HEPARIN-BINDING EPIDERMAL GROWTH FACTOR-LIKE GROWTH FACTOR ANTIGEN BINDING PROTEINS

BACKGROUND

[0001]The human epidermal growth factor receptor (HER) family comprises four distinct receptor tyrosine kinases referred to as HER1 (or erbB1), HER2 (or erbB2), HER3 (or erbB3), and HER4 (or erbB4). HER1 is also commonly referred to as epidermal growth factor receptor (EGFR). With the exception of HER3, these receptors have phospho-acceptor target specific intrinsic protein tyrosine kinase activities. Members of the HER family are expressed in most epithelial cells as well as in a number of different tumor cell types. For example, receptors of the HER family are expressed in tumor cells of epithelial origin, and of mesenchymal origin. Moreover, HER receptor tyrosine kinases are involved in cell proliferation and angiogenesis, which are associated with diseases such as cancer. For example, EGFR is frequently over-expressed or aberrantly activated in breast cancers, liver cancers, kidney cancers, leukemia, bronchial cancers, pancreatic cancers and gastrointestinal cancers such as colon, rectal or stomach cancers. High levels of the EGF receptor also correlate with poor prognosis and response to treatment (Wright *et al.*, 1992, *Br. J. Cancer* 65:118-121). Thus, disruption of signal transduction from and to these kinases would have an anti-proliferative, and as such, therapeutic effect upon a number of cancer and tumor cell types.

[0002]The enzymatic activity of receptor tyrosine kinases can be stimulated by over-expression and/or by ligand-mediated dimerization (Heldin, 1995, *Cell* 80:213-223). Activation of receptor homodimers and heterodimers results in phosphorylation of tyrosine residues on the receptors, which in turn phosphorylate tyrosine residues of other molecules, including intracellular proteins. (Ullrich *et al.*, 1990, *Cell* 61:203-212). This is followed by the activation of intracellular signaling pathways such as those involving the mitogen-activated protein kinase (MAP kinase) (Dhillon et al., 2007, *Oncogene* 26: 3279-3290) and the phosphatidylinositol 3-kinase (PI3 kinase). While activation of these pathways has been shown to increase cell proliferation and inhibit apoptosis, inhibition of signaling mediated by HER family members by either small molecule inhibitors or monoclonal antibodies has been shown to inhibit cell proliferation and promote apoptosis (Prenzel *et al.*, 2001, *Endocr. Relat. Cancer* 8: 11-31)

[0003]Heparin-binding epidermal growth factor-like growth factor (HB-EGF) is a 22 kDa, O-glycosylated protein (Higahiyama *et al.*, 1992, *J Biol Chem* 267: 6205-6212). In its mature form, HB-EGF binds to and activates the EGF receptor and HER4 (Elenius et al., 1997, EMBO 16:1268-1278). HB-EGF is the key mediator of G-protein coupled receptor (GPCR) induced cell proliferation *via* a process called triple-membrane passing signaling (TMPS) (Prenzel *et al.*,

1999, *Nature* 402:884-888, review in Fischer *et al.* 2003, *Biochem. Soc. Trans.* 31:1203-1208). It has been shown that HB-EGF promotes cellular proliferation as well as angiogenesis (Zushi *et al.*, 1997, *Int J Cancer* 73:917-923; Abramovitch *et al.*, 1998, *FEBS letters* 425:441-447). HB-EGF also has been demonstrated to play a key role in a number of cancers, *i.e.,* it has been linked to the aggressive behavior of ovarian tumors (Tanaka *et al.*, 2005, *Clin. Cancer Res.* 11:4783-4792). Moreover, HB-EGF is essential for xenograft tumor formation by ovarian cancer cell lines. Over-expression of HB-EGF (*wild type* or a secreted form) accelerates tumor formation in SKOV3 and RMG-1 cells. Knockdown of endogenous HB-EGF using siRNA, yet, abolished or delayed tumor formation by SKOV3 and RMG-1 cells. Miyamoto, 2004, *Cancer Res.* 64:5720. As suggested by the above evidence, inhibition of HB-EGF expression or activity may inhibit tumor formation.

[0004]Similarly, HB-EGF is a marker of poor prognosis in some cancers, including human bladder cancers (Thogersen *et al.*, 2001, *Cancer Res.* 61:6227-6233). *In vitro* studies indicate that human EJ bladder cells that were engineered to express HB-EGF (*wild type*, soluble or non-cleavable) exhibit an increase in growth, anchorage independent growth, and production of VEGF, and enhanced migration. When these HB-EGF-expressing EJ bladder cells were transplanted into nude mice, an increase in tumor formation, size and density of blood vessels was observed in those tumors. (Ongusaha, 2004, *Cancer Res.* 64:5283-5290).

SUMMARY

[0005]Provided herein are isolated antigen binding proteins that bind HB-EGF. Some of these antigen binding proteins comprise A) one or more light chain complementary determining regions (CDRLs) consisting of: (i) a CDRL1 selected from SEQ ID NOs:**189-217**; (ii) a CDRL2 selected from from SEQ ID NOs:**218-233**; (iii) a CDRL3 selected from SEQ ID NO:**234-274**; or (iv) a CDRL of (i), (ii) or (iii) that contains one or more amino acid substitutions, deletions or insertions of no more than four amino acids. Alternatively, the HB-EGF antigen binding protein may comprise B) one or more heavy chain complementary determining regions (CDRHs) consisting of: (i) a CDRH1 selected from SEQ ID NO:**275-299**; (ii) a CDRH2 selected from SEQ ID NO:**300-331**; (iii) a CDRH3 selected from SEQ ID NO:**332-372**; or (iv) a CDRH of (i), (ii) or (iii) that contains one or more amino acid substitutions, deletions or insertions of no more than four amino acids.

[0006]In one embodiment, the isolated antigen binding protein may comprise one, two or more of the aforementioned light chain CDRLs and one, two or more of the aforementioned heavy chain CDRHs. In one aspect, the isolated antigen binding protein comprises CDRH1, CDRH2, CDRH3, CDRL1, CDRL2 and CDRL3. In another aspect, the isolated antigen binding protein of A), *supra*, is selected from the group consisting of: a CDRL1 from SEQ ID NOs:**189-217**; a CDRL2 from SEQ ID NOs:**218-233**; a CDRL3 from SEQ ID NOs:**234-274**; and a CDRL of the

any of the aforementioned (i), (ii) or (ii) that contains one or more amino acid substitutions, deletions or insertions of no more than two amino acids. In addition, said heavy chain CDRH of B), *supra*, is selected from a CDRH1 from SEQ ID NOs:**275-299**; a CDRH2 from SEQ ID NOs:**300-331**; a CDRH3 amino acid sequence from SEQ ID NOs:**332-372** and a CDRH of the aforementioned that contains one or more amino acid substitutions, deletions or insertions of no more than two amino acids. Furthermore, the isolated antigen binding protein may comprise or one or more light chain CDRLs of A), *supra*; and one or more heavy chain CDRHs of B), *supra*.

[0007]In another embodiment, the antigen binding protein comprises a CDRL selected from the following: a CDRL1 from SEQ ID NOs:**189-217**; a CDRL2 from SEQ ID NOs:**218-233**; and a CDRL3 from SEQ ID NOs:**234-274**. The antigen binding protein may also comprise a CDRH selected from one of the following: a CDRH1 from SEQ ID NOs:**275-299**; a CDRH2 from SEQ ID NOs:**300-331**; and a CDRH3 selected from SEQ ID NOs:**332-372**. Alternatively, the isolated antigen binding protein may comprise one or more light chain CDRLs listed in A), *supra*, and one or more heavy chain CDRHs of B), *supra*. In particular, the isolated antigen binding protein may comprise a CDRL1 of SEQ ID NOs:**189-217**, a CDRL2 of SEQ ID NOs:**218-233,** and a CDRL3 of SEQ ID NOs:**234-274** and/or a CDRH1 of SEQ ID NOs:**275-299**, a CDRH2 of SEQ ID NOs:**300-331**, and a CDRH3 of SEQ ID NO:**332-372**.

[0008]In one aspect, the isolated antigen binding protein comprises a light chain variable region (V_L) having at least 80%, 90% or 100% sequence identity with an amino acid sequence selected from SEQ ID NOs:**94-141**. In another aspect, the isolated antigen binding protein comprises a heavy chain variable region (V_H) having at least 80%, 90% or 100% sequence identity with an amino acid sequence from SEQ ID NOs:**142-186**.

[0009]In another embodiment, the isolated antigen binding protein specifically recognizes at least an IHGE containing epitope and/or an EGF-like domain of HB-EGF.

[00010]Provided herein, in addition, is an isolated antigen binding protein that competes for binding with the isolated antigen binding protein that binds HB-EGF, as described above.

[00011]Also provided herein is an isolated antigen binding protein which binds HB-EGF and comprises A) one or more light chain CDRs (CDRLs) from the group consisting of: (i) a CDRL1 with at least 80%, or at least 90% sequence identity to SEQ ID NOs:**189-217**; (ii) a CDRL2 with at least 80%, or at least 90% sequence identity to SEQ ID NOs:**218-233**; and (iii) a CDRL3 with at least 80%, or at least 90% sequence identity to SEQ ID NOs:**234-274**. Alternatively, the isolated antigen binding protein which binds HB-EGF, comprises B) one or more heavy chain CDRs (CDRHs) from the group consisting of (i) a CDRH1 with at least 80%, or at least 90% sequence identity to SEQ ID NOs:**275-299**; (ii) a CDRH2 with at least 80%, or at least 90% sequence identity to SEQ ID NOs:**300-331**; and (iii) a CDRH3 with at least 80%, or at least 90%

sequence identity to SEQ ID NOs:**332-372**. The isolated antigen binding protein may also comprise C) one or more light chain CDRLs of A) and one or more heavy chain CDRHs of B).

[00012]In another embodiment, the isolated antigen binding protein binds HB-EGF and comprises: A) a light chain complementary determining region (CDRL) selected from: (i) a CDRL3 selected from the group consisting of SEQ ID NOs:**234-274**; (ii) a CDRL3 that differs in amino acid sequence from the CDRL3 of (i) by an amino acid addition, deletion or substitution of not more than two amino acids; and (iii) a CDRL3 amino acid sequence selected from the following:

$X_1QX_2X_3X_4X_5PX_6X_7$ (SEQ ID NO:1046), wherein

X_1 is I or M,

X_2 is A, G or S,

X_3 is I or T,

X_4 is H or Q,

X_5 is F, L or W,

X_6 is C, I, H, L or T,

X_7 is S or T;

$QQX_1X_2X_3X_4X_5IT$ (SEQ ID NO:1047), wherein

X_1 is I or S,

X_2 is F or Y,

X_3 is F, I, S or Y,

X_4 is A, S or T,

X_5 is P or S;

$X_1X_2X_3X_4X_5X_6X_7X_8T$ (SEQ ID NO:1048), wherein

X_1 is L or Q,

X_2 is K, N or Q,

X_3 is A, H, S or Y,

X_4 is H, N or Y,

X_5 is N, S or T,

X_6 is A, F, I, T, V or Y,

X_7 is P or no amino acid,

X_8 is F, L or P;

$QX_1X_2DX_3LPX_4X_5$ (SEQ ID NO:1049), wherein

X_1 is H or Q,

X_2 is C or Y,

X_3 is D, I, N, S or Y,

X_4 is F, I or L,

X_5 is A, S or T;

$QQX_1X_2X_3X_4PX_5X_6X_7$ (SEQ ID NO:1050), wherein

X_1 is H or Y,

X_2 is G or N,

X_3 is N or S,

X_4 is S or W,

X_5 is P or no amino acid,

X_6 is R or W,

X_7 is S or T; or

$X_1QYX_2X_3X_4X_5X_6X_7F$ (SEQ ID NO:1051), wherein

X_1 is H or Q,

X_2 is F or Y,

X_3 is G, I or S,

X_4 is F, I or T,

X_5 is M, P, S or T,

X_6 is F, L, R or W,

X_7 is S or T.

[00013]The isolated antigen binding protein may also comprise B) a heavy chain complementary determining region (CDRH) selected from the group consisting of: (i) a CDRH3 selected from the group consisting of SEQ ID NOs:**332-372**; (ii) a CDRH3 that differs in amino acid sequence from the CDRH3 of (i) by an amino acid addition, deletion or substitution of not more than two amino acids; and iii) a CDRH3 amino acid sequence selected from the following:

$X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}DX_{12}$ (SEQ ID NO:1065), wherein

X_1 is E or S,

X_2 is D, G or no amino acid,

X_3 is D, N or no amino acid,

X_4 is G or no amino acid,

X_5 is G or no amino acid,

X_6 is W, Y or no amino acid,

X_7 is I, N or Y,

X_8 is A or Y,

X_9 is G, V or Y,

X_{10} is A, F or G,

X_{11} is F, L or M,

X_{12} is V or Y;

$QX_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}YX_{12}X_{13}X_{14}DX_{15}$ (SEQ ID NO:1066), wherein

X_1 is G or no amino acid,

X_2 is K, L or Y,

X_3 is A, G or S,

X_4 is S, V or Y,

X_5 is A or G,

X_6 is G or no amino acid,

X_7 is T or no amino acid,

X_8 is S or no amino acid,

X_9 is Y or no amino acid,

X_{10} is W or Y,

X_{11} is G, S or Y,

X_{12} is F or Y,

X_{13} is G or no amino acid,

X_{14} is M or no amino acid,

X_{15} is V or Y;

$X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}X_{14}$ (SEQ ID NO:1067), wherein

X_1 is D, G, L, S or no amino acid,

X_2 is G, H, W, Y or no amino acid,

X_3 is A, F, W, Y or no amino acid,

X_4 is D, G, Q, T or no amino acid,

X_5 is G, I, Q, S or no amino acid,

X_6 is A, D, N, Q, S or no amino acid,

X_7 is G, Y or no amino acid,

X_8 is D, Y or no amino acid,

X_9 is Y or no amino acid,

X_{10} is A, E, N or Y,

X_{11} is G, P, T, V or Y,

X_{12} is F or I,

X_{13} is D or Q,

X_{14} is C, H, V or Y;

$X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}X_{14}X_{15}X_{16}X_{17}DX_{18}$ (SEQ ID NO:1068), wherein

X_1 is E, D or no amino acid,

X_2 is G, R or no amino acid,

X_3 is I, V, Y or no amino acid,

X_4 is A, G, L or N,

X_5 is A, G, V or W,

X_6 is A, N, R or T,

X_7 is G, N, P or no amino acid,

X_8 is G, T or no amino acid,

X_9 is A or no amino acid,

X_{10} is D, E or no amino acid,

X_{11} is S, Y or no amino acid,

X_{12} is G, Y or no amino acid,

X_{13} is N, Y or no amino acid,

X_{14} is Y or no amino acid,

X_{15} is D, Y or no amino acid,

X_{16} is A, G or no amino acid,

X_{17} is F or M,

X_{18} is I, V or Y;

$X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}X_{14}X_{15}X_{16}X_{17}X_{18}X_{19}X_{20}X_{21}X_{22}X_{23}$ (SEQ ID NO:1069), wherein

X_1 is A, D, G, S or T,

X_2 is A, E, G, L, N, R, Y or no amino acid,

X_3 is A, G, L, N, R, T, Y or no amino acid,

X_4 is D, G, R, S, V, Y or no amino acid,

X_5 is A, G, I, S, V, Y or no amino acid,

X_6 is F, G, L, R, V or no amino acid,

X_7 is L, T, Y or no amino acid,

X_8 is Y or no amino acid,

X_9 is Y or no amino acid,

X_{10} is D or no amino acid,

X_{11} is S or no amino acid,

X_{12} is S or no amino acid,

X_{13} is G or no amino acid,

X_{14} is D, L, M, S, Y or no amino acid,

X_{15} is H, I, P, V, W or no amino acid,

X_{16} is F, G, L, R, S, Y or no amino acid,

X_{17} is D, F, V, W, Y or no amino acid,

X_{18} is C, F, L, P, S or Y,

X_{19} is D, F, G or Y,

X_{20} is A, C, G, P, R, V or Y,

X_{21} is F, L, M, S or no amino acid,

X_{22} is A, D or no amino acid,

X_{23} is I, L, V, Y or no amino acid;

$X_1YSSGWX_2X_3YGX_4X_5DX_6$ (SEQ ID NO:1070), wherein

X_1 is M or V,

X_2 is S or no amino acid,

X_3 is F or no amino acid,

X_4 is V or no amino acid,

X_5 is F or M,

X_6 is V or Y; or

$RX_1X_2X_3PFX_4Y$ (SEQ ID NO:1071), wherein

X_1 is G, H, L, N or R,

X_2 is E, T or W,

X_3 is L, N, T or V,

X_4 is D or E.

[00014]In another aspect, the isolated antigen binding protein may further comprise A) a CDRL selected from: (i) a CDRL1 selected from SEQ ID NOs:**189-217**; (ii) a CDRL1 that differs in amino acid sequence from the CDRH1 of (i) by an amino acid addition, deletion or substitution of not more than two amino acids; or (iii) a CDRL1 amino acid sequence from the following:

$X_1SSQSLX_2X_3SDGX_4TYLX_5$ (SEQ ID NO:1035), wherein

X_1 is K or R,

X_2 is L or V,

X_3 is H or Y,

X_4 is K or N,

X_5 is N, S or Y;

$RASQX_1ISX_2YLN$ (SEQ ID NO:1036), wherein

X_1 is R, S or T,

X_2 is R or S;

$RASQX_1IX_2X_3X_4LX_5$ (SEQ ID NO:1037), wherein

X_1 is D, G, S or T,

X_2 is A, R or S,

X_3 is H, I, N, R, S or T,

X_4 is D, W or Y,

X_5 is A, G or N;

$QASQDIX_1X_2X_3LN$ (SEQ ID NO:1038), wherein

X_1 is S or T,

X_2 is D or N,

X_3 is S or Y;

$RASQX_1VX_2X_3X_4X_5LA$ (SEQ ID NO:1039), wherein

X_1 is S or T,

X_2 is I or S,

X_3 is R or S,

X_4 is S, N or no amino acid,

X_5 is Y or no amino acid; or

$KSSQX_1X_2LX_3X_4SNNKNYLX_5$ (SEQ ID NO:1040), wherein

X_1 is N or S,

X_2 is I or V,

X_3 is D or Y,

X_4 is N, R or S,

X_5 is A or V;

(iv) a CDRL2 from the group consisting of SEQ ID NOs:**218-233**; (v) a CDRL2 that differs in amino acid sequence from the CDRL2 of (iv) by an amino acid addition, deletion or substitution of not more than two amino acids; or (vi) a CDRL2 amino acid sequence from the following:

$X_1X_2SNX_3X_4S$ (SEQ ID NO:1041), wherein

X_1 is E or K,

X_2 is I or V,

X_3 is R or W,

X_4 is D or F;

$X_1X_2SX_3LQS$ (SEQ ID NO:1042), wherein

X_1 is A or T,

X_2 is A, E or V,

X_3 is S or T;

X_1ASX_2LQS (SEQ ID NO:1043), wherein

X_1 is A or V,

X_2 is S or T;

$DASX_1LET$ (SEQ ID NO:1044), wherein

X_1 is I or N;

GASSRAT (SEQ ID NO:223); or

$WASX_1RES$ (SEQ ID NO:1045), wherein

X_1 is A or T.

[00015]The isolated antigen binding proteins may further comprise B) a CDRH from the group consisting of: (i) a CDRH1 from the group consisting of SEQ ID NOs:**275-299**; (ii) a CDRH1 that differs in amino acid sequence from the CDRH1 of (i) by an amino acid addition, deletion or substitution of not more than two amino acids; (iii) a CDRH1 amino acid sequence selected from:

$GYTX_1TX_2X_3X_4X_5X_6$ (SEQ ID NO:1052), wherein

X_1 is F or L,

X_2 is E, G or S,

X_3 is H, L or Y,

X_4 is G, S or Y,

X_5 is I or M,

X_6 is H or S;

$GYX_1FTSYWIG$ (SEQ ID NO:1053), wherein

X_1 is R or S;

$GFTFX_1SX_2X_3MH$ (SEQ ID NO:1054), wherein

X_1 is R or S,

X_2 is H or Y,

X_3 is D or G;

$GFX_1FSX_2YX_3MX_4$ (SEQ ID NO:1055), wherein

X_1 is P or T,

X_2 is A, R or S,

X_3 is A or S,

X_4 is N or S;

$GX_1SX_2SX_3X_4X_5X_6X_7WX_8$ (SEQ ID NO:1056), wherein

X_1 is D or G,

X_2 is F, I or V,

X_3 is R, S or no amino acid,

X_4 is G, Y or no amino acid,

X_5 is D, G, S or no amino acid,

X_6 is A, S or Y,

X_7 is A or Y,

X_8 is N or S;

GFSLSNARMGVS (SEQ ID NO:279); or

$GFSLX_1TGGVGVG$ (SEQ ID NO:1057), wherein

X_1 is S or N;

[00016](iv) a CDRH2 selected from the group consisting of SEQ ID NOs:**300-331**; (v) a CDRH2 that differs in amino acid sequence from the CDRH2 of (iv) by an amino acid addition, deletion or substitution of not more than two amino acids; or (vi) a CDRH2 amino acid sequence from the following:

$X_1X_2X_3X_4X_5X_6GX_7TX_8X_9X_{10}QKX_{11}X_{12}$ (SEQ ID NO:1058), wherein

X_1 is S or W,

X_2 is F or I,

X_3 is D, N or S,

X_4 is A or P,

X_5 is E, N or S,

X_6 is D, N or S,

X_7 is E, G or N,

X_8 is I or N,

X_9 is C, H or Y,

X_{10} is A or T,

X_{11} is F or L,

X_{12} is D or G;

IIYPX_1DSDX_2RYSPSFQG (SEQ ID NO:1059), wherein

X_1 is D or G,

X_2 is A, I or T;

X_1IX_2X_3DGS$X_4X_5X_6$YX_7DSVX_8G (SEQ ID NO:1060), wherein

X_1 is F or V,

X_2 is S or W,

X_3 is D, S or Y,

X_4 is I, N or T,

X_5 is K or Q,

X_6 is N, R or Y,

X_7 is A, T or V,

X_8 is K or R;

X_1ISX_2S$X_3X_4X_5X_6$YYADSVKG (SEQ ID NO:1061), wherein

X_1 is A, H or Y,

X_2 is G, R or S,

X_3 is G or S,

X_4 is G, R or S,

X_5 is S, T or Y,

X_6 is I or T;

$X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}YX_{12}X_{13}SX_{14}KS$ (SEQ ID NO:1062), wherein

X_1 is E, R or Y,

X_2 is I or T,

X_3 is H, N or Y,

X_4 is C, H, S, T or Y,

X_5 is S or R,

X_6 is G or S,

X_7 is G, K, S or T,

X_8 is T or W,

X_9 is N or Y,

X_{10} is N or no amino acid,

X_{11} is D or no amino acid,

X_{12} is A or N,

X_{13} is P or V,

X_{14} is L or V;

X_1IFSNDEKSYSTSLKS (SEQ ID NO:1063), wherein

X_1 is H or LI; or

LIYWNX_1X_2KRYSPSLX_3S (SEQ ID NO:1064), wherein

X_1 is D or V,

X_2 is D or E,

X_3 is K or R.

[00017]In yet another embodiment, the isolated antigen binding protein described hereinabove comprises the first amino acid sequence and the second amino acid sequence, both sequences of which are covalently bonded to each other. The first amino acid sequence also comprises CDRL3 of SEQ ID NOs:**234-274**, CDRL2 of SEQ ID NOs:**218-233**, and CDRL1 of SEQ ID NOs:**189-217**, and the second amino acid sequence comprises said CDRH3 of SEQ ID NOs:**332-372**, CDRH2 of SEQ ID NOs:**300-331**, and CDRH1 of SEQ ID NOs:**275-299**.

