

DESCRIPTION

EGFL6 SPECIFIC MONOCLONAL ANTIBODIES AND METHODS OF THEIR USE

[0001] This application claims the benefit of United States Provisional Patent Application No 62/291,987, filed February 5, 2016, the entirety of which is incorporated
5 herein by reference.

BACKGROUND OF THE INVENTION

1. Field of the Invention

[0002] The present invention relates generally to the field of cancer biology. More particularly, it concerns EGFL6 targeting monoclonal antibodies for the treatment and
10 detection of cancer.

2. Description of Related Art

[0003] Human epidermal growth factor (EGF)-like domain multiple 6 (EGFL6) was first discovered in tumors and fetal tissues and is a member of the EGF repeat superfamily. EGFL6 was identified as a secreted protein with four and one-half EGF-like repeat domains,
15 two N-linked glycosylation sites, one integrin association motif (RGD), a tyrosine phosphorylation site, and a MAM domain (Yeung *et al.*, 1999). Studies have shown that high expression of EGFL6 is associated with tumor tissues in certain cancer types such as ovarian cancer and lung cancer, while limited expression was found in healthy adult tissue (Buckanovich *et al.*, 2007; Chim *et al.*, 2011; and Oberauer *et al.*, 2010). However, there
20 remains a need for reagents and the therapeutics for the treatment of EGFL6-positive cancers.

SUMMARY

[0003a] In one aspect, the present disclosure refers to an isolated antibody or antigen-binding fragment thereof that specifically binds to EGFL6 and comprises:

- (a) a first V_H CDR is identical to SEQ ID NO: 64;
- (b) a second V_H CDR is identical to SEQ ID NO:65;
- (c) a third V_H CDR is identical to SEQ ID NO: 66;
- (d) a first V_L CDR is identical to SEQ ID NO: 126;
- (e) a second V_L CDR is identical to SEQ ID NO: 127; and
- (f) a third V_L CDR is identical to SEQ ID NO: 128.

[0003b] In another aspect, the present disclosure refers to a composition comprising the antibody or antigen-binding fragment as disclosed herein in a pharmaceutically acceptable carrier.

[0003c] In yet another aspect, the present disclosure refers to recombinant polypeptide that specifically binds to EGFL6 comprising:

an antibody V_H domain comprising CDRs 1-3 of the V_H domain of E2-135 (SEQ ID NOs: 64, 65, and 66) and

an antibody V_L domain CDRs 1-3 of the V_L domain of E2-135 (SEQ ID NOs: 126, 127, and 128).

[0004] Described herein are EGFL6 monoclonal antibodies that bind to EGFL6. In further examples, provided EGFL6-binding antibodies reduce EGFL6 signaling and can be used to inhibit cancer cell proliferation. Thus, in a first example, there is provided an isolated or recombinant monoclonal antibody that specifically binds to an EGFL6. In certain examples, an antibody that competes for the binding of an EGFL6 with the E1-33, E1-34, E1-80, E1-89, E2-93, E1-38, E1-52, E2-36, E1-95, E2-116, E2-135, or E1-142 monoclonal antibody is provided. In certain examples, the antibody may comprise all or part of the heavy chain variable region and/or light chain variable region of the E1-33, E1-34, E1-80, E1-89, E2-93,

E1-38, E1-52, E2-36, E1-95, E2-116, E2-135, or E1-142 monoclonal antibodies. In a further example, the antibody may comprise an amino acid sequence that corresponds to a first, second, and/or third complementarity determining region (CDR) from the light variable and/or heavy variable chain of the E1-33, E1-34, E1-80, E1-89, E2-93, E1-38, E1-52, E2-36, E1-95, E2-116, E2-135, or E1-142 monoclonal antibodies of the present examples.

