

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
5 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).

10 **[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-
15 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.

[0049] Throughout this application, the term “about” is used to indicate that a value includes the inherent variation of error for the device, the method being employed to determine the value, or the variation that exists among the study subjects.

[0050] Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0051] The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein. The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[0052] **FIG. 1.** A schematic diagram illustrating the working principles of ADC (antibody-drug conjugate). Upon binding to its target antigen, the MAb-antigen complex is internalized into endosomes which is then fused with lysosomes where the MAb is degraded and the drug is released.

[0053] **FIG. 2.** Detection of high binding EGFL6 antibodies by ELISA. Human (left bars) or mouse (right bars) EGFL6 protein (Sino Biologicals) was coated on a 96-well high binding plate over night at 4°C in PBS. B cell culture supernatants (5 μ l medium and 95 μ l of PBS) were added at for binding to EGFL6 antigen coated on the plate. Bound antibody was detected using a secondary antibody against rabbit IgG conjugated with HRP and TMB substrate. Experiments were repeated 2 times for confirmation.

[0054] **FIG. 3.** Determination of binding affinities of EGFL6 antibodies in ELISA. A series of antibody concentrations was assayed in ELISA and 4-parameter fitting was used

to calculate binding affinity of the antibodies. Experiments have 3 repeats and error bars indicate standard deviation.

[0055] FIGS. 4A-D. EGFL6 is upregulated in tumor associated endothelial cells but not in normal ovary and wound healing tissue. A) Summary of isolation of endothelial cells. B) Gene microarray of endothelial cells from normal ovary, healing wound tissue and ovarian tumor associated endothelial cells. C) Expression of EGFL6, CD31 and VEGF in ovarian patients. D) Validation of gene microarray data using Q - RT PCR. Scale bar = 100 μ m.

[0056] FIGS. 5A-H. EGFL6 gene silencing did not compromise wound healing but reduced the tumor burden in A2780ip2 ovarian orthotopic mouse model. A) Expression of EGFL6 in siControl and siEGFL6 treated dermal endothelial cells. B) Effect of EGFL6 silencing on wound healing in vitro. C) Bar graph represent wound healing area. D) Effect of EGFL6 silencing on wound healing E) wound volume F) Representative images of tumor burden G) tumor weight and H) tumor nodules.

[0057] FIGS. 6A-K. TWIST1 induces EGFL6 expression in hypoxia. A,B) EGFL6 promoter reporter analysis under normoxia and hypoxic condition. C) TWIST1 Increases the expression of EGFL6 under hypoxic condition D) TWIST1 binds to promoter region of EGFL6. E and F) Ectopic expression of TWIST1 increases EGFL6 expression in RF24 cells. G) ChIP analysis of TWIST1 binding to EGFL6 promoter region in hypoxia compared to normoxia. EGFL6 and a ChIP assay of TWIST1 binding to EGFL6 promoter in human ovarian endothelial cells (RF24). Cross-linked chromatin from RF24 cells treated with TWIST1 and immunoprecipitated with EGFL6 or IgG control antibodies. The input and immunoprecipitated DNA was subjected to PCR using primers corresponding to the base pairs upstream of EGFL6 transcription start site. PCR products were examined on ethidium bromide-stained agarose gel. H) EGFL6 gene silencing using siRNA leads to increased cell death in hypoxia condition. I) Hind limb ischemia. After arterial ligation, the femoral artery was excised and mice were separated into 3 groups (n = 5): normal, ischemia-24h and ischemia-96h. Blood flow was monitored before and after femoral artery ligation using serial laser Doppler. At each time point, tissue was harvested and frozen to perform immunofluorescence. J,K) EGFL6 expression was increased in endothelial cells in ischemic (hypoxic) condition compared to normal condition.

[0058] FIGS. 7A-K. Treatment of endothelial cells with EGFL6 activates of PI3kinase/AKT signaling. A) RPPA analysis in control and EGFL6 treated RF24 cells. B) Western blotting of EGFL6 mediated activation of PI3Kinase/AKT signaling. C) Western blotting of EGFL6 mediated IGF-R, EGFR, and Tie2 receptor activation. D) Tie2 antibody pull-downed intergrin proteins. E) Tie2 and AKT signal pathway in cytosol and membrane fractioned proteins. F) Tie2 and AKT signal pathway in siTGB1 and siTie2 treated RF24cells. G, H) Silencing of integrin and Tie2 using specific siRNAs decreases the EGFL6 mediated tube formation (G) and migration (H) in endothelial cells. I) RGD blocking peptide decreases the integrin-mediated signal pathway J) migration K) and tube formation in endothelial cells.

[0059] FIGS. 8A-G. EGFL6 functional blocking antibody reduces angiogenesis and tumor growth. A) Line graph represent antibody binding affinity. B) Effect of EGFL6 blocking antibodies on Tie2/AKT activation in RF24 cells. Control, mAb93 and mAb135 working concentration is 10ug/ml. C) Effect of EGFL6 blocking antibodies on wound healing assay with dermal endothelial cells. D) Effect of EGFL6 blocking antibodies on tube formation and E) migration in RF24 cells. F) Effect of EGFL6 blocking antibodies on SKOV3ip1 tumor bearing mice tumor weight, tumor nodules. G) Ki67 and CD31 expression shown the cell proliferation and vessel density. Seven days following tumor cell injection, mice were randomly divided into three groups (10 mice/group) to receive therapy: (1) Control Ab (5mg/kg), (2) EGFL6 Ab 93 (5 mg/kg), and (3) EGFL6 Ab 135 (5 mg/kg). Antibody was given once a week. Tumors were harvested as described in the Examples herein. Wound was created and tumors were harvested as described in the Examples herein. Error bars indicates SEM. * $P < 0.05$ vs. Control Ab.

[0060] FIG. 8H. Inhibition of tube formation of endothelial cells (RF24) by EGFL6 antibodies. Antibodies (at 5 μ g/ml concentration) were added to the cell culture (RF24) in comparison with a control antibody. The number of tubes was counted after 48 hour treatment in a 96-well assay plate. FIG. 8H shows a representative for each group, and the bar graph illustrates the average of tube numbers in each treated group. Error bars indicate the standard error and $n=3$.

[0061] FIGS. 9A-C. Generation of *Tie2-cre; EGFL6^{ff}* knockout mice. A) Generation of Tie2 cre; EGFL6 knockout mouse. B) CD31 expression in isolated endothelial

cell with littermate and EGFL6 knockout mouse. C) EGFL6 expression in isolated endothelial cells.

[0062] **FIGS. 9D-G.** EGFL6 gene silencing reduces the tumor burden and angiogenesis in SKOV3ip1 ovarian orthotopic mouse models. D) Expression of EGFL6 in various ovarian cancer cells. E, F) Effects of endothelial cell (mEGFL6 siRNA) or tumor (hEGFL6 siRNA) targeted EGFL6 siRNA on tumor weight and tumor nodules in SKOV3ip1 orthotopic mouse models of ovarian cancer. Seven days following tumor cell injection, mice were randomly divided into four groups (10 mice/group) to receive therapy: (1) Control siRNA, (2) mEGFL6 siRNA, (3) hEGFL6 siRNA (4) mEGFL6 siRNA + hEGFL6 siRNA. Mice were sacrificed when any animals in a control or treatment group became moribund (after 3-4 weeks of therapy) and tumor weight (E) and number of tumor nodules (F) were recorded. Error bars indicates SEM. G) Effect of targeted EGFL6 siRNAs on proliferation and microvessel density. Harvested tumors were stained for Ki67 proliferation and CD31. Scale bar = 50 μ m. The bars in the graphs correspond sequentially to the labeled columns of images at left. Error bars indicates SEM.

[0063] **FIGS. 10A-E.** EGFL6 regulates tumor angiogenesis. A) Human normal ovary, ovarian tumor, and healing wound tissues were dissociated, and isolated endothelial cells and samples were processed for microarray. B) Expression of VEGF in human normal ovary, wound, and ovarian tumor samples. C), D), E) Control siRNA- and *EGFL6* siRNA-treated RF24 cells and characterized tube formation and migration. Representative images of human ovarian cancer vasculature with low or high immunohistochemical staining for EGFL6. Scale bar = 200 μ m. Error bars indicates SEM. * $p < 0.05$ vs. Control siRNA.

[0064] **FIGS. 11A-B.** Animals were treated with either Control siRNA-CH or mEGFL6 siRNA-CH with or without wound. Harvested tumors were stained for Ki67 (proliferation) and CD31 (microvessel). Error bars indicates SEM.

[0065] **FIGS. 12A-D.** Treatment of endothelial cells with EGFL6 activates PI3K/AKT signaling. A) Heat map representation of RPPA analysis showing protein expression change in Control and EGFL6-treated RF24 endothelial cells. B) Heat map representation of RPPA analysis showing protein expression change in control and EGFL6-treated RMG2 ovarian cancer cells. C), D) EGFL6-mediated migration and tube formation (lower panel) reduced by PI3K inhibition in endothelial cells.

DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

[0066] EGFL6 is a member of the EGF repeat superfamily that is involved in wound healing. However, elevated EGFL6 has also been found in variety of cancer cell types, such as ovarian cancer and lung cancer. Studies herein demonstrate that inhibition of EGFL6 activity is effective for inhibiting cancer cell proliferation and angiogenesis in tumor tissues. Moreover, EGFL6-binding antibodies provided here were found to be effective for inhibiting EGFL6 activity and cancer cell growth. Thus, antibodies of the embodiments provide new effective methods for treating cancers and inhibiting angiogenesis.

I. Antibodies of the Embodiments

[0067] In certain embodiments, an antibody or a fragment thereof that binds to at least a portion of EGFL6 protein and inhibits EGFL6 signaling and cancer cell proliferation are contemplated. As used herein, the term “antibody” is intended to refer broadly to any immunologic binding agent, such as IgG, IgM, IgA, IgD, IgE, and genetically modified IgG as well as polypeptides comprising antibody CDR domains that retain antigen binding activity. The antibody may be selected from the group consisting of a chimeric antibody, an affinity matured antibody, a polyclonal antibody, a monoclonal antibody, a humanized antibody, a human antibody, or an antigen-binding antibody fragment or a natural or synthetic ligand. Preferably, the anti-EGFL6 antibody is a monoclonal antibody or a humanized antibody.

[0068] Thus, by known means and as described herein, polyclonal or monoclonal antibodies, antibody fragments, and binding domains and CDRs (including engineered forms of any of the foregoing) may be created that are specific to EGFL6 protein, one or more of its respective epitopes, or conjugates of any of the foregoing, whether such antigens or epitopes are isolated from natural sources or are synthetic derivatives or variants of the natural compounds.

[0069] Examples of antibody fragments suitable for the present embodiments include, without limitation: (i) the Fab fragment, consisting of V_L , V_H , C_L , and C_{H1} domains; (ii) the “Fd” fragment consisting of the V_H and C_{H1} domains; (iii) the “Fv” fragment consisting of the V_L and V_H domains of a single antibody; (iv) the “dAb” fragment, which consists of a V_H domain; (v) isolated CDR regions; (vi) $F(ab')_2$ fragments, a bivalent fragment comprising two linked Fab fragments; (vii) single chain Fv molecules (“scFv”), wherein a V_H domain

and a V_L domain are linked by a peptide linker that allows the two domains to associate to form a binding domain; (viii) bi-specific single chain Fv dimers (see U.S. Pat. No. 5,091,513); and (ix) diabodies, multivalent or multispecific fragments constructed by gene fusion (US Patent App. Pub. 20050214860). Fv, scFv, or diabody molecules may be stabilized by the incorporation of disulphide bridges linking the V_H and V_L domains. Minibodies comprising a scFv joined to a CH3 domain may also be made (Hu *et al.*, 1996).

[0070] Antibody-like binding peptidomimetics are also contemplated in embodiments. Liu *et al.* (2003) describe “antibody like binding peptidomimetics” (ABiPs), which are peptides that act as pared-down antibodies and have certain advantages of longer serum half-life as well as less cumbersome synthesis methods.

[0071] Animals may be inoculated with an antigen, such as a EGFL6 extracellular domain (ECD) protein, in order to produce antibodies specific for EGFL6 protein. Frequently an antigen is bound or conjugated to another molecule to enhance the immune response. As used herein, a conjugate is any peptide, polypeptide, protein, or non-proteinaceous substance bound to an antigen that is used to elicit an immune response in an animal. Antibodies produced in an animal in response to antigen inoculation comprise a variety of non-identical molecules (polyclonal antibodies) made from a variety of individual antibody producing B lymphocytes. A polyclonal antibody is a mixed population of antibody species, each of which may recognize a different epitope on the same antigen. Given the correct conditions for polyclonal antibody production in an animal, most of the antibodies in the animal's serum will recognize the collective epitopes on the antigenic compound to which the animal has been immunized. This specificity is further enhanced by affinity purification to select only those antibodies that recognize the antigen or epitope of interest.

[0072] A monoclonal antibody is a single species of antibody wherein every antibody molecule recognizes the same epitope because all antibody producing cells are derived from a single B-lymphocyte cell line. The methods for generating monoclonal antibodies (MAbs) generally begin along the same lines as those for preparing polyclonal antibodies. In some embodiments, rodents such as mice and rats are used in generating monoclonal antibodies. In some embodiments, rabbit, sheep, or frog cells are used in generating monoclonal antibodies. The use of rats is well known and may provide certain advantages. Mice (e.g., BALB/c mice) are routinely used and generally give a high percentage of stable fusions.

[0073] Hybridoma technology involves the fusion of a single B lymphocyte from a mouse previously immunized with a EGFL6 antigen with an immortal myeloma cell (usually mouse myeloma). This technology provides a method to propagate a single antibody-producing cell for an indefinite number of generations, such that unlimited quantities of structurally identical antibodies having the same antigen or epitope specificity (monoclonal antibodies) may be produced.