[00018]In one aspect, the isolated antigen binding proteins can be a monoclonal antibody, a polyclonal antibody, a recombinant antibody, a human antibody, a humanized antibody, a chimeric antibody, a multispecific antibody, or an antibody fragment thereof. The antibody fragment may be a Fab fragment, a Fab' fragment, a $F(ab')_2$ fragment, a Fv fragment, a diabody, or a single chain antibody molecule. In one embodiment, the isolated antigen binding protein of the present invention is a human antibody. In another embodiment, the isolated antigen binding protein is a monoclonal antibody.

[00019]The isolated antigen binding proteins as described herein, may be of any of the following types: IgG1-, IgG2- IgG3- or IgG4-type. In one embodiment, the antigen binding protein is of

the IgG2- or IgG4- type. Furthermore, the antigen binding protein may be coupled to a labeling group. These labeling groups may be, for example, a radioisotope, radionuclide, a fluorescent group, an enzymatic group, a chemiluminescent group, a biotinyl group, or a predetermined polypeptide group.

[00020]In another embodiment, the isolated antigen binding protein is coupled to an effector group such as, for example, a radioisotope, a radionuclide, a toxin, a therapeutic group, or a chemotherapeutic group. The chemotherapeutic groups may be, for example, calicheamicin, auristatin-PE, geldanamycin, maytanasine, or derivatives thereof.

[00021]In yet another embodiment, the isolated antigen binding protein competes for binding to human HB-EGF with a antigen binding protein as described and claimed herein. This competing antigen binding protein may be, for example, a monoclonal antibody, a polyclonal antibody, a recombinant antibody, a human antibody, a humanized antibody, a chimeric antibody, a multispecific antibody, or an antibody fragment thereof. The antibody fragment may be, for example, a Fab fragment, a Fab' fragment, a $F(ab')_2$ fragment, a Fv fragment, a diabody, or a single chain antibody molecule. In one embodiment, the isolated binding protein is a human antibody. In another embodiment, the isolated antigen binding protein is a monoclonal antibody. In another embodiment, this isolated antigen binding protein is of the IgG1-, IgG2- IgG3- or IgG4-type. In one embodiment, the antigen binding protein is of the IgG2- or IgG4- type. In another embodiment, the antigen binding proteins as described herein can be coupled to a labeling group. Examples of labeling groups are: a radioisotope, radionuclide, a fluorescent group, an enzymatic group, a chemiluminescent group, a biotinyl group, or a predetermined polypeptide group. In another embodiment, the isolated antigen binding protein is coupled to an effector group such as, for example, a radioisotope, a radionuclide, a toxin, a therapeutic group, or a chemotherapeutic group. Examples of the therapeutic or chemotherapeutic groups include, for example, calicheamicin, auristatin-PE, geldanamycin, maytanasine, or derivatives thereof.

[00022]In one aspect, an isolated antigen binding protein is provided that reduces, at least partially, HB-EGF-mediated signal transduction.

[00023]Also presented herein is a nucleic acid molecule encoding the isolated antigen binding protein previously described, wherein the nucleic acid molecule is operably linked to a control sequence. In one aspect, a vector comprising the aforementioned nucleic acid molecule is provided. In another aspect, a host cell is provided that comprises the aforementioned nucleic acid molecule and/or vector.

[00024]In one embodiment, a method for making the antigen binding protein is provided that includes the step of preparing said antigen binding protein from a host cell that secretes said antigen binding protein.

[00025]In yet another embodiment, a pharmaceutical composition is provided comprising at least one of the aforementioned antigen binding proteins of the present invention and a pharmaceutically acceptable carrier, diluent or adjuvant. In one embodiment, the pharmaceutical composition may comprise an additional active agent, such as an anti-neoplastic agent. The anti-neoplastic agent may be, for example, an anti-tumor antibody. Examples of an anti-tumor antibody may be, for example, antibodies directed against receptor tyrosine kinase or EGFR.

[00026]In one aspect, the pharmaceutical composition is used for diagnosis, prevention or treatment of a hyperproliferative disease. In a further aspect, the hyperproliferative disease is associated with HB-EGF expression. In another aspect, the hyperproliferative disease is associated with or accompanied by a disturbed, (*e.g.*pathologically enhanced), growth factor receptor activation, wherein said pathologically enhanced growth factor receptor activation is associated with or caused by a pathological increase in the activity of a G protein and/or a G protein coupled receptor.

[00027]In one embodiment, the pharmaceutical composition comprises at least one antigen binding protein and pharmaceutically acceptable carrier, diluents and/or adjuvants for the diagnosis, prevention or treatment of cancer, such as, for example, breast cancer, gastrointestinal cancer, pancreas cancer, prostate cancer, ovarian cancer, stomach cancer, endometrial cancer, salivary gland cancer, lung cancer, kidney cancer, colon cancer, colorectal cancer, thyroid cancer, bladder cancer, glioma, melanoma, other HB-EGF expressing or overexpressing cancers, and formation of tumor metastases.

[00028]In another embodiment, antigen binding proteins as described herein are used for the manufacture of a pharmaceutical composition for the diagnosis, prevention or treatment of a hyperproliferative disease. In a further embodiment, the hyperproliferative disease is associated with HB-EGF expression.

[00029]One embodiment describes a method for diagnosing a condition associated with the expression of HB-EGF, the method comprising the step of contacting a sample an isolated antigen binding proteins as described herein, and determining the presence of HB-EGF in said sample. In a further embodiment, the condition is a hyperproliferative disease associated with HB-EGF expression.

[00030]Another aspect describes a method for preventing or treating a condition associated with the expression of HB-EGF in a patient, comprising administering to a patient in need thereof an effective amount of a antigen binding protein as described herein. In a further aspect, the condition is a hyperproliferative disease associated with HB-EGF expression. In yet another aspect, the patient is a mammalian patient.

[00031]In one embodiment, a kit is provided that comprises a antigen binding protein, a nucleic acid molecule, or a vector as described above. In a further embodiment, the kit comprises at least one further active agent, wherein the further active agent is an anti-neoplastic agent.

[00032]These and other aspects of the invention will be described in greater detail herein. Each of the aspects of the invention can encompass various embodiment of the present invention. It is therefore anticipated that each of the embodiments of the invention involving one element or combinations of elements can be included in each aspect of the invention. Other features, objects, and advantages of the present invention are apparent in the detailed description that follows.

DESCRIPTION OF THE FIGURES

[00033]FIGURES 1A-1P depict various light chain variable regions of the antigen binding proteins. The CDR1, CDR2 and CDR3 regions are indicated in boxes.

[00034]FIGURES 2A-2O depict various heavy chain variable regions of the antigen binding proteins. The CDR1, CDR2 and CDR3 regions are indicated in boxes.

[00035]FIGURES 3A-3K depict the amino acid sequences of various light chains of the antigen binding proteins.

[00036]FIGURES 4A-4O depict the amino acid sequences of various heavy chains of the antigen binding proteins.

[00037]FIGURE 5A depicts the amino acid sequence of an exemplary light chain constant region of the antigen binding proteins.

[00038]FIGURE 5B depicts the amino acid sequence of an exemplary heavy chain constant region of the antigen binding proteins.

[00039]FIGURES 6A-6F depict the amino acid sequences for various CDR regions of the light chain variable regions of the antigen binding proteins.

[00040]FIGURES 7A-7E depict the amino acid sequences for various CDR regions of the heavy chain variable regions of the antigen binding proteins.

[00041]FIGURES 8A-8H depict the amino acid sequences for various FR regions of the light chain variable regions of the antigen binding proteins.

[00042]FIGURES 9A-9F depict the amino acid sequences for various FR regions of the heavy chain variable regions of the antigen binding proteins.

[00043]FIGURES 10A and 10B depict an alignment of the amino acid sequences of the light chain variable sequences of the antigen binding proteins. The CDR1, CDR2 and CDR3 regions are shown in boxes.

[00044]FIGURES 11A and 11B depict an alignment of the amino acid sequences of the heavy chain variable sequences of the antigen binding proteins. The CDR1, CDR2 and CDR3 regions are shown in boxes.

[00045]FIGURE 12A depicts a cladogram showing the relatedness of the light chain variable regions of the antigen binding proteins.

[00046]FIGURE 12B depicts a cladogram showing the relatedness of the light chain CDRL3 regions of the antigen binding proteins.

[00047]FIGURE 12C depicts a cladogram showing the relatedness of the heavy chain variable regions of the antigen binding proteins.

[00048]FIGURES 13A-13V depict the nucleotide sequences of various light chain variable regions of the antigen binding proteins.

[00049]FIGURES 14A-14AC depict the nucleotide sequences of various heavy chain variable regions of the antigen binding proteins.

[00050]FIGURES 15A-15M depict the nucleotide sequences of the various light chains of the antigen binding proteins.

[00051]FIGURES 16A-16L depict the nucleotide sequences of the various heavy chains of the antigen binding proteins.

[00052]FIGURE 17A depicts the nucleotide sequence of the light chain constant region of the antigen binding proteins.

[00053]FIGURE 17B depicts the nucleotide sequence of the heavy chain constant region of the antigen binding proteins.

[00054]FIGURES 18A-18F depict the nucleotide sequences for various CDR regions of the light chain variable regions of the antigen binding proteins.

[00055]FIGURES 19A-19G depict the nucleotide sequences for various CDR regions of the heavy chain variable regions of the antigen binding proteins.

[00056]FIGURES 20A-20K depict the nucleotide sequences for various FR regions of the light chain variable regions of the antigen binding proteins.

[00057]FIGURES 21A-21K depict the nucleotide sequences for various FR regions of the heavy chain variable regions of the antigen binding proteins.

[00058]FIGURE 22A graphically illustrates the degree to which different anti-HB-EGF IgG2 antibody preparations provided herein inhibit HB-EGF-induced epidermal growth factor receptor (EGFR) tyrosine phosphorylation. The results for preparations of antibodies U2-1 to U2-68 are provided. As illustrated, monoclonal antibody preparations U2-18, U2-24, U2-19 and U2-42 strongly inhibit EGFR tyrosine phosphorylation.

[00059]FIGURE 22B graphically illustrates the degree to which different anti-HB-EGF IgG4 antibody preparations provided herein inhibit HB-EGF-induced epidermal growth factor receptor (EGFR) tyrosine phosphorylation. The results for preparations of antibodies U2-2 to U2-66 are provided. As illustrated, monoclonal antibody preparations U2-39, U2-34, U2-45 and U2-6 strongly inhibit EGFR tyrosine phosphorylation.

[00060]FIGURE 23 illustrates that the antibodies inhibit lysophosphatidic acid (LPA)-induced EGFR tyrosine phosphorylation in COS-7 cells. LPA is a GPCR ligand that activates the TMPS pathway, resulting in release of HB-EGF with consequent EGFR tyrosine phosphorylation. COS-7 cells were pretreated with antibodies as indicated and stimulated with LPA, then cell lysates were prepared and lysate proteins were separated by polyacrylamide gel electrophoresis. After preparation of the blot, an anti-phosphotyrosine antibody was used to detect phosphorylated EGFR. As a control, total EGFR was detected as shown at the bottom, using a WB anti-EGFR antibody. As illustrated, anti-HB-EGF antibody preparations U2-24, U2-19 and U2-42 strongly inhibit LPA-induced EGFR phosphorylation.

[00061]FIGURE 24 graphically illustrates dose-dependent inhibition of HB-EGF-induced EGF receptor tyrosine phosphorylation by various antibodies provided herein. Different concentrations of the candidate U2-39, U2-42 and U2-45 antibody preparations were preincubated with HB-EGF prior to stimulation of SCC9 squamous cancer cells and the amount of EGFR tyrosine phosphorylation was detected. As shown, antibody U2-42 and U2-39 achieved up to 111% inhibition. IC50 values determined for the antibodies were 0.167 nM (U2-39), 1 nM (U2-42) and 2 nM (U2-45), respectively.

[00062]FIGURE 25 graphically illustrates dose-dependent inhibition of thrombin-induced EGFR phosphorylation *via* TMPS in MDA-MB231 cells by anti-HB-EGF antibody preparations. MDA-MB231 cells were incubated with candidate U2-42, U2-39 and U2-45 antibody preparations in the presence of thrombin and the amount of EGFR tyrosine phosphorylation was detected using a procedure described in Example 6. As shown, antibodies U2-42 and U2-39 achieving 100% inhibition.

[00063]FIGURE 26 illustrates dose-dependent inhibition of LPA-induced EGFR tyrosine phosphorylation *via* TMPS in PPC-1 cells by anti-HB-EGF antibody preparations. PPC-1 cells were incubated with candidate U2-42, U2-39 and U2-45 antibody preparations and the amount of EGFR tyrosine phosphorylation following LPA stimulation was detected using a procedure described in Example 4. As shown, antibodies U2-42, U2-39 and U2-45 achieved 100% inhibition.

[00064]FIGURE 27 illustrates that anti-HB-EGF antibody preparations inhibited by up to 100% the induction of MDA-MB231 breast cancer cell migration by sphingosine-1-phosphate. Candidate anti-HB-EGF antibody preparations U2-42, U2-39 and U2-45 were tested for cell migration inhibition using of collagen I-coated transwells (BD Falcon, 8 μm pores). As shown, anti-HB-EGF antibody preparation U2-42 inhibited sphingosine-1-phosphate-induced MDA-MB231 cell migration by about 70% while the U2-39 and U2-45 anti-HB-EGF antibody preparations inhibited MDA-MB231 cell migration by about 100%. Thus, the anti-HB-EGF antibodies provided herein strongly inhibit MDA-MB231 cell migration.

[00065]FIGURE 28 graphically illustrates that HB-EGF-induced migration of MCF-7 breast cancer cells is inhibited by three anti-HB-EGF antibody preparations (the U2-42, the U2-39 and the U2-45 monoclonal antibody preparations).

[00066]FIGURE 29 illustrates the dose-dependent inhibition of HB-EGF-induced tyrosine phosphorylation of HER4 by anti-HB-EGF antibody preparations. U2-42.1 or U2-39.1 anti-HB-EGF antibody preparations were incubated with HB-EGF prior to stimulation and detection of HER4 tyrosine phosphorylation. As shown, 100% inhibition of HER4 tyrosine phosphorylation was observed. Note that the amount of antibody shown on the x-axis decreases logarithmically.

[00067]FIGURE 30A shows that monoclonal antibody preparations cross-react with HB-EGF from cynomolgus monkeys as assessed by flow cytometry (FACS) using HEK-293 cells transfected with a DNA vector expressing cynomolgus HB-EGF. As shown, very low X-mean values (1-2) are observed for HEK-293 control cells that were transfected with an empty vector control. In contrast, X-mean values of 250 or more were observed when HEK-293 cells were transfected with an expression cassette encoding cynomolgus monkey HB-EGF.

[00068]FIGURE 30B shows that monoclonal antibody U2-45 preparation cross-reacts with HB-EGF from mouse as assessed by flow cytometry (FACS) using HEK-293 cells transfected with a DNA vector expressing mouse HB-EGF. X-mean values of 33.7 were observed when HEK-293 cells were transfected with an expression cassette encoding mouse HB-EGF.

[00069]FIGURE 30C shows the degree of cross-reactivity of HB-EGF antibodies with amphiregulin.

[00070]FIGURE 31 shows that HB-EGF is expressed on human vascular endothelial cells (HUVECs), as detected by FACS analysis.

[00071]FIGURES 32A - 32B show that while HB-EGF stimulates HUVEC cellular proliferation, anti-HB-EGF antibody preparations inhibited basal proliferation by about 8% to 14%. HB-EGF stimulates HUVEC cellular proliferation by about 38% (FIGURE 32A). However, upon addition of anti-HB-EGF antibody preparations U2-42, U2-39 or U2-45, basal cellular proliferation is inhibited by about 8% to 14% (FIGURE 32B).

[00072]FIGURES 33A-33L illustrate that anti-HB-EGF antibodies accelerate HUVEC tube regression. HUVEC tube formation is a model system for endothelial cell angiogenesis. FIGURES 33A-33C provide control assays that were performed without anti-HB-EGF antibodies. As shown, HUVEC cells join to form many circular structures or "tubes." FIGURES 33D-33F illustrate the effects of adding the anti-HB-EGF U2-39 antibody preparation upon tube formation. FIGURES 33G-33I illustrate the effects of adding the anti-HB-EGF U2-42 antibody preparation upon tube formation. FIGURES 33J-33L illustrate the effects of adding the anti-HB-EGF U2-45 antibody preparation upon tube formation. As shown, fewer HUVEC tubes are visible and the

network is diminished when the U2-42, U2-39 and U2-45 anti-HB-EGF antibody preparations are present.

[00073]FIGURE 33M graphically illustrates a quantitative evaluation of HUVEC tube formation after adding the anti-HB-EGF antibody preparations provided herein, supporting the utility of HB-EGF antibodies for inhibiting angiogenesis. The number of tubes or closed cell structures per microscopic field is plotted for the U2-42, U2-39 or U2-45 anti-HB-EGF antibody preparations. As shown, while approximately 10 HUVEC tubes were visible per field when no anti-HB-EGF antibodies were present, only about 4 HUVEC tubes were observed per microscopic field when the U2-39 anti-HB-EGF antibody preparation was added. In the presence of the U2-42 anti-HB-EGF antibody preparation only about 1 HUVEC tube was observed. When the U2-45 anti-HB-EGF antibody preparation was present only 6 HUVEC tubes were observed.

[00074]FIGURE 34A illustrates that anti-HB-EGF antibodies inhibit HB-EGF-stimulated colony formation of OVCAR-8 ovarian cancer cells. As shown, HB-EGF stimulated OVCAR-8 cells to form a significantly larger mean colony size than control OVCAR-8 cells cultured without HB-EGF. However, when OVCAR-8 cells were cultured with anti-HB-EGF U2-39 antibodies in the presence of HB-EGF, mean colony size was reduced to the baseline size observed for control cells without HB-EGF treatment.

[00075]FIGURE 34B illustrates that anti-HB-EGF antibodies inhibit HB-EGF-stimulated colony formation of BM1604 prostate cancer cells in soft agar. As shown, HB-EGF stimulated BM1604 cells to form a larger number of colonies per well than control BM1604 cells cultured without HB-EGF. However, when BM1604 cells were cultured with anti-HB-EGF U2-39 antibodies in the presence of HB-EGF, mean colony size was reduced to a size similar to that observed for control cells without HB-EGF treatment. Anti-HB-EGF U2-45 and U2-42 antibodies partially inhibited colony formation, while, in this assay, the U2-39 anti-HB-EGF antibody completely inhibited colony formation.

[00076]FIGURE 34C illustrates that anti-HB-EGF antibodies inhibit HB-EGF-stimulated colony formation of NCI-H226 lung carcinoma cells. As shown, HB-EGF stimulated NCI-H226 cells to form a significantly larger mean colony size than control NCI-H226 cells cultured without HB-EGF. However, when NCI-H226 cells were cultured with anti-HB-EGF U2-39 antibodies in the presence of HB-EGF, mean colony size was reduced to the baseline size observed for control cells without HB-EGF treatment.

[00077]FIGURE 34D illustrates that anti-HB-EGF antibodies inhibit basal colony formation of SkOV-3 HB-EGF clone 71 cells, derived from SkOV-3 ovarian cancer cells transfected with an HB-EGF expression vector to cause constitutive over-expression of HB-EGF. As shown, control SkOV-3 HB-EGF clone 71 cells formed large numbers of colonies. However, when SkOV-3 HB-

EGF cl. 71 cells were cultured with either anti-HB-EGF U2-42 or U2-39 antibodies, the number of colonies was dramatically reduced.

[00078]FIGURE 34E illustrates that anti-HB-EGF antibodies inhibit basal colony formation of SkOV-3 HB-EGFclone 74 cells, derived from SkOV-3 ovarian cancer cells transfected with an HB-EGF expression vector to cause constitutive over-expression of HB-EGF. As shown, control SkOV-3 HB-EGF clone 74 cells formed large numbers of colonies. However, when SkOV-3 HB-EGF clone 74 cells were cultured with anti-HB-EGF U2-39 antibodies, the number of colonies was dramatically reduced.

[00079]FIGURE 34F illustrates that anti-HB-EGF antibodies inhibit basal colony formation of BxPC3 pancreatic adenocarcinoma cells grown in soft agar. As shown, control BxPC3 cells formed large numbers of colonies. However, when BxPC3 cells were cultured with either anti-HB-EGF U2-42 or U2-39 antibodies in the presence of HB-EGF, the number of colonies was dramatically reduced.

[00080]FIGURE 35 illustrates that anti-HB-EGF antibodies inhibit basal colony formation of EFO-27 HB-EGF clone 58 ovarian cancer cells overexpressing HB-EGF grown in soft agar. As shown, control cells formed large numbers of colonies. However, when EFO-27 HB-EGF cl. 58 cells were cultured with either anti-HB-EGF U2-42, U2-39 or U2-45 antibodies the number of colonies was dramatically reduced. Moreover, combination therapy of anti-HB-EGF antibodies with the anti-EGFR antibody Erbitux completely inhibited the colony formation.

[00081]FIGURE 36 shows that anti-HB-EGF antibodies inhibit HB-EGF induced angiogenesis *in vivo*. Angiogenic network formation in a mouse matrigel plug assay could be blocked in a dose-dependent manner by antibodies U2-42, U2-39 and U2-45.

[00082]FIGURE 37 illustrates inhibition of the growth of established BxPC3 tumors in mouse xenograft models by antibodies U2-42 and U2-39.

[00083]FIGURES 38A-38C illustrate inhibition of the growth of established EFO-27 HB-EGF clone 58 tumors in mouse xenograft models by antibodies U2-42, U2-39 and U2-45 (FIGURE 38A). As shown in FIGURE 38B, efficacy of inhibition of the xenograft tumor growth by antibodies U2-42 and U2-39 was shown to be dose dependent. Moreover, combination treatment with the anti-EGFR antibody Erbitux leads to complete regression of tumor growth and shows the potent synergistic activity of the anti-HB-EGF antibodies as agents for combination therapy (FIGURE 38C).

[00084]FIGURES 39A-39B illustrate the use of the human anti-HB-EGF antibodies for detection of HB-EGF in human tissue by immunohistochemistry (FIGURE 39A) and by ELISA (FIGURE 39B).

[00085]FIGURE 40A illustrates a scratch assay indicating the inhibition of HB-EGF-induced migration of CLS354 epithelial squamous carcinoma cells (mouth).

[00086]FIGURE 40B illustrates a transmigration assay indicating the inhibition of HB-EGF-induced migration of Detroit 562 epithelial carcinoma cells (pharynx).

[00087]FIGURE 41 illustrates a spheroid-based cellular angiogenesis assay indicating the inhibition of VEGF-stimulated endothelial cell sprouting.

[00088]FIGURE 42 illustrates immunohistochemistry (IHC) analysis of human tumor xenograft samples indicating the inhibition of CD31 staining of tumor in vivo.

[00089]FIGURE 43 illustrates in vivo ovarian tumor xenograft model indicating combination treatment of U2-39 with Cisplatin and Avastin.

DETAILED DESCRIPTION

[00090]The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

[00091]Unless otherwise defined herein, scientific and technical terms used in connection with the present application shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular.

[00092]Generally, nomenclatures used in connection with, and techniques of, cell and tissue culture, molecular biology, immunology, microbiology, genetics and protein and nucleic acid chemistry and hybridization described herein are those well known and commonly used in the art. The methods and techniques of the present application are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification unless otherwise indicated. *See, e.g.,* Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, 3rd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (2001) and Ausubel *et al.*, Current Protocols in Molecular Biology, Greene Publishing Associates (1992), and Harlow and Lane Antibodies: A Laboratory Manual Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1990), which are incorporated herein by reference. Enzymatic reactions and purification techniques are performed according to manufacturer's specifications, as commonly accomplished in the art or as described herein. The terminology used in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well known and commonly used in the art. Standard techniques can be used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients.

[00093]It should be understood that this invention is not limited to the particular methodology, protocols, and reagents, etc., described herein and as such may vary. The terminology used

herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the disclosed, which is defined solely by the claims.

[00094]Other than in the operating examples, or where otherwise indicated, all numbers expressing quantities of ingredients or reaction conditions used herein should be understood as modified in all instances by the term "about." The term "about" when used in connection with percentages may mean ±1%.