[0005] In certain examples, the isolated antibody comprises CDR sequences at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the CDR regions of the E1-33, E1-34, E1-80, E1-89, E2-93, E1-38, E1-52, E2-36, E1-95, E2-116, E2-135, or E1-142 heavy and light chain amino acid sequences. In further examples, an antibody comprises CDR regions identical to the E1-33, E1-34, E1-80, E1-89, E2-93, E1-38, E1-52, E2-36, E1-95, E2-116, E2-135, or E1-142 CDR regions, except for one or two amino acid substitutions, deletions, or insertions at one or more of the CDRs. For example, the antibody can comprise CDRs wherein the CDR sequences comprise 1 or 2 amino acid substitutions in the V_H CDR1, V_H CDR2, V_H CDR3, V_L CDR1, V_L CDR2 and/or V_L CDR3 relative to the CDRs of a E1-33, E1-34, E1-80, E1-89, E2-93, E1-38, E1-52, E2-36, E1-95, E2-116, E2-135, or E1-142 monoclonal antibody. Thus, in some specific examples, an antibody of the examples comprises (a) a first V_H CDR at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to V_H CDR1 of E1-33 (SEQ ID NO: 4), E1-34 (SEQ ID NO: 10), E1-80 (SEQ ID NO: 16), E1-89 (SEQ ID NO: 22), E2-93 (SEQ ID NO: 28), E1-38 (SEQ ID NO: 34), E1-52 (SEQ ID NO: 40), E2-36 (SEQ ID NO: 46), E1-95 (SEQ ID NO: 52), E2-116 (SEQ ID NO: 58), E2-135 (SEQ ID NO: 64), or E1-142 (SEQ ID NO: 70); (b) a second V_H CDR at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to V_H CDR2 of E1-33 (SEQ ID NO: 5), E1-34 (SEQ ID NO: 11), E1-80 (SEQ ID NO: 17), E1-89 (SEQ ID NO: 23), E2-93 (SEQ ID NO: 29), E1-38 (SEQ ID NO: 35), E1-52 (SEQ ID NO: 41), E2-36 (SEQ ID NO: 47), E1-95 (SEQ ID NO: 53), E2-116 (SEQ ID NO: 59), E2-135 (SEQ ID NO: 65), or E1-142 (SEQ ID NO: 71); (c) a third V_H CDR at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to V_H CDR3 of E1-33 (SEQ ID NO: 6), E1-34 (SEQ ID NO: 12), E1-80 (SEQ ID NO: 18), E1-89 (SEQ ID NO: 24), E2-93 (SEQ ID NO: 30), E1-38 (SEQ ID NO: 36), E1-52 (SEQ ID NO: 42), E2-36 (SEQ ID NO: 48), E1-95 (SEQ ID NO: 54), E2-116 (SEQ ID NO: 60), E2-135 (SEQ ID NO: 66), or E1-142 (SEQ ID NO: 72); (d) a first V_L CDR at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to V_L CDR1 of E1-33 (SEQ ID NO: 76), E1-34 (SEQ ID NO: 82), E1-80 (SEQ ID

NO: 88), E1-89 (SEQ ID NO: 93), E2-93 (SEQ ID NO: 99), E1-38 (SEQ ID NO: 104), E1-52 (SEQ ID NO: 108), E2-36 (SEQ ID NO: 113), E1-95 (SEQ ID NO: 117), E2-116 (SEQ ID NO: 121), E2-135 (SEQ ID NO: 126), or E1-142 (SEQ ID NO: 131); (e) a second V_L CDR at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to V_L CDR2 of E1-33 (SEQ ID NO: 77), E1-34 (SEQ ID NO: 83), E1-80 (SEQ ID NO: 77), E1-89 (SEQ ID NO: 94), E2-93 (SEQ ID NO: 100), E1-38 (SEQ ID NO: 100), E1-52 (SEQ ID NO: 77), E2-36 (SEQ ID NO: 83), E1-95 (SEQ ID NO: 83), E2-116 (SEQ ID NO: 100), E2-135 (SEQ ID NO: 127), or E1-142 (SEQ ID NO: 100); and (f) a third V_L CDR at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to V_L CDR3 of E1-33 (SEQ ID NO: 78), E1-34 (SEQ ID NO: 84), E1-80 (SEQ ID NO: 89), E1-89 (SEQ ID NO: 95), E2-93 (SEQ ID NO: 101), E1-38 (SEQ ID NO: 105), E1-52 (SEQ ID NO: 109), E2-36 (SEQ ID NO: 114), E1-95 (SEQ ID NO: 118), E2-116 (SEQ ID NO: 122), E2-135 (SEQ ID NO: 128), or E1-142 (SEQ ID NO: 132). In certain examples, such an antibody is a humanized or de-immunized antibody comprising the foregoing CDRs on a human IgGs (*e.g.*, IgG1, IgG2, IgG4, or a genetically modified IgG) backbone.