[0074] Plasma B cells (CD45+CD5-CD19+) may be isolated from freshly prepared rabbit peripheral blood mononuclear cells of immunized rabbits and further selected for EGFL6 binding cells. After enrichment of antibody producing B cells, total RNA may be isolated and cDNA synthesized. DNA sequences of antibody variable regions from both heavy chains and light chains may be amplified, constructed into a phage display Fab expression vector, and transformed into *E. coli*. EGFL6 specific binding Fab may be selected out through multiple rounds enrichment panning and sequenced. Selected EGFL6 binding hits may be expressed as full length IgG in rabbit and rabbit/human chimeric forms using a mammalian expression vector system in human embryonic kidney (HEK293) cells (Invitrogen) and purified using a protein G resin with a fast protein liquid chromatography (FPLC) separation unit.

[0075] In one embodiment, the antibody is a chimeric antibody, for example, an antibody comprising antigen binding sequences from a non-human donor grafted to a heterologous non-human, human, or humanized sequence (e.g., framework and/or constant domain sequences). Methods have been developed to replace light and heavy chain constant domains of the monoclonal antibody with analogous domains of human origin, leaving the variable regions of the foreign antibody intact. Alternatively, "fully human" monoclonal antibodies are produced in mice transgenic for human immunoglobulin genes. Methods have also been developed to convert variable domains of monoclonal antibodies to more human form by recombinantly constructing antibody variable domains having both rodent, for example, mouse, and human amino acid sequences. In "humanized" monoclonal antibodies, only the hypervariable CDR is derived from mouse monoclonal antibodies, and the framework and constant regions are derived from human amino acid sequences (see U.S. Pat. Nos. 5,091,513 and 6,881,557). It is thought that replacing amino acid sequences in the antibody that are characteristic of rodents with amino acid sequences found in the corresponding position of human antibodies will reduce the likelihood of adverse immune

reaction during therapeutic use. A hybridoma or other cell producing an antibody may also be subject to genetic mutation or other changes, which may or may not alter the binding specificity of antibodies produced by the hybridoma.

[0076] Methods for producing polyclonal antibodies in various animal species, as well as for producing monoclonal antibodies of various types, including humanized, chimeric, and fully human, are well known in the art and highly predictable. For example, the following U.S. patents and patent applications provide enabling descriptions of such methods: U.S. Patent Application Nos. 2004/0126828 and 2002/0172677; and U.S. Pat. Nos. 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,196,265; 4,275,149; 4,277,437; 4,366,241; 4,469,797; 4,472,509; 4,606,855; 4,703,003; 4,742,159; 4,767,720; 4,816,567; 4,867,973; 4,938,948; 4,946,778; 5,021,236; 5,164,296; 5,196,066; 5,223,409; 5,403,484; 5,420,253; 5,565,332; 5,571,698; 5,627,052; 5,656,434; 5,770,376; 5,789,208; 5,821,337; 5,844,091; 5,858,657; 5,861,155; 5,871,907; 5,969,108; 6,054,297; 6,165,464; 6,365,157; 6,406,867; 6,709,659; 6,709,873; 6,753,407; 6,814,965; 6,849,259; 6,861,572; 6,875,434; and 6,891,024. All patents, patent application publications, and other publications cited herein and therein are hereby incorporated by reference in the present application.

[0077] Antibodies may be produced from any animal source, including birds and mammals. Preferably, the antibodies are ovine, murine (e.g., mouse and rat), rabbit, goat, guinea pig, camel, horse, or chicken. In addition, newer technology permits the development of and screening for human antibodies from human combinatorial antibody libraries. For example, bacteriophage antibody expression technology allows specific antibodies to be produced in the absence of animal immunization, as described in U.S. Pat. No. 6,946,546, which is incorporated herein by reference. These techniques are further described in: Marks (1992); Stemmer (1994); Gram et al. (1992); Barbas et al. (1994); and Schier et al. (1996).

[0078] It is fully expected that antibodies to EGFL6 will have the ability to neutralize or counteract the effects of EGFL6 regardless of the animal species, monoclonal cell line, or other source of the antibody. Certain animal species may be less preferable for generating therapeutic antibodies because they may be more likely to cause allergic response due to activation of the complement system through the "Fc" portion of the antibody. However, whole antibodies may be enzymatically digested into "Fc" (complement binding) fragment, and into antibody fragments having the binding domain or CDR. Removal of the Fc portion reduces the likelihood that the antigen antibody fragment will elicit an undesirable

immunological response, and thus, antibodies without Fc may be preferential for prophylactic or therapeutic treatments. As described above, antibodies may also be constructed so as to be chimeric or partially or fully human, so as to reduce or eliminate the adverse immunological consequences resulting from administering to an animal an antibody that has been produced in, or has sequences from, other species.

[0079] Substitutional variants typically contain the exchange of one amino acid for another at one or more sites within the protein, and may be designed to modulate one or more properties of the polypeptide, with or without the loss of other functions or properties. Substitutions may be conservative, that is, one amino acid is replaced with one of similar shape and charge. Conservative substitutions are well known in the art and include, for example, the changes of: alanine to serine; arginine to lysine; asparagine to glutamine or histidine; aspartate to glutamate; cysteine to serine; glutamine to asparagine; glutamate to aspartate; glycine to proline; histidine to asparagine or glutamine; isoleucine to leucine or valine; leucine to valine or isoleucine; lysine to arginine; methionine to leucine or isoleucine; phenylalanine to tyrosine, leucine or methionine; serine to threonine; threonine to serine; tryptophan to tyrosine; tyrosine to tryptophan or phenylalanine; and valine to isoleucine or leucine. Alternatively, substitutions may be non-conservative such that a function or activity of the polypeptide is affected. Non-conservative changes typically involve substituting a residue with one that is chemically dissimilar, such as a polar or charged amino acid for a nonpolar or uncharged amino acid, and vice versa.

[0080] Proteins may be recombinant, or synthesized in vitro. Alternatively, a non-recombinant or recombinant protein may be isolated from bacteria. It is also contemplated that a bacteria containing such a variant may be implemented in compositions and methods. Consequently, a protein need not be isolated.

[0081] It is contemplated that in compositions there is between about 0.001 mg and about 10 mg of total polypeptide, peptide, and/or protein per ml. Thus, the concentration of protein in a composition can be about, at least about or at most about 0.001, 0.010, 0.050, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, 10.0 mg/ml or more (or any range derivable therein). Of this, about, at least about, or at most about 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, 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, or 100% may be an antibody that binds EGFL6.

[0082] An antibody or preferably an immunological portion of an antibody, can be chemically conjugated to, or expressed as, a fusion protein with other proteins. For purposes
5 of this specification and the accompanying claims, all such fused proteins are included in the definition of antibodies or an immunological portion of an antibody.

[0083] Embodiments provide antibodies and antibody-like molecules against EGFL6, polypeptides and peptides that are linked to at least one agent to form an antibody conjugate or payload. In order to increase the efficacy of antibody molecules as diagnostic or
10 therapeutic agents, it is conventional to link or covalently bind or complex at least one desired molecule or moiety. Such a molecule or moiety may be, but is not limited to, at least one effector or reporter molecule. Effector molecules comprise molecules having a desired activity, e.g., cytotoxic activity. Non-limiting examples of effector molecules that have been attached to antibodies include toxins, therapeutic enzymes, antibiotics, radio-labeled
15 nucleotides and the like. By contrast, a reporter molecule is defined as any moiety that may be detected using an assay. Non-limiting examples of reporter molecules that have been conjugated to antibodies include enzymes, radiolabels, haptens, fluorescent labels, phosphorescent molecules, chemiluminescent molecules, chromophores, luminescent molecules, photoaffinity molecules, colored particles or ligands, such as biotin.

[0084] Several methods are known in the art for the attachment or conjugation of an
20 antibody to its conjugate moiety. Some attachment methods involve the use of a metal chelate complex employing, for example, an organic chelating agent such as diethylenetriaminepentaacetic acid anhydride (DTPA); ethylenetriaminetetraacetic acid; N-chloro-p-toluenesulfonamide; and/or tetrachloro-3,6-bis(diphenylglycouril)-3 attached to the
25 antibody. Monoclonal antibodies may also be reacted with an enzyme in the presence of a coupling agent such as glutaraldehyde or periodate. Conjugates with fluorescein markers are prepared in the presence of these coupling agents or by reaction with an isothiocyanate.

II. Treatment of Diseases

[0085] Certain aspects of the present embodiments can be used to prevent or treat a
30 disease or disorder associated with EGFL6 signaling. Signaling of EGFL6 may be reduced

by any suitable drugs to prevent cancer cell proliferation. Preferably, such substances would be an anti-EGFL6 antibody.

[0086] “Treatment” and “treating” refer to administration or application of a therapeutic agent to a subject or performance of a procedure or modality on a subject for the purpose of obtaining a therapeutic benefit of a disease or health-related condition. For example, a treatment may include administration of a pharmaceutically effective amount of an antibody that inhibits the EGFL6 signaling.

[0087] “Subject” and “patient” refer to either a human or non-human, such as primates, mammals, and vertebrates. In particular embodiments, the subject is a human.

[0088] The term “therapeutic benefit” or “therapeutically effective” as used throughout this application refers to anything that promotes or enhances the well-being of the subject with respect to the medical treatment of this condition. This includes, but is not limited to, a reduction in the frequency or severity of the signs or symptoms of a disease. For example, treatment of cancer may involve, for example, a reduction in the size of a tumor, a reduction in the invasiveness of a tumor, reduction in the growth rate of the cancer, or prevention of metastasis. Treatment of cancer may also refer to prolonging survival of a subject with cancer.

A. Pharmaceutical Preparations

[0089] Where clinical application of a therapeutic composition containing an inhibitory antibody is undertaken, it will generally be beneficial to prepare a pharmaceutical or therapeutic composition appropriate for the intended application. In certain embodiments, pharmaceutical compositions may comprise, for example, at least about 0.1% of an active compound. In other embodiments, an active compound may comprise between about 2% to about 75% of the weight of the unit, or between about 25% to about 60%, for example, and any range derivable therein.

[0090] The therapeutic compositions of the present embodiments are advantageously administered in the form of injectable compositions either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid prior to injection may also be prepared. These preparations also may be emulsified.

[0091] The phrases “pharmaceutical or pharmacologically acceptable” refers to molecular entities and compositions that do not produce an adverse, allergic, or other untoward reaction when administered to an animal, such as a human, as appropriate. The preparation of a pharmaceutical composition comprising an antibody or additional active ingredient will be known to those of skill in the art in light of the present disclosure. Moreover, for animal (e.g., human) administration, it will be understood that preparations should meet sterility, pyrogenicity, general safety, and purity standards as required by FDA Office of Biological Standards.

[0092] As used herein, “pharmaceutically acceptable carrier” includes any and all aqueous solvents (e.g., water, alcoholic/aqueous solutions, saline solutions, parenteral vehicles, such as sodium chloride, Ringer's dextrose, etc.), non-aqueous solvents (e.g., propylene glycol, polyethylene glycol, vegetable oil, and injectable organic esters, such as ethyloleate), dispersion media, coatings, surfactants, antioxidants, preservatives (e.g., antibacterial or antifungal agents, anti-oxidants, chelating agents, and inert gases), isotonic agents, absorption delaying agents, salts, drugs, drug stabilizers, gels, binders, excipients, disintegration agents, lubricants, sweetening agents, flavoring agents, dyes, fluid and nutrient replenishers, such like materials and combinations thereof, as would be known to one of ordinary skill in the art. The pH and exact concentration of the various components in a pharmaceutical composition are adjusted according to well-known parameters.

[0093] The term “unit dose” or “dosage” refers to physically discrete units suitable for use in a subject, each unit containing a predetermined quantity of the therapeutic composition calculated to produce the desired responses discussed above in association with its administration, i.e., the appropriate route and treatment regimen. The quantity to be administered, both according to number of treatments and unit dose, depends on the effect desired. The actual dosage amount of a composition of the present embodiments administered to a patient or subject can be determined by physical and physiological factors, such as body weight, the age, health, and sex of the subject, the type of disease being treated, the extent of disease penetration, previous or concurrent therapeutic interventions, idiopathy of the patient, the route of administration, and the potency, stability, and toxicity of the particular therapeutic substance. For example, a dose may also comprise from about 1 $\mu\text{g/kg/body weight}$ to about 1000 mg/kg/body weight (this such range includes intervening doses) or more per administration, and any range derivable therein. In non-limiting examples

of a derivable range from the numbers listed herein, a range of about 5 µg/kg/body weight to about 100 mg/kg/body weight, about 5 µg/kg/body weight to about 500 mg/kg/body weight, etc., can be administered. The practitioner responsible for administration will, in any event, determine the concentration of active ingredient(s) in a composition and appropriate dose(s) for the individual subject.

[0094] The active compounds can be formulated for parenteral administration, e.g., formulated for injection via the intravenous, intramuscular, sub-cutaneous, or even intraperitoneal routes. Typically, such compositions can be prepared as either liquid solutions or suspensions; solid forms suitable for use to prepare solutions or suspensions upon the addition of a liquid prior to injection can also be prepared; and, the preparations can also be emulsified.

[0095] The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions; formulations including sesame oil, peanut oil, or aqueous propylene glycol; and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases the form must be sterile and must be fluid to the extent that it may be easily injected. It also should be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi.

[0096] The proteinaceous compositions may be formulated into a neutral or salt form. Pharmaceutically acceptable salts, include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like.

[0097] A pharmaceutical composition can include a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion, and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various

antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

B. Combination Treatments

[0098] In certain embodiments, the compositions and methods of the present embodiments involve an antibody or an antibody fragment against EGFL6 to inhibit its activity in cancer cell proliferation, in combination with a second or additional therapy. Such therapy can be applied in the treatment of any disease that is associated with EGFL6-mediated cell proliferation. For example, the disease may be cancer.