[00095]

A. General Overview

[00096]Antigen binding proteins that bind HB-EGF protein, in particular human HB-EGF (hHB-EGF) protein are provided herein. The antigen binding proteins provided are polypeptides into which one or more complementary determining regions (CDRs), as described herein, are embedded and/or joined. In some antigen binding proteins, the CDRs are embedded into a "framework" region, which orients the CDR(s) such that the proper antigen binding properties of the CDR(s) is achieved. In general, antigen binding proteins that are provided can interfere with, block, reduce or modulate the interaction between HB-EGF and its cognate receptors, including EGF-R and HER4.

[00097]Certain antigen binding proteins described herein are antibodies or are derived from antibodies. In certain embodiments, the polypeptide structure of the antigen binding proteins is based on antibodies, including, but not limited to, monoclonal antibodies, bispecific antibodies, minibodies, domain antibodies, synthetic antibodies (sometimes referred to herein as "antibody mimetics"), chimeric antibodies, humanized antibodies, human antibodies, antibody fusions (sometimes referred to herein as "antibody conjugates"), and fragments thereof, respectively. The various structures are further described herein below.

[00098]The antigen binding proteins provided herein have been demonstrated to bind to several epitopes of HB-EGF, in particular human HB-EGF. As demonstrated in the examples, the ability of HB-EGF to bind to its cognate receptors is reduced or inhibited. As a consequence, the antigen binding proteins provided herein are capable of inhibiting the activity of HB-EGF. In particular, antigen binding proteins binding to these epitopes can have one or more of the following activities: inhibiting, *inter alia*, EGF-R and HER4 autophosphorylation, induction of EGF-R and HER4 signal transduction pathway, EGF-R and HER4 induced cell growth, and other physiological effects induced by EGF-R and HER4 upon HB-EGF binding.

[00099]The antigen binding proteins that are disclosed herein have a variety of utilities. Some of the antigen binding proteins, for instance, are useful in specific binding assays, affinity purification of HB-EGF, in particular hHB-EGF and in screening assays to identify pof such receptors. In additiojn, the disclosed antigen binding proteins may be used for the diagnosis

and/or treatment of disease, such as proliferative disorders. These include, but are not limited to, various types of cancer.

B. Heparin-Binding Epidermal Growth Factor-like Growth Factor (HB-EGF)

[000100]HB-EGF is produced by various tumor cells and acts as an autocrine tumor growth factor. Davis-Fleischer *et al.*, 1998, *Front Biosci.* 3:288-299; Iwamoto & Mekada, 2000, *Cytokine Growth Factor Rev.* 11:335-344. HB-EGF has a strong affinity for heparin which can increase the biological activity of HB-EGF. HB-EGF is produced as a transmembrane protein which is proteolytically cleaved by metalloproteinases to yield the mature soluble form of the growth factor.

[000101]HB-EGF was first identified from supernatants of cultured human macrophages in a soluble, secreted form. On human cells, the precursor proHB-EGF, acts as the diphtheria toxin receptor. Various cell types, including epithelial cells, keratinocytes, monocytes, mesangial cells, lymphoid cells, and skeletal muscle cells, produce HB-EGF. It is a potent mitogen and chemotactic factor for epithelial cells, fibroblasts, smooth muscle cells and various human cancer cells.

[000102]The transmembrane form of HB-EGF is synthesized by many cell types as a 208-amino acid transmembrane precursor (tm-HB-EGF) containing EGF, heparin-binding, transmembrane, and cytoplasmic domains. The extracellular domain can be released as a 12- to 22-kDa soluble form of HB-EGF (sol-HB-EGF) through the action of metalloproteinases, which is regulated by different G protein-coupled receptors (GPCRs) or tumor promoters such as tetradecanoyl phorbol acetate (TPA). Typically, a substantial amount of transmembrane HB-EGF precursor remains uncleaved on the cell surface.

[000103]Both tm-HB-EGF and sol-HB-EGF are biologically active. The biological functions of both sol- and tm-HB-EGF are mediated by the EGF receptor (EGFR; HER1) and ErbB4 (HER4). Activation of these types of these receptors is believed to occur as a consequence of ligand-induced receptor homo- or hetero-dimerization. Upon activation, the EGF receptor has been demonstrated to increase cell growth, increase cell motility, inhibit apoptosis and increase cellular transformation.

[000104]EGFR-dependent signaling pathways can be transactivated upon stimulation of G-protein-coupled receptors (GPCR). Ligand activation of heterotrimeric G proteins by interaction with a GPCR results in an intracellular signal that induces the extracellular activity of a transmembrane metalloproteinase. Ligands that activate the GPCR pathway include LPA (lysophosphatidic acid), thrombin, carbachol, bombesin, and endothelin. Such activation leads to extracellular processing of a transmembrane growth factor precursor and release of the mature factor which, directly or through the proteoglycan matrix, interacts with the ectodomain of

EGFR and activates it through tyrosine phosphorylation. *See*, Prenzel *et al.*, 1999, *Nature* 402:884-888. Thus, HB-EGF is a component of a triple membrane-passing signal (TMPS) mechanism whereby a GPCR activates a membrane-bound metalloproteinase, which cleaves proHB-EGF to release the soluble growth factor, which subsequently activates the EGF receptor. EGFR transactivation has been linked to various disease states such as cardiac hypertrophy (reviewed in Shah BH, Catt KJ. Trends Pharmacol Sci. 2003 May;24(5):239-244), vascular remodeling (reviewed in Eguchi *et al.*, 2003, *Biochem Soc Trans. 2003 Dec;31(Pt 6):1198-202.*) and cancer (reviewed in Fischer *et al.*, 2003, *supra*).

[000105]Sequences for HB-EGF proteins and nucleic acids encoding those proteins are available to one of skill in the art. For example, such HB-EGF sequences can be found in the database provided by the National Center for Biotechnology Information (NCBI) (*see,* http://www.ncbi.nlm.nih.gov/). One example of a sequence for a HB-EGF is the amino acid sequence at NCBI accession numbers NM 001945 and NP_001936 (gi:4503413). This sequence is provided below for easy reference (SEQ ID NO:1072):

[000106]MKLLPSVVLKLFLAAVLSALVTGESLERLRRGLAAGTSNPDPPTVSTDQLLPLGGGRD RKVRDLQEADLDLLRVTLSSKPQALATPNKEEHGKRKKKGKGLGKKRDPCLRKYKDFCIHGEC KYVKELRAPSCICHPGYHGERCHGLSLPVENRLYTYDHTTILAVVAVVLSSVCLLVIVG LLMFRYHRRG GYDVENEEKVKLGMTNSH.

[000107]Note that the HB-EGF sequence shown above (SEQ ID NO:1072) has the nineteen amino acid signal peptide (MKLLPSVVLK LFLAAVLSA, SEQ ID NO:1073).

[000108]The soluble extracellular domain consists of amino acids 1-149 of the above HB-EGF sequence. This sequence for the HB-EGF soluble extracellular domain is provided below as SEQ ID NO:1074:

[000109]MKLLPSVVLKLFLAAVLSALVTGESLERLRRGLAAGTSNPDPPTVSTDQLLPLGGGRD RKVRDLQEADLDLLRVTLSSKPQALATPNKEEHGKRKKKGKGLGKKRDPCLR KYKDFCIHGECKYVKELRAPSCICHPGYHGERCHGLSLP.

[000110]Upon cleavage, a mature HB-EGF is generated that consists of amino acids 63-149 (87 amino acids). This sequence for the mature HB-EGF is provided below as SEQ ID NO:1075:

[000111]DLQEADLDLLRVTLSSKPQALATPNKEEHGKRKKKGKGLGKKRDPCLRKYKDFCIHG ECKYVKELRAPSCICHPGYHGERCHGLSLP.

[000112]HB-EGF interacts with and activates the.. epidermal growth factor receptor (EGFR). EGFR is a 170 kDa transmembrane glycoprotein consisting of an extracellular ligand-binding domain, a transmembrane region and an intracellular domain with tyrosine kinase activity. Binding of growth factors to the EGFR results in internalization of the ligand-receptor complex, autophosphorylation of the receptor and other protein substrates, leading ultimately to DNA

synthesis and cell division. The external ligand binding domain is not only stimulated by HB-EGF, but also by EGF, TGFα and amphiregulin (AR).

[000113]Overexpression of the EGFR is often accompanied by the co-expression of EGF-like growth factors, suggesting that an autocrine pathway for control of growth may play a major part in the progression of tumors. It is now widely believed that this is a mechanism by which tumor cells can escape normal physiological control.

C. HB-EGF Receptor Antigen Binding Proteins

[000114]A variety of selective binding agents useful for regulating the activity of HB-EGF are provided. These agents include, for instance, antigen binding proteins that contain an antigen binding domain (*e.g.*, single chain antibodies, domain antibodies, inmmunoadhesions, and polypeptides with an antigen binding region) and specifically bind to a HB-EGF polypeptide, in particular human HB-EGF. Some of the agents, for example, are useful in inhibiting the binding of HB-EGF to its receptors, and can thus be used to inhibit, interfere with, or modulate one or more activities associated with HB-EGF-mediated signaling.

[000115]In general, the antigen binding proteins that are provided typically comprise one or more CDRs as described herein (*e.g.*, 1, 2, 3, 4, 5 or 6). In some instances, the antigen binding protein comprises (a) a polypeptide structure and (b) one or more CDRs that are inserted into and/or joined to the polypeptide structure. The polypeptide structure can take a variety of different forms. For example, it can be, or comprise, the framework of a naturally occurring antibody, or fragment or variant thereof, or may be completely synthetic in nature. Examples of various polypeptide structures are further described below.

[000116]In certain embodiments, the polypeptide structure of the antigen binding proteins is an antibody or is derived from an antibody, including, but not limited to, monoclonal antibodies, bispecific antibodies, minibodies, domain antibodies, synthetic antibodies (sometimes referred to herein as "antibody mimetics"), chimeric antibodies, humanized antibodies, antibody fusions (sometimes referred to as "antibody conjugates"), and portions or fragments of each, respectively. In some instances, the antigen binding protein is an immunological fragment of an antibody (*e.g.*, a Fab, a Fab', a $F(ab')_2$, or a scFv). The various structures are further described and defined herein.

[000117]Certain of the antigen binding proteins as provided herein specifically bind to human HB-EGF. In a specific embodiment, the antigen binding protein specifically binds to human HB-EGF protein having the amino acid sequence of SEQ ID NO:1072.

[000118]In embodiments where the antigen binding protein is used for therapeutic applications, an antigen binding protein can inhibit, interfere with or modulate one or more biological activities of HB-EGF. In this case, an antigen binding protein binds specifically to and/or substantially

inhibits binding of human HB-EGF to its receptor when an excess of antibody reduces the quantity of human HB-EGF bound to its receptor, or *vice versa*, by at least about 20%, 40%, 60%, 80%, 85%, or more (for example by measuring binding in an *in vitro* competitive binding assay). HB-EGF has many distinct biological effects, which can be measured in many different assays in different cell types; examples of such assays are provided herein.

1. Naturally Occurring Antibody Structure

[000119]Some of the antigen binding proteins that are provided have the structure typically associated with naturally occurring antibodies. The structural units of these antibodies typically comprise one or more tetramers, each composed of two identical couplets of polypeptide chains, though some species of mammals also produce antibodies having only a single heavy chain. In a typical antibody, each pair or couplet includes one full-length "light" chain (in certain embodiments, about 25 kDa) and one full-length "heavy" chain (in certain embodiments, about 50-70 kDa). Each individual immunoglobulin chain is composed of several "immunoglobulin domains", each consisting of roughly 90 to 110 amino acids and expressing a characteristic folding pattern. These domains are the basic units of which antibody polypeptides are composed. The amino-terminal portion of each chain typically includes a variable domain that is responsible for antigen recognition. The carboxy-terminal portion is more conserved evolutionarily than the other end of the chain and is referred to as the "constant region" or "C region". Human light chains generally are classified as kappa and lambda light chains, and each of these contains one variable domain and one constant domain. Heavy chains are typically classified as mu, delta, gamma, alpha, or epsilon chains, and these define the antibody's isotype as IgM, IgD, IgG, IgA, and IgE, respectively. IgG has several subtypes, including, but not limited to, IgG1, IgG2, IgG3, and IgG4. IgM subtypes include IgM1, and IgM2. IgA subtypes include IgA1 and IgA2. In humans, the IgA and IgD isotypes contain four heavy chains and four light chains; the IgG and IgE isotypes contain two heavy chains and two light chains; and the IgM isotype contains five heavy chains and five light chains. The heavy chain C region typically comprises one or more domains that may be responsible for effector function. The number of heavy chain constant region domains will depend on the isotype. IgG heavy chains, for example, contain three C region domains known as C_H1, C_H2 and C_H3. The antibodies that are provided can have any of these isotypes and subtypes. In certain embodiments, the HB-EGF antibody is of the IgG1, IgG2, or IgG4 subtype.

[000120]In full-length light and heavy chains, the variable and constant regions are joined by a "J" region of about twelve or more amino acids, with the heavy chain also including a "D" region of about ten more amino acids. *See, e.g.,* Fundamental Immunology, 2nd ed., Ch. 7 (Paul, W., ed.) 1989, New York: Raven Press (hereby incorporated by reference in its entirety for all

purposes). The variable regions of each light/heavy chain pair typically form the antigen binding site.

[000121]One example of a kappa Light Constant domain of an exemplary HB-EGF monoclonal antibody has the amino acid sequence:

[000122]RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS TYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:187).

[000123]One example of an IgG2 heavy constant domain of an exemplary HB-EGF monoclonal antibody has the amino acid sequence:

[000124]ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS LSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKG LPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPML DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:188).

[000125]Variable regions of immunoglobulin chains generally exhibit the same overall structure, comprising relatively conserved framework regions (FR) joined by three hypervariable regions, more often called "complementarity determining regions" or CDRs. The CDRs from the two chains of each heavy chain/light chain pair mentioned above typically are aligned by the framework regions to form a structure that binds specifically to a specific epitope on the target protein (*e.g.*, HB-EGF). From N-terminal to C-terminal, naturally-occurring light and heavy chain variable regions both typically conform with the following order of these elements: FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. A numbering system has been devised for assigning numbers to amino acids that occupy positions in each of these domains. This numbering system is defined in Kabat Sequences of Proteins of Immunological Interest (1987 and 1991, NIH, Bethesda, MD), or Chothia & Lesk, 1987, *J. Mol. Biol.* 196:901-917; Chothia *et al.*, 1989, *Nature* 342:878-883.

[000126]The various light chain and heavy chain variable regions provided herein are depicted in FIGURES 1A-1P, and FIGURES 2A-2O, respectively. Each of these variable regions may be attached to the above heavy and light chain constant regions to form a complete antibody heavy and light chain, respectively. Further, each of the so generated heavy and light chain sequences may be combined to form a complete antibody structure.

[000127]Specific examples of some of the full length light and heavy chains of the antibodies that are provided and their corresponding amino acid sequences are summarized in FIGURES 3A-3K and FIGURES 4A-4O, respectively.

[000128]Again, each of the exemplary light chains (U_L-1, U_L-2, U_L-3 etc.) listed in FIGURES 3A-3K can be combined with any of the exemplary heavy chains shown in FIGURES 4A-4O to form an antibody. Examples of such combinations include U_L-1 combined with any of U_H-1 through

U_H-58; U_L-2 combined with any of U_H-1 through U_H-58; or U_L-3 combined with any of U_H-1 through U_H-58, and so on. In some instances, the antibodies include at least one light chain and one heavy chain from those listed in FIGURES 3A-3K and FIGURES 4A-4O, respectively. In other instances, the antibodies contain two identical light chains and two identical heavy chains. As an example, an antibody or immunologically functional fragment may include two U_L-1 light chains and two U_H-1 heavy chains, or two U_L-2 light chains and two U_H-2 heavy chains, or two U_L-3 light chains and two U_H-3 heavy chains and other similar combinations of pairs of light chains and pairs of heavy chains as listed in FIGURES 3A-3K and FIGURES 4A-4O, respectively.

[000129]Other antibodies that are provided are variants of antibodies formed by combination of the heavy and light chains shown in FIGURES 3A-3K and FIGURES 4A-4O, respectively, and comprise light and/or heavy chains that each have at least 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to the amino acid sequences of these chains. In some instances, such antibodies include at least one light chain and one heavy chain, whereas in other instances the variant forms contain two identical light chains and two identical heavy chains.

2. Variable Domains of Antibodies

[000130]Also provided are antigen binding proteins that contain an antibody light chain variable region selected from the group consisting of U-V_L1, U-V_L2, U-V_L3, U-V_L4, U-V_L5, U-V_L6, U-V_L7, U-V_L8, U-V_L9, U-V_L10, U-V_L11, U-V_L12, U-V_L13, U-V_L14, U-V_L15, U-V_L16, U-V_L17, U-V_L18, U-V_L19, U-V_L20, U-V_L21, U-V_L22, U-V_L23, U-V_L24, U-V_L25, U-V_L26, U-V_L27, U-V_L28, U-V_L29, U-V_L30, U-V_L31, U-V_L32, U-V_L33, U-V_L34, U-V_L35, U-V_L36, U-V_L37, U-V_L38, U-V_L39, U-V_L40, U-V_L41, U-V_L42, U-V_L43, U-V_L44, U-V_L45, U-V_L46, U-V_L47, U-V_L48, U-V_L49, U-V_L50, U-V_L51, U-V_L52, U-V_L54, U-V_L55, U-V_L56, U-V_L57, U-V_L58, U-V_L59, U-V_L60, U-V_L61, U-V_L62, U-V_L64, and U-V_L65; and/or an antibody light chain variable region selected from the group consisting of U-V_H1, U-V_H2, U-V_H3, U-V_H4, U-V_H5, U-V_H6, U-V_H7, U-V_H8, U-V_H9, U-V_H10, U-V_H11, U-V_H12, U-V_H13, U-V_H14, U-V_H15, U-V_H16, U-V_H17, U-V_H18, U-V_H19, U-V_H20, U-V_H21, U-V_H22, U-V_H23, U-V_H24, U-V_H25, U-V_H26, U-V_H27, U-V_H28, U-V_H29, U-V_H30, U-V_H31, U-V_H32, U-V_H33, U-V_H34, U-V_H35, U-V_H36, U-V_H37, U-V_H38, U-V_H39, U-V_H40, U-V_H41, U-V_H42, U-V_H43, U-V_H44, U-V_H45, U-V_H46, U-V_H47, U-V_H48, U-V_H49, U-V_H50, U-V_H51, U-V_H52, U-V_H53, U-V_H54, U-V_H55, U-V_H56, U-V_H57, and U-V_H58, as shown in FIGURES 1A-1P, and FIGURES 2A-2O, respectively, and immunologically functional fragments, derivatives, muteins and variants of these light chain and heavy chain variable regions.

[000131]Sequence alignments of the various light and heavy chain variable regions, respectively, are provided in FIGURES 10A and 10B, and FIGURES 11 A and 11B, respectively.

[000132]Antigen binding proteins of this type can generally be designated by the formula " V_Hx/ V_Ly," where "x" corresponds to the number of heavy chain variable regions and "y" corresponds to the number of the light chain variable regions (in general, x and y are each 1 or 2).

[000133]Each of the light chain variable regions listed in FIGURES 1A-1P may be combined with any of the light chain variable regions shown in FIGURES 2A-2O to form an antigen binding protein. Examples of such combinations include U-V_L1 combined with any of U-V_H1, U-V_H2, U-V_H3, U-V_H4, U-V_H5, U-V_H6, U-V_H7, U-V_H8, U-V_H9, U-V_H10, U-V_H11, U-V_H12, U-V_H13, U-V_H14, U-V_H15, U-V_H16, U-V_H17, U-V_H18, U-V_H19, U-V_H20, U-V_H21, U-V_H22, U-V_H23, U-V_H24, U-V_H25, U-V_H26, U-V_H27, U-V_H28, U-V_H29, U-V_H30, U-V_H31, U-V_H32, U-V_H33, U-V_H34, U-V_H35, U-V_H36, U-V_H37, U-V_H38, U-V_H39, U-V_H40, U-V_H41, U-V_H42, U-V_H43, U-V_H44, U-V_H45, U-V_H46, U-V_H47, U-V_H48, U-V_H49, U-V_H50, U-V_H51, U-V_H52, U-V_H53, U-V_H54, U-V_H55, U-V_H56, U-V_H57, or U-V_H58, or U-V_L2 combined with any of U-V_H1, U-V_H2, U-V_H3, U-V_H4, U-V_H5, U-V_H6, U-V_H7, U-V_H8, U-V_H9, U-V_H10, U-V_H11, U-V_H12, U-V_H13, U-V_H14, U-V_H15, U-V_H16, U-V_H17, U-V_H18, U-V_H19, U-V_H20, U-V_H21, U-V_H22, U-V_H23, U-V_H24, U-V_H25, U-V_H26, U-V_H27, U-V_H28, U-V_H29, U-V_H30, U-V_H31, U-V_H32, U-V_H33, U-V_H34, U-V_H35, U-V_H36, U-V_H37, U-V_H38, U-V_H39, U-V_H40, U-V_H41, U-V_H42, U-V_H43, U-V_H44, U-V_H45, U-V_H46, U-V_H47, U-V_H48, U-V_H49, U-V_H50, U-V_H51, U-V_H52, U-V_H53, U-V_H54, U-V_H55, U-V_H56, U-V_H57, or U-V_H58, etc.

[000134]In some instances, the antigen binding protein includes at least one heavy chain variable region and/or one light chain variable region from those listed in FIGURES 1A-1P, and FIGURES 2A-2O, respectively. In some instances, the antigen binding protein includes at least two different heavy chain variable regions and/or light chain variable regions from those listed in FIGURES 1A-1P, and FIGURES 2A-2O, respectively. An example of such an antigen binding protein comprises (a) one U-V_L1, and (b) one of U-V_L2, U-V_L3, U-V_L4, U-V_L5, U-V_L6, U-V_L7, U-V_L8, U-V_L9, U-V_L10, U-V_L11, U-V_L12, U-V_L13, U-V_L14, U-V_L15, U-V_L16, U-V_L17, U-V_L18, U-V_L19, U-V_L20, U-V_L21, U-V_L22, U-V_L23, U-V_L24, U-V_L25, U-V_L26, U-V_L27, U-V_L28, U-V_L29, U-V_L30, U-V_L31, U-V_L32, U-V_L33, U-V_L34, U-V_L35, U-V_L36, U-V_L37, U-V_L38, U-V_L39, U-V_L40, U-V_L41, U-V_L42, U-V_L43, U-V_L44, U-V_L45, U-V_L46, U-V_L47, U-V_L48, U-V_L49, U-V_L50, U-V_L51, U-V_L52, U-V_L54, U-V_L55, U-V_L56, U-V_L57, U-V_L58, U-V_L59, U-V_L60, U-V_L61, U-V_L62, U-V_L64, and U-V_L65. Again another example of such an antigen binding protein comprises (a) one U-V_L2, and (b) one of U-V_L1, U-V_L3, U-V_L4, U-V_L5, U-V_L6, U-V_L7, U-V_L8, U-V_L9, U-V_L10, U-V_L11, U-V_L12, U-V_L13, U-V_L14, U-V_L15, U-V_L16, U-V_L17, U-V_L18, U-V_L19, U-V_L20, U-V_L21, U-V_L22, U-V_L23, U-V_L24, U-V_L25, U-V_L26, U-V_L27, U-V_L28, U-V_L29, U-V_L30, U-V_L31, U-V_L32, U-V_L33, U-V_L34, U-V_L35, U-V_L36, U-V_L37, U-V_L38, U-V_L39, U-V_L40, U-V_L41, U-V_L42, U-V_L43, U-V_L44, U-V_L45, U-V_L46, U-V_L47, U-V_L48, U-V_L49, U-V_L50, U-V_L51, U-V_L52, U-V_L54, U-V_L55, U-V_L56, U-V_L57, U-V_L58, U-V_L59, U-V_L60, U-V_L61, U-V_L62, U-V_L64, and U-V_L65. Again another example of such an antigen binding protein comprises (a) one U-V_L3, and (b) one of U-V_L1, U-V_L2, U-V_L4, U-V_L5, U-V_L6, U-

V_L7, U-V_L8, U-V_L9, U-V_L10, U-V_L11, U-V_L12, U-V_L13, U-V_L14, U-V_L15, U-V_L16, U-V_L17, U-V_L18, U-V_L19, U-V_L20, U-V_L21, U-V_L22, U-V_L23, U-V_L24, U-V_L25, U-V_L26, U-V_L27, U-V_L28, U-V_L29, U-V_L30, U-V_L31, U-V_L32, U-V_L33, U-V_L34, U-V_L35, U-V_L36, U-V_L37, U-V_L38, U-V_L39, U-V_L40, U-V_L41, U-V_L42, U-V_L43, U-V_L44, U-V_L45, U-V_L46, U-V_L47, U-V_L48, U-V_L49, U-V_L50, U-V_L51, U-V_L52, U-V_L54, U-V_L55, U-V_L56, U-V_L57, U-V_L58, U-V_L59, U-V_L60, U-V_L61, U-V_L62, U-V_L64, and U-V_L65, etc.