[0006] In further examples, the isolated antibody comprises a first V_H, a second V_H, a third V_H, a first V_L, a second V_L, and a third V_L CDR sequence at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the corresponding CDR sequence of monoclonal antibody E1-33, which are represented by SEQ ID NOs: 4, 5, 6, 76, 77, and 78, respectively. In one example, the isolated antibody comprises CDR sequences that are identical to the CDR sequences of monoclonal antibody E1-33.

[0007] In another example, the isolated antibody comprises a V_H domain at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the V_H domain of E1-33 (SEQ ID NO: 157) or the humanized V_H domain of E1-33 mAB; and a V_L domain at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the V_L domain of E1-33 (SEQ ID NO: 158) or the humanized V_L domain of E1-33 mAB. For example, the antibody can comprise a V_H domain at least 95% identical to the V_H domain of the humanized E1-33 mAB and a V_L domain at least 95% identical to the V_L domain of the humanized E1-33 mAB. Thus, in some examples, an antibody comprises a V_H domain identical to the V_H domain of humanized E1-33 mAB and a V_L domain identical to the V_L domain of the humanized E1-33 mAB. In a specific example, the

isolated antibody can comprise V_H and V_L domains identical to those of monoclonal antibody E1-33.

[0008] In further examples, the isolated antibody comprises a first V_H, a second V_H, a third V_H, a first V_L, a second V_L, and a third V_L CDR sequence at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the corresponding CDR sequence of monoclonal antibody E1-34, which are represented by SEQ ID NOs: 10, 11, 12, 82, 83, and 84, respectively. In one example, the isolated antibody comprises CDR sequences that are identical to the CDR sequences of monoclonal antibody E1-34.

[0009] In another example, the isolated antibody comprises a V_H domain at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the V_H domain of E1-34 (SEQ ID NO: 159) or the humanized V_H domain of E1-34 mAB; and a V_L domain at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the V_L domain of E1-34 (SEQ ID NO: 160) or the humanized V_L domain of E1-34 mAB. For example, the antibody can comprise a V_H domain at least 95% identical to the V_H domain of the humanized E1-34 mAB and a V_L domain at least 95% identical to the V_L domain of the humanized E1-34 mAB. Thus, in some examples, an antibody comprises a V_H domain identical to the V_H domain of humanized E1-34 mAB and a V_L domain identical to the V_L domain of the humanized E1-34 mAB. In a specific example, the isolated antibody can comprise V_H and V_L domains identical to those of monoclonal antibody E1-34.

[0010] In further examples, the isolated antibody comprises a first V_H, a second V_H, a third V_H, a first V_L, a second V_L, and a third V_L CDR sequence at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the corresponding CDR sequence of monoclonal antibody E1-80, which are represented by SEQ ID NOs: 16, 17, 18, 88, 77, and 89, respectively. In one example, the isolated antibody comprises CDR sequences that are identical to the CDR sequences of monoclonal antibody E1-80.

[0011] In another example, the isolated antibody comprises a V_H domain at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the V_H domain of E1-80 (SEQ ID NO: 161) or the humanized V_H domain of E1-80 mAB; and a V_L domain at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the V_L domain of E1-80 (SEQ ID NO: 162) or the humanized V_L

domain of E1-80 mAB. For example, the antibody can comprise a V_H domain at least 95% identical to the V_H domain of the humanized E1-80 mAB and a V_L domain at least 95% identical to the V_L domain of the humanized E1-80 mAB. Thus, in some examples, an antibody comprises a V_H domain identical to the V_H domain of humanized E1-80 mAB and a V_L domain identical to the V_L domain of the humanized E1-80 mAB. In a specific example, the isolated antibody can comprise V_H and V_L domains identical to those of monoclonal antibody E1-80.

[0012] In further examples, the isolated antibody comprises a first V_H, a second V_H, a third V_H, a first V_L, a second V_L, and a third V_L CDR sequence at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the corresponding CDR sequence of monoclonal antibody E1-89, which are represented by SEQ ID NOs: 22, 23, 24, 93, 94, and 95, respectively. In one example, the isolated antibody comprises CDR sequences that are identical to the CDR sequences of monoclonal antibody E1-89.