[0099] The methods and compositions, including combination therapies, enhance the therapeutic or protective effect, and/or increase the therapeutic effect of another anti-cancer or anti-hyperproliferative therapy. Therapeutic and prophylactic methods and compositions can be provided in a combined amount effective to achieve the desired effect, such as the killing of a cancer cell and/or the inhibition of cellular hyperproliferation. This process may involve contacting the cells with both an antibody or antibody fragment and a second therapy. A tissue, tumor, or cell can be contacted with one or more compositions or pharmacological formulation(s) comprising one or more of the agents (i.e., antibody or antibody fragment or an anti-cancer agent), or by contacting the tissue, tumor, and/or cell with two or more distinct compositions or formulations, wherein one composition provides 1) an antibody or antibody fragment, 2) an anti-cancer agent, or 3) both an antibody or antibody fragment and an anti-cancer agent. Also, it is contemplated that such a combination therapy can be used in conjunction with chemotherapy, radiotherapy, surgical therapy, or immunotherapy.

[00100] The terms “contacted” and “exposed,” when applied to a cell, are used herein to describe the process by which a therapeutic construct and a chemotherapeutic or radiotherapeutic agent are delivered to a target cell or are placed in direct juxtaposition with the target cell. To achieve cell killing, for example, both agents are delivered to a cell in a combined amount effective to kill the cell or prevent it from dividing.

[00101] An inhibitory antibody may be administered before, during, after, or in various combinations relative to an anti-cancer treatment. The administrations may be in

intervals ranging from concurrently to minutes to days to weeks. In embodiments where the antibody or antibody fragment is provided to a patient separately from an anti-cancer agent, one would generally ensure that a significant period of time did not expire between the time of each delivery, such that the two compounds would still be able to exert an advantageously combined effect on the patient. In such instances, it is contemplated that one may provide a patient with the antibody therapy and the anti-cancer therapy within about 12 to 24 or 72 h of each other and, more particularly, within about 6-12 h of each other. In some situations it may be desirable to extend the time period for treatment significantly where several days (2, 3, 4, 5, 6, or 7) to several weeks (1, 2, 3, 4, 5, 6, 7, or 8) lapse between respective administrations.

[00102] In certain embodiments, a course of treatment will last 1-90 days or more (this such range includes intervening days). It is contemplated that one agent may be given on any day of day 1 to day 90 (this such range includes intervening days) or any combination thereof, and another agent is given on any day of day 1 to day 90 (this such range includes intervening days) or any combination thereof. Within a single day (24-hour period), the patient may be given one or multiple administrations of the agent(s). Moreover, after a course of treatment, it is contemplated that there is a period of time at which no anti-cancer treatment is administered. This time period may last 1-7 days, and/or 1-5 weeks, and/or 1-12 months or more (this such range includes intervening days), depending on the condition of the patient, such as their prognosis, strength, health, etc. It is expected that the treatment cycles would be repeated as necessary.

[00103] Various combinations may be employed. For the example below an antibody therapy is “A” and an anti-cancer therapy is “B”:

A/B/A B/A/B B/B/A A/A/B A/B/B B/A/A A/B/B/B B/A/B/B
 B/B/B/A B/B/A/B A/A/B/B A/B/A/B A/B/B/A B/B/A/A
 B/A/B/A B/A/A/B A/A/A/B B/A/A/A A/B/A/A A/A/B/A

[00104] Administration of any compound or therapy of the present embodiments to a patient will follow general protocols for the administration of such compounds, taking into account the toxicity, if any, of the agents. Therefore, in some embodiments there is a step of monitoring toxicity that is attributable to combination therapy.

i. Chemotherapy

[00105] A wide variety of chemotherapeutic agents may be used in accordance with the present embodiments. The term “chemotherapy” refers to the use of drugs to treat cancer. A “chemotherapeutic agent” is used to connote a compound or composition that is administered in the treatment of cancer. These agents or drugs are categorized by their mode of activity within a cell, for example, whether and at what stage they affect the cell cycle. Alternatively, an agent may be characterized based on its ability to directly cross-link DNA, to intercalate into DNA, or to induce chromosomal and mitotic aberrations by affecting nucleic acid synthesis.

[00106] Examples of chemotherapeutic agents include alkylating agents, such as thiotepa and cyclophosphamide; alkyl sulfonates, such as busulfan, improsulfan, and piposulfan; aziridines, such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines, including altretamine, triethylenemelamine, trietylenephosphoramide, triethylenethiophosphoramide, and trimethylolomelamine; acetogenins (especially bullatacin and bullatacinone); a camptothecin (including the synthetic analogue topotecan); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CB1-TM1); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards, such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, and uracil mustard; nitrosureas, such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimustine; antibiotics, such as the enediyne antibiotics (e.g., calicheamicin, especially calicheamicin gammall and calicheamicin omegaII); dynemicin, including dynemicin A; bisphosphonates, such as clodronate; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antibiotic chromophores, aclacinomysins, actinomycin, authrarnycin, azaserine, bleomycins, cactinomycin, carabycin, carminomycin, carzinophilin, chromomycinis, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin (including morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins, such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin,

tubercidin, ubenimex, zinostatin, and zorubicin; anti-metabolites, such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues, such as denopterin, pteropterin, and trimetrexate; purine analogs, such as fludarabine, 6-mercaptopurine, thiamiprine, and thioguanine; pyrimidine analogs, such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, and floxuridine; androgens, such as calusterone, dromostanolone propionate, epitio stanol, mepitio stanane, and testolactone; anti-adrenals, such as mitotane and trilostane; folic acid replenisher, such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elformithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids, such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidanmol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK polysaccharide complex; razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2''-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; taxoids, e.g., paclitaxel and docetaxel; gemcitabine; 6-thioguanine; mercaptopurine; platinum coordination complexes, such as cisplatin, oxaliplatin, and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine; vinorelbine; novantrone; teniposide; edatrexate; daunomycin; aminopterin; xeloda; ibandronate; irinotecan (e.g., CPT-11); topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoids, such as retinoic acid; capecitabine; carboplatin, procarbazine, plicomycin, gemcitabine, navelbine, farnesyl-protein transferase inhibitors, transplatin, and pharmaceutically acceptable salts, acids, or derivatives of any of the above.

ii. Radiotherapy

[00107] Other factors that cause DNA damage and have been used extensively include what are commonly known as γ -rays, X-rays, and/or the directed delivery of radioisotopes to tumor cells. Other forms of DNA damaging factors are also contemplated, such as microwaves, proton beam irradiation (U.S. Patents 5,760,395 and 4,870,287), and UV-irradiation. It is most likely that all of these factors affect a broad range of damage on DNA, on the precursors of DNA, on the replication and repair of DNA, and on the assembly and maintenance of chromosomes. Dosage ranges for X-rays range from daily doses of 50 to

200 roentgens for prolonged periods of time (3 to 4 wk), to single doses of 2000 to 6000 roentgens. Dosage ranges for radioisotopes vary widely, and depend on the half-life of the isotope, the strength and type of radiation emitted, and the uptake by the neoplastic cells.

iii. Immunotherapy

5 **[00108]** The skilled artisan will understand that additional immunotherapies may be used in combination or in conjunction with methods of the embodiments. In the context of cancer treatment, immunotherapeutics, generally, rely on the use of immune effector cells and molecules to target and destroy cancer cells. Rituximab (RITUXAN®) is such an example. The immune effector may be, for example, an antibody specific for some
10 marker on the surface of a tumor cell. The antibody alone may serve as an effector of therapy or it may recruit other cells to actually affect cell killing. The antibody also may be conjugated to a drug or toxin (chemotherapeutic, radionuclide, ricin A chain, cholera toxin, pertussis toxin, etc.) and serve as a targeting agent. Alternatively, the effector may be a lymphocyte carrying a surface molecule that interacts, either directly or indirectly, with a
15 tumor cell target. Various effector cells include cytotoxic T cells and NK cells

[00109] Antibody-drug conjugates have emerged as a breakthrough approach to the development of cancer therapeutics. Cancer is one of the leading causes of deaths in the world. Antibody–drug conjugates (ADCs) comprise monoclonal antibodies (MAbs) that are covalently linked to cell-killing drugs (FIG. 1). This approach combines the high specificity
20 of MAbs against their antigen targets with highly potent cytotoxic drugs, resulting in “armed” MAbs that deliver the payload (drug) to tumor cells with enriched levels of the antigen (Carter *et al.*, 2008; Teicher 2014; Leal *et al.*, 2014). Targeted delivery of the drug also minimizes its exposure in normal tissues, resulting in decreased toxicity and improved therapeutic index. The approval of two ADC drugs, ADCETRIS® (brentuximab vedotin) in
25 2011 and KADCYLA® (trastuzumab emtansine or T-DM1) in 2013 by FDA validated the approach. There are currently more than 30 ADC drug candidates in various stages of clinical trials for cancer treatment (Leal *et al.*, 2014). As antibody engineering and linker-payload optimization are becoming more and more mature, the discovery and development of new ADCs are increasingly dependent on the identification and validation of new targets that
30 are suitable to this approach (Teicher 2009) and the generation of targeting MAbs. Two criteria for ADC targets are upregulated/high levels of expression in tumor cells and robust internalization.

[00110] In one aspect of immunotherapy, the tumor cell must bear some marker that is amenable to targeting, i.e., is not present on the majority of other cells. Many tumor markers exist and any of these may be suitable for targeting in the context of the present embodiments. Common tumor markers include CD20, carcinoembryonic antigen, tyrosinase (p97), gp68, TAG-72, HMFG, Sialyl Lewis Antigen, MucA, MucB, PLAP, laminin receptor, erb B, and p155. An alternative aspect of immunotherapy is to combine anticancer effects with immune stimulatory effects. Immune stimulating molecules also exist including: cytokines, such as IL-2, IL-4, IL-12, GM-CSF, gamma-IFN, chemokines, such as MIP-1, MCP-1, IL-8, and growth factors, such as FLT3 ligand.

[00111] Examples of immunotherapies currently under investigation or in use are immune adjuvants, e.g., Mycobacterium bovis, Plasmodium falciparum, dinitrochlorobenzene, and aromatic compounds (U.S. Patents 5,801,005 and 5,739,169; Hui and Hashimoto, 1998; Christodoulides et al., 1998); cytokine therapy, e.g., interferons α , β and γ , IL-1, GM-CSF, and TNF (Bukowski et al., 1998; Davidson et al., 1998; Hellstrand et al., 1998); gene therapy, e.g., TNF, IL-1, IL-2, and p53 (Qin et al., 1998; Austin-Ward and Villaseca, 1998; U.S. Patents 5,830,880 and 5,846,945); and monoclonal antibodies, e.g., anti-CD20, anti-ganglioside GM2, and anti-p185 (Hollander, 2012; Hanibuchi et al., 1998; U.S. Patent 5,824,311). It is contemplated that one or more anti-cancer therapies may be employed with the antibody therapies described herein.

iv. Surgery

[00112] Approximately 60% of persons with cancer will undergo surgery of some type, which includes preventative, diagnostic or staging, curative, and palliative surgery. Curative surgery includes resection in which all or part of cancerous tissue is physically removed, excised, and/or destroyed and may be used in conjunction with other therapies, such as the treatment of the present embodiments, chemotherapy, radiotherapy, hormonal therapy, gene therapy, immunotherapy, and/or alternative therapies. Tumor resection refers to physical removal of at least part of a tumor. In addition to tumor resection, treatment by surgery includes laser surgery, cryosurgery, electrosurgery, and microscopically-controlled surgery (Mohs' surgery).

[00113] Upon excision of part or all of cancerous cells, tissue, or tumor, a cavity may be formed in the body. Treatment may be accomplished by perfusion, direct injection, or local application of the area with an additional anti-cancer therapy. Such

treatment may be repeated, for example, every 1, 2, 3, 4, 5, 6, or 7 days, or every 1, 2, 3, 4, and 5 weeks or every 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months. These treatments may be of varying dosages as well.

v. Other Agents

5 **[00114]** It is contemplated that other agents may be used in combination with certain aspects of the present embodiments to improve the therapeutic efficacy of treatment. These additional agents include agents that affect the upregulation of cell surface receptors and GAP junctions, cytostatic and differentiation agents, inhibitors of cell adhesion, agents that increase the sensitivity of the hyperproliferative cells to apoptotic inducers, or other
10 biological agents. Increases in intercellular signaling by elevating the number of GAP junctions would increase the anti-hyperproliferative effects on the neighboring hyperproliferative cell population. In other embodiments, cytostatic or differentiation agents can be used in combination with certain aspects of the present embodiments to improve the anti-hyperproliferative efficacy of the treatments. Inhibitors of cell adhesion are
15 contemplated to improve the efficacy of the present embodiments. Examples of cell adhesion inhibitors are focal adhesion kinase (FAKs) inhibitors and Lovastatin. It is further contemplated that other agents that increase the sensitivity of a hyperproliferative cell to apoptosis, such as the antibody c225, could be used in combination with certain aspects of the present embodiments to improve the treatment efficacy.

20 III. Kits and Diagnostics

[00115] In various aspects of the embodiments, a kit is envisioned containing therapeutic agents and/or other therapeutic and delivery agents. In some embodiments, the present embodiments contemplates a kit for preparing and/or administering a therapy of the embodiments. The kit may comprise one or more sealed vials containing any of the
25 pharmaceutical compositions of the present embodiments. The kit may include, for example, at least one EGFL6 antibody as well as reagents to prepare, formulate, and/or administer the components of the embodiments or perform one or more steps of the inventive methods. In some embodiments, the kit may also comprise a suitable container, which is a container that will not react with components of the kit, such as an eppendorf tube, an assay plate, a syringe,
30 a bottle, or a tube. The container may be made from sterilizable materials such as plastic or glass.

[00116] The kit may further include an instruction sheet that outlines the procedural steps of the methods set forth herein, and will follow substantially the same procedures as described herein or are known to those of ordinary skill in the art. The instruction information may be in a computer readable media containing machine-readable instructions that, when executed using a computer, cause the display of a real or virtual procedure of delivering a pharmaceutically effective amount of a therapeutic agent.