[000135]Again another example of such an antigen binding protein comprises (a) one U-V_H1, and (b) one of U-V_H2, U-V_H3, U-V_H4, U-V_H5, U-V_H6, U-V_H7, U-V_H8, U-V_H9, U-V_H10, U-V_H11, U-V_H12, U-V_H13, U-V_H14, U-V_H15, U-V_H16, U-V_H17, U-V_H18, U-V_H19, U-V_H20, U-V_H21, U-V_H22, U-V_H23, U-V_H24, U-V_H25, U-V_H26, U-V_H27, U-V_H28, U-V_H29, U-V_H30, U-V_H31, U-V_H32, U-V_H33, U-V_H34, U-V_H35, U-V_H36, U-V_H37, U-V_H38, U-V_H39, U-V_H40, U-V_H41, U-V_H42, U-V_H43, U-V_H44, U-V_H45, U-V_H46, U-V_H47, U-V_H48, U-V_H49, U-V_H50, U-V_H51, U-V_H52, U-V_H53, U-V_H54, U-V_H55, U-V_H56, U-V_H57, and U-V_H58. Another example comprises (a) one U-V_H2, and (b) one of U-V_H1, U-V_H3, U-V_H4, U-V_H5, U-V_H6, U-V_H7, U-V_H8, U-V_H9, U-V_H10, U-V_H11, U-V_H12, U-V_H13, U-V_H14, U-V_H15, U-V_H16, U-V_H17, U-V_H18, U-V_H19, U-V_H20, U-V_H21, U-V_H22, U-V_H23, U-V_H24, U-V_H25, U-V_H26, U-V_H27, U-V_H28, U-V_H29, U-V_H30, U-V_H31, U-V_H32, U-V_H33, U-V_H34, U-V_H35, U-V_H36, U-V_H37, U-V_H38, U-V_H39, U-V_H40, U-V_H41, U-V_H42, U-V_H43, U-V_H44, U-V_H45, U-V_H46, U-V_H47, U-V_H48, U-V_H49, U-V_H50, U-V_H51, U-V_H52, U-V_H53, U-V_H54, U-V_H55, U-V_H56, U-V_H57, and U-V_H58. Again another example comprises (a) one U-V_H3, and (b) one of U-V_H1, U-V_H2, U-V_H4, U-V_H5, U-V_H6, U-V_H7, U-V_H8, U-V_H9, U-V_H10, U-V_H11, U-V_H12, U-V_H13, U-V_H14, U-V_H15, U-V_H16, U-V_H17, U-V_H18, U-V_H19, U-V_H20, U-V_H21, U-V_H22, U-V_H23, U-V_H24, U-V_H25, U-V_H26, U-V_H27, U-V_H28, U-V_H29, U-V_H30, U-V_H31, U-V_H32, U-V_H33, U-V_H34, U-V_H35, U-V_H36, U-V_H37, U-V_H38, U-V_H39, U-V_H40, U-V_H41, U-V_H42, U-V_H43, U-V_H44, U-V_H45, U-V_H46, U-V_H47, U-V_H48, U-V_H49, U-V_H50, U-V_H51, U-V_H52, U-V_H53, U-V_H54, U-V_H55, U-V_H56, U-V_H57, and U-V_H58, etc.

[000136]The various combinations of heavy chain variable regions may be combined with any of the various combinations of light chain variable regions.

[000137]In other instances, the antigen binding protein contains two identical light chain variable regions and/or two identical heavy chain variable regions. As an example, the antigen binding protein may be an antibody or immunologically functional fragment that includes two light chain variable regions and two heavy chain variable regions in combinations of pairs of light chain variable regions and pairs of heavy chain variable regions as listed in FIGURES 1A-1P, and FIGURES 2A-2O, respectively.

[000138]Some antigen binding proteins that are provided comprise a light chain variable domain comprising a sequence of amino acids that differs from the sequence of a light chain variable domain selected from U-V_L1, U-V_L2, U-V_L3, U-V_L4, U-V_L5, U-V_L6, U-V_L7, U-V_L8, U-V_L9, U-V_L10, U-V_L11, U-V_L12, U-V_L13, U-V_L14, U-V_L15, U-V_L16, U-V_L17, U-V_L18, U-V_L19, U-V_L20, U-V_L21, U-

V_L22, U-V_L23, U-V_L24, U-V_L25, U-V_L26, U-V_L27, U-V_L28, U-V_L29, U-V_L30, U-V_L31, U-V_L32, U-V_L33, U-V_L34, U-V_L35, U-V_L36, U-V_L37, U-V_L38, U-V_L39, U-V_L40, U-V_L41, U-V_L42, U-V_L43, U-V_L44, U-V_L45, U-V_L46, U-V_L47, U-V_L48, U-V_L49, U-V_L50, U-V_L51, U-V_L52, U-V_L54, U-V_L55, U-V_L56, U-V_L57, U-V_L58, U-V_L59, U-V_L60, U-V_L61, U-V_L62, U-V_L64, or U-V_L65 at only 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 amino acid residues, wherein each such sequence difference is independently either a deletion, insertion or substitution of one amino acid. The light chain variable region in some antigen binding proteins comprises a sequence of amino acids that has at least 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% sequence identity to the amino acid sequences of the light chain variable region of U-V_L1, U-V_L2, U-V_L3, U-V_L4, U-V_L5, U-V_L6, U-V_L7, U-V_L8, U-V_L9, U-V_L10, U-V_L11, U-V_L12, U-V_L13, U-V_L14, U-V_L15, U-V_L16, U-V_L17, U-V_L18, U-V_L19, U-V_L20, U-V_L21, U-V_L22, U-V_L23, U-V_L24, U-V_L25, U-V_L26, U-V_L27, U-V_L28, U-V_L29, U-V_L30, U-V_L31, U-V_L32, U-V_L33, U-V_L34, U-V_L35, U-V_L36, U-V_L37, U-V_L38, U-V_L39, U-V_L40, U-V_L41, U-V_L42, U-V_L43, U-V_L44, U-V_L45, U-V_L46, U-V_L47, U-V_L48, U-V_L49, U-V_L50, U-V_L51, U-V_L52, U-V_L54, U-V_L55, U-V_L56, U-V_L57, U-V_L58, U-V_L59, U-V_L60, U-V_L61, U-V_L62, U-V_L64, or U-V_L65.

[000139]Certain antibodies comprise a heavy chain variable domain comprising a sequence of amino acids that differs from the sequence of a heavy chain variable domain selected from U-V_H1, U-V_H2, U-V_H3, U-V_H4, U-V_H5, U-V_H6, U-V_H7, U-V_H8, U-V_H9, U-V_H10, U-V_H11, U-V_H12, U-V_H13, U-V_H14, U-V_H15, U-V_H16, U-V_H17, U-V_H18, U-V_H19, U-V_H20, U-V_H21, U-V_H22, U-V_H23, U-V_H24, U-V_H25, U-V_H26, U-V_H27, U-V_H28, U-V_H29, U-V_H30, U-V_H31, U-V_H32, U-V_H33, U-V_H34, U-V_H35, U-V_H36, U-V_H37, U-V_H38, U-V_H39, U-V_H40, U-V_H41, U-V_H42, U-V_H43, U-V_H44, U-V_H45, U-V_H46, U-V_H47, U-V_H48, U-V_H49, U-V_H50, U-V_H51, U-V_H52, U-V_H53, U-V_H54, U-V_H55, U-V_H56, U-V_H57, or U-V_H58 at only 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 amino acid residues, wherein each such sequence difference is independently either a deletion, insertion or substitution of one amino acid. The heavy chain variable region in some antigen binding proteins comprises a sequence of amino acids that has at least 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% sequence identity to the amino acid sequences of the heavy chain variable region of U-V_H1, U-V_H2, U-V_H3, U-V_H4, U-V_H5, U-V_H6, U-V_H7, U-V_H8, U-V_H9, U-V_H10, U-V_H11, U-V_H12, U-V_H13, U-V_H14, U-V_H15, U-V_H16, U-V_H17, U-V_H18, U-V_H19, U-V_H20, U-V_H21, U-V_H22, U-V_H23, U-V_H24, U-V_H25, U-V_H26, U-V_H27, U-V_H28, U-V_H29, U-V_H30, U-V_H31, U-V_H32, U-V_H33, U-V_H34, U-V_H35, U-V_H36, U-V_H37, U-V_H38, U-V_H39, U-V_H40, U-V_H41, U-V_H42, U-V_H43, U-V_H44, U-V_H45, U-V_H46, U-V_H47, U-V_H48, U-V_H49, U-V_H50, U-V_H51, U-V_H52, U-V_H53, U-V_H54, U-V_H55, U-V_H56, U-V_H57, or U-V_H58.

[000140]Still other antigen binding proteins, *e.g.*, antibodies or immunologically functional fragments include variant forms of a variant light chain and a variant heavy chain as just described.

3. CDRs

[000141]The antigen binding proteins disclosed herein are polypeptides into which one or more CDRs are grafted, inserted and/or joined. An antigen binding protein can have 1, 2, 3, 4, 5 or 6 CDRs. An antigen binding protein thus can have, for example, one light chain CDR1 ("CDRL1"), and/or one light chain CDR2 ("CDRL2"), and/or one light chain CDR3 ("CDRL3"), and/or one heavy chain CDR1 ("CDRH1"), and/or one heavy chain CDR2 ("CDRH2"), and/or one heavy chain CDR3 ("CDRH3"). Some antigen binding proteins inlcude both a CDRL3 and a CDRH3. Specific CDRs are identified in FIGURES 6A-6F, and FIGURES 7A-7E.

[000142]Complementarity determining regions (CDRs) and framework regions (FR) (examples of light and heavy chain FR amino acid sequences are given in FIGURES 8A-8H and FIGURES 9A-9F, respectively) of a given antibody may be identified using the system described by Kabat *et al.* in Sequences of Proteins of Immunological Interest, 5th Ed., US Dept. of Health and Human Services, PHS, NIH, NIH Publication No. 91-3242, 1991. Certain antibodies that are disclosed herein comprise one or more amino acid sequences that are identical or have substantial sequence identity to the amino acid sequences of one or more of the CDRs presented in FIGURES 6A-6F (CDRLs) and FIGURES 7A-7E (CDRHs).

[000143]The structure and properties of CDRs within a naturally occurring antibody has been described, *supra*. Briefly, in a traditional antibody, the CDRs are embedded within a framework in the heavy and light chain variable region where they constitute the regions responsible for antigen binding and recognition. A variable region comprises at least three heavy or light chain CDRs, *see, supra* (Kabat *et al.*, 1991, *Sequences of Proteins of Immunological Interest*, Public Health Service N.I.H., Bethesda, MD; *see,* also Chothia and Lesk, 1987, *J. Mol. Biol.* 196:901-917; Chothia *et al.*, 1989, *Nature* 342: 877-883), within a framework region (designated framework regions 1-4, FR1, FR2, FR3, and FR4, by Kabat *et al.*, 1991, *supra*; *see,* also Chothia and Lesk, 1987, *supra*). The CDRs provided herein, however, may not only be used to define the antigen binding domain of a traditional antibody structure, but may be embedded in a variety of other polypeptide structures, as described herein.

[000144]In one aspect, the CDRs provided are a (a) a CDRL selected from the group consisting of (i) a CDRL1 selected from the group consisting of SEQ ID NOs:189-217; (ii) a CDRL2 selected from the group consisting of SEQ ID NO:218-233; (iii) a CDRL3 selected from the group consisting of SEQ ID NO:234-274; and (iv) a CDRL of (i), (ii) and (iii) that contains one or more amino acid substitutions, deletions or insertions of no more than five, four, three, two, or one amino acids; (B) a CDRH selected from the group consisting of (i) a CDRH1 selected from the group consisting of SEQ ID NO:275-299; (ii) a CDRH2 selected from the group consisting of SEQ ID NO:300-331; (iii) a CDRH3 selected from the group consisting of SEQ ID NO:332-372;

and (iv) a CDRLHof (i), (ii) and (iii) that contains one or more amino acid substitutions, deletions or insertions of no more than five, four, three, two, or one amino acids amino acids.

[000145]In yet another aspect, variant forms of the CDRs are provided that have at least 80%, 85%, 90% or 95% sequence identity to a CDR sequence listed in FIGURES 6A-6F and FIGURES 7A-7E.

[000146]

[000147]In yet another aspect, the CDRs disclosed herein include consensus sequences derived from groups of related monoclonal antibodies. As described herein, a "consensus sequence" refers to amino acid sequences having conserved amino acids common among a number of sequences and variable amino acids that vary within a given amino acid sequences. The CDR consensus sequences provided include CDRs corresponding to each of CDRL1, CDRL2, CDRL3, CDRH1, CDRH2 and CDRH3.

[000148]Consensus sequences were determined using a standard phylogenic analysis approach of the CDRs corresponding to the U-V_L and U-V_H of anti-HB-EGF antibodies. First, in this approach, amino acid sequences corresponding to the entire variable domains of either U-V_L or U-V_H were converted to FASTA formatting for ease in processing comparative alignments and inferring phylogenies. Based on this comparison, each the light and heavy chain variable regions, respectivey were divided in phylogenetically related groups, *i.e.*, the light chain variable regions were devided into six groups A, B, C, D, E, and F (*see*, FIGURE 12A and 12B), and the heavy chain variable regions were ddivided into seven groups A, B, C, D, E, F, and G (*see*, FIGURE 12C). Then, within each of these groups, comparison of each of the the CDRL1, CDRL2, CDRL3, CDRH1, CDRH2, CDRH3 regions was used to define consensus collections.

[000149]Group A of the light chain CDRs includes the following consensus collections:

a. a CDRL1 of the generic formula $X_1SSQSLX_2X_3SDGX_4TYLX_5$ (SEQ ID NO:1035), wherein

X_1 is K or R,

X_2 is L or V,

X_3 is H or Y,

X_4 is K or N,

X_5 is N, S or Y.

b. a CDRL2 of the generic formula $X_1X_2SNX_3X_4S$ (SEQ ID NO:1041), wherein

X_1 is E or K,

X_2 is I or V,

X_3 is R or W,

X_4 is D or F.

c. a CDRL3 of the generic formula $X_1QX_2X_3X_4X_5PX_6X_7$ (SEQ ID NO:1046), wherein

X_1 is I or M,

X_2 is A, G or S,

X_3 is I or T,

X_4 is H or Q,

X_5 is F, L or W,

X_6 is C, I, H, L or T,

X_7 is S or T.

[000150]Group B of the light chain CDRs includes the following consensus collections:

a. a CDRL1 of the generic formula $RASQX_1ISX_2YLN$ (SEQ ID NO:1036), wherein

X_1 is R, S or T,

X_2 is R or S.

b. a CDRL2 of the generic formula $X_1X_2SX_3LQS$ (SEQ ID NO:1042), wherein

X_1 is A or T,

X_2 is A, E or V,

X_3 is S or T.

c. a CDRL3 of the generic formula $QQX_1X_2X_3X_4X_5IT$ (SEQ ID NO:1047), wherein

X_1 is I or S,

X_2 is F or Y,

X_3 is F, I, S or Y,

X_4 is A, S or T,

X_5 is P or S.

[000151]Group C of the light chain CDRs includes the following consensus collections:

a. a CDRL1 of the generic formula $RASQX_1IX_2X_3X_4LX_5$ (SEQ ID NO:1037), wherein

X_1 is D, G, S or T,

X_2 is A, R or S,

X_3 is H, I, N, R, S or T,

X_4 is D, W or Y,

X_5 is A, G or N.

b. a CDRL2 of the generic formula X_1ASX_2LQS (SEQ ID NO:1043), wherein

X_1 is A or V,

X_2 is S or T.

c. a CDRL3 of the generic formula $X_1X_2X_3X_4X_5X_6X_7X_8T$ (SEQ ID NO:1048), wherein

X_1 is L or Q,

X_2 is K, N or Q,

X_3 is A, H, S or Y,

X_4 is H, N or Y,

X_5 is N, S or T,

X_6 is A, F, I, T, V or Y,

X_7 is P or no amino acid,

X_8 is F, L or P.

[000152]Group D of the light chain CDRs includes the following consensus collections:

a. a CDRL1 of the generic formula $QASQDIX_1X_2X_3LN$ (SEQ ID NO:1038), wherein

X_1 is S or T,

X_2 is D or N,

X_3 is S or Y.

b. a CDRL2 of the generic formula $DASX_1LET$ (SEQ ID NO:1044), wherein

X_1 is I or N.

c. a CDRL3 of the generic formula $QX_1X_2DX_3LPX_4X_5$ (SEQ ID NO:1049), wherein

X_1 is H or Q,

X_2 is C or Y,

X_3 is D, I, N, S or Y,

X_4 is F, I or L,

X_5 is A, S or T.

[000153]Group E of the light chain CDRs includes the following consensus collections:

a. a CDRL1 of the generic formula $RASQX_1VX_2X_3X_4X_5LA$ (SEQ ID NO:1039), wherein

X_1 is S or T,

X_2 is I or S,

X_3 is R or S,

X_4 is S, N or no amino acid,

X_5 is Y or no amino acid.

b. a CDRL2 of the generic formula GASSRAT (SEQ ID NO:223)

c. a CDRL3 of the generic formula $QQX_1X_2X_3X_4PX_5X_6X_7$ (SEQ ID NO:1050), wherein

X_1 is H or Y,

X_2 is G or N,

X_3 is N or S,

X_4 is S or W,

X_5 is P or no amino acid,

X_6 is R or W,

X_7 is S or T.

[000154]Group F of the light chain CDRs includes the following consensus collections:

a. a CDRL1 of the generic formula $KSSQX_1X_2LX_3X_4SNNKNYLX_5$ (SEQ ID NO:1040), wherein

X_1 is N or S,

X_2 is I or V,

X_3 is D or Y,

X_4 is N, R or S,

X_5 is A or V.

b. a CDRL2 of the generic formula $WASX_1RES$ (SEQ ID NO:1045), wherein

X_1 is A or T.

c. a CDRL3 of the generic formula $X_1QYX_2X_3X_4X_5X_6X_7F$ (SEQ ID NO:1051), wherein

X_1 is H or Q,

X_2 is F or Y,

X_3 is G, I or S,

X_4 is F, I or T,

X_5 is M, P, S or T,

X_6 is F, L, R or W,

X_7 is S or T

[000155]Group A of the heavy chain CDRs includes the following consensus collections:

a. a CDRH1 of the generic formula $GYTX_1TX_2X_3X_4X_5X_6$ (SEQ ID NO:1052), wherein

X_1 is F or L,

X_2 is E, G or S,

X_3 is H, L or Y,

X_4 is G, S or Y,

X_5 is I or M,

X_6 is H or S.

b. a CDRH2 of the generic formula $X_1X_2X_3X_4X_5X_6GX_7TX_8X_9X_{10}QKX_{11}X_{12}$ (SEQ ID NO:1058), wherein

X_1 is S or W,

X_2 is F or I,

X_3 is D, N or S,

X_4 is A, P,

X_5 is E, N or S,

X_6 is D, N or S,

X_7 is E, G or N,

X_8 is I or N,

X_9 is C, H or Y,

X_{10} is A or T,

X_{11} is F or L,

X_{12} is D or G.

c. a CDRH3 of the generic formula $X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}DX_{12}$ (SEQ ID NO:1065), wherein

X_1 is E or S,

X_2 is D, G or no amino acid,

X_3 is D, N or no amino acid,

X_4 is G or no amino acid,

X_5 is G or no amino acid,

X_6 is W, Y or no amino acid,

X_7 is I, N or Y,

X_8 is A or Y,

X_9 is G, V or Y,

X_{10} is A, F or G,

X_{11} is F, L or M,

X_{12} is V or Y.

[000156]Group B of the heavy chain CDRs includes the following consensus collections:

a. a CDRH1 of the generic formula $GYX_1FTSYWIG$ (SEQ ID NO:1053), wherein

X_1 is R or S.

b. a CDRH2 of the generic formula $IIYPX_1DSDX_2RYSPSFQG$ (SEQ ID NO:1059), wherein

X_1 is D or G,

X_2 is A, I or T.

c. a CDRH3 of the generic formula $QX_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}YX_{12}X_{13}X_{14}DX_{15}$ (SEQ ID NO:1066), wherein

X_1 is G or no amino acid,

X_2 is K, L or Y,

X_3 is A, G or S,

X_4 is S, V or Y,

X_5 is A or G,

X_6 is G or no amino acid,

X_7 is T or no amino acid,

X_8 is S or no amino acid,

X_9 is Y or no amino acid,

X_{10} is W or Y,

X_{11} is G, S or Y,

X_{12} is F or Y,

X_{13} is G or no amino acid,

X_{14} is M or no amino acid,

X_{15} is V or Y.

[000157]Group C of the heavy chain CDRs includes the following consensus collections:

a. a CDRH1 of the generic formula $GFTFX_1SX_2X_3MH$ (SEQ ID NO:1054), wherein

X_1 is R or S,

X_2 is H or Y,

X_3 is D or G.

b. a CDRH2 of the generic formula $X_1IX_2X_3DGSX_4X_5X_6YX_7DSVX_8G$ (SEQ ID NO:1060), wherein

X_1 is F or V,

X_2 is S or W,

X_3 is D, S or Y,

X_4 is I, N or T,

X_5 is K or Q,

X_6 is N, R or Y,

X_7 is A, T or V,

X_8 is K or R.

c. a CDRH3 of the generic formula $X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}X_{14}$ (SEQ ID NO:1067), wherein

X_1 is D, G, L, S or no amino acid,

X_2 is G, H, W, Y or no amino acid,

X_3 is A, F, W, Y or no amino acid,

X_4 is D, G, Q, T or no amino acid,

X_5 is G, I, Q, S or no amino acid,

X_6 is A, D, N, Q, S or no amino acid,

X_7 is G, Y or no amino acid,

X_8 is D, Y or no amino acid,

X_9 is Y or no amino acid,

X_{10} is A, E, N or Y,

X_{11} is G, P, T, V or Y,

X_{12} is F or I,

X_{13} is D or Q,

X_{14} is C, H, V or Y.

[000158]Group D of the heavy chain CDRs includes the following consensus collections:

a. a CDRH1 of the generic formula $GFX_1FSX_2YX_3MX_4$ (SEQ ID NO:1055), wherein

X_1 is P or T,

X_2 is A, R or S,

X_3 is A or S,

X_4 is N or S.

b. a CDRH2 of the generic formula $X_1ISX_2SX_3X_4X_5X_6YYADSVKG$ (SEQ ID NO:1061), wherein

X_1 is A, H or Y,

X_2 is G, R or S,

X_3 is G or S,

X_4 is G, R or S,

X_5 is S, T or Y,

X_6 is I or T.

c. a CDRH3 of the generic formula $X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}X_{14}X_{15}X_{16}X_{17}DX_{18}$ (SEQ ID NO:1068), wherein

X_1 is E, D or no amino acid,

X_2 is G, R or no amino acid,

X_3 is I, V, Y or no amino acid,

X_4 is A, G, L or N,

X_5 is A, G, V or W,

X_6 is A, N, R or T,

X_7 is G, N, P or no amino acid,

X_8 is G, T, no amino acid,

X_9 is A or no amino acid,

X_{10} is D, E or no amino acid,

X_{11} is S, Y or no amino acid,

X_{12} is G, Y or no amino acid,

X_{13} is N, Y or no amino acid,

X_{14} is Y or no amino acid,

X_{15} is D, Y or no amino acid,

X_{16} is A, G or no amino acid,

X_{17} is F or M,

X_{18} is I, V or Y.