[0013] In another example, the isolated antibody comprises a V_H domain at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the V_H domain of E1-89 (SEQ ID NO: 163) or the humanized V_H domain of E1-89 mAB; and a V_L domain at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the V_L domain of E1-89 (SEQ ID NO: 164) or the humanized V_L domain of E1-89 mAB. For example, the antibody can comprise a V_H domain at least 95% identical to the V_H domain of the humanized E1-89 mAB and a V_L domain at least 95% identical to the V_L domain of the humanized E1-89 mAB. Thus, in some examples, an antibody comprises a V_H domain identical to the V_H domain of humanized E1-89 mAB and a V_L domain identical to the V_L domain of the humanized E1-89 mAB. In a specific example, the isolated antibody can comprise V_H and V_L domains identical to those of monoclonal antibody E1-89.

[0014] In further examples, the isolated antibody comprises a first V_H, a second V_H, a third V_H, a first V_L, a second V_L, and a third V_L CDR sequence at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the corresponding CDR sequence of monoclonal antibody E2-93, which are represented by SEQ ID NOs: 28, 29, 30, 99, 100, and 101, respectively. In one example, the isolated antibody comprises CDR sequences that are identical to the CDR sequences of monoclonal antibody E2-93.

[0015] In another example, the isolated antibody comprises a V_H domain at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the V_H domain of E2-93 (SEQ ID NO: 165) or the humanized V_H domain of E2-93 mAB; and a V_L domain at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the V_L domain of E2-93 (SEQ ID NO: 166) or the humanized V_L domain of E2-93 mAB. For example, the antibody can comprise a V_H domain at least 95% identical to the V_H domain of the humanized E2-93 mAB and a V_L domain at least 95% identical to the V_L domain of the humanized E2-93 mAB. Thus, in some examples, an antibody comprises a V_H domain identical to the V_H domain of humanized E2-93 mAB and a V_L domain identical to the V_L domain of the humanized E2-93 mAB. In a specific example, the isolated antibody can comprise V_H and V_L domains identical to those of monoclonal antibody E2-93.

[0016] In further examples, the isolated antibody comprises a first V_H, a second V_H, a third V_H, a first V_L, a second V_L, and a third V_L CDR sequence at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the corresponding CDR sequence of monoclonal antibody E1-38, which are represented by SEQ ID NOs: 34, 35, 36, 104, 100, and 105, respectively. In one example, the isolated antibody comprises CDR sequences that are identical to the CDR sequences of monoclonal antibody E1-38.

[0017] In another example, the isolated antibody comprises a V_H domain at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the V_H domain of E1-38 (SEQ ID NO: 167) or the humanized V_H domain of E1-38 mAB; and a V_L domain at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the V_L domain of E1-38 (SEQ ID NO: 168) or the humanized V_L domain of E1-38 mAB. For example, the antibody can comprise a V_H domain at least 95% identical to the V_H domain of the humanized E1-38 mAB and a V_L domain at least 95% identical to the V_L domain of the humanized E1-38 mAB. Thus, in some examples, an antibody comprises a V_H domain identical to the V_H domain of humanized E1-38 mAB and a V_L domain identical to the V_L domain of the humanized E1-38 mAB. In a specific example, the isolated antibody can comprise V_H and V_L domains identical to those of monoclonal antibody E1-38.

[0018] In further examples, the isolated antibody comprises a first V_H, a second V_H, a third V_H, a first V_L, a second V_L, and a third V_L CDR sequence at least 80%, 85%, 90%,

91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the corresponding CDR sequence of monoclonal antibody E1-52, which are represented by SEQ ID NOs: 40, 41, 42, 108, 77, and 109, respectively. In one example, the isolated antibody comprises CDR sequences that are identical to the CDR sequences of monoclonal antibody E1-52.

5 **[0019]** In another example, the isolated antibody comprises a V_H domain at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the V_H domain of E1-52 (SEQ ID NO: 169) or the humanized V_H domain of E1-52 mAB; and a V_L domain at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the V_L domain of E1-52 (SEQ ID NO: 170) or the
10 humanized V_L domain of E1-52 mAB. For example, the antibody can comprise a V_H domain at least 95% identical to the V_H domain of the humanized E1-52 mAB and a V_L domain at least 95% identical to the V_L domain of the humanized E1-52 mAB. Thus, in some examples, an antibody comprises a V_H domain identical to the V_H domain of humanized E1-52 mAB and a V_L domain identical to the V_L domain of the humanized E1-52 mAB. In a specific
15 example, the isolated antibody can comprise V_H and V_L domains identical to those of monoclonal antibody E1-52.