IV. Examples

[00117] The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

Example 1 – Generation and cloning of monoclonal antibodies targeting human EGFL6

[00118] EGFL6 (Genebank accession # Q8IUX8) protein was used to immunize New Zealand rabbits at RevMAb Biosciences USA, Inc. Titer of anti-EGFL6 sera was determined by series of dilutions of serum in ELISA for binding by coating EGFL6 protein on 96-well plates (max-sorb plates, Nunc) and were detected with an anti-rabbit antibody conjugated with horseradish peroxidase (HRP) and TMB substrate. After 2-3 immunization boosts, the titer reached $>10^6$ and peripheral blood samples were collected from the immunized rabbits for B cells (CD45+CD5-CD19+) isolation from the freshly prepared peripheral blood mononuclear cells (PBMCs) using a fluorescence assisted cell sorting (FACS) instrument (BD FACS Aria™ III, BD Biosciences). The isolated B cells were plated as single B cells and cultured for 7-10 days. The culture supernatants were assayed for EGFL6 binding. Cells from the positives wells were lysed, total RNA was isolated, and cDNA was synthesized using a superscript reverse transcriptase II (Invitrogen) according to manufacturer's suggestion. DNA sequences of antibody variable region from both heavy chains and light chains were amplified by polymerase chain reaction (PCR) using a set of designed primers and sequenced. Both DNA and amino acid sequences are listed in Section

V. below. CDRs of the anti-EGFL6 monoclonal antibodies were identified using the IMGT program and are listed in **Tables 1 and 2.**

Table 1. CDRs of heavy chain variable sequences of EGFL6 antibodies.

mAb	Heavy Chain		
	CDR1	CDR2	CDR3
E1-33	ggactcgacctcagtagctactactac (SEQ ID NO: 1)	atttatctgtagtagtggtgacct (SEQ ID NO: 2)	gcgagagggtgtagtagtactatgctcaatatlttaacttg (SEQ ID NO: 3)
	GLDLSSYY (SEQ ID NO: 4)	IYAGSSGST (SEQ ID NO: 5)	ARGGGSTY AQYFNL (SEQ ID NO: 6)
E1-34	ggattctcttcagtagtatttattgg (SEQ ID NO: 7)	attcagattactagtggtatcact (SEQ ID NO: 8)	agaaggggataatggcgctatgctggtagtgggctctgacttg (SEQ ID NO: 9)
	GFSFSSIYW (SEQ ID NO: 10)	IQITSGIT (SEQ ID NO: 11)	RRGYGAYAGTGASDL (SEQ ID NO: 12)
E1-80	ggattcacccctcaatagttattat (SEQ ID NO: 13)	attgatagtgatagtcctactacg (SEQ ID NO: 14)	gcgagaggctatggctctgttcgattggatctc (SEQ ID NO: 15)
	GFTLNSYY (SEQ ID NO: 16)	IDS DSPPT (SEQ ID NO: 17)	ARGYGPVRLDL (SEQ ID NO: 18)
E1-89	ggattctcttcagtagcggtactgg (SEQ ID NO: 19)	atttatctgtagtagtggtggcac (SEQ ID NO: 20)	tgtacaagagataatattgggtgggtgtctgcttccaaattg (SEQ ID NO: 21)
	GFSFSSGYW (SEQ ID NO: 22)	IYAGSSGGH (SEQ ID NO: 23)	CTRDNYGGGGSASKL (SEQ ID NO: 24)
E2-93	ggattctcttcagtagttatgga (SEQ ID NO: 25)	attggtcttagtagtagatc (SEQ ID NO: 26)	gtgagagatcttatacatagtaattgggtg (SEQ ID NO: 27)
	GFSFSSYG (SEQ ID NO: 28)	IGLSSEI (SEQ ID NO: 29)	VRDLYHSNGL (SEQ ID NO: 30)
E1-38	ggattctcttcagtagcggtactgg (SEQ ID NO: 31)	atctatactagtagtctactggtgcc (SEQ ID NO: 32)	tgtacaagagataatattgggtgggtgtctgcttccaaattg (SEQ ID NO: 33)
	GFSFNSGYW (SEQ ID NO: 34)	IYTSSPTGA (SEQ ID NO: 35)	CTRDNFGGGSASKL (SEQ ID NO: 36)
E1-52	ggattcacccctcagtagctactac (SEQ ID NO: 37)	attgatctgataatgatattagg (SEQ ID NO: 38)	gggagaggctatgggtcgctcggtggatctc (SEQ ID NO: 39)
	GFTLSSYY (SEQ ID NO: 40)	IDTDNDIR (SEQ ID NO: 41)	GRGYGALRLDL (SEQ ID NO: 42)
E2-36	ggattctcttcagtagctaccac (SEQ ID NO: 43)	attaataatattgggccaca (SEQ ID NO: 44)	gccagaagtcctgggattcctgggttaattacg (SEQ ID NO: 45)

	GFSLSYYH (SEQ ID NO: 46)	INNYGAT (SEQ ID NO: 47)	ARSPGIPGYN (SEQ ID NO: 48)
E1-95	ggattctctcagtagcaatca (SEQ ID NO: 49)	attgtagtagtagtagtagt (SEQ ID NO: 50)	gcgagagattcggtaatcgtagctttagcggcgactttaacttg (SEQ ID NO: 51)
	GFSFSSNS (SEQ ID NO: 52)	IASSSSHS (SEQ ID NO: 53)	ARDSGNRGYLYAGDFNL (SEQ ID NO: 54)
E2-116	ggatcgactcagtagctctactac (SEQ ID NO: 55)	attgacggtgggggtagccact (SEQ ID NO: 56)	gcgagacgagatcgtagctgggaacgccttagcttg (SEQ ID NO: 57)
	GFDLSSYY (SEQ ID NO: 58)	IDGGGEPT (SEQ ID NO: 59)	ARRDAGAGNAFSL (SEQ ID NO: 60)
E2-135	ggatcgactcagtagcagctactt (SEQ ID NO: 61)	atttactgttagtagcgaagact (SEQ ID NO: 62)	gcgagatcggaacaattgaaagattggatctc (SEQ ID NO: 63)
	GFDSSSYF (SEQ ID NO: 64)	IYTVISRKT (SEQ ID NO: 65)	ARSATIERLDL (SEQ ID NO: 66)
E1-142	ggattcaccatcaataactacaac (SEQ ID NO: 67)	atttgaatggtagggcagc (SEQ ID NO: 68)	gcgagaaattttaacttg (SEQ ID NO: 69)
	GFTINNYN (SEQ ID NO: 70)	IWNGDGS (SEQ ID NO: 71)	ARNFNL (SEQ ID NO: 72)

Table 2. CDRs of light chain variable sequences of EGFL6 antibodies.

	Light Chain		
	CDR1	CDR2	CDR3
E1-33	ccgagtggttaggcactac (SEQ ID NO: 73)	tggtctcc (SEQ ID NO: 74)	gcagggaatatgctagtagtagataatcat (SEQ ID NO: 75)
	PSVYRHY (SEQ ID NO: 76)	WAS (SEQ ID NO: 77)	AGEYASDSDNH (SEQ ID NO: 78)
E1-34	cagagtggttataataacaac (SEQ ID NO: 79)	gaagcatcc (SEQ ID NO: 80)	gcaggcgttagctggctacattgggct (SEQ ID NO: 81)
	QSVYNNNN (SEQ ID NO: 82)	EAS (SEQ ID NO: 83)	AGGYAGYIWA (SEQ ID NO: 84)
E1-80	aagaacgcctattatcctactac (SEQ ID NO: 85)	tggtctcc (SEQ ID NO: 86)	gcagggaatatagtagtagtagataatgggt (SEQ ID NO: 87)

[00120]

	KNAYLSYY (SEQ ID NO: 88)	WAS (SEQ ID NO: 77)	AAEYNSDSNG (SEQ ID NO: 89)
E1-89	cagagtgtttatagtaacaaccgc (SEQ ID NO: 90)	tatgcagcc (SEQ ID NO: 91)	gcaggataaaaactcgtgattctgatggattgct (SEQ ID NO: 92)
	QSVYNNR (SEQ ID NO: 93)	YAA (SEQ ID NO: 94)	AGYKTADSDGIA (SEQ ID NO: 95)
E2-93	gagagcgtttataataaaccgc (SEQ ID NO: 96)	tatgcatcc (SEQ ID NO: 97)	gtagcctttaaagggtatggtacgacggcaatgct (SEQ ID NO: 98)
	ESVYNNR (SEQ ID NO: 99)	YAS (SEQ ID NO: 100)	VAFKGYGTDGNA (SEQ ID NO: 101)
E1-38	gagagtgtttatagtaacaaccgc (SEQ ID NO: 102)	tatgcatcc (SEQ ID NO: 97)	gcaggataaagactgccgattctgatggcttggt (SEQ ID NO: 103)
	ESVYNNR (SEQ ID NO: 104)	YAS (SEQ ID NO: 100)	AGYKTADSDGLG (SEQ ID NO: 105)
E1-52	ccgagtgtttatagtcactac (SEQ ID NO: 106)	tgggttcc (SEQ ID NO: 86)	gcagggaatatgctagtagtgataatcat (SEQ ID NO: 107)
	PSVYRHY (SEQ ID NO: 108)	WAS (SEQ ID NO: 77)	AGEYASDSNH (SEQ ID NO: 109)
E2-36	cagaatgtttatagtiacaaccgc (SEQ ID NO: 110)	gaagcatcc (SEQ ID NO: 111)	gcaggcgggtatgtagtaggagttctgatgtagct (SEQ ID NO: 112)
	QNVYNNR (SEQ ID NO: 113)	EAS (SEQ ID NO: 83)	AGGYDCRSSDCDA (SEQ ID NO: 114)
E1-95	cagagcattatagttgg (SEQ ID NO: 115)	gaagcatcc (SEQ ID NO: 111)	caacagggttatagttatagtaattgataataatatt (SEQ ID NO: 116)
	QSINSW (SEQ ID NO: 117)	EAS (SEQ ID NO: 83)	QQGYSYNNVDNNI (SEQ ID NO: 118)
E2-116	caaagtgtttatctcagaacaac (SEQ ID NO: 119)	tatgcatcc (SEQ ID NO: 97)	caggcgggttacagtgagataatcaattct (SEQ ID NO: 120)
	QSVYLQNN (SEQ ID NO: 121)	YAS (SEQ ID NO: 100)	QGGYSGYINS (SEQ ID NO: 122)

E2-135	gagagtgtttataataactaccgc (SEQ ID NO: 123)	gctgcaccc (SEQ ID NO: 124)	gtaggataaaaagtggttatattgtagtattcct (SEQ ID NO: 125)
	ESVYNNYR (SEQ ID NO: 126)	AAS (SEQ ID NO: 127)	VGYSKSGYIDSIP (SEQ ID NO: 128)
E1-142	gcgagtgtttatagtaacaactac (SEQ ID NO: 129)	tatgcaccc (SEQ ID NO: 97)	gcaggcgattatagtagtagtgatagtgtatt (SEQ ID NO: 130)
	ASVYSNNY (SEQ ID NO: 131)	YAS (SEQ ID NO: 100)	AGDYSSSSDMCI (SEQ ID NO: 132)

[00121] Selected EGFL6 binding hits were expressed as rabbit or rabbit/human chimeric IgGs using a mammalian expression vector system in human embryonic kidney (HEK293) cells (Invitrogen). Antibodies were purified using a column with protein A resin by a fast protein liquid chromatography (FPLC) separation unit. Purified EGFL6 binding
5 antibodies were characterized for their biological properties.

Example 2 – Binding affinity of anti-EGFL6 monoclonal antibodies to the EGFL6 protein

[00122] Binding of EGFL6 by monoclonal antibodies was first screened by ELISA using supernatants collected from the B cell cultures (**FIG. 2**). ELISA titration was
10 used to determine the binding affinity of a panel of monoclonal antibodies to EGFL6 antigen (**FIG. 3**). Binding constants (K_D and/or EC 50) of a panel of monoclonal antibodies were estimated using the 4 parameter curve fitting with Prism GraphPad program. For Biacore analysis, all experiments were performed at 25°C at a flow rate of 45 μ l/min. An anti-human IgG Fc antibody (from ThermoFisher, at 50 μ g/ml each in acetate buffer, pH 5.0) was
15 immobilized onto a carboxymethyl dextran sensorchip (CM5) using amine coupling procedures based on instruction from the manufacturer. Purified rabbit/human chimeric antibody to be tested was diluted at a concentration of 5 μ g/ml in 0.5% P20, HBS-EP buffer and injected on FC2 to reach 500 to 1000 RU. FC1 was used as the reference cell. Specific signals correspond to the difference of signals obtained on FC2 *versus* FC1. The analyte
20 (recombinant human EGFL6, apparent molecular weight 60 kDa on SDS-PAGE gel) was injected during 90 sec at series of concentration dilutions (100, 50, 25, 12.5, 6.25, and 3.13, 1.56 nM) in 0.5 % P20, HBS-EP buffer. These concentrations were prepared from stock solution in 0.5 % P20, HBS-EP. The dissociation phase of the analyte was monitored over a 30 minutes period. Running buffer was also injected under the same conditions as a double
25 reference. After each running cycle, both flow cells were regenerated by injecting 20 to 45 μ l of Glycine-HCl buffer pH 1.5. Binding K_D on EGFL6 was calculated by k_{off}/k_{on} kinetic rate for each EGFL6 monoclonal antibodies (**Table 3**).

[00123] **Table 3.** EGFL6 antibody binding affinities determined by ELISA or Biacore method.

Antibody name	EC50 (ng/ml)
E1-34	0.78
E1-38	5.81
E2-93	0.37
E1-142	1.91
E2-135	0.44

Example 3 – Experimental Procedures and Methods

5 **[00124]** **Cell lines and culture:** Human epithelial ovarian cancer cell lines, SKOV3ip1 and A2780ip2 were maintained as described (Sood, A.K. *et al.* Molecular determinants of ovarian cancer plasticity. *American Journal of Pathology* 158, 1279-1288, 2001). Human immortalized umbilical endothelial cells (RF24) were grown in MEM medium with supplements (sodium pyruvate, non-essential amino acids, MEM vitamins and
10 glutamine; Life Technologies). The derivation and characterization of the mouse ovarian endothelial cells (MOEC) has been described previously (Langley, R.R. *et al.* Tissue-specific microvascular endothelial cell lines from H-2K(b)-tsA58 mice for studies of angiogenesis and metastasis. *Cancer Research* 63, 2971-2976, 2003). Cell cultures were maintained at 37°C in a 5% CO₂ incubator with 95% humidity. For *in vivo* injections, cells were
15 trypsinized and centrifuged at 1,200 rpm for 5 min at 4°C, washed twice with PBS, and reconstituted in serum-free Hank's balanced salt solution (Life Technologies, Grand Island, NY, USA). Only single-cell suspensions with more than 95% viability (as determined by trypan blue exclusion) were used for *in vivo* intra-peritoneal injections.