[000159]Group E of the heavy chain CDRs includes the following consensus collections:

a. a CDRH1 of the generic formula $GX_1SX_2SX_3X_4X_5X_6X_7WX_8$ (SEQ ID NO:1056), wherein

X_1 is D or G,

X_2 is F, I or V,

X_3 is R, S or no amino acid,

X_4 is G, Y or no amino acid,

X_5 is D, G, S or no amino acid,

X_6 is A, S or Y,

X_7 is A or Y,

X_8 is N or S.

b. a CDRH2 of the generic formula $X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}YX_{12}X_{13}SX_{14}KS$ (SEQ ID NO:1062), wherein

X_1 is E, R or Y,

X_2 is I or T,

X_3 is H, N or Y,

X_4 is C, H, S, T or Y,

X_5 is S or R,

X_6 is G or S,

X_7 is G, K, S or T,

X_8 is T or W,

X_9 is N or Y,

X_{10} is N or no amino acid,

X_{11} is D or no amino acid,

X_{12} is A or N,

X_{13} is P or V,

X_{14} is L or V.

c. a CDRH3 of the generic formula $X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}X_{14}X_{15}X_{16}X_{17}X_{18}X_{19}X_{20}X_{21}X_{22}X_{23}$ (SEQ ID NO:1069), wherein

X_1 is A, D, G, S or T,

X_2 is A, E, G, L, N, R, Y or no amino acid,

X_3 is A, G, L, N, R, T, Y or no amino acid,

X_4 is D, G, R, S, V, Y or no amino acid,

X_5 is A, G, I, S, V, Y or no amino acid,

X_6 is F, G, L, R, V or no amino acid,

X_7 is L, T, Y or no amino acid,

X_8 is Y or no amino acid,

X_9 is Y or no amino acid,

X_{10} is D or no amino acid,

X_{11} is S or no amino acid,

X_{12} is S or no amino acid,

X_{13} is G or no amino acid,

X_{14} is D, L, M, S, Y or no amino acid,

X_{15} is H, I, P, V, W or no amino acid,

X_{16} is F, G, L, R, S, Y or no amino acid,

X_{17} is D, F, V, W, Y or no amino acid,

X_{18} is C, F, L, P, S or Y,

X_{19} is D, F, G or Y,

X_{20} is A, C, G, P, R, V or Y,

X_{21} is F, L, M, S or no amino acid,

X_{22} is A, D or no amino acid,

X_{23} is I, L, V, Y or no amino acid.

[000160]Group F of the heavy chain CDRs includes the following consensus collections:

a. a CDRH1 of the generic formula GFSLSNARMGVS (SEQ ID NO:279).

b. a CDRH2 of the generic formula X_1IFSNDEKSYSTSLKS (SEQ ID NO:1063), wherein

X_1 is H or L.

c. a CDRH3 of the generic formula X_1YSSGWX_2X_3YGX_4X_5DX_6 (SEQ ID NO:1070), wherein

X_1 is M or V,

X_2 is S or no amino acid,

X_3 is F or no amino acid,

X_4 is V or no amino acid,

X_5 is F or M,

X_6 is V or Y.

[000161]Group G of the heavy chain CDRs includes the following consensus collections:

a. a CDRH1 of the generic formula GFSLX_1TGGVGVG (SEQ ID NO:1057), wherein

X_1 is S or N.

b. a CDRH2 of the generic formula LIYWNX_1X_2KRYSPSLX_3S (SEQ ID NO:1064), wherein

X_1 is D or V,

X_2 is D or E,

X_3 is K or R.

c. a CDRH3 of the generic formula R$X_1X_2X_3$PFX_4Y (SEQ ID NO:1071), wherein

X_1 is G, H, L, N or R,

X_2 is E, T or W,

X_3 is L, N, T or V,

X_4 is D or E.

[000162]In another approach, consensus sequences may be determined by keeping the CDRs contiguous within the same sequence corresponding to a U-V_L or U-V_H. Briefly, in this approach, amino acid sequences corresponding to the entire variable domains of either U-V_L or U-V_H are converted to FASTA formatting for ease in processing comparative alignments and inferring phylogenies. Next, framework regions of these sequences are replaced with an artificial linker sequence so that examination of the CDRs alone is performed without introducing any amino acid position weighting bias due to coincident events (*e.g.*, such as unrelated antibodies that serendipitously share a common germline framework heritage) whilst still keeping CDRs contiguous within the same sequence corresponding to a U-V_l or U-V_H. U-V_L or U-V_H sequences of this format are then subjected to sequence similarity alignment interrogation using a program that employs a standard ClutalW-like algorithm (*see*, Thompson *et al.*, 1994, *Nucleic Acids Res.* 22:4673-4680). This program likewise generates phylograms (phylogenic tree illustrations) based on sequence similarity alignments using either UPGMA (unweighted pair group method using arithmetic averages) or Neighbor-Joining methods (*see*, Saitou and Nei, 1987, *Molecular Biology and Evolution* 4:406-425) to construct and illustrate similarity and distinction of sequence groups *via* branch length comparison and grouping. Both methods produce similar results to determine consensus sequence collections within the individual groups.

[000163]In some cases the antigen binding protein comprises at least one CDRL1, CDRL2, or CDRL3 having one of the above consensus sequences. In some cases, the antigen binding protein comprises at least one CDRH1, CDRH2, or CDRH3 having one of the above consensus sequences. In other cases, the antigen binding protein comprises at least two CDRLs according to the above consensus sequences, and/or at least two CDRHs according to the above consensus sequences. In one aspect, the CDRLs and/or CDRHs are derived from different groups. In other cases, the antigen binding protein comprises at least two CDRLs from the same group A, B, C, D, E, or F and/or at least two CDRHs from the same group A, B, C, D, E, F, or G. In other aspects, the antigen binding protein comprises all three CDRL1, CDRL2, and CDRL3 sequences from the same of the above groups A, B, C, D, E, or F, and/or all three CDRH1, CDRH2, and CDRH3 sequence from the same of the above groups A, B, C, D, E, F, or G.

[000164]

D. Exemplary Antigen Binding Proteins

[000165] According to one aspect, an isolated antigen binding protein is provided that binds HB-EGF comprising (A) one or more light chain complementary determining regions (CDRLs) selected from the group consisting of: (i) a CDRL1 selected from the group consisting of SEQ ID NO:189-217; (ii) a CDRL2 selected from the group consisting of SEQ ID NO:218-233; (iii) a

CDRL3 selected from the group consisting of SEQ ID NO:234-274; and (iv) a CDRL of (i), (ii) and (iii) that contains one or more amino acid substitutions, deletions or insertions of no more than five, four, three, four, two or one amino acids; (B) one or more heavy chain complementary determining regions (CDRHs) selected from the group consisting of: (i) a CDRH1 selected from the group consisting of SEQ ID NO:275-299; (ii) a CDRH2 selected from the group consisting of SEQ ID NO:300-331; (iii) a CDRH3 selected from the group consisting of SEQ ID NO:332-372; and (iv) a CDRH of (i), (ii) and (iii) that contains one or more amino acid substitutions, deletions or insertions of no more than five, four, three, four, two or one amino acids; or (C) one or more light chain CDRLs of (A); and (D) one or more heavy chain CDRHs of (B).

[000166]In yet another embodiment, the isolated antigen binding protein may comprise (A) a CDRL selected from the group consisting of (i) a CDRL1 selected from the group consisting of SEQ ID NO:189-217; (ii) a CDRL2 selected from the group consisting of SEQ ID NO:218-233; and (iii) a CDRL3 selected from the group consisting of SEQ ID NO:234-274; (B) a CDRH selected from the group consisting of (i) a CDRH1 selected from the group consisting of SEQ ID NO:275-299; (ii) a CDRH2 selected from the group consisting of SEQ ID NO:300-331; and (iii) a CDRH3 selected from the group consisting of SEQ ID NO:332-372; or (C) one or more light chain CDRLs of (A); and (D) one or more heavy chain CDRLs of (B). In one embodiment, the isolated antigen binding protein may include (A) a CDRL1 of SEQ ID NO:189-217, a CDRL2 of SEQ ID NO:218-233, and a CDRL3 of SEQ ID NO:234-274, and (B) a CDRH1 of SEQ ID NO:275-299, a CDRH2 of SEQ ID NO:300-331, and a CDRH3 of SEQ ID NO:332-372.

[000167]In another embodiment, the antigen binding protein comprises a variable light chain (V_L) has at least 80%, 85%, 90% or 95% sequence identity with an amino acid sequence selected from the group consisting of SEQ ID NO:94-141, and/or the variable heavy chain (V_H) has at least 80%, 85%, 90% or 95% sequence identity with an amino acid sequence selected from the group consisting of SEQ ID NO:142-186. In a further embodiment, the V_L is selected from the group consisting of SEQ ID NO:94-141, and/or the V_H is selected from the group consisting of SEQ ID NO:142-186.

[000168]In another aspect, also provided is an isolated antigen binding protein that specifically binds to an epitope containing at least one IHGE-containing epitope and/or EGF-like epitope of HB-EGF.

[000169]In a further aspect, there is a provision of an isolated antigen binding protein that binds HB-EGF, the antigen binding protein including (A) a light chain complementary determining region (CDRL) selected from the group consisting of (i) a CDRL3 selected from the group consisting of SEQ ID NO:234-274, (ii) a CDRL3 that differs in amino acid sequence from the CDRL3 of (i) by an amino acid addition, deletion or substitution of not more than two amino acids; (iii) a CDRL3 amino acid sequence selected from the group consisting of

$X_1QX_2X_3X_4X_5PX_6X_7$ (SEQ ID NO:1046), wherein X_1 is selected from the group consisting of I and M, X_2 is selected from the group consisting of A, G and S, X_3 is selected from the group consisting of I and T, X_4 is selected from the group consisting of H and Q, X_5 is selected from the group consisting of F, L and W, X_6 is selected from the group consisting of C, I, H, L and T, X_7 is selected from the group consisting of S and T; $QQX_1X_2X_3X_4X_5IT$ (SEQ ID NO:1047), wherein X_1 is selected from the group consisting of I and S, X_2 is selected from the group consisting of F and Y, X_3 is selected from the group consisting of F, I, S and Y, X_4 is selected from the group consisting of A, S and T, X_5 is selected from the group consisting of P and S; $X_1X_2X_3X_4X_5X_6X_7X_8T$ (SEQ ID NO:1048), wherein X_1 is selected from the group consisting of L and Q, X_2 is selected from the group consisting of K, N and Q, X_3 is selected from the group consisting of A, H, S and Y, X_4 is selected from the group consisting of H, N and Y, X_5 is selected from the group consisting of N, S and T, X_6 is selected from the group consisting of A, F, I, T, V and Y, X_7 is selected from the group consisting of P and no amino acid, X_8 is selected from the group consisting of F, L and P; $QX_1X_2DX_3LPX_4X_5$ (SEQ ID NO:1049), wherein X_1 is selected from the group consisting of H and Q, X_2 is selected from the group consisting of C and Y, X_3 is selected from the group consisting of D, I, N, S and Y, X_4 is selected from the group consisting of F, I and L, X_5 is selected from the group consisting of A, S and T; $QQX_1X_2X_3X_4PX_5X_6X_7$ (SEQ ID NO:1050), wherein X_1 is selected from the group consisting of H and Y, X_2 is selected from the group consisting of G and N, X_3 is selected from the group consisting of N and S, X_4 is selected from the group consisting of S and W, X_5 is selected from the group consisting of P and no amino acid, X_6 is selected from the group consisting of R and W, X_7 is selected from the group consisting of S and T; and $X_1QYX_2X_3X_4X_5X_6X_7F$ (SEQ ID NO:1051), wherein X_1 is selected from the group consisting of H and Q, X_2 is selected from the group consisting of F and Y, X_3 is selected from the group consisting of G, I and S, X_4 is selected from the group consisting of F, I and T, X_5 is selected from the group consisting of M, P, S and T, X_6 is selected from the group consisting of F, L, R and W, X_7 is selected from the group consisting of S and T; and/or (B) a heavy chain complementary determining region (CDRH) selected from the group consisting of (i) a CDRH3 selected from the group consisting of SEQ ID NOs:332-372, (ii) a CDRH3 that differs in amino acid sequence from the CDRH3 of (i) by an amino acid addition, deletion or substitution of not more than two amino acids; and (iii) a CDRH3 amino acid sequence selected from the group consisting of $X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}DX_{12}$ (SEQ ID NO:1065), wherein X_1 is selected from the group consisting of E and S, X_2 is selected from the group consisting of D, G and no amino acid, X_3 is selected from the group consisting of D, N and no amino acid, X_4 is selected from the group consisting of G and no amino acid, X_5 is selected from the group consisting of G and no amino acid, X_6 is selected from the group consisting of W, Y and no amino acid, X_7 is selected from the group consisting of I, N and Y, X_8

is selected from the group consisting of A and Y, X_9 is selected from the group consisting of G, V and Y, X_{10} is selected from the group consisting of A, F and G, X_{11} is selected from the group consisting of F, L and M, X_{12} is selected from the group consisting of V and Y; $QX_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}YX_{12}X_{13}X_{14}DX_{15}$ (SEQ ID NO:1066), wherein X_1 is selected from the group consisting of G and no amino acid, X_2 is selected from the group consisting of K, L and Y, X_3 is selected from the group consisting of A, G and S, X_4 is selected from the group consisting of S, V and Y, X_5 is selected from the group consisting of A and G, X_6 is selected from the group consisting of G and no amino acid, X_7 is selected from the group consisting of T and no amino acid, X_8 is selected from the group consisting of S and no amino acid, X_9 is selected from the group consisting of Y and no amino acid, X_{10} is selected from the group consisting of W and Y, X_{11} is selected from the group consisting of G, S and Y, X_{12} is selected from the group consisting of F and Y, X_{13} is selected from the group consisting of G and no amino acid, X_{14} is selected from the group consisting of M and no amino acid, X_{15} is selected from the group consisting of V and Y; $X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}X_{14}$ (SEQ ID NO:1067), wherein X_1 is selected from the group consisting of D, G, L, S and no amino acid, X_2 is selected from the group consisting of G, H, W, Y and no amino acid, X_3 is selected from the group consisting of A, F, W, Y and no amino acid, X_4 is selected from the group consisting of D, G, Q, T and no amino acid, X_5 is selected from the group consisting of G, I, Q, S and no amino acid, X_6 is selected from the group consisting of A, D, X_8 is selected from the group consisting of D, Y and no amino acid, X_9 is selected from the group consisting of Y and no amino acid, X_{10} is selected from the group consisting of A, E, N and Y, X_{11} is selected from the group consisting of G, P, T, V and Y, X_{12} is se X_{14} is selected from the group consisting of C, H, V and Y; $X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}X_{14}X_{15}X_{16}X_{17}DX_{18}$ (SEQ ID NO:1068), wherein X_1 is selected from the group consisting of E, D and no amino acid, X_2 is selected from the group consisting of G, R and no amino acid, X_3 is selected from the group consisting of I, V, Y and no amino acid, X_4 is selected from the group consisting of A, G, L and N, X_5 is selected from the group consisting of A, G, V and W, X_6 is selected from the group consisting of A, N, R and T, X_7 is selected from the group consisting of G, N, P and no amino acid, X_8 is selected from the group consisting of G, T and no amino acid, X_9 is selected from the group consisting of A and no amino acid, X_{10} is selected from the group consisting of D, E and no amino acid, X_{11} is selected from the group consisting of S, Y and no amino acid, X_{12} is selected from the group consisting of G, Y and no amino acid, X_{13} is selected from the group consisting of N, Y and no amino acid, X_{14} is selected from the group consisting of Y and no amino acid, X_{15} is selected from the group consisting of D, Y and no amino acid, X_{16} is selected from the group consisting of A, G and no amino acid, X_{17} is selected from the group consisting of F and M, X_{18} is selected from the group consisting of I, V and Y; $X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}X_{14}X_{15}X_{16}X_{17}X_{18}X_{19}X_{20}X_{21}X_{22}X_{23}$ (SEQ ID NO:1069),

wherein X_1 is selected from the group consisting of A, D, G, S and T, X_2 is selected from the group consisting of A, E, G, L, N, R, Y and no amino acid, X_3 is selected from the group consisting of A, G, L, N, R, T, Y and no amino acid, X_4 is selected from the group consisting of D, G, R, S, V, Y and no amino acid, X_5 is selected from the group consisting of A, G, I, S, V, Y and no amino acid, X_6 is selected from the group consisting of F, G, L, R, V and no amino acid, X_7 is selected from the group consisting of L, T, Y and no amino acid, X_8 is selected from the group consisting of Y and no amino acid, X_9 is selected from the group consisting of Y and no amino acid, X_{10} is selected from the group consisting of D and no amino acid, X_{11} is selected from the group consisting of S and no amino acid, X_{12} is selected from the group consisting of S and no amino acid, X_{13} is selected from the group consisting of G and no amino acid, X_{14} is selected from the group consisting of D, L, M, S, Y and no amino acid, X_{15} is selected from the group consisting of H, I, P, V, W and no amino acid, X_{16} is selected from the group consisting of F, G, L, R, S, Y and no amino acid, X_{17} is selected from the group consisting of D, F, V, W, Y and no amino acid, X_{18} is selected from the group consisting of C, F, L, P, S and Y, X_{19} is selected from the group consisting of D, F, G and Y, X_{20} is selected from the group consisting of A, C, G, P, R, V and Y, X_{21} is selected from the group consisting of F, L, M, S and no amino acid, X_{22} is selected from the group consisting of A, D and no amino acid, X_{23} is selected from the group consisting of I, L, V, Y and no amino acid; $X_1YSSGWX_2X_3YGX_4X_5DX_6$ (SEQ ID NO:1070), wherein X_1 is selected from the group consisting of M and V, X_2 is selected from the group consisting of S and no amino acid, X_3 is selected from the group consisting of F and no amino acid, X_4 is selected from the group consisting of V and no amino acid, X_5 is selected from the group consisting of F and M, X_6 is selected from the group consisting of V and Y; and $RX_1X_2X_3PFX_4Y$ (SEQ ID NO:1071), wherein X_1 is selected from the group consisting of G, H, L, N and R, X_2 is selected from the group consisting of E, T and W, X_3 is selected from the group consisting of L, N, T and V, X_4 is selected from the group consisting of D and E.

[000170]In one embodiment, the isolated antigen binding protein further comprises (A) a CDRL selected from the group consisting of: (i) a CDRL1 selected from the group consisting of SEQ ID NO:189-217; (ii) a CDRL1 that differs in amino acid sequence from the CDRL1 of (i) by an amino acid addition, deletion or substitution of not more than two amino acids; (iii) a CDRL1 amino acid sequence selected from the group consisting of $X_1SSQSLX_2X_3SDGX_4TYLX_5$ (SEQ ID NO:1035), wherein X_1 is selected from the group consisting of K and R, X_2 is selected from the group consisting of L and V, X_3 is selected from the group consisting of H and Y, X_4 is selected from the group consisting of K and N, X_5 is selected from the group consisting of N, S and Y; $RASQX_1ISX_2YLN$ (SEQ ID NO:1036), wherein X_1 is selected from the group consisting of R, S and T, X_2 is selected from the group consisting of R and S; $RASQX_1IX_2X_3X_4LX_5$ (SEQ ID NO:1037), wherein X_1 is selected from the group consisting of D, G, S and T, X_2 is selected from

the group consisting of A, R and S, X_3 is selected from the group consisting of H, I, N, R, S and T, X_4 is selected from the group consisting of D, W and Y, X_5 is selected from the group consisting of A, G and N; $QASQDIX_1X_2X_3LN$ (SEQ ID NO:1038), wherein X_1 is selected from the group consisting of S and T, X_2 is selected from the group consisting of D and N, X_3 is selected from the group consisting of S and Y; $RASQX_1VX_2X_3X_4X_5LA$ (SEQ ID NO:1039), wherein X_1 is selected from the group consisting of S and T, X_2 is selected from the group consisting of I and S, X_3 is selected from the group consisting of R and S, X_4 is selected from the group consisting of S, N and no amino acid, X_5 is selected from the group consisting of Y and no amino acid; and $KSSQX_1X_2LX_3X_4SNNKNYLX_5$ (SEQ ID NO:1040), wherein X_1 is selected from the group consisting of N and S, X_2 is selected from the group consisting of I and V, X_3 is selected from the group consisting of D and Y, X_4 is selected from the group consisting of N, R and S, X_5 is selected from the group consisting of A and V; or (iv) a CDRL2 selected from the group consisting of SEQ ID NO:218-233; (v) a CDRH2 that differs in amino acid sequence from the CDRL2 of (iv) by an amino acid addition, deletion or substitution of not more than two amino acids; or (vi) a CDRl2 amino acid sequence selected from the group consisting of $X_1X_2SNX_3X_4S$ (SEQ ID NO:1041), wherein X_1 is selected from the group consisting of E and K, X_2 is selected from the group consisting of I and V, X_3 is selected from the group consisting of R and W, X_4 is selected from the group consisting of D and F; $X_1X_2SX_3LQS$ (SEQ ID NO:1042), wherein X_1 is selected from the group consisting of A and T, X_2 is selected from the group consisting of A, E and V, X_3 is selected from the group consisting of S and T; X_1ASX_2LQS (SEQ ID NO:1043), wherein X_1 is selected from the group consisting of A and V, X_2 is selected from the group consisting of S and T; $DASX_1LET$ (SEQ ID NO:1044), wherein X_1 is selected from the group consisting of I and N; GASSRAT (SEQ ID NO:223); and $WASX_1RES$ (SEQ ID NO:1045), wherein X_1 is selected from the group consisting of A and T; or B) a CDRH selected from the group consisting of: (i) a CDRH1 selected from the group consisting of SEQ ID NO:275-299; (ii) a CDRH1 that differs in amino acid sequence from the CDRH1 of (i) by an amino acid addition, deletion or substitution of not more than two amino acids; (iii) a CDRH1 amino acid sequence selected from the group consisting of $GYTX_1TX_2X_3X_4X_5X_6$ (SEQ ID NO:1052), wherein X_1 is selected from the group consisting of F and L, X_2 is selected from the group consisting of E, G and S, X_3 is selected from the group consisting of H, L and Y, X_4 is selected from the group consisting of G, S and Y, X_5 is selected from the group consisting of I and M, X_6 is selected from the group consisting of H and S; $GYX_1FTSYWIG$ (SEQ ID NO:1053), wherein X_1 is selected from the group consisting of R and S; $GFTFX_1SX_2X_3MH$ (SEQ ID NO:1054), wherein X_1 is selected from the group consisting of R and S, X_2 is selected from the group consisting of H and Y, X_3 is selected from the group consisting of D and G; $GFX_1FSX_2YX_3MX_4$ (SEQ ID NO:1055), wherein X_1 is selected from the group consisting of P and T, X_2 is selected from the group

consisting of A, R and S, X_3 is selected from the group consisting of A and S, X_4 is selected from the group consisting of N and S; $GX_1SX_2SX_3X_4X_5X_6X_7WX_8$ (SEQ ID NO:1056), wherein X_1 is selected from the group consisting of D and G, X_2 is selected from the group consisting of F, I and V, X_3 is selected from the group consisting of R, S and no amino acid, X_4 is selected from the group consisting of G, Y and no amino acid, X_5 is selected from the group consisting of D, G, S and no amino acid, X_6 is selected from the group consisting of A, S and Y, X_7 is selected from the group consisting of A and Y, X_8 is selected from the group consisting of N and S; GFSLSNARMGVS (SEQ ID NO:279); and $GFSLX_1TGGVGVG$ (SEQ ID NO:1057), wherein X_1 is selected from the group consisting of S and N; (iv) a CDRH2 selected from the group consisting of SEQ ID NO:300-331; (v) a CDRH2 that differs in amino acid sequence from the CDRH2 of (iv) by an amino acid addition, deletion or substitution of not more than two amino acids; or (vi) a CDRH2 amino acid sequence selected from the group consisting of $X_1X_2X_3X_4X_5X_6GX_7TX_8X_9X_{10}QKX_{11}X_{12}$ (SEQ ID NO:1058), wherein X_1 is selected from the group consisting of S and W, X_2 is selected from the group consisting of F and I, X_3 is selected from the group consisting of D, N and S, X_4 is selected from the group consisting of A and P, X_5 is selected from the group consisting of E, N and S, X_6 is selected from the group consisting of D, N and S, X_7 is selected from the group consisting of E, G and N, X_8 is selected from the group consisting of I and N, X_9 is selected from the group consisting of C, H and Y, X_{10} is selected from the group consisting of A and T, X_{11} is selected from the group consisting of F and L, X_{12} is selected from the group consisting of D and G; $IIYPX_1DSDX_2RYSPSFQG$ (SEQ ID NO:1059), wherein X_1 is selected from the group consisting of D and G, X_2 is selected from the group consisting of A, I and T; $X_1IX_2X_3DGSX_4X_5X_6YX_7DSVX_8G$ (SEQ ID NO:1060), wherein X_1 is selected from the group consisting of F and V, X_2 is selected from the group consisting of S and W, X_3 is selected from the group consisting of D, S and Y, X_4 is selected from the group consisting of I, N and T, X_5 is selected from the group consisting of K and Q, X_6 is selected from the group consisting of N, R and Y, X_7 is selected from the group consisting of A, T and V, X_8 is selected from the group consisting of K and R; $X_1ISX_2SX_3X_4X_5X_6YYADSVKG$ (SEQ ID NO:1061), wherein X_1 is selected from the group consisting of A, H and Y, X_2 is selected from the group consisting of G, R and S, X_3 is selected from the group consisting of G and S, X_4 is selected from the group consisting of G, R and S, X_5 is selected from the group consisting of S, T and Y, X_6 is selected from the group consisting of I and T; $X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}YX_{12}X_{13}SX_{14}KS$ (SEQ ID NO:1062), wherein X_1 is selected from the group consisting of E, R and Y, X_2 is selected from the group consisting of I and T, X_3 is selected from the group consisting of H, N and Y, X_4 is selected from the group consisting of C, H, S, T and Y, X_5 is selected from the group consisting of S and R, X_6 is selected from the group consisting of G and S, X_7 is selected from the group consisting of G, K, S and T, X_8 is selected

from the group consisting of T and W, X_9 is selected from the group consisting of N and Y, X_{10} is selected from the group consisting of N and no amino acid, X_{11} is selected from the group consisting of D and no amino acid, X_{12} is selected from the group consisting of A and N, X_{13} is selected from the group consisting of P and V, X_{14} is selected from the group consisting of L and V; X_1IFSNDEKSYSTSLKS (SEQ ID NO:1063), wherein X_1 is selected from the group consisting of H and LI; and LIYWNX_1X_2KRYSPSLX_3S (SEQ ID NO:1064), wherein X_1 is selected from the group consisting of D and V, X_2 is selected from the group consisting of D and E, X_3 is selected from the group consisting of K and R.