[0020] In further examples, the isolated antibody comprises a first V_H, a second V_H, a third V_H, a first V_L, a second V_L, and a third V_L CDR sequence at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the corresponding CDR
20 sequence of monoclonal antibody E2-36, which are represented by SEQ ID NOs: 46, 47, 48, 113, 83, and 114, respectively. In one example, the isolated antibody comprises CDR sequences that are identical to the CDR sequences of monoclonal antibody E2-36.

[0021] In another example, the isolated antibody comprises a V_H domain at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the V_H
25 domain of E2-36 (SEQ ID NO: 171) or the humanized V_H domain of E2-36 mAB; and a V_L domain at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the V_L domain of E2-36 (SEQ ID NO: 172) or the humanized V_L domain of E2-36 mAB. For example, the antibody can comprise a V_H domain at least 95% identical to the V_H domain of the humanized E2-36 mAB and a V_L domain at least 95% identical to the V_L
30 domain of the humanized E2-36 mAB. Thus, in some examples, an antibody comprises a V_H domain identical to the V_H domain of humanized E2-36 mAB and a V_L domain identical to the V_L domain of the humanized E2-36 mAB. In a specific example, the

isolated antibody can comprise V_H and V_L domains identical to those of monoclonal antibody E2-36.

[0022] In further examples, the isolated antibody comprises a first V_H, a second V_H, a third V_H, a first V_L, a second V_L, and a third V_L CDR sequence at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the corresponding CDR sequence of monoclonal antibody E1-95, which are represented by SEQ ID NOs: 52, 53, 54, 117, 83, 119, respectively. In one example, the isolated antibody comprises CDR sequences that are identical to the CDR sequences of monoclonal antibody E1-95.

[0023] In another example, the isolated antibody comprises a V_H domain at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the V_H domain of E1-95 (SEQ ID NO: 173) or the humanized V_H domain of E1-95 mAB; and a V_L domain at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% to the V_L domain of E1-95 (SEQ ID NO: 174) or the humanized V_L domain of E1-95 mAB. For example, the antibody can comprise a V_H domain at least 95% identical to the V_H domain of the humanized E1-95 mAB and a V_L domain at least 95% identical to the V_L domain of the humanized E1-95 mAB. Thus, in some examples, an antibody comprises a V_H domain identical to the V_H domain of humanized E1-95 mAB and a V_L domain identical to the V_L domain of the humanized E1-95 mAB. In a specific example, the isolated antibody can comprise V_H and V_L domains identical to those of monoclonal antibody E1-95.

[0024] In further examples, the isolated antibody comprises a first V_H, a second V_H, a third V_H, a first V_L, a second V_L, and a third V_L CDR sequence at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the corresponding CDR sequence of monoclonal antibody E2-116, which are represented by SEQ ID NOs: 58, 59, 60, 121, 100, and 122, respectively. In one example, the isolated antibody comprises CDR sequences that are identical to the CDR sequences of monoclonal antibody E2-116.

[0025] In another example, the isolated antibody comprises a V_H domain at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the V_H domain of E2-116 (SEQ ID NO: 175) or the humanized V_H domain of E2-116 mAB; and a V_L domain at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the V_L domain of E2-116 (SEQ ID NO: 176) or the humanized V_L domain of E2-116 mAB. For example, the antibody can comprise a V_H domain at least 95%

identical to the V_H domain of the humanized E2-116 mAB and a V_L domain at least 95% identical to the V_L domain of the humanized E2-116 mAB. Thus, in some examples, an antibody comprises a V_H domain identical to the V_H domain of humanized E2-116 mAB and a V_L domain identical to the V_L domain of the humanized E2-116 mAB. In a specific example, the isolated antibody can comprise V_H and V_L domains identical to those of monoclonal antibody E2-116.

[0026] In further examples, the isolated antibody comprises a first V_H, a second V_H, a third V_H, a first V_L, a second V_L, and a third V_L CDR sequence at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the corresponding CDR sequence of monoclonal antibody E2-135, which are represented by SEQ ID NOs: 64, 65, 66, 126, 127, and 128, respectively. In one example, the isolated antibody comprises CDR sequences that are identical to the CDR sequences of monoclonal antibody E2-135.