[00125] **Endothelial cell isolation:** Fresh tissue samples (5 normal ovaries, 5
20 wound tissue and 10 epithelial high-grade, stage III or IV invasive serous ovarian cancers) were obtained from patients undergoing primary surgical exploration at the M. D. Anderson Cancer Center after approval from the Institutional Review Board. Total RNA from purified endothelial cells was subjected to microarray analysis using the Affymetrix Human U133 plus 2.0 GeneChip platform (Lu, C. *et al.* Gene alterations identified by expression profiling
25 in tumor-associated endothelial cells from invasive ovarian carcinoma. *Cancer Research* 67, 1757-1768, 2007).

[00126] **Quantitative real-time PCR validation:** Quantitative real-time RT-PCR was performed using 50 ng of total RNA from purified endothelial cells was isolated

using the RNeasy mini kit (Qiagen) according to the manufacturer's instructions. Complementary DNA (cDNA) was synthesized from 0.5-1µg of total RNA using Verso cDNA kit (Thermo Scientific). Quantitative PCR (qPCR) analysis was performed in triplicate using the SYBR Green ER qPCR SuperMix Universal (Invitrogen) and Bio-Rad (Bio-Rad Laboratories, Hercules, CA, USA). Relative quantification was calculated using the $2^{-\Delta\Delta CT}$ method normalizing to control for percent fold changes (Donninger, H. *et al.* Whole genome expression profiling of advance stage papillary serous ovarian cancer reveals activated pathways. *Oncogene* **23**, 8065-8077, 2004).

[00127] SiRNA constructs and delivery: SiRNAs were purchased from Sigma-Aldrich (The Woodlands, TX, USA). A non-silencing siRNA that did not share sequence homology with any known human mRNA based on a BLAST search was used as control for target siRNA. *In vitro* transient transfection was performed as described (Landen, C.N., Jr. *et al.* Therapeutic EphA2 gene targeting *in vivo* using neutral liposomal small interfering RNA delivery. *Cancer Research* **65**, 6910-6918, 2005). Briefly, siRNA (4 µg) was incubated with 10 µL of Lipofectamine 2000 transfection reagent (Lipofectamine) for 20 min at room temperature according to manufacturer's instructions and added to cells in culture at 80% confluence in 10 cm culture plates.

[00128] Reverse phase protein array (RPPA) and Western blot analysis: RF24 and OVCAR3 cells in the presence or absence of human recombinant EGFL6 protein were subjected to RPPA analysis. Western blot analysis was performed as previously (Landen, C.N., Jr. *et al.* 2005, *ibid*; Halder, J. *et al.* Focal adhesion kinase targeting using *in vivo* short interfering RNA delivery in neutral liposomes for ovarian carcinoma therapy. *Clinical Cancer Research: an official journal of the American Association for Cancer Research* **12**, 4916-4924, 2006). Cell lysate of RF24 cells treated with human recombinant EGFL6 protein or anti-EGFL6 antibodies and checked for activation of PI3kinase and AKT signaling using anti-human EGFL6, PI3kinase and AKT antibodies followed by secondary antibodies conjugated with horseradish peroxidase (HRP).

[00129] Cell migration assay: Using modified Boyden chambers coated with 0.1% gelatin, migration of the RF24 cells was assessed in the presence or absence of hEGFL6 siRNA. After post transfection of 48h with hEGFL6 or integrin siRNAs or with EGFL6 antibody or PI3kinase inhibitor for 6h, RF24 cells (1.0×10^5) in MEM serum free medium were seeded into the upper chamber of the Transwell pore Polycarbonate Membrane insert

(Corning, Lowell, MA, USA). The chamber was placed in a 24-well plate containing MEM medium with 15% serum in the lower chamber as chemo-attractant. Cells were allowed to migrate in a humidified incubator for 6h. Cells that had migrated were stained using hematoxylin staining and counted by light microscopy in five random fields (200x original
5 magnification) per sample. Experiments were done in duplicate and performed three times.

[00130] Tube formation assay: Matrigel (12.5 mg/mL) was thawed at 4°C and 50 µL were quickly added to each well of a 96-well plate and allowed to solidify for 10 min at 37°C. The wells were then incubated for 6 h at 37°C with RF24 cells (20,000 per well), which had previously been treated with EGFL6 or integrin siRNA (for 48 h) or
10 EGFL6 antibody or PI3kinase inhibitor (for 6h). Experiments were performed in triplicate and repeated twice. Using an Olympus IX81 inverted microscope, five images per well were taken at × 100 magnification. The amount of nodes (defined as when at least three cells formed a single point) per image was quantified. To account for cell clumping, the highest and lowest value was removed from each group.

[00131] Promoter analysis and chromatin immunoprecipitation (ChIP) assay: RF24 cells were cultured in low serum medium (0.5% serum) for 18 h and then treated with either EGFL6 or HIF1α (50 ng/mL) for 6 hours. After treatment, ChIP assays were performed using EZ ChIP™ kit (Millipore, Temecula, CA, USA) as described by the manufacturer. Briefly, cross-linked cells were collected, lysed, sonicated and subsequently
20 subjected to immunoprecipitation with EGFL6 (Abchem) antibody or IgG control. Immunocomplexes were collected with protein G agarose beads and eluted. Cross-links were reversed by incubating at 65 °C. DNA then was extracted and purified for PCR using primers pairs upstream of the EGFL6 transcription start site.

[00132] Flow cytometric analysis: RF24 cells were washed with PBS and
25 harvested with PBS-EDTA 5mM. Cells were then immunolabeled with different integrin primary antibodies (Sigma-Aldrich) and subsequently stained with secondary antibodies (Invitrogen). Samples were acquired on a FACSCalibur with Cell Quest software and data were analyzed with FlowJo software.

[00133] Orthotopic *in vivo* model of ovarian cancer and tissue processing:
30 Female athymic nude mice (NCR-nu) were purchased from the NCI-Frederick Cancer Research and Development Center (Frederick, MD, USA) and maintained as previously

described (Landen, C.N., Jr. *et al.* 2005, *ibid*). All mouse studies were approved by the Institutional Animal Care and Use Committee. Mice were cared for in accordance with guidelines set forth by the American Association for Accreditation of Laboratory Animal Care and the US Public Health Service Policy on Human Care and Use of Laboratory Animals. For tumor cells injection, A2780ip2 or SKOV3ip1 or OVCAR3 cells (1×10^6) were injected intra peritoneal (i.p). For therapy experiments, each siRNA was given twice weekly at a dose of 150 μ g/kg body weight. At the time of sacrifice, mouse and tumor weight, number and distribution of tumors were recorded. Individuals who performed the necropsies were blinded to the treatment group assignments. Tissue specimens were fixed either with formalin, OCT (Miles, Inc., Elkhart, IN, USA) or snap frozen in liquid nitrogen. For off-target effects, SKOV3ip1 tumor bearing mice were treated with two different EGFL6 siRNA sequences same as mentioned above.

[00134] Immunohistochemical and Immunofluorescence staining of xenografts: IHC analysis for cell proliferation (Ki67, 1:200, Zymed), microvessel density (MVD, CD31, 1:500, Pharmingen), and hypoxia (carbonic anhydrase Anti-CA9, 1:500, Novus), were all performed as described (Thaker, P.H. *et al.* Chronic stress promotes tumor growth and angiogenesis in a mouse model of ovarian carcinoma. *Nature Medicine* **12**, 939-944, 2006; Lu, C. *et al.* Regulation of tumor angiogenesis by EZH2. *Cancer Cell* **18**, 185-197, 2010). For statistical analyses, sections from five randomly selected tumors per group were stained and 5 random fields per tumor were scored. Pictures were taken at x200 or x100 magnification. To quantify MVD in the mouse tumor samples, the number of blood vessels staining positive for CD31 was recorded in 10 random 0.159-mm² fields at x200 magnification. To quantify PCNA expression, the number of positive cells (3,3'-diaminobenzidine staining) was counted in 10 random 0.159-mm² fields at $\times 100$ magnification (Thaker, P.H. *et al.* 2006, *ibid* ; Lu, C. *et al.* 2010, *ibid*). All staining was quantified by 2 investigators in a blinded fashion. Staining for EGFL6 (Santa Cruz) and CD31 was performed using frozen tissue as described (Lu, C. *et al.* 2010, *ibid*).

[00135] Matrigel plug assay: An *in-vivo* matrigel plug assay was performed by injecting matrigel plugs into mice subcutaneously. The matrigel plug included either serum free MEM complete medium (as negative control), VEGF (as positive control) or EGFL6 (as test group). After 6h post injection, animals were sacrificed and the matrigel was collected and hemoglobin assay was performed.

[00136] **Wound healing assay:** On day 1, A2780 ip2 cells were injected into nude mice and on day 2 a wound was created on back of the tumor bearing mice. Animals received veterinary care and were maintained in individual cages. Mice were divided into two groups (n=10).

5 [00137] **CH/ControlsiRNA and CH/mEGFL6 siRNA nanoparticles:** siRNA treatment was started on day 3 and given twice a week (150 µg/kg). Wound was measured on day 0, 1, 3, 5, 7, 9, 11, 13, and 15 (till the completion of wound healing). The tumors were harvested when animals in any group became moribund.

[00138] **Hind-Limb Ischemia:** Critical hind-limb ischemia as described previously (Baluk, P., Hashizume, H. & McDonald, D.M. Cellular abnormalities of blood vessels as targets in cancer. *Current Opinion in Genetics & Development* **15**, 102-111, 2005) was induced in female nude mice after being anesthetized with ketamine (100 mg/kg) by intraperitoneal injection and the femoral artery was excised from its proximal origin as a branch of the external iliac artery to the distal point where it bifurcates into the saphenous and popliteal arteries. After arterial ligation, mice were immediately assigned to the following experimental groups (n= 5): control group, ischemia-24h and ischemia-96h. Serial laser Doppler imaging analysis (Moor Instruments, Devon, UK) was performed to monitor the blood flow of hind-limbs before and after femoral artery ligation (after 24h and 96h). The digital color-coded images were analyzed to quantify the blood flow in the region from the knee to the toe; the mean values of perfusion were calculated. At each time point, tissue from the ischemic limb was harvested and frozen in OCT medium. Mouse monoclonal anti-CD31 was used to determine the MVD and mouse polyclonal anti-EGFL6 antibody for EGFL6 expression on frozen embedded tissues using standard immunostaining procedure.

10
15
20

[00139] **Human ovarian cancer specimens:** Following approval by the Institutional Review Board, 180 paraffin-embedded epithelial ovarian cancer specimens (collected between 1985-2004) with available clinical outcome data and confirmed diagnosis by a board-certified gynecologic pathologist were obtained from the Karmanos Cancer Institute tumor bank.

25

[00140] For human ovarian cancer samples, immunohistochemistry for EGFL6, CD34, and VEGF, was performed, as described previously (Ali-Fehmi, R. *et al.* Expression of cyclooxygenase-2 in advanced stage ovarian serous carcinoma: correlation with tumor cell

30

proliferation, apoptosis, angiogenesis, and survival. *American Journal of Obstetrics and Gynecology* **192**, 819-825, 2005). EGFL6 staining was performed using an anti-human EGFL6 antibody (Sigma-Aldrich). In brief, formalin- fixed, paraffin-embedded tissue sections were de-paraffinized and rehydrated. After antigen retrieval with Diva solution, the endogenous peroxidase was blocked with 3% hydrogen peroxide in methanol for 15 min. After washing with PBS, sections were blocked with protein block (5% normal horse serum and 1% goat serum) for 20 min at room temperature (RT), followed by incubation with the anti-EGFL6 antibody (Sigma-Aldrich) overnight at 4 °C. After washing with PBS, sections were incubated with horseradish peroxidase (HRP)-conjugated goat anti-rabbit (1:250, Jackson ImmunoResearch) for 1h at RT. Finally, visualization was attained with 3, 3'-diaminobenzidine (Research Genetics) and counter-staining with Gill's hematoxylin (BioGenex Laboratories). Negative staining was reported as score 0, scores 1-4 were used for increasing intensity of EGFL6. The stained slides were scored by two investigators on the basis of the histochemical score (H-score; >100 defined as high expression and ≤100, low expression), according to the method described previously (Ali-Fehmi, *et al.*, 2005 *ibid*), which considers both the intensity of staining and the percentage of cells stained.

[00141] Statistical analysis: For animal experiments, ten mice were assigned per treatment group. This sample size gave 80% power to detect a 50% reduction in tumor weight with 95% confidence. Tumor weights and the number of tumor nodules for each group were compared using Student's *t*-test (for comparisons of two groups). A P-value less than 0.05 were deemed statistically significant. All statistical tests were two-sided and were performed using SPSS version 12 for Windows statistical software (SPSS, Inc., Chicago, IL, USA).

Example 4 – EGFL6 expression upregulated in tumor associated endothelial cells

[00142] Five normal ovaries, five wound tissue samples and 10 invasive epithelial ovarian tumors were obtained and subjected to negative and positive immunoselection. Prior to carrying out microarray analysis, the purity of all samples for endothelial cells was established using the endothelial cell markers P1H12 and von Willebrand factor (FIG. 4A). Immunostaining revealed that the immunopurification technique had yielded endothelial cell purity of >95% in all samples. Analysis of the data revealed that 375 genes were upregulated in ovarian tumor endothelial cells compared to normal and wound endothelial cells (FIG. 4B). Among them, EGFL6 showed the highest

differential expression in tumor endothelial cells compared to normal and wound endothelial cells (FIG. 4B). Expression of EGFL6, VEGF and CD31 in ovarian patient samples was determined (FIG. 4C). To further validate this result, endothelial cells were isolated from normal ovary, ovarian tumor and wound healing tissue and the expression of EGFL6 was determined using PCR. EGFL6 predominantly overexpressed only in tumor endothelial cells as compared to normal or wound endothelial cells (FIG. 4D). EGFL6 upregulation in tumor endothelial cells was also demonstrated. EGFL6 was expressed in endothelial cells and most of the ovarian cancer cells tested. To demonstrate the role of EGFL6 in tumor angiogenesis, RF24 cells were treated with siEGFL6 which resulted in a greater than 80% knockdown in protein levels at 72hrs as compared to control cells. EGFL6 siRNA treated cells showed significantly less migration and tube formation compared to control siRNA treated cells indicating the importance of EGFL6 in angiogenesis.