[000171]In some embodiments, at least two of, or all three of CDRL1, CDRL2, and CDRL3 sequences are derived from the same group A, B, C, D, E, or F of consensus sequences, and/or at least two, or all three of, CDRH1, CDRH2, and CDRH3 sequences are derived from the same group A, B, C, D, E, F, or G. In other cases CDRs from different consensus sequence groups are mixed and matched.

[000172]In yet another embodiment, the isolated antigen binding protein described hereinabove comprises the first amino acid sequence and the second amino acid sequence, both sequences of which are covalently bonded to each other. In a further embodiment, the first amino acid sequence of the isolated antigen binding protein includes the CDRL3 of SEQ ID NO:234-274, CDRL2 of SEQ ID NO:218-233, and CDRL1 of SEQ ID NO:189-217. On the other hand, the second amino acid sequence of the isolated antigen binding protein comprises the CDRH3 of SEQ ID NO:332-372, CDRH2 of SEQ ID NO:300-331, and CDRH1 of SEQ ID NO:275-299.

[000173]In one aspect, the isolated antigen binding proteins provided herein can be a monoclonal antibody, a polyclonal antibody, a recombinant antibody, a human antibody, a humanized antibody, a chimeric antibody, a multispecific antibody, or an antibody fragment thereof.

[000174]In another embodiment, the antibody fragment of the isolated antigen binding proteins provided herein can be a Fab fragment, a Fab' fragment, an $F(ab')_2$ fragment, an Fv fragment, a diabody, or a single chain antibody molecule.

[000175]In a further embodiment, the isolated antigen binding protein provided herein is a human antibody and can be of the IgG1-, IgG2- IgG3- or IgG4-type.

[000176]In yet another aspect, the isolated antigen binding protein provided herein can be coupled to a labeling group, such as radioisotope, radionuclide, a fluorescent group, an enzymatic group, a chemiluminescent group, a biotinyl group, or a predetermined polypeptide group, or an effector group, such as a radioisotope, a radionuclide, a toxin, a therapeutic group, or a chemotherapeutic group. Examples of a therapeutic or chemotherapeutic group are calicheamicin, auristatin-PE, geldanamycin, maytanasine, or derivatives thereof.

[000177]In yet other aspects, the invention includes antigen binding proteins competing with any of the above described antigen binding proteins.

[000178]As will be appreciated by those in the art, for any antigen binding protein with more than one CDR from the depicted sequences, any combination of CDRs independently selected from the depicted sequences is useful. Thus, antigen binding proteins with one, two, three, four, five or six of independently selected CDRs can be generated. However, as will be appreciated by those in the art, specific embodiments generally utilize combinations of CDRs that are non-repetitive, *e.g.*, antigen binding proteins are generally not made with two CDRH2 regions, etc.

[000179]Some of the antigen binding proteins provided are discussed in more detail below.

1. Antigen Binding Proteins And Binding Epitopes

[000180]When an antigen binding protein is said to bind an epitope within specified residues of a polypeptide, such as HB-EGF, for example, what is meant is that the antigen binding protein specifically binds to a polypeptide consisting of the specified residues (*e.g.*, a specified segment of HB-EGF). Such an antigen binding protein typically does not contact every residue within HB-EGF. Nor does every single amino acid substitution or deletion within HB-EGF, or the extracellular domain of HB-EGF, necessarily significantly affect binding affinity. Epitope specificity of an antigen binding protein can be determined in variety of ways. One approach, for example, involves testing a collection of overlapping peptides of about 15 amino acids spanning the sequence of the antigen and differing in increments of a small number of amino acids (*e.g.*, three amino acids). The peptides are immobilized within the wells of a microtiter dish. Immobilization can be effected by biotinylating one terminus of the peptides. Optionally, different samples of the same peptide can be biotinylated at the amino- and the carboxy-terminus and immobilized in separate wells for purposes of comparison. This is useful for identifying end-specific antigen binding proteins. Optionally, additional peptides can be included by terminating at a particular amino acid of interest. This approach is useful for identifying end-specific antigen binding proteins to internal fragments of HB-EGF. An antigen binding protein or immunologically functional fragment is screened for specific binding to each of the various peptides. The epitope is defined as occurring with a segment of amino acids that is common to all peptides to which the antigen binding protein shows specific binding. Details regarding a specific approach for defining an epitope are set forth in Example 23.

[000181]As demonstrated in Example 23, the antigen binding proteins provided herein are capable of binding at least one IHGE-containing epitope and/or an EGF-like domain of HB-EGF.

2. Competing Antigen Binding Proteins

[000182]In another aspect, antigen binding proteins are provided that compete with one of the exemplified antibodies or functional fragments binding to the epitope described above for specific binding to HB-EGF. Such antigen binding proteins may also bind to the same epitope as one of the herein exemplified antigen binding proteins, or an overlapping epitope. Antigen binding proteins and fragments that compete with or bind to the same epitope as the exemplified antigen binding proteins are expected to show similar functional properties. The exemplified antigen binding proteins and fragments include those described above, including those with the heavy and light chains, variable region domains and CDRs included in FIGURES 1, 2, 3, 4, 6, and 7.

3. Human Antibodies and Humanization of Antibodies

[000183]In one embodiment, the HB-EGF antigen binding proteins are human or humanized antibodies. Human antibodies avoid many of the problems associated with antibodies that possess murine or rat variable and/or constant regions. The presence of such murine or rat derived proteins can lead to the rapid clearance of the antibodies or can lead to the generation of an immune response against the antibody by a patient. In order to avoid the utilization of murine or rat derived antibodies, fully human antibodies have been generated through the introduction of functional human antibody genetic loci into a rodent, other mammal or animal so that the rodent, other mammal or animal produces fully human antibodies.

[000184]One method for generating fully human antibodies is through the use of XenoMouse® strains of mice that have been engineered to contain up to but less than 1000 kb-sized germline configured fragments of the human heavy chain locus and kappa light chain locus. *See,* Mendez *et al.*, 1997, *Nature Genetics* 15:146-156, and Green and Jakobovits, 1998, *J. Exp. Med.* 188:483-495. The XenoMouse® strains are available from Abgenix, Inc. (Fremont, CA).

[000185]The production of the XenoMouse® strains of mice is discussed and delineated in U.S. Patent Application Serial Nos. 07/466,008, filed January 12, 1990, 07/610,515, filed November 8, 1990, 07/919,297, filed July 24, 1992, 07/922,649, filed July 30, 1992, 08/031,801, filed March 15, 1993, 08/112,848, filed August 27, 1993, 08/234,145, filed April 28, 1994, 08/376,279, filed January 20, 1995, 08/430, 938, filed April 27, 1995, 08/464,584, filed June 5, 1995, 08/464,582, filed June 5, 1995, 08/463,191, filed June 5, 1995, 08/462,837, filed June 5, 1995, 08/486,853, filed June 5, 1995, 08/486,857, filed June 5, 1995, 08/486,859, filed June 5, 1995, 08/462,513, filed June 5, 1995, 08/724,752, filed October 2, 1996, 08/759,620, filed December 3, 1996, U.S. Publication 2003/0093820, filed November 30, 2001 and U.S. Patent Nos. 6,162,963, 6,150,584, 6,114,598, 6,075,181, and 5,939,598 and Japanese Patent Nos. 3 068 180 B2, 3 068 506 B2, and 3 068 507 B2. *See, also* European Patent No., EP 0 463 151 B1, grant

published June 12, 1996, International Patent Application No., WO 94/02602, published February 3, 1994, International Patent Application No., WO 96/34096, published October 31, 1996, WO 98/24893, published June 11, 1998, WO 00/76310, published December 21, 2000. The disclosures of each of the above-cited patents, applications, and references are hereby incorporated by reference in their entirety.

[000186]In an alternative approach, others, including GenPharm International, Inc., have utilized a "minilocus" approach. In the minilocus approach, an exogenous Ig locus is mimicked through the inclusion of pieces (individual genes) from the Ig locus. Thus, one or more V_H genes, one or more D_H genes, one or more J_H genes, a mu constant region, and usually a second constant region (preferably a gamma constant region) are formed into a construct for insertion into an animal. This approach is described in U.S. Patent No. 5,545,807 to Surani *et al.* and U.S. Patent Nos. 5,545,806, 5,625,825, 5,625,126, 5,633,425, 5,661,016, 5,770,429, 5,789,650, 5,814,318, 5,877,397, 5,874,299, and 6,255,458 each to Lonberg and Kay, U.S. Patent No. 5,591,669 and 6,023.010 to Krimpenfort and Berns, U.S. Patent Nos. 5,612,205, 5,721,367, and 5,789,215 to Berns *et al.*, and U.S. Patent No. 5,643,763 to Choi and Dunn, and GenPharm International U.S. Patent Application Serial Nos. 07/574,748, filed August 29, 1990, 07/575,962, filed August 31, 1990, 07/810,279, filed December 17, 1991, 07/853,408, filed March 18, 1992, 07/904,068, filed June 23, 1992, 07/990,860, filed December 16, 1992, 08/053,131, filed April 26, 1993, 08/096,762, filed July 22, 1993, 08/155,301; filed November 18, 1993, 08/161,739, filed December 3, 1993, 08/165,699, filed December 10, 1993, 08/209,741, filed March 9, 1994, the disclosures of which are hereby incorporated by reference. *See, also* European Patent No. 0 546 073 B1, International Patent Application Nos. WO 92/03918, WO 92/22645, WO 92/22647, WO 92/22670, WO 93/12227, WO 94/00569, WO 94/25585, WO 96/14436, WO 97/13852, and WO 98/24884 and U.S. Patent No. 5,981,175, the disclosures of which are hereby incorporated by reference in their entirety.

[000187]Kirin has also demonstrated the generation of human antibodies from mice in which, through microcell fusion, large pieces of chromosomes, or entire chromosomes, have been introduced. *See,* European Patent Application Nos. 773 288 and 843 961, the disclosures of which are hereby incorporated by reference. Additionally, KM™ mice, which are the result of cross-breeding of Kirin's Tc mice with Medarex's minilocus (Humab) mice have been generated. These mice possess the human IgH transchromosome of the Kirin mice and the kappa chain transgene of the Genpharm mice (Ishida *et al.*, 2002, *Cloning Stem Cells* 4:91-102).

[000188]Human antibodies can also be derived by *in vitro* methods. Suitable examples include but are not limited to phage display (CAT, Morphosys, Dyax, Biosite/Medarex, Xoma, Symphogen, Alexion (formerly Proliferon), Affimed) ribosome display (CAT), yeast display, and the like.

E. Preparation of Antibodies

[000189]Antibodies, as described herein, were prepared through the utilization of the XenoMouse® technology, as described herein. Such mice are capable of producing human immunoglobulin molecules and antibodies and are substantially deficient in the production of murine immunoglobulin molecules and antibodies. Technologies utilized for achieving production of human antibodies are disclosed in the patents, applications, and references disclosed herein. In some embodiments, transgenic production of mice and human antibodies is performed as disclosed in U.S. Patent Application Serial No. 08/759,620, filed December 3, 1996 and International Patent Application Nos. WO 98/24893, published June 11, 1998 and WO 00/76310, published December 21, 2000, the disclosures of which are hereby incorporated by reference. *See, also* Mendez *et al.*, 1997, *Nature Genetics* 15:146-156, the disclosure of which is hereby incorporated by reference.

[000190]Through the use of such technology, fully human monoclonal antibodies to a variety of antigens have been produced. Essentially, XenoMouse® lines of mice are immunized with an antigen of interest (*e.g.*,HB-EGF), lymphatic cells (such as B-cells) are recovered from the hyper-immunized mice, and the recovered lymphocytes are fused with a myeloid-type cell line to prepare immortal hybridoma cell lines. These hybridoma cell lines are screened and selected to identify hybridoma cell lines that produced antibodies specific to the antigen of interest. The supernatants might also be screened for immunoreactivity against fragments of HB-EGF to further map the different antibodies for binding to domains of functional interest on HB-EGF. The antibodies may also be screened for binding to other ligand of EGFR or its family members, other related human chemokines and against the rat, the mouse, and non-human primate, such as cynomolgus monkey, orthologues of HB-EGF, the last to determine species cross-reactivity. Provided herein are methods for the production of multiple hybridoma cell lines that produce antibodies specific to HB-EGF. Further, provided herein are characterization of the antibodies produced by such cell lines, including nucleotide and amino acid sequence analyses of the heavy and light chains of such antibodies.

[000191]Alternatively, instead of being fused to myeloma cells to generate hybridomas, B cells can be directly assayed. For example, B cells can be isolated from hyperimmune XenoMouse® mice and allowed to proliferate and differentiate into antibody-secreting plasma cells. Antibodies from the cell supernatants are then screened by ELISA for reactivity against the HB-EGF immunogen. The supernatants might also be screened for immunoreactivity against fragments of HB-EGF to further map the different antibodies for binding to domains of functional interest on HB-EGF. The antibodies may also be screened for binding to other ligands of EGF receptor, or its family members, other related human chemokines and against the rat, the mouse, and non-

human primate, such as cynomolgus monkey, orthologues of HB-EGF, the last to determine species cross-reactivity. B cells from wells containing antibodies of interest may be immortalized by various methods including fusion to make hybridomas either from individual or from pooled wells, or by infection with EBV or transfection by known immortalizing genes and then plating in suitable medium. Alternatively, single plasma cells secreting antibodies with the desired specificities are then isolated using an HB-EGF-specific hemolytic plaque assay (Babcook *et al.*, 1996, *Proc. Natl. Acad. Sci. USA* 93:7843-48). Cells targeted for lysis are preferably sheep red blood cells (SRBCs) coated with the HB-EGF antigen.

[000192]As discussed, *supra*, there are a number of isotypes of antibodies including without limitation the following: human IgG1, IgG2, IgG3 and IgG4. It will be appreciated that antibodies that are generated need not initially possess such an isotype but, rather the antibody as generated can possess any isotype and that the antibody can be isotype-switched by using the molecularly cloned V region genes or cloned constant region genes or cDNAs in appropriate expression vectors using conventional molecular biological techniques that are well known in the art and then expressing the antibodies in host cells using techniques known in the art

[000193]In general, antibodies produced by the fused hybridomas were either human IgG2 heavy chains or human IgG4 heavy chains with fully human kappa chains. Antibodies can also be of other human isotypes, including IgG1 or IgG3. The antibodies possessed high affinities, typically possessing a K_D of from about 10^{-6} through about 10^{-12} M or below, when measured by solid phase and solution phase techniques. Antibodies possessing a K_D of at least 10^{-9} M are preferred to inhibit the activity of HB-EGF. Antibodies possessing a K_D of at least 10^{-10} M are also preferred to inhibit the activity of HB-EGF. Antibodies possessing a K_D of at least 10^{-11} M are also preferred to inhibit the activity of HB-EGF.

[000194]As will be appreciated, anti-HB-EGF antibodies can be expressed in cell lines other than hybridoma cell lines. Sequences encoding particular antibodies can be used to transfect a suitable mammalian host cell. During construction of appropriate vectors for transfection and subsequent expression of antibody, the antibody may be class-switched from one isotype to another, *e.g.*, IgG4 antibodies may be class-switched to IgG2, by techniques known in the art. Transfection can be by any known method for introducing polynucleotides into a host cell, including, for example packaging the polynucleotide in a virus (or into a viral vector) and transducing a host cell with the virus (or vector) or by transfection procedures known in the art, as exemplified by U.S. Patent Nos. 4,399,216, 4,912,040, 4,740,461, and 4,959,455 (which patents are hereby incorporated herein by reference). The transformation procedure used depends upon the host to be transformed. Methods for introducing heterologous polynucleotides into mammalian cells are well known in the art and include dextran-mediated transfection, calcium phosphate precipitation, polybrene mediated transfection, protoplast fusion,

electroporation, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the DNA into nuclei.

[000195]Mammalian cell lines available as hosts for expression are well known in the art and include many immortalized cell lines available from the American Type Culture Collection (ATCC), including but not limited to Chinese hamster ovary (CHO) cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells (*e.g.*, Hep G2), human epithelial kidney 293 cells, and a number of other cell lines. Cell lines of particular preference are selected through determining which cell lines have high expression levels and produce high amounts of anti-HB-EGF antibodies.

[000196]Alternatively, these antibodies may be prepared from animals genetically engineered to make fully human antibodies or from an antibody display library made in bacteriophage, yeast, ribosome or *E. coli*. *See, e.g.,*Clackson *et al.*,1991, *Nature* 352:624-628, Marks *et al.*,1991, *J. Mol. Biol.* 222: 581-597, Feldhaus and Siegel, 2004, *J. Immunol. Methods.* 290:69-80, Groves and Osbourn, 2005, *Expert Opin Biol Ther.* 5:125-135 and Jostock and Dubel, 2005, *Comb Chem High Throughput Screen.* 8:127-133.

[000197]Another aspect relates to an isolated nucleic acid molecule encoding an HB-EGF antigen binding protein such as an antibody. Within the context herein, the term "isolated nucleic acid molecule", as used herein, means a polynucleotide of genomic, cDNA, or synthetic origin or some combination thereof, which by virtue of its origin, the "isolated nucleic acid molecule" (1) is not associated with all or a portion of a polynucleotide in which the "isolated polynucleotide" is found in nature, (2) is operably linked to a polynucleotide which it is not linked to in nature, or (3) does not occur in nature as part of a larger sequence. Further, the term "nucleic acid molecule", as referred to herein, means a polymeric form of nucleotides of at least 10 bases in length, either ribonucleotides or deoxynucleotides or a modified form of either type of nucleotide, such as nucleotides with modified or substituted sugar groups and the like. The term also includes single and double stranded forms of DNA. Exemplatory nucleic acids encoding antigen binding proteins or portions thereof are described in more detail, *infra*.

[000198]In a one embodiment, a nucleic acid molecule is operably linked to a control sequence. The term "control sequence", as used herein, refers to polynucleotide sequences that are necessary to effect the expression and processing of coding sequences to which they are ligated. The nature of such control sequences differs depending upon the host organism. In prokaryotes, such control sequences generally include promoters, ribosomal binding sites, and transcription termination sequences. In eukaryotes, generally, such control sequences include promoters and transcription termination sequences. The term "control sequence" is intended to include, at a minimum, all components whose presence is essential for expression and processing, and can also include additional components whose presence is advantageous, for

example, leader sequences and fusion partner sequences. Furthermore, the term "operably linked", as used herein, refers to positions of components so described which are in a relationship permitting them to function in their intended manner. Moreover, as provided herein, an expression control sequence operably linked to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under conditions compatible with the expression control sequence.

[000199]A further aspect is a vector comprising a nucleic acid molecule that encodes an HB-EGF antigen binding protein provided herein. The nucleic acid molecule can be operably linked to a control sequence. Furthermore, the vector may additionally contain a replication origin or a selection marker gene. Examples of vectors that may be used are *e.g.*,plasmids, cosmids, phages, viruses, etc.

F. Antigen Binding Proteins Based On Basic Antibody Structure

[000200]As discussed, *supra*, the basic antibody structural unit is known to comprise a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kDa) and one "heavy" chain (about 50-70 kDa). The amino-terminal portion of each chain includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The carboxy-terminal portion of each chain defines a constant region responsible for dimerization effector function, circulating half-life and other functions. Human light chains are classified as kappa and lambda light chains. Heavy chains are classified as mu, delta, gamma, alpha, or epsilon, and define the antibody's isotype as IgM, IgD, IgG, IgA, and IgE, respectively. Within light and heavy chains, the variable and constant regions are joined by a "J" region of about 12 or more amino acids, with the heavy chain also including a "D" region of about 10 more amino acids. *See,* generally, Fundamental Immunology Ch. 7 (Paul, W., ed., 2nd ed. Raven Press, N.Y. (1989)) (incorporated by reference in its entirety for all purposes). The variable regions of each light/heavy chain pair form the antigen binding site of the antibody.

[000201]Thus, an intact IgG antibody has two binding sites. Except in bifunctional or bispecific antibodies, the two binding sites are the same.

[000202]The variable regions of the chains all exhibit the same general structure of relatively conserved framework regions (FR) joined by three hyper variable regions, also called complementarity determining regions or CDRs. The CDRs from the two variable regions of each pair are aligned by the framework regions, enabling binding to a specific epitope on the antigen. From N-terminal to C-terminal, the variable regions of both light and heavy chains comprise the domains FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. The assignment of amino acids to each domain is in accordance with the definitions of Kabat Sequences of Proteins of

Immunological Interest (National Institutes of Health, Bethesda, Md. (1987 and 1991)), or Chothia & Lesk, 1897, *J. Mol. Biol.* 196:901-917; Chothia *et al.*, 1989, *Nature* 342:878-883.