[0027] In another example, the isolated antibody comprises a V_H domain at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the V_H domain of E2-135 (SEQ ID NO: 177) or the humanized V_H domain of E2-135 mAB; and a V_L domain at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the V_L domain of E2-135 (SEQ ID NO: 178) or the humanized V_L domain of E2-135 mAB. For example, the antibody can comprise a V_H domain at least 95% identical to the V_H domain of the humanized E2-135 mAB and a V_L domain at least 95% identical to the V_L domain of the humanized E2-135 mAB. Thus, in some examples, an antibody comprises a V_H domain identical to the V_H domain of humanized E2-135 mAB and a V_L domain identical to the V_L domain of the humanized E2-135 mAB. In a specific example, the isolated antibody can comprise V_H and V_L domains identical to those of monoclonal antibody E2-135.

[0028] In further examples, the isolated antibody comprises a first V_H, a second V_H, a third V_H, a first V_L, a second V_L, and a third V_L CDR sequence at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the corresponding CDR sequence of monoclonal antibody E1-142, which are represented by SEQ ID NOs: 70, 71, 72, 131, 100, and 132, respectively. In one example, the isolated antibody comprises CDR sequences that are identical to the CDR sequences of monoclonal antibody E1-142.

[0029] In another example, the isolated antibody comprises a V_H domain at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the the V_H domain of E1-142 (SEQ ID NO: 179) or the humanized V_H domain of E1-142 mAB; and a V_L domain at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical to the V_L domain of E1-142 (SEQ ID NO: 180) or the humanized V_L domain of E1-142 mAB. For example, the antibody can comprise a V_H domain at least 95% identical to the V_H domain of the humanized E1-142 mAB and a V_L domain at least 95% identical to the V_L domain of the humanized E1-142 mAB. Thus, in some examples, an antibody comprises a V_H domain identical to the V_H domain of humanized E1-142 mAB and a V_L domain identical to the V_L domain of the humanized E1-142 mAB. In a specific example, the isolated antibody can comprise V_H and V_L domains identical to those of monoclonal antibody E1-142.

[0030] In some examples, an antibody of the examples may be an IgG (*e.g.*, IgG1, IgG2, IgG3 or IgG4), IgM, IgA, genetically modified IgG isotype, or an antigen binding fragment thereof. The antibody may be a Fab', a F(ab')₂ a F(ab')₃, a monovalent scFv, a bivalent scFv, a bispecific or a single domain antibody. The antibody may be a human, humanized, or de-immunized antibody. In a further example, the isolated antibody is the E1-33, E1-34, E1-80, E1-89, E2-93, E1-38, E1-52, E2-36, E1-95, E2-116, E2-135, or E1-142 antibody.

[0031] In some examples, the antibody may be conjugated to an imaging agent, a chemotherapeutic agent, a toxin, or a radionuclide. In specific examples, the antibody may be conjugated to auristatin or to monomethyl auristatin E (MMAE) in particular.

[0032] In one example, there is provided a recombinant polypeptide comprising an antibody V_H domain comprising CDRs 1-3 of the V_H domain of E1-33 (SEQ ID NOs: 4, 5, and 6); CDRs 1-3 of the V_H domain of E1-34 (SEQ ID NOs: 10, 11, and 12); CDRs 1-3 of the V_H domain of E1-80 (SEQ ID NOs: 16, 17, and 18); CDRs 1-3 of the V_H domain of E1-89 (SEQ ID NOs: 22, 23, and 24); CDRs 1-3 of the V_H domain of E2-93 (SEQ ID NOs: 28, 29, and 30); CDRs 1-3 of the V_H domain of E1-38 (SEQ ID NOs: 34, 35, and 36); CDRs 1-3 of the V_H domain of E1-52 (SEQ ID NOs: 40, 41, and 42); CDRs 1-3 of the V_H domain of E2-36 (SEQ ID NOs: 46, 47, and 48); CDRs 1-3 of the V_H domain of E1-95 (SEQ ID NOs: 52, 53, and 54); CDRs 1-3 of the V_H domain of E2-116 (SEQ ID NOs: 58, 59, and 60); CDRs 1-3 of the V_H domain of E2-135 (SEQ ID NOs: 64, 65, and 66); or CDRs 1-3 of the V_H