Example 5 - EGFL6 silencing did not affect wound healing in mice

[00143] The role of EGFL6 in wound healing was addressed using wounds generated using human dermal microvascular endothelial cells (HDMECs). Effects on wound healing were conducted using following procedures. On day 1, SKOV3ip1 cells were injected into nude mice and on day 2 wound was created on back of the tumor bearing mice (2cm x 2cm). Animals were randomly divided into two groups (n=10), one with administration of control antibody and the other group of mice were treated with EGFL6 antibody. Antibody treatment was started on day 3 and given once a week (5mg/kg). Wound (area=length X width) was monitored for 2 weeks until completion of wound healing. EGFL6 antibody did not prevent wound healing when tested using a wound healing *in vivo* study (**FIG. 9C**).

[00144] In a wound-healing assay revealed that after 24hrs, siControl treated cells and siEGFL6 treated cells had no effect on wound healing capacity (FIGS. 5A-5C). Moreover, similar wounds generated on tumor bearing mice were used to determine the effect on wound healing of EGFL6 silencing in endothelial cell compartment using murine siRNA sequence. As shown in FIG. 5D and 5E, no significant difference was observed in the wound healing of the animals treated with control siRNA or mouse EGFL6 siRNAs and both groups also showed similar patterns of wound healing.

[00145] However, animals treated with mouse EGFL6 siRNA showed a significant reduction in tumor burden (FIGS. 5F-5H), suggesting that silencing of EGFL6 in the endothelial cell compartment significantly affects tumor growth but does not compromise

wound healing. EGFL6 gene silencing also resulted in significant reduction in proliferation of tumor blood vessels (FIGS. 11A-11B).

Example 6 - EGFL6 enhances angiogenesis in endothelial cells

[00146] To establish that EGFL6 leads to increased survival of endothelial cells under hypoxic conditions, EGFL6 was silenced in hypoxic RF24 cells in hypoxia using EGFL6 siRNA and cell death was examined. As shown in FIG. 6A, almost 50% of the cells survived hypoxia even after 5 days compared to normoxia. In contrast to this, EGFL6 gene silencing in hypoxia resulted in 75% cell death compared to untreated cells under hypoxia and normoxia condition (FIG. 6H). hind limb ischemia was created in mice by excising the femoral artery on the hind limb of the mouse, which resulted in shut off of blood and oxygen supply to the hind limb (FIG. 6I). As shown in FIG. 6J-6K, ischemic mice showed significant reduction in MVD (blood vessels) and increase in EGFL6 expression in endothelial cells. The migration (FIG. 12C) and tube formation (FIG. 12D) of RF24 cells increased after treatment with EGFL6.

Example 7 – EGFL6 silencing inhibits tumor growth and angiogenesis

[00147] The therapeutic efficacy of EGFL6 in gene silencing was studied using two orthotopic ovarian cancer tumor models, SKOV3ip1 and OVCAR5. Female athymic nude mice (NCr-nu) were purchased from the NCI-Frederick Cancer Research and Development Center (Frederick, MD) and all mouse studies were approved by the Institutional Animal Care and Use Committee. Mice were cared for in accordance with guidelines set forth by the American Association for Accreditation of Laboratory Animal Care and the US Public Health Service Policy on Human Care and Use of Laboratory Animals. For tumor cells injection, SKOV3ip1 cells (1×10^6) were injected through intra peritoneal (i.p) route. For antibody treatment groups, purified monoclonal antibody was dosed weekly for 5 weeks at 5 mg/kg body weight. At the time of sacrifice, mouse and tumor weight, number and distribution of tumors were recorded. Individuals who performed the necropsies were blinded to the treatment group assignments. Tissue specimens were fixed either with formalin, OCT (Miles, Inc., Elkhart, IN) or snap frozen in liquid nitrogen.

[00148] As shown in FIG. 8F, treatment of SKOV3ip1 tumor bearing animals with mouse EGFL6 siRNA alone and in combination with human EGFL6 siRNA resulted in a significant reduction in tumor growth as compared to tumor bearing mice that were treated

with control siRNA. Human EGFL6 siRNA alone did not have much effect on tumor reduction. The effect of EGFL6 on the number of tumor nodules and observed tumor burden are shown in FIG. 8F. OVCAR5 tumor bearing mice treated with mouse EGFL6 siRNA alone and in combination with human EGFL6 siRNA also showed significant reduction in tumor weight and nodules.

[00149] SKOV3ip1 tumor bearing animals treated with mEGFL6 siRNA and the combination of mouse and human EGFL6siRNA showed a significant reduction in proliferating cells and microvessel density as compared to animals treated with the control siRNA (FIGS. 8C-8D). OVCAR5 tumor bearing animals also showed similar results. To determine the off-target effects of mouse EGFL6 siRNA sequences, the effect of EGFL6 gene silencing on SKOV3ip1 tumor growth was checked using two other mouse siRNA sequences and both sequences showed substantial reduction in tumor growth and tumor nodules.

[00150] Treatments with anti-EGFL6 antibodies Mab #135 and #93 (E2-135 & E2-93) greatly suppressed tumor growth (FIG. 8F) and only residue cancer cells were detected in EGFL6 antibody treated mice, but un-treated control mice had large tumor load and tumor spread as indicated as number of tumor nodules. Treatment with anti-EGFL6 antibodies (Mab E2-93 and E2-135) also inhibited cancer cell proliferation (Ki67 staining) and reduced micro-vasculature density (tumor angiogenesis, CD31 IHC staining) when compared to the control antibody treated groups (FIG. 8G).

Example 8 – Anti-EGFL6 blocking antibody reduces angiogenesis in endothelial cells

[00151] To demonstrate that EGFL6 blocking would affect its angiogenic mediated functions an EGFL6 functional blocking antibody was developed and tested for its activity on angiogenesis. Several EGFL6 antibody clones bound to human and mouse EGFL6 were screened with comparable affinities. Two antibodies (93 and 135) met all binding affinity and *in vitro* activity criteria were chosen to carry out for further studies. As shown in FIG. 8B, treatment of endothelial cells with EGFL6 recombinant protein increased the expression of both phosphorylated Tie2 and AKT proteins. In contrast to this, EGFL6 blocking antibodies 93 and 135 resulted in reduction in expression of both phosphorylated proteins. As shown in FIGS. 8D-E, treatment of endothelial cells with EGFL6 recombinant

protein enhanced the migration and tube formation in these cells. However, the EGFL6 mediated functional effects of both tube formation and migration was significantly reduced by EGFL6 blocking antibodies.

[00152] One of the antibodies was subject to humanization in which it was placed within the human IgG1 backbone to enable use in human cancer patients, and it was demonstrated that the binding affinity and *in vitro* activities of antibody were preserved after humanization.

Example 9 - Anti-EGFL6 blocking antibody had anti-angiogenesis and anti-tumor effects in ovarian cancer models

[00153] The *in vitro* activity of EGFL6 reported above indicated that blocking EGFL6 function would enhance the ability to damage tumor vessels, thereby increasing the anti-tumor efficacy. To test this, the ability of EGFL6 antibody to block the activity of EGFL6 and to inhibit angiogenesis and tumor growth and angiogenesis was investigated.

[00154] SKOV3-ip1 tumor-bearing mice were treated with control antibody and anti-EGFL6 antibodies. After 5 weeks of treatment, tumors were harvested and analyzed for anti-tumor and anti-angiogenic activity. Treatments with anti-EGFL6 antibodies resulted in potent anti-tumor activity as compared to treatments with control antibody. Treatment with both anti-EGFL6 antibodies 93 and 135 resulted in significant reduction in tumor weight and tumor nodules (FIGS. 9E and 9F). Animals treated with EGFL6 blocking antibody also showed decreased MVD compared to the control antibody treated groups (FIG. 9F), indicating that blocking EGFL6 activity inhibited tumor growth and angiogenesis. EGFL6 antibody did not prevent wound healing *in vitro* or *in vivo* (FIG. 9G), illustrating that it could regulate tumor angiogenesis, without affecting normal tissue repair.

V. Antibody Variable Sequences

[00155] Variable DNA sequences of anti-EGFL6 antibodies are shown below.

[00156] >E1-33H

CAGTCGCTGGAGGAGTCCGAGGGAGGCCTGGTCCAGCCTGAGGGATCCCTGACA
CTCACCTGCAAAGCCTCTGGACTCGACCTCAGTAGCTACTACTACATGTGCTGGG
TCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGATCGCATGCATTTATGCTGGTA

GTAGTGGTAGCACTTACTACGCGAGCTGGGCGAAAGGCCGATTCACCATCTCCA
AAACCTCGTCGACCACGGTGACTCTGCAAATGACCAGTCTGACAGCCGCGGACA
CGGCCACCTATTTCTGTGCGAGAGGTGGTGGTAGTACTTATGCTCAATATTTTAA
CTTGTGGGGCCCAGGCACCCTGGTCACCATCTCCTCAG (SEQ ID NO: 133)

5 [00157] >E1-33K

GAGCTCGATATGACCCANACACCAGCCTCCGTGTCTGCAGCTGTGGGAGGCACA
GTCAGCATCAATTGCCAGTCCAGTCCGAGTGTTTATAGGCACTACTTATCCTGGT
ATCAGCAGAAACCAGGGCAGCCTCCCAAGCTCCTGATCTACTGGGCTTCCACTCT
GGCATCTGGGGTCCCATCGCGGTTTCAGCGGCAGTGGATCTGGGACAGAGTTCCT
10 CTCACCATCAGCGGCGTGCAGTGTGACGATGCTGCCACTTACTACTGTGCAGGCG
AATATGCTAGTGATAGTGATAATCATTTTCGGCGGAGGGACCGAGCTGGAGATCC
TAG (SEQ ID NO: 134)

[00158] >E1-34H

GAGCAGTCGGTGAAGGAGTCCGGGGGAGGCCTGGTCCAGCCTGAGGGATCCCTG
15 ACACTCACCTGCACAGCTTCTGGATTCTCCTTCAGTAGTATTTATTGGATATGCTG
GGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTTGATCGCATGCATTCAGATTAC
TAGTGGTATCACTTACTACGCGAGCTGGGCGAAAGGCCGATTCACCATCTCCAAA
ATGTCGTCGACCACGGTGACTCTGCAAATGACCAGTCTGACAGTCGCGGACACG
GCCACCTATTTCTGTGGGAGAAGGGGATATGGTGCCTATGCTGGTACTGGTGCCT
20 CTGACTTGTGGGGCCCAGGCACCCTGGTCACCGTCTCTTCAG (SEQ ID NO: 135)

[00159] >E1-34K

GAGCTCGATCTGACCCAGACTGCATCGTCCGTGTCTGCAGCTGTGGGAGGCACCG
TCACCATCAATTGCCAGTCCAGTCAGAGTGTTTATAATAACAACAACCTTAGCCTG
GTATCAGCAGAAACCAGGGCAGCCTCCCAAGCTCCTGATCTACGAAGCATCCAA
25 ACTGGCATCTGGGGTCCCATCGCGGTTCAAAGGCAGTGGATCTGGGACACAGTTC
ACTCTCACCATCAGCGGCGTGCAGTGTGACGATGCTGCCACTTACTATTGTGCAG
GCGGTTATGCTGGCTACATTTGGGCTTTCGGCGGAGGGACCGAGGTGGTGGTCAA
AG (SEQ ID NO: 136)

[00160] >E1-80H

GAGCAGTCGGTGGAGGAGTCCGGGGGAGGCCTGTTCCAGCCTGGGGGATCCCTG
GCACTCACCTGCAAAGCCTCTGGATTACCCCTCAATAGTTATTATATGTCCTGGG
TCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGATCGGATGCATTGATAGTGATA
GTCCTACTACGACTGCCTACGCGAACTGGGCGAGAGGCCGATTACCATCTCCAA
5 GACCTCGTTCGACCACGGTGACTCTGCAAATGACCAGTCTGACAGCCGCGGACAC
GGCCACCTATTTCTGTGCGAGAGGCTATGGTCCTGTTTCGATTGGATCTCTGGGGC
CAGGGCACCCCTGGTCACCGTCTCTTCAG (SEQ ID NO: 137)

[00161] >E1-80K

ACCCAGACACCAGCCTCCGTGTCTGCAGCTGTGGGAGGCACAGTCAGCATCAATT
10 GCCAGTCCAGTCAGAGTGTTTATAAGAACGCCTATTTATCCTACTACTTAGCCTG
GTATCAGCAGAAACCAGGGCAGCCTCCCAAGCTCCTGATCTACTGGGCTTCCACT
CTGGCATCTGGGGTCCCATCGCGGTTCAAAGGCAGTGGATCTGGGACACAGTTCA
CTCTCACCATCAGCGACGTGCAGTGTGACGATGCTGCCACTTACTACTGTGCAGC
CGAATATAGTAATGATAGTGATAATGGTTTCGGCGGAGGGACCGAGGTGGAAAT
15 CAAAG (SEQ ID NO: 138)