[000203]Thus, the antibodies provided herein may include at least one variable region polypeptide chain of the formula: FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4, wherein FR1 is a first human framework region, CDR1 is a first complementarity determining region, FR2 is a second human framework region, CDR2 is a second complementarity determining region, FR3 is a third human framework region, CDR3 is a third complementarity determining region, and FR4 is a fourth human framework region. The CDR3 is generally the most diverse region of the antibody variable region.

[000204]In some embodiments, the FR1 region includes but is not limited to any one of amino acid sequences SEQ ID NOs:373-393 and/or 453-469; the CDR1 region includes but is not limited to any one of amino acid sequences SEQ ID NOs:189-217 and/or 275-299; the FR2 region includes but is not limited to any one of amino acid sequences SEQ ID NOs:394-414 and/or 470-481; the CDR2 region includes but is not limited to any one of amino acid sequences SEQ ID NOs:218-233 and/or 300-331; the FR3 region includes but is not limited to any one of amino acid sequences SEQ ID NOs:415-440 and/or 482-511; the CDR3 region includes but is not limited to any one of amino acid sequences SEQ ID NOs:234-274 and/or 332-372; and the FR4 region includes but is not limited to any one of amino acid sequences SEQ ID NOs:441-452 and/or 512-517. Thus, in some embodiments, the human antibodies provided herein have one or more of the amino acid sequences provided herein.

[000205]It is to be understood, that the amino acid sequence of the antibodies provided herein is not limited to the twenty conventional amino acids (*See, Immunology - A Synthesis* (2nd Edition, E.S. Golub and D.R. Gren, Eds., Sinauer Associates, Sunderland, Mass. (1991)), which is incorporated herein by reference). For example, the amino acids may include stereoisomers (*e.g.*,D-amino acids) of the twenty conventional amino acids, unnatural amino acids such as α-,α-disubstituted amino acids, N-alkyl amino acids, lactic acid, and other unconventional amino acids. Examples of unconventional amino acids, which may also be suitable components for the antibody provided, include: 4-hydroxyproline, γ-carboxyglutamate, ε-N,N,N-trimethyllysine, ε-N-acetyllysine, O-phosphoserine, N-acetylserine, N-formylmethionine, 3-methylhistidine, 5-hydroxylysine, σ-N-methylarginine, and other similar amino acids and imino acids, *e.g.*,4-hydroxyproline.

[000206]Furthermore, minor variations in the amino acid sequences shown in SEQ ID NOs:1-517 and 1035-1071 are contemplated as being encompassed, providing that the variations in the amino acid sequence maintain at least 75%, more preferably at least 80%, 90%, 95%, and most preferably 99% of the sequences shown in SEQ ID NOs:1-517 and 1035-1071. Preferred variations in the amino acid sequences shown in SEQ ID Nos:1-517 and 1035-1071, *i.e.*,

deletions, insertions and/or replacements of at least one amino acid, occur near boundaries of functional domains. Structural and functional domains can be identified by comparison of the nucleotide and/or amino acid sequence data to public or proprietary sequence databases. Computerized comparison methods can be used to identify sequence motifs or predicted protein conformation domains that occur in other antibodies of known structure and/or function. Methods to identify protein sequences that fold into a known three-dimensional structure are known. *See, e.g.,* Bowie *et al.*,1991, *Science* 253:164; *Proteins, Structures and Molecular Principles* (Creighton, Ed., W. H. Freeman and Company, New York (1984)); *Introduction to Protein Structure* (C. Branden and J. Tooze, eds., Garland Publishing, New York, N.Y. (1991)); and Thornton *et al.*, 1991, *Nature* 354:105, which are all incorporated herein by reference. Thus, those of skill in the art can recognize sequence motifs and structural conformations that may be used to define structural and functional domains.

[000207]Especially preferred variations in the amino acid sequences shown in SEQ ID NOs:1-517 and 1035-1071 are those that lead to a reduced susceptibility to proteolysis or oxidation, alter glycosylation patterns or alter binding affinities or confer or modify other physicochemical or functional properties of the antibody. In particular, conservative amino acid replacements are contemplated. Conservative replacements are those that take place within a family of amino acids that are related in their side chains. Preferred amino acid families are the following: acidic family = aspartate, glutamate; basic family = lysine, arginine, histidine; non-polar family = alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan; and uncharged polar family = glycine, asparagine, glutamine, cysteine, serine, threonine, tyrosine. More preferred families are: aliphatic-hydroxy family = serine and threonine; amide-containing family = asparagine and glutamine; aliphatic family = alanine, valine, leucine and isoleucine; and aromatic family = phenylalanine, tryptophan, and tyrosine. For example, it is reasonable to expect that an isolated replacement of a leucine with an isoleucine or valine, an aspartate with a glutamate, a threonine with a serine, or a similar replacement of an amino acid with a structurally related amino acid will not have a major effect on the binding or properties of the resulting antibody, especially if the replacement does not involve an amino acid within a framework site. However, all other possible amino acid replacements are also encompassed. Whether an amino acid change results in a functional antibody, *i.e.,* in a antibody that binds to HB-EGF and reduces, neutralizes or substantially inhibits the function of HB-EGF, can readily be determined by assaying the specific activity of the resulting antibody in ELISA or FACS for binding to HB-EGF or *in vitro* or *in vivo* functional assay.

[000208]A reduction, neutralization or substantially inhibition of HB-EGF mediated signal transduction may be caused by influencing, *e.g.,* decreasing or inhibiting, the binding of HB-EGF to its receptor, *e.g.,* to the EGFR or HER4.

[000209]The term "antibody" or "anti-HB-EGF antibody", as used herein, means a monoclonal antibody, a polyclonal antibody, a recombinant antibody, a humanized antibody (Jones *et al.*, 1986, *Nature* 321:522-525; Riechmann *et al.*, 1988, *Nature* 332:323-329; and Presta, 1992, *Curr. Op. Struct. Biol.* 2: 593-596), a chimeric antibody (Morrison *et al.*, 1984, *Proc. Natl. Acad. Sci. U.S.A.* 81: 6851-6855), a multispecific antibody (*e.g.*,a bispecific antibody) formed from at least two antibodies, or an antibody fragment thereof. The term "antibody fragment" comprises any portion of the afore-mentioned antibodies, preferably at least one of their antigen binding or variable regions. Examples of antibody fragments include Fab fragments, Fab' fragments, $F(ab')_2$ fragments, Fv fragments, diabodies (Hollinger *et al.*, 1993, *Proc. Natl. Acad. Sci. U.S.A.* 90: 6444-6448), single chain antibody molecules (Plückthun in: The Pharmacology of Monoclonal Antibodies 113, Rosenburg and Moore, EDS, Springer Verlag, N.Y. (1994), 269-315) and other fragments as long as they exhibit the desired capability of binding to HB-EGF.

[000210]In addition, the term "antibody" or "anti-HB-EGF antibody", as used herein, may include antibody-like molecules that contain engineered sub-domains of antibodies or naturally occurring antibody variants. These antibody-like molecules may be single-domain antibodies such as VH-only or VL-only domains derived either from natural sources such as camelids (Muyldermans *et al.*, 2001, *Reviews in Molecular Biotechnology* 74, 277-302) or through *in vitro* display of libraries from humans, camelids or other species (Holt *et al.*, 2003,*Trends Biotechnol.* 21:484-90).

[000211]A "Fv fragment" is the minimum antibody fragment that contains a complete antigen-recognition and -binding site. This region consists of a dimer of one heavy- and one light-chain variable domain in tight, non-covalent association. It is in this configuration that the three CDR´s of each variable domain interact to define an antigen-binding site on the surface of the V_H-V_L dimer. Collectively, the six CDR´s confer antigen binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDR´s specific for an antigen) has the ability to recognize and bind the antigen, although usually at a lower affinity than the entire binding site. The "Fab fragment" also contains the constant domain of the light chain and the first constant domain (C_H1) of the heavy chain. The "Fab fragment" differs from the "Fab' fragment" by the addition of a few residues at the carboxy terminus of the heavy chain C_H1 domain including one or more cysteines from the antibody hinge region. The "$F(ab')_2$ fragment" originally is produced as a pair of "Fab' fragments" which have hinge cysteines between them. Methods of preparing such antibody fragments, such as papain or pepsin digestion, are known to those skilled in the art.

[000212]An antibody provided herein may fix complement (CDC) or activate antibody-dependent cellular cytotoxicity (ADCC), especially an IgG1 antibody, IgG1 variant class-switched from IgG2 or IgG4 or another isotype of human or mammalian origin, by molecular biology or generated de

novo from human IgG1 producing mice. Other methods may also be used. An antibody provided herein may sometimes be coupled to a labelling group or an effector group, *e.g.*,a toxin, chemotherapeutic agent, reporter molecule or imaging agent.

G. Further types of HB-EGF Binding Proteins

[000213]In another aspect, the HB-EGF antigen binding protein as provided herein is a scaffold protein having an antibody like binding activity, *i.e.,* binds to HB-EGF.

[000214]Within the context of the present invention, the term "scaffold protein", as used herein, means a polypeptidic framework with a high tolerance of its fold for modifications such as multiple insertions, deletions or substitutions. This intrinsic conformational stability enables the directed randomization and drastic changes within a defined region of the protein. Thus, it acquires certain novel properties, whereas its overall structural integrity and original physicochemical behaviour remains conserved. This *de novo* adopted property mostly, but not exclusively, comprises the binding specificity for a pre-defined target molecule.

[000215]Currently, a broad variety of scaffold proteins are in use that imitate the binding principle of a conventional antibody to different degrees (Hey et al., 2005, *Trends in Biotechnol.* 23:514-22). Examples of scaffold proteins that can be used in accordance with the present invention can be subdivided into different groups. Antibody related scaffolds, which are defined as derivatives of antibodies that are either naturally smaller in size and simpler in structure, or have been engineered in this way. This group includes for example so called Nanobodies (reviewed in Hey et al., Trends in Biotechnol. 2005; 23 (10); 514-522), domain antibodies (Holt et al., 2003, *Trends in Biotechnol.* 21:484-489) or shark antigen reactive proteins (Holt et al.,Trends in Biotechnol. 2003; 21; 484-489). A second group of scaffold proteins are rigid protein folds which tolerate the insertion or randomization of single loop peptides like the Kunitz-type domain (Dennis *et al.*, 1995; *J. Biol. Cell.* 270:25411-25417), huma transferrin (Ali *et al.*, 1999; *J. Biol. Cell.* 274:24066-24074) or cystein-knot structural motives (Christmann *et al.*, 1999, *Protein Eng.* 12:797-806). Proteins that share the principle of multiple hypervariable loops on a rigid conserved framework in analogy to antibodies are represented by human CTLA-4 (Hufton *et al.*, 2000 *FEBS Lett.* 475:225-231), human fibronectin type III domains (Koide *et al.*, 1998; *J. Mol. Biol.* 284:1141-1151), C-type lectin like domains or lipocalins (Skerra, 2001, *J. Biotechnol.* 74:257-275). Scaffolds with the binding specificity accomplished through aminoacid residues which are positioned partially or completely within the rigid secondary structure of the protein are for example ankyrin repeat proteins (Binz, 2003, *J. Mol. Biol.* 332:489-503), the Z domain of proteinA (Nord *et al.*, 1995, *Protein Eng.* 8:601-608) or γ-crystalline (Fiedler and Rudolph, 2001, International Patent Application WO 01/04144). Scaffold proteins and peptides and applications

thereof are reviewed in Hey *et al.*, 2005, *Trends in Biotechnol.* 23:514-522; Binz *et al.*, 2005, *Nature Biotechnol.* 23:1257-1268) and Holliger *et al.*, 2005, *Nature Biotechnol.* 23:11261136.

[000216]Engineering of a scaffold protein can be regarded as grafting or integrating an affinity function onto or into the structural framework of a polypeptidic framework with a high tolerance of its fold for modifications. Affinity function means a protein binding affinity according to the present invention. A scaffold can be structurally separable from the amino acid sequences conferring binding specificity. In general, proteins appearing suitable for the development of such artificial affinity reagents may be obtained by rational, or most commonly, combinatorial protein engineering techniques such as panning against an antigen, *e.g* , HB-EGF, either purified protein or protein displayed on the cell surface, for binding agents in an artificial scaffold library displayed in vitro, using skills which are known in the art (Skerra, 2000, *J. Mol. Recog.* Jul-Aug;13(4):167-87; Binz and Plückthun, 2005, Aug;16(4):459-69). In addition, a scaffold protein having an antibody like binding activity can be derived from an acceptor polypeptide, e.g one of the foregoing proteins, containing the scaffold domain, which can be grafted with binding domains of a donor polypeptide to confer the binding specificity of the donor polypeptide onto the scaffold domain containing the acceptor polypeptide. Said inserted binding domains may be, for example, one or more of the complementarity determining region (CDR) of an antibody, in particular an HB-EGF antibody. Preferably the CDR is a CDR3. Insertion can be accomplished by various methods known to those skilled in the art including, for example, polypeptide synthesis, nucleic acid synthesis of an encoding amino acid as well by various forms of recombinant methods well known to those skilled in the art.

H. HB-EGF Antigen Binding Protein Conjugates

[000217]In another embodiment, an HB-EGF antigen binding protein, *e.g.*,an antibody provided herein is coupled to a labelling group. Such a labelled antigen binding protein is particularly suitable for diagnostic applications. As used herein, the term "labelling group" refers to a detectable marker, *e.g.*,a radiolabelled amino acid or biotinyl moiety that can be detected by marked avidin (*e.g.*,streptavidin bound to a fluorescent marker or enzymatic activity that can be detected by optical or colorimetric methods). Various methods for labelling polypeptides and glycoproteins, such as antibodies, are known in the art and may be used. Examples of suitable labelling groups include, but are not limited to, the following: radioisotopes or radionuclides (*e.g.*, ^{3}H, ^{14}C, ^{15}N, ^{35}S, ^{90}Y, ^{99}Tc, ^{111}In, ^{125}I, ^{131}I), fluorescent groups (*e.g.*,FITC, rhodamine, lanthanide phosphors), enzymatic groups (*e.g.*, horseradish peroxidase, β-galactosidase, luciferase, alkaline phosphatase), chemiluminescent groups, biotinyl groups, or predetermined polypeptide epitopes recognized by a secondary reporter (*e.g.*,leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags). In certain

respects, it may be desirable that the labelling groups are attached by spacer arms of various lengths to reduce potential steric hindrance.

[000218]Alternatively, an HB-EGF antigen binding protein provided herein, such as an antibody, may be coupled to an effector group. Such an effector-modified antigen binding protein is especially suitable for therapeutic applications. As used herein, the term "effector group" refers to a cytotoxic group such as a radioisotope or radionuclide, a toxin, a therapeutic group or other effector group known in the art. Examples for suitable effector groups are radioisotopes or radionuclides (*e.g.*, ^{3}H, ^{14}C, ^{15}N, ^{35}S, ^{90}Y, ^{99}Tc, ^{111}In, ^{125}I, ^{131}I), calicheamicin, dolastatin analogs such as auristatins, and chemotherapeutic agents such as geldanamycin and maytansine derivates, including DM1. In certain respects, it may be desirable that the effector groups are attached by spacer arms of various lengths to reduce potential steric hindrance.

[000219]Also as described herein, many of the highly useful HB-EGF antigen binding protein, *e.g.*,antibody preparations provided herein recognize epitopes within the EGF-like domain of HB-EGF, which includes residues 106-149 of the protein, for example, with the following sequence:

[000220]PCLRKYKDFCIHGECKYVKELRAPSCICHPGY HGERCHGLSLP (SEQ ID NO:1076).

In some embodiments, the epidermal pgrowth factor epitope recognized by the antigen binding proteins provided herein includes amino acid sequence IHGE. Accordingly, antibody preparations are provided that bind to and recognize an IHGE-containing epitope and/or an EGF-like domain of HB-EGF, for example, SEQ ID NO:1076.

[000221]Anti-HB-EGF antigen binding proteins are useful in the detection of HB-EGF in patient samples and accordingly are useful as diagnostics for disease states as described herein. In addition, based on their ability to significantly inhibit HB-EGF and/or EGF receptor activity (as demonstrated in the Examples below), HB-EGF antigen binding proteins have therapeutic effects in treating symptoms and conditions resulting from HB-EGF expression and/or HB-EGF activity. In specific embodiments, the antigen binding proteins and methods herein relate to the treatment of symptoms resulting from HB-EGF-associated diseases, HER4-associated diseases or EGF receptor-associated disease states, for example, cancerous conditions. Further embodiments involve using the antigen binding proteins and methods described herein to treat undesired angiogenesis, neoplastic diseases, such as, melanoma, non-small cell lung cancer, glioma, hepatocellular (liver) carcinoma, thyroid tumor, gastric (stomach) cancer, prostrate cancer, breast cancer, ovarian cancer, bladder cancer, lung cancer, glioblastoma, endometrial cancer, kidney cancer, colon cancer, and pancreatic cancer.

[000222]

I. Nucleic Acids Encoding HB-EGF Antigen Binding Proteins

[000223]Nucleic acids that encode for the antigen binding proteins described herein, or portions thereof, are also provided, including nucleic acids encoding one or both chains of an antibody, or

a fragment, derivative, mutein, or variant thereof, polynucleotides encoding heavy chain variable regions or only CDRs, polynucleotides sufficient for use as hybridization probes, PCR primers or sequencing primers for identifying, analyzing, mutating or amplifying a polynucleotide encoding a polypeptide, anti-sense nucleic acids for inhibiting expression of a polynucleotide, and complementary sequences of the foregoing. The nucleic acids can be any length. They can be, for example, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 75, 100, 125, 150, 175, 200, 250, 300, 350, 400, 450, 500, 750, 1,000, 1,500, 3,000, 5,000 or more nucleotides in length, and/or can comprise one or more additional sequences, for example, regulatory sequences and/or be part of a larger nucleic acid, for example, a vector. The nucleic acids can be single-stranded or double-stranded and can comprise RNA and/or DNA nucleotides, and artificial variants thereof (*e.g.*, peptide nucleic acids). FIGURES 15A - 15V depict the nucleotide sequences for the various light chains of the antigen binding proteins. FIGURES 16A – 16AC deptice the nucleotide sequences for the various heavy chains of the antigen binding proteins. FIGURES 13A - 13M depict the nucleotide sequences of various light chain variable regions of the antigen binding proteins. FIGURES 14A – 14L depict the nucleotide sequences of various heavy chain variable regions of the antigen binding proteins. FIGURES 18A - 18F depict the nucleotide sequences for various CDR regions of the light chain variable regions of the antigen binding proteins. FIGURES 19A-19G depict the nucleotide sequences for various CDR regions of the heavy chain variable regions of the antigen binding proteins. FIGURES 20A – 20K depict the nucleotide sequences for various FR regions of the light chain variable regions of the antigen binding proteins. FIGURES 21A – 21K depict the nucleotide sequences for various FR regions of the heavy chain, variable regions of the antigen binding proteins. FIGURE 17A depicts the nucleotide sequence of the light chain constant region of the antigen binding proteins. Finally, FIGURE 17B depicts the nucleotide sequence of the heavy chain constant region of the antigen binding proteins.

[000224]Nucleic acids encoding certain antigen binding proteins, or portions thereof (*e.g.*, full length antibody, heavy or light chain variable domain or CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, or CDRL3) may be isolated from B-cells of mice that have been immunized with HB-EGF or an immunogenic fragment thereof. The nucleic acid may be isolated by conventional procedures such as polymerase chain reaction (PCR). Phage display is another example of a known technique whereby derivatives of antibodies and other antigen binding proteins may be prepared. In one approach, polypeptides that are components of an antigen binding protein of interest are expressed in any suitable recombinant expression system, and the expressed polypeptides are allowed to assemble to form antigen binding protein molecules.

[000225]An aspect further provides nucleic acids that hybridize to other nucleic acids (*e.g.*, nucleic acids comprising a nucleotide sequence depicted in FIGURES 13 through 21) under

particular hybridization conditions. Methods for hybridizing nucleic acids are well-known in the art. *See, e.g.*, Current Protocols in Molecular Biology, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. As defined herein, a moderately stringent hybridization condition uses a prewashing solution containing 5x sodium chloride/sodium citrate (SSC), 0.5% SDS, 1.0 mM EDTA (pH 8.0), hybridization buffer of about 50% formamide, 6x SSC, and a hybridization temperature of 55°C (or other similar hybridization solutions, such as one containing about 50% formamide, with a hybridization temperature of 42°C), and washing conditions of 60°C, in 0.5x SSC, 0.1% SDS. A stringent hybridization condition hybridizes in 6x SSC at 45°C, followed by one or more washes in 0.1 x SSC, 0.2% SDS at 68°C. Furthermore, one of skill in the art can manipulate the hybridization and/or washing conditions to increase or decrease the stringency of hybridization such that nucleic acids comprising nucleotide sequences that are at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or 99% identical to each other typically remain hybridized to each other.

[000226]The basic parameters affecting the choice of hybridization conditions and guidance for devising suitable conditions are set forth by, for example, Sambrook, Fritsch, and Maniatis (2001, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., *supra* and Current Protocols in Molecular Biology, 1995, Ausubel *et al.*, eds., John Wiley & Sons, Inc., sections 2.10 and 6.3-6.4), and can be readily determined by those having ordinary skill in the art based on, *e.g.*, the length and/or base composition of the nucleic acid.

[000227]Changes can be introduced by mutation into a nucleic acid, thereby leading to changes in the amino acid sequence of a polypeptide (*e.g.*, an antibody or antibody derivative) that it encodes. Mutations can be introduced using any technique known in the art. In one embodiment, one or more particular amino acid residues are changed using, for example, a site-directed mutagenesis protocol. In another embodiment, one or more randomly selected residues is changed using, for example, a random mutagenesis protocol. However it is made, a mutant polypeptide can be expressed and screened for a desired property.

[000228]Mutations can be introduced into a nucleic acid without significantly altering the biological activity of a polypeptide that it encodes. For example, one can make nucleotide substitutions leading to amino acid substitutions at non-essential amino acid residues. Alternatively, one or more mutations can be introduced into a nucleic acid that selectively changes the biological activity of a polypeptide that it encodes. For example, the mutation can quantitatively or qualitatively change the biological activity. Examples of quantitative changes include increasing, reducing or eliminating the activity. Examples of qualitative changes include changing the antigen specificity of an antibody.

[000229]Another aspect provides nucleic acid molecules that are suitable for use as primers or hybridization probes for the detection of nucleic acid sequences. A nucleic acid molecule can

comprise only a portion of a nucleic acid sequence encoding a full-length polypeptide, for example, a fragment that can be used as a probe or primer or a fragment encoding an active portion (*e.g.*, a HB-EGF binding portion) of a polypeptide.

[000230]Probes based on the sequence of a nucleic acid can be used to detect the nucleic acid or similar nucleic acids, for example, transcripts encoding a polypeptide. The probe can comprise a label group, *e.g.*, a radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor. Such probes can be used to identify a cell that expresses the polypeptide.

[000231]Another aspect provides vectors comprising a nucleic acid encoding a polypeptide or a portion thereof (*e.g.*, a fragment containing one or more CDRs or one or more variable region domains). Examples of vectors include, but are not limited to, plasmids, viral vectors, non-episomal mammalian vectors and expression vectors, for example, recombinant expression vectors. The recombinant expression vectors can comprise a nucleic acid in a form suitable for expression of the nucleic acid in a host cell. The recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, which is operably linked to the nucleic acid sequence to be expressed. Regulatory sequences include those that direct constitutive expression of a nucleotide sequence in many types of host cells (*e.g.*, SV40 early gene enhancer. Rous sarcoma virus promoter and cytomegalovirus promoter), those that direct expression of the nucleotide sequence only in certain host cells (*e.g.*, tissue-specific regulatory sequences, *see*, Voss *et al.*, 1986 *Trends Biochem. Sci.* 11:287, Maniatis *et al.*, 1987, *Science* 236:1237, incorporated by reference herein in their entireties), and those that direct inducible expression of a nucleotide sequence in response to particular treatment or condition (*e.g.*, the metallothionin promoter in mammalian cells and the tet-responsive and/or streptomycin responsive promoter in both prokaryotic and eukaryotic systems (*see*, *id.*). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc. The expression vectors can be introduced into host cells to thereby produce proteins or peptides, including fusion proteins or peptides, encoded by nucleic acids as described herein.