domain of E1-142 (SEQ ID NOs: 70, 71, and 72). In another example, there is provided a recombinant polypeptide comprising an antibody V_L domain comprising CDRs 1-3 of the V_L domain of E1-33 (SEQ ID NOs: 76, 77, and 78); CDRs 1-3 of the V_L domain of E1-34 (SEQ ID NOs: 82, 83, and 84); CDRs 1-3 of the V_L domain of E1-80 (SEQ ID NOs: 88, 77, and 89); CDRs 1-3 of the V_L domain of E1-89 (SEQ ID NOs: 93, 94, and 95); CDRs 1-3 of the V_L domain of E2-93 (SEQ ID NOs: 99, 100, and 101); CDRs 1-3 of the V_L domain of E1-38 (SEQ ID NOs: 104, 100, and 105); CDRs 1-3 of the V_L domain of E1-52 (SEQ ID NOs: 108, 77, and 109); CDRs 1-3 of the V_L domain of E2-36 (SEQ ID NOs: 113, 83, and 114); CDRs 1-3 of the V_L domain of E1-95 (SEQ ID NOs: 117, 83, and 118); CDRs 1-3 of the V_L domain of E2-116 (SEQ ID NOs: 121, 100, and 122); CDRs 1-3 of the V_L domain of E2-135 (SEQ ID NOs: 126, 127, and 128); or CDRs 1-3 of the V_L domain of E1-142 (SEQ ID NOs: 131, 100, and 132).

[0033] In some examples, there is provided an isolated polynucleotide molecule comprising nucleic acid sequence encoding an antibody or a polypeptide comprising an antibody V_H or V_L domain disclosed herein.

[0034] In further examples, a host cell is provided that produces a monoclonal antibody or recombinant polypeptide of the examples. In some examples, the host cell is a mammalian cell, a yeast cell, a bacterial cell, a ciliate cell, or an insect cell. In certain examples, the host cell is a hybridoma cell.

[0035] In still further examples, there is provided a method of manufacturing an antibody of the present disclosure comprising expressing one or more polynucleotide molecule(s) encoding a V_L or V_H chain of an antibody disclosed herein in a cell and purifying the antibody from the cell.

[0036] In additional examples, there are pharmaceutical compositions comprising an antibody or antibody fragment as discussed herein. Such a composition further comprises a pharmaceutically acceptable carrier and may or may not contain additional active ingredients.

[0037] In examples of the present disclosure, there is provided a method for treating a subject having a cancer comprising administering an effective amount of an antibody disclosed herein. In certain examples, the antibody is a monoclonal antibody of the examples herein, such as the E1-33, E1-34, E1-80, E1-89, E2-93, E1-38, E1-52, E2-36,

E1-95, E2-116, E2-135, or E1-142 antibody or a recombinant polypeptide comprising antibody segment derived therefrom.

[0038] In certain examples, the cancer may be a breast cancer, lung cancer, head & neck cancer, prostate cancer, esophageal cancer, tracheal cancer, brain cancer, liver cancer, bladder cancer, stomach cancer, pancreatic cancer, ovarian cancer, uterine cancer, cervical cancer, testicular cancer, colon cancer, rectal cancer or skin cancer. In specific examples, the cancer is an epithelial cancer. In other examples, cancer may be a colorectal adenocarcinoma, lung adenocarcinoma, lung squamous cell carcinoma, breast cancer, hepatocellular carcinoma, ovarian cancer, kidney renal clear cell carcinoma, lung cancer or kidney cancer.

[0039] In one example, the antibody may be administered systemically. In additional examples, the antibody may be administered intravenously, intradermally, intratumorally, intramuscularly, intraperitoneally, subcutaneously, or locally. The method may further comprise administering at least a second anticancer therapy to the subject. Examples of the second anticancer therapy include, but are not limited to, surgical therapy, chemotherapy, radiation therapy, cryotherapy, hormonal therapy, immunotherapy, or cytokine therapy.

[0040] In further examples, the method may further comprise administering a composition of the present disclosure more than one time to the subject, such as, for example, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20 or more times.

[0041] In another example, there is provided a method for detecting a cancer in a subject comprising testing for the presence of elevated EGFL6 relative to a control in a sample from the subject, wherein the testing comprises contacting the sample with an antibody disclosed herein. For example, the method may be an *in vitro* or *in vivo* method.

[0042] Certain examples are directed to an antibody or recombinant polypeptide composition comprising an isolated and/or recombinant antibody or polypeptide that specifically binds EGFL6. In certain examples the antibody or polypeptide has a sequence that is, is at least, or is at most 80, 85, 90, 95, 96, 97, 98, 99, or 100% identical (or any range derivable therein) to all or part of any monoclonal antibody provided herein. In still further examples the isolated and/or recombinant antibody or polypeptide has, has at least, or has at most 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82,

83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100 or more contiguous amino acids from any of the sequences provided herein or a combination of such sequences.