[00162] >E1-89H

GAGCAGTCGTTGGAGGAGTCCGGGGGAGACCTGGTCAAGCCTGAGGGATCCCTG
ACACTCACCTGCGCAGCCTCTGGATTCTCCTTCAGTAGCGGCTACTGGATATGCT
GGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGATCGGATGCATTTATGCTG
20 GTAGTAGTGGTGGGCACATTTATTACGCGACCTGGGCGAAAGGCCGATTACCAT
CTCCCAAACCTCGTCGACCACGGTGACTCTGCAAATGACCAGTCTGACAGCCGCG
GACACGGCCACATATTTCTGTACAAGAGATAATTATGGTGGTGGTGGTTCTGCTT
CCAAATTGTGGGGCCCAGGCACCCTGGTCACCATCTCTTCAG (SEQ ID NO: 139)

[00163] >E1-89K

25 GAGCTCGTGATGACCCAGACTCCATCCCCCGTGTCTGCAGCTGTGGGAGGCACAG
TCACCATCAACTGCCAGTCCAGTCAGAGTGTTTATAGTAACAACCGCTTAGCCTG
GTATCAGCAGAAACCAGGGCAGCCTCCCAAGCTCCTGGTCTATTATGCAGCCACT
CTGGCATCTGGGGTCCCGTCGCGGTTCAAAGGCAGTGGATATGGGACACAGTCC
ACTCTCACCATCGCCGATGTGGTGTGTGACGATGCTGCCACTTACTACTGTGCAG

GATATAAACTGCTGATTCTGATGGTATTGCTTTCGGCGGAGGGACCGAGGTGGA
AATCAAAG (SEQ ID NO: 140)

[00164] >E2-93H

CAGTCGGTGAAGGAGTCCGAGGGAGGCCTGGTCCAGCCTGAGGGATCCCTGACA
5 CTCACCTGCAAAGCCTCTGGATTCTCCTTCAGTAGTTATGGAGTGAAGTGGGTCC
GCCAGGCTCCAGGGAAGGGGCTGGAGTGGATCGCGTATATTGGTCTTAGTAGTG
AGATCACTTACTACGCGGGCTGGGCGAAAGGCCGATTCACCATCTCCAAGCCCTC
GTCGACCACGGTGACTCTGCAAATGACCAGTCTGACAGCCGCGGACACGGCCAC
CTATTTCTGTGTGAGAGATCTTTATCATAGTAATGGTTTGTGGGGCCCAGGCACC
10 CTGGTCACCATCTCTTCAG (SEQ ID NO: 141)

[00165] >E2-93K

GAGCTCGATCTGACCCAGACTCCATCCCCCGTGTCTGCAGCTGTGGGAGGCACAG
TCACCGTCAGTTGCCAGGCCAGTGAGAGCGTTTATAATAATAACCGCTTATCCTG
GTATCAGCAGAAACCAGGGCAGCCTCCCAAGCTCCTGATCTATTATGCATCCACT
15 CTGGCATCTGGGGTCCCATCGCGGTTTCAGCGGCAGTGGATCTGGGACACAGTTCA
CTCTCACCATCAGCAGCGTGCAATGTGCTGATGCTGCCACGTATTATTGTGTAGC
CTTTAAAGGTTATGGTACTGACGGCAATGCTTTCGGCGGAGGGACCGAGGTGGA
AATCAAAG (SEQ ID NO: 142)

[00166] >E1-38H

20 GAGCAGTCGGTGAAGGAGTCCGGGGGAGACCTGGTCAAGCCTGAGGGATCCCTG
ACACTCACCTGCACAGCCTCTGGATTCTCCTTCAATAGCGGCTACTGGGTATGCT
GGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGATCGCTTGCATCTATACTA
GTAGTCCTACTGGTGCCATATACTACGCGACCTGGGCGAAAGGCCGATTCACCAT
CTCCCAAACCTCGTCGACCACGGTGACTCTGCAAATGACCAGTCTGACAGCCGCG
25 GACACGGCCACCTATTTCTGTACAAGAGATAATTTTGGTGGTGGTGGTTCTGCTT
CCAAATTGTGGGGCCCAGGCACCCTGGTCACCATCTCTTCAG (SEQ ID NO: 143)

[00167] >E1-38K

GAGCTCGTGATGACCCAGACTCCATCTTCCAAGTCTGTCCCTGTGGGAGGCACAG
TCACCATCGATTGCCAGGCCAGTGAGAGTGTTTATAGTAACAACCGCTGTGCCTG

GTATCAGCAGAAACCAGGGCAGCCTCCCAAGCTCCTGATCTATTATGCATCCACT
CTGGCATCTGGGGTCCCGTCGCGGTTCAAATGCAGTGGATCTGGGACACGGTTCA
CTCTCACCATCAGCGGCGTGCAGTGTGAAGATGCTGCCACTTACTACTGTGCAGG
ATATAAGACTGCCGATTCTGATGGTCTTGGTTTCGGCGGAGGGACCGAGGTGGA
5 AATCAAA (SEQ ID NO: 144)

[00168] >E1-52H

GAGCAGTCGGTGAAGGAGTCCGAGGGAGACCTGGTCAAGCCTGAGGGATCCCTG
ACACTCGCCTGCACAGCTTCTGGATTACCCCTCAGTAGCTACTACATGTGCTGGG
TCCGCCAGGCTCCAGGGAAGGGGCTGGAATGGATCGCATGCATTGATACTGATA
10 ATGATATTAGGACTGCCTACGCGAGCTGGGCGAGGGGCGGATTACCATCTCCA
GGACCTCGTCGACCACGGTGACTCTGCAAATGACCAGTCTGACAGCCGCGGACA
CGGCCACCTATTTCTGTGGGAGAGGCTATGGTGCGCTTCGGTTGGATCTCTGGGG
CCAGGGCCCCTGGTCACCGTCTCTTCAG (SEQ ID NO: 145)

[00169] >E1-52K

GAGCTCGATCTGACCCAGACACCAGCCTCCGTGTCTGCAGCTGTGGGAGGCACA
GTCAGCATCAATTGCCAGTCCAGTCCGAGTGTATTATAGGCACTACTTATCCTGGT
ATCAGCAGAAACCAGGGCAGCCTCCCAAGCTCCTGATCTACTGGGCTTCCACTCT
GGCATCTGGGGTCCCATCGCGGTTTCAGCGGCAGTGGATCTGGGACAGAGTTCCT
CTCACCATCAGCGGCGTGCAGTGTGACGATGCTGCCACTTACTACTGTGCAGGCG
20 AATATGCTAGTGATAGTGATAATCATTTCGGCGGAGGGACCGAGGTGGAAATCA
AAG (SEQ ID NO: 146)

[00170] >E2-36H

CAGTCGGTGAAGGAGTCCGAGGGTCGCCTGGTCAAGCCTGGGACACCCCTGACA
CTCACCTGCACAGTCTCTGGATTCTCCCTCAGTAGCTACCACATGGGCTGGGTCC
25 GCCAGGCTCCAGGGAAGGGGCTGGAATACATCGGAATCATTAAATAATTATGGTG
CCACATACTACGCGAGCTGGGCAAAAGGCCGATTACCATCTCCAGAACCTCGA
CCACGGTGGATCTGAAAATGACCAGTCTGACAACCGAGGACACGGCCACCTATT
TCTGTGCCAGAAGTCCTGGGATTCTGGTTATAATTCGTGGGGCCCAGGCACCCT
GGTCACCATCTCCTCAG (SEQ ID NO: 147)

[00171] >E2-36K

GAGCTCGATCTGACCCAGACTCCATCTTCCACGTCTGCGGCTGTGGGAGGCACAG
TCACCATCAACTGCCAGTCCAGTCAGAATGTTTATAGTTACAACCGCTTATCCTG
GTTTCAGCAGAAACCAGGGCAGCCTCCCAAGCTCCTGATCTACGAAGCATCCAA
5 ACTGGCATCTGGGGTCCCATCGCGGTTCAAAGGCAGTGGATCTGGGACACAGTTC
ACTCTCACCATCAGCGGCGTGCAGTGTGACGATGCTGCCACTTACTACTGTGCAG
GCGGTTATGATTGTAGGAGTTCTGATTGTGATGCTTTCGGCGGAGGGACCGAGGT
GGAAATCAAAC (SEQ ID NO: 148)

[00172] >E1-95H

10 AGCAGTTCGGTGGAGGAGTCCGGGGGAGACCTGGTCAAGCCCGGGGCATCCCTG
ACACTCACCTGCACAGCCTCTGGATTCTCCTTCAGTAGCAATTCAATGTGCTGGG
TCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGATCGGATGCATTGCTAGTAGTA
GTAGTCATAGTACTTACTACGCGAGCTGGGCGAAAGGCCGATTACCATCTCCAA
AACCTCGTCGACCACGGTGA CTCTGCAAATGACCAGTCTGACAGCCGCGGACAT
15 GGCCACCTATTTCTGTGCGAGAGATTCTGGTAATCGTGGTTACCTTTATGCGGGC
GACTTTAACTTGTGGGGGCCAGGCACCCTGGTCACCGTCTCTTCAG (SEQ ID NO:
149)

[00173] >E1-95K

GAGCTCGTGCTGACCCAGACTCCAGCCTCTGTGGAGGTAGCTGTGGGAGGCACA
20 GTCACCATCAATTGCCAGGCCAGTCAGAGCATTAAATAGTTGGTTATCCTGGTATC
AGCAGAAACCAGGGCAGCGTCCCAAACCTCCTGATCTACGAAGCATCCACTCTGG
CATCTGGGGTCTCATCGCGGTTCA GTGGCAGTGGATCTGGGACACAGTTC ACTCT
CACCATCAGCGGCGTGCAGTGTGACGATGCTGCCACTTACTACTGTCAACAGGGT
TATAGTTATAGTAATGTTGATAATAATATTTTCGGCGGAGGGACCGAGGTGGTGG
25 TCAAAG (SEQ ID NO: 150)

[00174] >E2-116H

CAGTCGTTGGAGGAGTCCGGGGGAGGCCTGGTCAAGCCTGAGGGATCCCTGACA
CTCACCTGCACAGCCTCTGGATTCTGACCTCAGTAGCTCCTACTACATGTGCTGGG
TCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGATCGTCTGTATTGACGGTGGTG

GGGGTGAGCCCACTGCCTACCCGAGCTGGGCGAAAGGCCGATTCACCGTCTCCA
AAACCTCGTCGACCACGGTGACTCTTCAAATGACCAGTCTGACAGTCGCGGACAC
GGCCACGTATTTCTGTGCGAGACGAGATGCTGGTGCTGGGAACGCCTTTAGCTTG
TGGGGCCCAGGCACCCTGGTCACCATCTCCTCAG (SEQ ID NO: 151)

5 [00175] >E2-116K

GAGCTCGATATGACCCAGACTCCATCCCCCGTGTCTGCAGCTGTGGGAGGCACAG
TCACCATCAGTTGCCAGTCCAGTCAAAGTGTTTATCTTCAGAACAACTTAGCCTG
GTATCAGCAGAAACCAGGGCAGCCTCCCAAGCTCCTGATCTATTATGCATCCACT
CTGGCATCTGGGGTCTCATCGCGGTTCAAAGGCAGTGGATCTGGGACACAGTTCA
10 CTCTCACCATCAGCGACCTGGAGTGTGACGATGCTGCCACTTACTACTGTCAGGG
CGGTTACAGTGGATATATCAATTCTTTCGGCGGAGGGACCGAGGTGGAAATCAA
AG (SEQ ID NO: 152)

[00176] >E2-135H

CAGTCGGTGAAGGAGTCCGAGGGAGACCTGGTCAAGCCTGGGGCATCCCTGACA
15 CTCACCTGCAAAGCCTCTGGATTCGACTTCAGTAGCAGCTACTTTATGTGCTGGG
TCCGCCAGGCTCCAGGGAGGGGGCTGGAGTGGATCGCATGCATTTATACTGTTAT
TAGTCGTAAGACTTATTACGCGAGCTGGGCGAAAGGCCGATTCACCATCTCCAAA
ACCTCGGCGACCACGGTGGATCTGCAAATGACCAGTCTGACAGCCGCGGACACG
GCCACCTATTTCTGTGCGAGATCGGCAACAATTGAAAGATTGGATCTCTGGGGCC
20 AGGGCACCTGGTCACCGTCTCCTCAG (SEQ ID NO: 153)

[00177] >E2-135K

GAGCTCGATCTGACCCAGACTCCATCGCCCGTGTCTGCACCTGTGGGAGGCACAG
TCACCATCAATTGCCAGGCCAGTGAGAGTGTTTATAATAACTACCGCTTATCCTG
GTATCAGCAGAAACCAGGGCAGCCTCCCAAGCTCCTAATCTATGCTGCATCCACT
25 CTGGCATCTGGGGTCCCATCGCGGTTCAAAGGCAGTGGATCTGGGACACAGTTCA
CTCTCGCCATCAGCGATGTGGTGTGTGACGATGCTGCCACTTACTACTGTGTAGG
ATATAAAAGTGGTTATATTGATAGTATTCCTTTTCGGCGGAGGGACCGAGGTGGTG
GTCAAAG (SEQ ID NO: 154)

[00178] >E1-142H

CAGTCGTTGGAGGAGTCCGGGGGAGACCTGGTCAAGCCTGGGGCATCCCTGACA
 CTCACCTGCACAGCTTCTGGATTCACCATCAATAACTACAACATTAAGTGGGTCC
 GCCAGGCTCCAGGGAAGGGGCTGGAGTGGATCGCACGTATTTGGAATGGTGATG
 GCAGCACATACTACGCGAGCTGGGCGAAAGGCCGATTACCATCTCCAAAACCT
 5 CGTCGACCACGGTGACTCTACAAATGACCAGTCTGACAGCCGCGGACACGGCCA
 CCTATTTCTGTGCGAGAAATTTTAACTTGTGGGGCCCAGGCACCCTGGTCACCAT
 CTCTTCAG (SEQ ID NO: 155)

[00179] >E1-142K

GAGCTCGTGCTGACCCAGACTCCATCTCCCGTGTCTGCAGCTGTGGGAGG
 10 CACAGTCACCATCAATTGCCAGTCCAGTGCGAGTGTTTATAGTAACAACACTACTTA
 TCCTGGTTTCAGCAGAAACCAGGGCAGCCTCCCAAGCCCCTGATCTATTATGCAT
 CCACTCTGGCATCTGGGGTCCCATCGCGGTTTAAAGGCAGTGGATCTGGGACACA
 GTTCACTCTCACCATCAGCGACGTGCAGTGTGACGATGCTGCCACTTACTACTGT
 GCAGGCGATTATAGTAGTAGTAGTGATATGTGTATTTTCGGCGGAGGGACCGAG
 15 CTGGAAATCAAAG (SEQ ID NO: 156)

[00180] Variable amino acid sequences of anti-EGFL6 antibodies are shown below.

