and a heavy chain variable region comprising an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO:62 or substantially identical to SEQ ID NO:62.

6. The antigen-binding agent of claim 3, wherein the antibody or antigen-binding fragment thereof comprises:

- 5 a. a light chain variable region comprising a sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO:7 or substantially identical to SEQ ID NO:7 and a heavy chain variable region comprising a sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO:12 or substantially identical to SEQ ID NO:12;
- 10 b. a light chain variable region comprising an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO: 17 or substantially identical to SEQ ID NO:17 and a heavy chain variable region comprising an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO:22 or substantially identical to SEQ ID NO:22;
- 15 c. a light chain variable region comprising an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO:27 or substantially identical to SEQ ID NO:27 and a heavy chain variable region comprising an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO:32 or substantially identical to SEQ ID NO:32;
- 20 d. a light chain variable region comprising an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO: 37 or substantially identical to SEQ ID NO:37 and a heavy chain variable region comprising an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO:42 or substantially identical to SEQ ID NO:42;
- 25 e. a light chain variable region comprising an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO:47 or substantially identical to SEQ ID NO:47 and a heavy chain variable region comprising an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO:52 or substantially identical to SEQ ID NO:52;
- 30 f. a light chain variable region comprising an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO:57 or substantially identical to SEQ ID NO:57 and a heavy chain variable region comprising an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO:62 or substantially identical to SEQ ID NO:62;

- 5 g. a light chain variable region comprising an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO:67 or substantially identical to SEQ ID NO:67 and a heavy chain variable region comprising an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO:77 or substantially identical to SEQ ID NO:77, the amino acid set forth in SEQ ID NO:92 or substantially identical to SEQ ID NO:92 or the amino acid sequence set forth in SEQ ID NO:102 or substantially identical to SEQ ID NO:102; or
- 10 h. a light chain variable region comprising an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO:72 or substantially identical to SEQ ID NO: 72 and a heavy chain variable region comprising an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO:77 or substantially identical to SEQ ID NO:77 or the amino acid set forth in SEQ ID NO:92 or substantially identical to SEQ ID NO:92.
- 15 7. The antigen-binding agent of any one of claims 3 to 6, wherein the antibody or antigen-binding fragment thereof comprises a human IgG1 constant region, a human IgG2 constant region, or both.
8. The antigen-binding agent of claim 3, wherein the antibody or antigen-binding fragment thereof comprises a sequence selected from the group consisting of:
- 20 a. A light chain comprising an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO:108 or substantially identical to SEQ ID NO:108 and a heavy chain comprising an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO:107 or substantially identical to SEQ ID NO:107; and
- 25 b. A light chain comprising an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO:110 or substantially identical to SEQ ID NO:110 and a heavy chain comprising an amino acid sequence at least 80% identical to the amino acid sequence set forth in SEQ ID NO:109 or substantially identical to SEQ ID NO:109.
- 30 9. The antigen-binding agent of any one of claims 3 to 8, wherein the antigen-binding fragment comprises a scFv, a Fab, a Fab' or a (Fab')₂.