[0043] In still further examples, an antibody or polypeptide of the examples comprises one or more amino acid segments of the any of the amino acid sequences disclosed herein. For example, the antibody or polypeptide can comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more amino acid segments comprising about, at least or at most 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 to 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199 or 200 amino acids in length, including all values and ranges there between, that are at least 80, 85, 90, 95, 96, 97, 98, 99, or 100% identical to any of the amino acid sequences disclosed herein. In certain examples the amino segment(s) are selected from one of the amino acid sequences of a EGFL6-binding antibody as provided herein.

[0044] In still further examples, an antibody or polypeptide of the examples comprises an amino acid segment of the any of the amino acid sequences disclosed herein, wherein the segment begins at amino acid position 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 to 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, or 200 in any sequence provided herein and ends at amino acid position 4, 5, 6, 7, 8, 9,

10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 to 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 5 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 10 196, 197, 198, 199, or 200 in the same provided sequence. In certain examples the amino segment(s), or portions thereof, are selected from one of the amino acid sequences of a EGFL6-binding antibody as provided herein.

[0045] In yet further examples, an antibody or polypeptide of the examples comprises an amino acid segment that is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 15 97%, 98%, 99% or 100% identical (or any range derivable therein) to a V, VJ, VDJ, D, DJ, J or CDR domain of a EGFL6-binding antibody (as provided in Tables 1 and 2). For example, a polypeptide may comprise 1, 2 or 3 amino acid segment that are at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical (or any range derivable therein) to CDRs 1, 2, and/or 3 a EGFL6-binding antibody as provided in Tables 1 and 2.

20 **[0046]** Embodiments discussed in the context of methods and/or compositions of the invention may be employed with respect to any other method or composition described herein. Thus, an embodiment pertaining to one method or composition may be applied to other methods and compositions of the invention as well.

25 **[0047]** As used herein the specification, “a” or “an” may mean one or more. As used herein in the claim(s), when used in conjunction with the word “comprising”, the words “a” or “an” may mean one or more than one.

30 **[0048]** The use of the term “or” in the claims is used to mean “and/or” unless explicitly indicated to refer to alternatives only or the alternatives are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and “and/or.” As used herein “another” may mean at least a second or more.

WHAT IS CLAIMED IS:

1. An isolated antibody or antigen-binding fragment thereof that specifically binds to EGFL6 and comprises:
 - (a) a first V_H CDR is identical to SEQ ID NO: 64;
 - (b) a second V_H CDR is identical to SEQ ID NO:65;
 - (c) a third V_H CDR is identical to SEQ ID NO: 66;
 - (d) a first V_L CDR is identical to SEQ ID NO: 126;
 - (e) a second V_L CDR is identical to SEQ ID NO: 127; and
 - (f) a third V_L CDR is identical to SEQ ID NO: 128.
2. The antibody or antigen-binding fragment of claim 1 comprising a V_H domain at least about 80% identical to the V_H domain of E2-135 (SEQ ID NO: 177) or the humanized V_H domain of E2-135 mAB; and a V_L domain at least about 80% identical to the V_L domain of E2-135 (SEQ ID NO: 178) or the humanized V_L domain of E2-135 mAB.
3. The antibody or antigen-binding fragment of claim 1 that is recombinant.
4. The antibody or antigen-binding fragment of claim 1 that is an IgG, IgM, or IgA.
5. The antibody or antigen-binding fragment of claim 1 that is an Fab', an F(ab')₂, an F(ab')₃, a monovalent scFv, or a bivalent scFv.
6. The antibody or antigen-binding fragment of claim 1 that is humanized.
7. The antibody or antigen-binding fragment of claim 1 that is conjugated to an imaging agent, a chemotherapeutic agent, a toxin or a radionuclide.
8. A composition comprising the antibody or antigen-binding fragment of claim 1 in a pharmaceutically acceptable carrier.
9. A recombinant polypeptide that specifically binds to EGFL6 comprising:
an antibody V_H domain comprising CDRs 1-3 of the V_H domain of E2-135 (SEQ ID NOs: 64, 65, and 66) and
an antibody V_L domain CDRs 1-3 of the V_L domain of E2-135 (SEQ ID NOs: 126, 127, and 128).