[00181] >E1-33H

QSLEESEGLVQPEGLTLTCKASGLDLSSYYMCWVRQAPGKGLEWCIY
 20 AGSSGSTYYASWAKGRFTISKTSSTTVTLQMTSLTAADTATYFCARGGGSTY
 AQYFNLWGPGTLVTISS (SEQ ID NO: 157)

[00182] >E1-33L

ELDMTTPASVSAAVGGTVSINCQSSPSVYRHYLSWYQQKPGQPPLLIY WAS
 TLASGVPSRFSGSGSGTEFTLTISGVQCDDAATYYCAGEYASDSDNHFGGGTE
 25 LEIL (SEQ ID NO: 158)

[00183] >E1-34H

EQSVKESGGGLVQPEGSLTLTCTASGFSFSSIWICWVRQAPGKGLELIACIQI
TSGITYYASWAKGRFTISKMSSTTVTLQMTSLTVADTATYFCGRRGYGAYAG
TGASDLWGPGLVTVSS (SEQ ID NO: 159)

[00184] >E1-34L

5 ELDLTQTASSVSAAVGGTVTINCQSSQSVYNNNNLAWYQQKPGQPPKLLIYE
ASKLASGVPSRFKGSSTGTQFTLTISGVQCDDAATYYCAGGYAGYIWAFFGGG
TEVVVK (SEQ ID NO: 160)

[00185] >E1-80H

10 EQSVEESGGGLFQPGGSLALTCKASGFTLNSYYMSWVRQAPGKGLEWIGCID
SDSPTTTAYANWARGRFTISKTSSTTVTLQMTSLTAADTATYFCARGYGPVR
LDLWGQGTLVTVSS (SEQ ID NO: 161)

[00186] >E1-80LK

15 TQTPASVSAAVGGTVSINCQSSQSVYKNAYLSYYLAWYQQKPGQPPKLLIYW
ASTLASGVPSRFKGSSTGTQFTLTISDVQCDDAATYYCAA EYSNDSNGFGG
GTEVEIK (SEQ ID NO: 162)

[00187] >E1-89H

EQSLEESGGDLVKPEGSLTLTCAASGFSFSSGYWICWVRQAPGKGLEWIGCIY
AGSSGGHIYYATWAKGRFTISQTSSTTVTLQMTSLTAADTATYFCTRDNYGG
GGSASKLWGPGLVTISS (SEQ ID NO: 163)

20 [00188] >E1-89L

ELVMTQTPSPVSAAVGGTVTINCQSSQSVYSNNRLAWYQQKPGQPPKLLVYY
AATLASGVPSRFKGSSTGTQSTLTIADVVCDDAATYYCAGYKTADSDGIAFG
GGTEVEIK (SEQ ID NO: 164)

[00189] >E2-93H

25 QSVKESEGGLVQPEGSLTLTCKASGFSFSSYGVNWVRQAPGKGLEWIAIYIGLS
SEITYYAGWAKGRFTISKPSSTTVTLQMTSLTAADTATYFCVRDLYHSNGLW
GPGTLVTISS (SEQ ID NO: 165)

[00190] >E2-93L

ELDLTQTPSPVSAAVGGTVTVSCQASESVYNNNRLSWYQQKPGQPPKLLIYY
ASTLASGVPSRFSGSGSGTQFTLTISVQCADAATYYCVAFKGYGTDGNAFG
GGTEVEIK (SEQ ID NO: 166)

5 [00191] >E1-38H

EQSVKESGGDLVKPEGSLTLTCTASGFSFNSGYWVCWVRQAPGKGLEWIACI
YTSSPTGAIYYATWAKGRFTISQTSSTTVTLQMTSLTAADTATYFCTRDNFGG
GGSASKLWGPGTLVTISS (SEQ ID NO: 167)

[00192] >E1-38L

10 ELVMTQTPSSKSPVGGTVTIDCQASESVYSNNRCAWYQQKPGQPPKLLIYY
ASTLASGVPSRFKCSGSGTRFTLTISGVQCEDAATYYCAGYKTADSDGLGFG
GGTEVEIK (SEQ ID NO: 168)

[00193] >E1-52H

15 EQSVKESEGLVKPEGSLTLACTASGFTLSSYYMCWVRQAPGKGLEWIACID
TDNDIRTAYASWARGRFTISRTSSTTVTLQMTSLTAADTATYFCGRGYGALR
LDLWGQGTLVTISS (SEQ ID NO: 169)

[00194] >E1-52L

20 ELDLTQTPASVSAAVGGTVSINCQSSPSVYRHYLSWYQQKPGQPPKLLIYWA
STLASGVPSRFSGSGSGTEFTLTISGVQCDDAATYYCAGEYASDSDNHFGGGT
EVEIK (SEQ ID NO: 170)

[00195] >E2-36H

QSVKESEGR LVTPGTPLTLTCTVSGFSLSSYHMGWVRQAPGKGLEYIGIINNY
GATYYASWAKGRFTISRTSTTVDLKMTSLTTEDTATYFCARSPGIPGYNSWGP
GTLVTISS (SEQ ID NO: 171)

25 [00196] >E2-36L

ELDLTQTPSSTSAAVGGTVTINCQSSQNVYSYNRLSWFQQKPGQPPKLLIYEA
SKLASGVPSRFKGS GSGTQFTLTISGVQCDDAATYYCAGGYDCRSSDCDAFG
GGTEVEIK (SEQ ID NO: 172)

[00197] >E1-95H

5 SSSVEESGGDLVKPGASLTCTASGFSFSSNSMCWVRQAPGKGLEWIGCIAS
SSSHSTYYASWAKGRFTISKTSSTTVTLQMTSLTAADMATYFCARDSGNRGY
LYAGDFNLWGPGLVTVSS (SEQ ID NO: 173)

[00198] >E1-95L

10 ELVLTQTPASVEVAVGGTVTINCQASQSINWSWYQQKPGQRPKLLIYEAST
LASGVSSRFSGSGSGTQFTLTISGVQCDDAATYYCQQGYSYSNVDNNIFGGGT
EVVVK (SEQ ID NO: 174)

[00199] >E2-116H

15 QSLEESGGGLVKPEGSLTCTASGFDLSSSYMCWVRQAPGKGLEWIVCID
GGGGEPTAYPSWAKGRFTVSKTSSTTVTLQMTSLTVADTATYFCARRDAGA
GNAFSLWGPGLVTISS (SEQ ID NO: 175)

[00200] >E2-116L

ELDMTQTPSPVSAVGGTVTISCQSSQSVYLQNNLAWYQQKPGQPPKLLIYY
ASTLASGVSSRFKGS GSGTQFTLTISDLECDDAATYYCQGGYSGYINSFGGGT
EVEIK (SEQ ID NO: 176)

20 [00201] >E2-135H

QSVKESEGDVVKPGASLTCTCKASGFDFSSSYFMCWVRQAPGRGLEWIACIY
TVISRKTTYASWAKGRFTISKTSATTVDLQMTSLTAADTATYFCARSATIERL
DLWGQGTGLVTVSS (SEQ ID NO: 177)

[00202] >E2-135L

25 ELDLTQTPSPVSAPVGGTVTINCQASESVYNNYRLSWYQQKPGQPPKLLIYAA
STLASGVPSRFKGS GSGTQFTLAISDVVCDDAATYYCVGYKSGYIDSIPFGGG
TEVVVK (SEQ ID NO: 178)

[00203] >E1-142H

QSLEESGGDLVKPGASLTLTCTASGFTINNYNINWVRQAPGKGLEWIARIWN
GDGSTYYASWAKGRFTISKTSSTTVTLQMTSLTAADTATYFCARNFNLWGPG
TLVTISS (SEQ ID NO: 179)

5 [00204] >E1-142L

ELVLTQTPSPVSAAVGGTVTINCQSSASVYSNNYLSWFQQKPGQPPKPLIYYA
STLASGVPSRFKGS GSGTQFTLTISDVQCDDAATYYCAGDYSSSSDMCIFGGG
TELEIK (SEQ ID NO: 180)

* * *

10 [00205] All of the methods disclosed and claimed herein can be made and executed
without undue experimentation in light of the present disclosure. While the compositions and
methods of this invention have been described in terms of preferred embodiments, it will be
apparent to those of skill in the art that variations may be applied to the methods and in the
steps or in the sequence of steps of the method described herein without departing from the
15 concept, spirit and scope of the invention. More specifically, it will be apparent that certain
agents which are both chemically and physiologically related may be substituted for the
agents described herein while the same or similar results would be achieved. All such similar
substitutes and modifications apparent to those skilled in the art are deemed to be within the
spirit, scope and concept of the invention as defined by the appended claims.

REFERENCES

The following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference.

U.S. Patent Application No. 2002/0172677

U.S. Patent Application No. 2004/0126828

U.S. Patent Application No. 2005/0214860

U.S. Patent No. 3,817,837

U.S. Patent No. 3,850,752

U.S. Patent No. 3,939,350

U.S. Patent No. 3,996,345

U.S. Patent No. 4,196,265

U.S. Patent No. 4,275,149

U.S. Patent No. 4,277,437

U.S. Patent No. 4,366,241

U.S. Patent No. 4,469,797

U.S. Patent No. 4,472,509

U.S. Patent No. 4,606,855

U.S. Patent No. 4,703,003

U.S. Patent No. 4,742,159

U.S. Patent No. 4,767,720

U.S. Patent No. 4,816,567

U.S. Patent No. 4,867,973

U.S. Patent No. 4,870,287

U.S. Patent No. 4,938,948

U.S. Patent No. 4,946,778

U.S. Patent No. 5,021,236

U.S. Patent No. 5,091,513

U.S. Patent No. 5,164,296

U.S. Patent No. 5,196,066

U.S. Patent No. 5,223,409

U.S. Patent No. 5,403,484

U.S. Patent No. 5,420,253

U.S. Patent No. 5,565,332

U.S. Patent No. 5,571,698

U.S. Patent No. 5,627,052

U.S. Patent No. 5,656,434

U.S. Patent No. 5,739,169

U.S. Patent No. 5,760,395

U.S. Patent No. 5,770,376

U.S. Patent No. 5,789,208

U.S. Patent No. 5,801,005

U.S. Patent No. 5,821,337

U.S. Patent No. 5,824,311

U.S. Patent No. 5,830,880

U.S. Patent No. 5,844,091

U.S. Patent No. 5,846,945

U.S. Patent No. 5,858,657

U.S. Patent No. 5,861,155

U.S. Patent No. 5,871,907

U.S. Patent No. 5,969,108

U.S. Patent No. 6,054,297

U.S. Patent No. 6,165,464

U.S. Patent No. 6,365,157

U.S. Patent No. 6,406,867

U.S. Patent No. 6,709,659

U.S. Patent No. 6,709,873

U.S. Patent No. 6,753,407

U.S. Patent No. 6,814,965

U.S. Patent No. 6,849,259

U.S. Patent No. 6,861,572

U.S. Patent No. 6,875,434

U.S. Patent No. 6,881,557

U.S. Patent No. 6,891,024

U.S. Patent No. 6,946,646

- Ali-Fehmi *et al.*, Expression of cyclooxygenase-2 in advanced stage ovarian serous carcinoma: correlation with tumor cell proliferation, apoptosis, angiogenesis, and survival. *American journal of obstetrics and gynecology* 192, 819-825, 2005.
- Baluk *et al.*, Cellular abnormalities of blood vessels as targets in cancer. *Current opinion in genetics & development* 15, 102-111, 2005.
- Buckanovich *et al.* Tumor vascular proteins as biomarkers in ovarian cancer. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 25, 852-861, 2007.
- Chim *et al.* EGFL6 promotes endothelial cell migration and angiogenesis through the activation of extracellular signal-regulated kinase. *The Journal of biological chemistry* 286, 22035-22046, 2011.
- Donninger *et al.*, Whole genome expression profiling of advance stage papillary serous ovarian cancer reveals activated pathways. *Oncogene* 23, 8065-8077, 2004.
- Halder *et al.*, Focal adhesion kinase targeting using in vivo short interfering RNA delivery in neutral liposomes for ovarian carcinoma therapy. *Clinical cancer research : an official journal of the American Association for Cancer Research* 12, 4916-4924, 2006.
- Landen *et al.*, Therapeutic EphA2 gene targeting in vivo using neutral liposomal small interfering RNA delivery. *Cancer research* 65, 6910-6918, 2005.
- Langley *et al.*, Tissue-specific microvascular endothelial cell lines from H-2K(b)-tsA58 mice for studies of angiogenesis and metastasis. *Cancer Research* 63, 2971-2976, 2003.
- Lu *et al.*, Gene alterations identified by expression profiling in tumor-associated endothelial cells from invasive ovarian carcinoma. *Cancer research* 67, 1757-1768, 2007.
- Lu *et al.*, Regulation of tumor angiogenesis by EZH2. *Cancer cell* 18, 185-197, 2010.
- Oberauer *et al.*, EGFL6 is increasingly expressed in human obesity and promotes proliferation of adipose tissue-derived stromal vascular cells. *Molecular and cellular biochemistry* 343, 257-269, 2010.
- Sood *et al.*, Molecular determinants of ovarian cancer plasticity. *American Journal of Pathology* 158, 1279-1288, 2001.

Thaker *et al.*, Chronic stress promotes tumor growth and angiogenesis in a mouse model of ovarian carcinoma. *Nature medicine* 12, 939-944, 2006.

Yeung *et al.*, Cloning of a novel epidermal growth factor repeat containing gene EGFL6: expressed in tumor and fetal tissues. *Genomics* 62, 304-307, 1999.

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).