10. The antigen-binding agent of any one of claims 3 to 9, wherein the antibody or antigen-binding fragment thereof is linked to a cargo molecule.
11. The antigen-binding agent of claim 10, wherein the cargo molecule comprises a therapeutic moiety.
- 5 12. The antigen-binding agent of claim 11, wherein the therapeutic moiety comprises a cytotoxic agent, a cytostatic agent, an anti-cancer agent or a radiotherapeutic.
13. A pharmaceutical composition comprising the antigen-binding agent of any one of claims 1 to 12 and a pharmaceutically acceptable carrier, diluent or excipient.
- 10 14. A nucleic acid molecule encoding a light chain variable region and/or a heavy chain variable region of the antigen-binding agent of any one of claims 1 to 9.
15. The nucleic acid molecule of claim 14, wherein the nucleic acid molecule is selected from the group consisting of:
- 15 a. a nucleic acid molecule comprising a sequence at least 80% identical to the nucleotide sequence set forth in SEQ ID NO:41 and/or a nucleic acid molecule comprising a sequence at least 80% identical to the nucleotide sequence set forth in SEQ ID NO:46;
 - 20 b. a nucleic acid molecule comprising a sequence at least 80% identical to the nucleotide sequence set forth in SEQ ID NO: 11 and/or a nucleic acid molecule comprising a sequence at least 80% identical to the nucleotide sequence set forth in SEQ ID NO:16;
 - 25 c. a nucleic acid molecule comprising a sequence at least 80% identical to the nucleotide sequence set forth in SEQ ID NO:21 and/or a nucleic acid molecule comprising a sequence at least 80% identical to the nucleotide sequence set forth in SEQ ID NO:26;
 - d. a nucleic acid molecule comprising a sequence at least 80% identical to the nucleotide sequence set forth in SEQ ID NO:31 and/or a nucleic acid molecule comprising a sequence at least 80% identical to the nucleotide sequence set forth in SEQ ID NO:36;
 - 30 e. a nucleic acid molecule comprising a sequence at least 80% identical to the nucleotide sequence set forth in SEQ ID NO:51 and/or a nucleic acid molecule

comprising a sequence at least 80% identical to the nucleotide sequence set forth in SEQ ID NO:56;

f. a nucleic acid molecule comprising a sequence at least 80% identical to the nucleotide sequence set forth in SEQ ID NO:61 and/or a nucleic acid molecule comprising a sequence at least 80% identical to the nucleotide sequence set forth in SEQ ID NO:66;

g. a nucleic acid molecule comprising the nucleotide sequence a sequence at least 80% identical to set forth in SEQ ID NO:71 and/or a nucleic acid comprising a sequence at least 80% identical to the nucleotide sequence set forth in SEQ ID NO:81;

h. a nucleic acid comprising the nucleotide sequence a sequence at least 80% identical to set forth in SEQ ID NO:76 and/or a nucleic acid molecule comprising a sequence at least 80% identical to the nucleotide sequence set forth in SEQ ID NO:81;

i. a nucleic acid molecule comprising a sequence at least 80% identical to the nucleotide sequence set forth in SEQ ID NO:86 and/or a nucleic acid molecule comprising a sequence at least 80% identical to the nucleotide sequence set forth in SEQ ID NO:96;

j. a nucleic acid molecule comprising a sequence at least 80% identical to the nucleotide sequence set forth in SEQ ID NO:91 and/or a nucleic acid molecule comprising a sequence at least 80% identical to the nucleotide sequence set forth in SEQ ID NO:96; and

k. a nucleic acid molecule comprising a sequence at least 80% identical to the nucleotide sequence set forth in SEQ ID NO:101 and/or a nucleic acid molecule comprising a sequence at least 80% identical to the nucleotide sequence set forth in SEQ ID NO:106.

16. Use of the antigen-binding agent of any one of claims 1 to 12 in the manufacture of a medicament for treating a cancer, wherein the cancer comprises cells expressing EGFRvIII.

17. Use of cells expressing the antigen-binding agent of claim 1 or 2 in the manufacture of a medicament for treating a subject having a cancer associated with EGFRvIII expression,

wherein the antigen-binding agent is a chimeric antigen receptor, a bi-specific T-cell engager, a bispecific killer cell engager or a trispecific killer cell engager.

18. An isolated cell population engineered to express the antigen-binding agent of claim 1 or 2.

5 19. The isolated cell population of claim 18, wherein the isolated cell population comprises T cells, Natural Killer (NK) cells, cytotoxic T cells, or regulatory T cells, or any combination thereof.

20. A pharmaceutical composition comprising the isolated cell population of claim 18 or 19 and a pharmaceutically acceptable buffer or excipient.

10