[000232]Another aspect provides host cells into which a recombinant expression vector has been introduced. A host cell can be any prokaryotic cell (for example, *E. coli*) or eukaryotic cell (for example, yeast, insect, or mammalian cells (*e.g.*, CHO cells)). Vector DNA can be introduced into prokaryotic or eukaryotic cells *via* conventional transformation or transfection techniques. For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (*e.g.*, for resistance to antibiotics) is generally introduced into the

host cells along with the gene of interest. Preferred selectable markers include those which confer resistance to drugs, such as G418, hygromycin and methotrexate. Cells stably transfected with the introduced nucleic acid can be identified by drug selection (*e.g.*, cells that have incorporated the selectable marker gene will survive, while the other cells die), among other methods.

J. Use of HB-EGF Antigen Binding Proteins for Diagnostic and Therapeutic Purposes

1. Indications

[000233]The HB-EGF antigen binding proteins as described herein can be used to detect or treat a number of diseases and disorders, including those involving excessive cellular proliferation, undesirable cellular migration and/or aberrant angiogenesis. For example, these HB-EGF antigen binding proteins can inhibit HB-EGF-induced EGFR and/or HER4 tyrosine phosphorylation in cancerous cells (FIGURES 22-29). Such inhibition can interrupt the cascade of the signaling events that drives cell proliferation and migration, and angiogenesis. Further, the HB-EGF antigen binding proteins can interfere with the transactivation of the EGFR. In addition, these antigen binding proteins inhibit basal HUVEC cell proliferation (FIGURE 32B), endothelial cell tube formation (FIGURE 33) and HB-EGF-induced vessel formation in a matrigel plug assay *in vivo* (FIGURE 36). These results indicate that the HB-EGF antigen binding proteins as described herein inhibit angiogenesis *in vitro* and *in vivo.* Moreover, these antigen binding proteins also inhibit anchorage independent cell growth (FIGURE 34 and FIGURE 35) and xenograft tumor growth in mice (FIGURE 37 and FIGURE 38). Significantly, the HB-EGF antigen binding proteins also inhibit migration of MCF-7 and MDA-MB231 cancer cells (*see,* FIGURES 27 and 27). Thus, the HB-EGF antigen binding proteins as described herein attack several steps in the development of tumors and other cancerous conditions, including signaling events that control cell proliferation, angiogenesis and cell migration associated with the spread and development of metastatic cancer. Such multi-faceted intervention is highly beneficial for controlling and inhibiting the process by which cancer develops. Furthermore, cancers at any stage of progression (*e.g.*, primary, metastatic and recurrent cancers) can be treated.

[000234]In addition to the HB-EGF antigen binding proteins' use in detecting and treating cancers in various stages of progression, these antigen binding proteins may also be useful for detecting a number of different types of cancer. For example, HB-EGF is expressed at very low levels in normal breast and pancreatic tissues. However, HB-EGF is expressed at high levels in about 55% pancreatic cancer cells and about 70% of breast cancer cells. Furthermore, as described in the Examples, HB-EGF expression was detected in various cancer cell lines,

indicating that the HB-EGF antigen binding proteins described herein can be used for detection of a variety of cancer types.

[000235]For example, cancers that can be detected or treated by the claimed antigen binding proteins include solid mammalian tumors as well as hematological malignancies. Solid mammalian tumors include cancers in children such as, for example, germ cell tumors, soft tissue sarcomas, primary brain tumors, neuroblastoma, nephroblastoma and carcinoma, in particular squamous carcinoma and epithelial carcinoma. Solid mammalian tumors may also include adult cancers such as, for example, tumors of unknown origin, primary brain cancer in adults, tumors of the pituitary gland, lip, oral cavity, Nasopharynx, larynx, maxillary sinus, Ethmoid sinus, salivary glands, thyroid gland (including para thyroid glands and carcinoid), esophagus, stomach, pancreas, small intestine, colon, rectum, anal canal, liver, gallbladder, extra hepatic bile ducts, ampulla of vater, carcinoid, endocrine tumors of gastro-entero-hepatic system, pheochromocytoma and paraganglioma, adrenal glands, lung, pleura, mediastinum, thymus, tumors of bone and soft tissue, skin tumors of lip, eyelid, external ear, other unspecified parts of the face, scalp and neck, trunk, upper limpb and shoulder, lower limb and hip, vulva, penis, scrotum, breast tumors, gynecological tumors of vulva, vagina, cervix uteri, corpus uteri, ovary, fallopian tube, gestational and trophoblastic tumors, penis, prostate, testis, kidney, renal pelvis and ureter, urinary bladder, urethra, ophthalmic tumors of eyelid, conjunctiva, uvea, retina, orbit and lacrimal gland. Hematological malignancies include childhood, for example, leukemia and lymphomas, acute and chronic leukemia (AML, ANLL, ALL, CML, MDS), Hodgkin's disease, B-Cell, T-Cell, large cell, follicular, indolent/low grade, aggressive/high grade lymphomas of lymphocytic and cutaneous origin, plasma cell neoplasm and cancers associated with AIDS.

[000236]In addition, the HB-EGF antigen binding proteins described herein may also be used to detect or treat cancerous conditions or neoplasia disorders, which include, for example, adenoma, tubulovillous adenoma, villous adenoma, angiofibroma, atypical proliferating mucinous neoplasias, Brenner tumor, carcinoid, cavernous hemangioma, cellular leiomyoma, chorangioma, congenital mesoblastic nephroma, mucinous cystadenoma, serous cystadenoma, dermoid, desmoid, fibroadenoma, fibroma, fibrothecoma, follicular adenoma, ganglioneuroma, giant cell tumor, granular cell tumor, granulosa cell tumor, hemangioma, intraductal papilloma, islet cell tumor, leiomyoma, lipoma, luteoma, meningioma, mole, myelolipoma, myxoma, neurofibroma, nevus, osteochondroma, pheochromocytoma, polyposis, schwannoma, serous cystadenoma, struma ovarii, synovial chrondromatosis, benign thymoma.

[000237]Further examples of the types of cancers that can be detected or treated with the HB-EGF proteins as described herein may be found, for example, from the American Cancer Society (www.cancer.org), or from Wilson *et al.* (1991) Harrison's Principles of Internal Medicine, 12th Edition, McGraw-Hill, Inc.

[000238]Therefore, the HB-EGF antigen binding proteins as described herein can be used to treat and/or prevent cancer, cancerous conditions, tumor growth, metastasis of cancer cells, angiogenic processes and/or neoplastic disorders. Thus, these antigen binding proteins provide a method of treating or preventing cancer in a subject that involves administering to the subject an effective amount of a composition comprising one of the human and/or monoclonal HB-EGF antigen binding protein preparations as described herein, or a combination thereof.

[000239]A high proportion of solid tumor diseases are often characterized by tumor angiogenesis, the excessive growth of (abnormal) vessels in the tumor tissue mediated by growth factors (*i.e.,* VEGF) and other factors (*i.e.,* HB-EGF). Targeting HB-EGF through a HB-EGF-specific antigen binding protein could prevent the formation of new vessels and therefore limit the expansion of existing tumors and the development of new tumors (*i.e.,* metastases).

[000240]Besides its role as a mitogenic and pro-invasive ligand several studies have substantiated the picture of HB-EGF as an important regulator of angiogenic processes in cancer. As illustrated in the Examples, a function of HB-EGF in the regulation of angiogenesis *in vivo* was shown. Thus, the antigen binding proteins as described herein can be used for treating diseases associated with or caused by angiogenesis, *e.g.,* cancerous or non-cancerous diseases.

[000241]For example HB-EGF antigen binding proteins as described herein may interfere with the communication between smooth muscle cells (SMCs) and endothelial cells, a fundamental process in the development and functionality of blood vessels in angiogenesis, *e.g.,* tumor angiogenesis.

[000242]Furthermore, these antigen binding proteins may at least partially inhibit the HB-EGF induced expression and release of VEGF from SMCs which acts subsequently as a powerful endothelial mitogen. Similarly, VEGF increases HB-EGF production in endothelial cells which therefore constitutes a pro-angiogenic feedback loop consisting of these two important ligands that can be disrupted by administering the HB-EGF antigen binding proteins as described herein. Recently, and in addition to VEGF, further critical pro-angiogenic constituents such as angiopoetin 1 and 2 (Ang-1 & 2) and their receptor TIE-2 as well as the potent smooth muscle cell GPCR stimulus angiotensin II (ATII) were identified. Interestingly, HB-EGF is a critical mediator of ATII-induced EGFR transactivation and downstream upregulation of VEGF and Ang-2 in endothelial cells. *In vivo*, ATII induces angiogenesis in an HB-EGF-dependent manner and enhanced the angiogenic activity of VEGF. These findings support that in parallel to VEGF, HB-EGF is able to activate additional angiogenic pathways *via* Ang2. Therefore, aberrant angiogenesis or cancer in a mammal may be treated with the administration of an effective amount of the HB-EGF antigen binding protein described herein.

[000243]Besides its role as an important regulator of angiogenic processes in cancer, various non-cancer indications with activated angiogenic pathways and the requirement of collateral blood flow are putative disease areas for HB-EGF antigen binding protein therapy.

[000244]Chronic inflammatory diseases (*e.g.*,nephritis, COPD, inflammatory bowel disease) including immune system mediated "inflammatory reactions" (*e.g.*,graft versus host disease, transplant rejection, restenosis), metabolic diseases (*e.g.*,diabetes), or chronic hypoxic conditions are often characterized by hyper- and/or neo-vascularization (*e.g.*,chronic ulcers). The described HB-EGF antigen binding proteins may at least partially inhibit the essential process for angiogenesis - the recruitment of vascular smooth muscle cells by endothelial cells induced by HB-EGF either produced by inflammatory cells or upregulated by hypoxia - and represent therefore an attractive route for interventional therapy of excessive/pathological vascularization.

[000245]In obese subjects, increased levels of HB-EGF derived from the accumulated fat which contribute to the higher incidence of vascular disease in obesity, can be neutralized by the HB-EGF antigen binding proteins described herein. A therapeutic intervention with an HB-EGF antigen binding protein may act therefore directly as an anti-adipocytokine in interrupting the adipovascular axis.

[000246]In fibroblasts, HB-EGF significantly downregulates elastin mRNA *via* activation of epidermal growth factor receptor. This effect provides an avenue of intervention in the development of lung fibrosis.

[000247]Moreover HB-EGF is a mitogen of keratinocytes involved in the pathogenesis of inflammatory diseases. In addition, expression of HB-EGF and co-localization with the EGFR may play an important role in the early pathogenesis of psoriasis which opens a potential therapeutic window in the treatment of cutaneous inflammatory diseases and specifically in the early treatment of psoriasis with the claimed antigen binding proteins.

[000248]Targeting HB-EGF with the described antigen binding proteins may also result in a treatment option for patients with proliferative vitreoretinopathy (PVR), since the development of PVR is accompanied by a significant upregulation of HB-EGF in PVR retinas. In addition HB-EGF expression in fibroproliferative tissue and its stimulatory effect on glial cell proliferation, chemotaxis, and VEGF secretion suggest that HB-EGF may be a factor mediating glial cell responses during PVR. These principles can also be applied to further angiogenesis-dependent eye diseases such as age-related and non age-related macular degeneration, diabetic retinopathy, rubeotic glaucoma, interstitial keratitis, retinopathy of prematurity and corneal graft failure.

[000249]GPCRs such as adrenoceptors and angiotensin receptors have been linked to the pathogenesis of hypertension due to their vasoconstrictive and growth promoting abilities. Since

HB-EGF is a critical mediator of these pathways, neutralizing antigen binding proteins are suitable for targeted intervention in hypertensive disorders such as cardiac hypertrophy and congestive heart failure, kidney failure or stroke.

[000250]HB-EGF, a potent mitogen and chemoattractant for smooth muscle cells (SMCs), was detected in atherosclerotic plaques of coronary arteries with intimal thickening and produced by SMCs and macrophages. Remnant lipoproteins which are known causative agents of atherosclerosis were furthermore shown to induce SMC proliferation *via* HB-EGF mediated EGFR transactivation. Therefore, the HB-EGF antigen binding proteins as described herein represent selective and efficient agents targeting atherosclerosis by blocking critical smooth muscle cell functions. In addition restenosis after percutaneous coronary intervention which is characterized by proliferation of smooth muscle cells might also be prevented by specific neutralization of HB-EGF function.

[000251]Thus, the HB-EGF antigen binding proteins as described herein can be used to treat a variety of diseases, including non-malignant proliferative diseases such as aberrant angiogenesis, leiomyoma (uterine fibrosis), benign smooth muscle cell tumors, glomerulosclerosis (hyperproliferation of mesangial cells), smooth muscle cell hyperplasia, atherosclerosis (hyperproliferation of vascular smooth muscle cells), rubeosis; neovascular glaucoma, diabetic retinopathy, diabetic blindness, macular degeneration, rheumatoid arthritis, cardiac hypertrophy, psoriasis, and the like.

[000252]In a further aspect, these HB-EGF antigen binding proteins can be used to treat disorders associated with or accompanied by a disturbed, *e.g.*,pathologically enhanced growth factor receptor activation.

[000253]In another aspect, this enhanced growth factor receptor activation may be associated with or caused by a pathological increase in the activity of a G protein and/or a G protein coupled receptor. It should be noted that disorders that are associated with or accompanied by a disturbed, *e.g.*,pathologically enhanced growth factor receptor activation and which are may be associated with or caused by a pathological increase in the activity of a G protein and/or a G protein coupled receptor, can be delimited from other disorders characterized by an enhanced activity of growth factor receptor activation in that a transactivation of the growth factor receptor *via* G protein coupled receptor takes place.

2. Diagnostic Methods

[000254]The HB-EGF antigen binding molecules as described herein may be used in a method for detecting cancer cells in a test sample that includes contacting the test sample with the antigen binding molecule and detecting whether the antibody binds to a cell expressing proHB-EGF or HB-EGF molecules in the sample. The degree to which binding occurs can be assessed

by use of a control sample. The test and control samples can, for example, be blood, serum, ascites, pleural effusion, cerebro-spinal fluid, tissue, cell, urine, lymph, saliva, milk or other samples. Such a control sample can be a non-cancerous sample of the fluid or tissue or cell type. In some embodiments, the control sample is a non-cancerous sample obtained from the same subject as the test sample. "Subject" or "patient" refers to a mammal, preferably a human, in need of treatment for or detection of a condition, disorder or disease.

[000255]The binding of antibody to components of the test sample can be detected by detecting a reporting molecule, imaging agent or label that is bound or can be selectively bound to a antigen binding protein as described herein. These HB-EGF antigen binding proteins can have one or more reporter molecules, labels or imaging agents. 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. The reporting molecule can provide a detectable signal. For example, the detectable signal can be a fluorescent, phosphorescent, chemiluminescent, electrochemiluminescent, electrochemical, color change or enzymatic signal. Anti-HB-EGF antibodies provided herein can be used as capturing antibodies for ELISA-based detection of the growth factor or immunohistochemical analysis of tissue samples as shown in FIGURE 39.

[000256]In some embodiments, the reporting molecule is covalently bound to a antigen binding protein as described herein. In other embodiments, the reporting molecule can be selectively bound to the HB-EGF antigen binding protein as described herein. Such selective binding of a reporting molecule to the described antigen binding protein can be accomplished by a secondary antibody with the label covalently bound thereto, where the secondary antibody selectively binds to an HB-EGB antigen binding protein as described herein.

3. Methods Of Treatment: Pharmaceutical Formulations, Routes Of Administration

[000257]Treatment of, or treating, cancer is intended to include the alleviation of or diminishment of at least one symptom typically associated with the disease. The treatment also includes alleviation or diminishment of more than one of the associated symptoms. The treatment may cure the cancer, *e.g.*, it may substantially eliminate the cancer cells and/or arrest the growth of the cancerous tumor. Alternatively, treatment may slow the progression of the cancer.

[000258]Anti-cancer activity can be evaluated against varieties of cancers or cancer cells using methods available to one of skill in the art. Anti-cancer activity, for example, can be determined by identifying the LD_{100} or ED_{50} of a preparation of the antigen binding proteins described herein

that prevents the growth of a cancer. In one embodiment, anti-cancer activity is the amount of antibody that kills 50% or 100% of the cancer cells when measured using standard dose response methods.

[000259]The HB-EGF antigen binding proteins as described herein can be administered alone, or in combination with antibodies, chemotherapeutic drugs or radiation therapy. Pharmaceutical compositions according to the invention may be administered as monotherapy or in combination with another pharmaceutical composition, preferably comprising another anti-neoplastic agent, in particular Cisplantin or Avastin. For example, the described HB-EGF antigen binding proteins can be co-administered with anti-tumor antibodies, (*e.g.*,chimeric, humanized or human anti-tumor antibodies), with antibodies that specifically bind to VEGF to further inhibit tumor angiogenesis, or with antibodies that specifically bind to a receptor tyrosine kinase such as HER2, HER4 or EGFR and therefore further inhibit tumor cell proliferation. In FIGURE 38, the synergistic effect of an anti-EGFR antibody and antiHB-EGF antibodies could be shown for both therapeutic antiHB-EGF antibodies tested. Further, the HB-EGF antigen binding proteins described herein may be co-administered with other anti-tumor agents. Specific examples of anti-tumor agents which can be co-administered with the antibodies provided herein include, for example, gefitinib, lapatinib, sunitinib, pemetrexed, bevacisumab, cetuximab, imatinib, trastuzumab, alemtuzumab, rituximab, erlotinib, bortezomib and the like. Any other anti-cancer agent or drug that can inhibit cancer or tumor cell proliferation can also be used in the compositions as described and claimed herein. For example, the claimed compositions can include chemotherapeutic agents such as capecitabine, daunorubicin, daunomycin, dactinomycin, doxorubicin, epirubicin, idarubicin, esorubicin, bleomycin, mafosfamide, ifosfamide, cytosine arabinoside, bis-chloroethylnitrosurea, busulfan, mitomycin C, actinomycin D, mithramycin, prednisone, hydroxyprogesterone, testosterone, tamoxifen, dacarbazine, procarbazine, hexamethylmelamine, pentamethylmelamine, mitoxantrone, amsacrine, chlorambucil, methylcyclohexylnitrosurea, nitrogen mustards, melphalan, cyclophosphamide, 6-mercaptopurine, 6-thioguanine, cytarabine (CA), 5-azacytidine, hydroxyurea, deoxycoformycin, 4-hydroxyperoxycyclophosphor-amide, 5-fluorouracil (5-FU), 5-fluorodeoxyuridine (5-FUdR), methotrexate (MTX), colchicine, taxol, vincristine, vinblastine, etoposide, trimetrexate, teniposide, cisplatin, avastin and diethylstilbestrol (DES). *See*, generally, The Merck Manual of Diagnosis and Therapy, 15th Ed. 1987, pp. 1206-1228, Berkow *et al.*, eds., Rahway, N.J. When used with the described HB-EGF antigen binding proteins, such chemotherapeutic agents may be used individually (*e.g.*, 5-FU and an antibody), sequentially (*e.g.*, 5-FU and an antibody for a period of time followed by MTX and an antibody), or in combination with one or more other such chemotherapeutic agents (*e.g.*, 5-FU, MTX and an antibody, or 5-FU, radiotherapy and an antibody).

[000260]Also provided is a method of evaluating a therapeutically effective dosage for treating a cancer with an antibody having an amino acid sequence disclosed herein, that includes determining the LD_{100} or ED_{50} of the HB-EGF antigen binding protein preparation *in vivo* or *in vitro*. Such a method permits calculation of the approximate amount of antigen binding protein needed per volume to inhibit cancer cell growth or metastasis by about 10% to 100%, or about 20% to 100%, or about 25% to 100%, or about 30% to 100%, or about 40% to 100%, or about 50% to 100%. In some embodiments, less than 100% inhibition of cancer cell growth or metastasis is observed. For example, cancer cell growth and/or metastasis is inhibited by about 90%, 80%, 70%, 60%, or 50%. Percentage inhibition can be determined, for example, by administration of the antigen binding protein preparation to SCID or nu/nu mice available in the art wherein tumor cells have been introduced and/or by standard methods using cultured cancer cells (*see,* FIGURE 38B). Several methods are described in the Examples.

[000261]Also included are sterile pharmaceutical formulations of the HB-EGF antigen binding proteins as described herein that are useful as treatments for diseases including, for example, cancer and/or aberrant angiogenesis. Such formulations would inhibit the binding of HB-EGF to its receptor, *e.g.,*EGFR or to HER4, thereby effectively treating pathological conditions where, for example, serum, cellular or tissue HB-EGF is abnormally elevated or where its receptors, *e.g.,*EGFR or HER4, are abnormally active. As illustrated herein the HB-EGF antigen binding proteins possess adequate affinity to potently neutralize HB-EGF and to modulate the signaling events associated with the HB-EGF receptors.

[000262]The HB-EGF antigen binding proteins as described herein are preferably humanized antigen binding proteins. Administration of these humanized antigen binding proteins reduce the probability of a negative side effect. Moreover, these antigen binding proteins are stable *in vivo*, for example, because they are recognized as normal human products, thereby minimizing the risk of immune system responses. Moreover, these antigen binding proteins are not prone to proteolytic destruction, improving their circulating half-lives. Hence, the HB-EGF preparations as described herein have an excellent half-life *in vivo* so that administration in humans is comparatively infrequent. Such a prolonged duration of action may allow for less frequent and more convenient dosing schedules by alternate parenteral routes such as subcutaneous or intramuscular injection.

[000263]Sterile formulations can be created, for example, by filtration through sterile filtration membranes, prior to or following lyophilization and reconstitution of the antibody. The antigen binding proteins as described herein are ordinarily stored in lyophilized form or in solution. Therapeutic antibody compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having an adapter that allows retrieval of the formulation, such as a stopper pierceable by a hypodermic injection needle.

[000264]The route of administration of the HB-EGF antigen binding proteins are in accord with known methods, *e.g.*, injection or infusion by intravenous, subcutaneous, intradermal, intraperitoneal, intracerebral, intramuscular, intraocular, intraarterial, intrathecal, intravesical, intra-cavernous, inhalation, intralesional routes, or by sustained release systems as noted below. In some embodiments, the antigen binding proteins as described herein are administered continuously by infusion or by bolus injection.

[000265]The HB-EGF antigen binding proteins, as described herein, can be prepared in a mixture with a pharmaceutically acceptable carrier. This therapeutic composition can be administered intravenously or through the nose or lung, preferably as a liquid or powder aerosol (lyophilized). The composition may also be administered parenterally or subcutaneously as desired. When administered systemically, the therapeutic composition should be sterile, pyrogen-free and in a parenterally acceptable solution with consideration for what are physiologically acceptable pH values, isotonicity, and stability. These conditions are known to those skilled in the art. Briefly, dosage formulations of the compounds described herein are prepared for storage or administration by mixing the compound having the desired degree of purity with physiologically acceptable carriers, excipients, or stabilizers. Such materials are non-toxic to the recipients at the dosages and concentrations employed, and include buffers such as TRIS HCl, phosphate, citrate, acetate and other organic acid salts; antioxidants such as ascorbic acid; low molecular weight (less than about ten residues) peptides such as polyarginine, proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidinone; amino acids such as glycine, glutamic acid, aspartic acid, or arginine; monosaccharides, disaccharides, and other carbohydrates including cellulose or its derivatives, glucose, mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; counterions such as sodium and/or nonionic surfactants such as TWEEN, PLURONICS or polyethyleneglycol.

[000266]Sterile compositions for injection can be formulated according to conventional pharmaceutical practice as described in *Remington: The Science and Practice of Pharmacy* (20th ed, Lippincott Williams & Wilkens Publishers (2003)). For example, dissolution or suspension of the active compound in a vehicle such as water or naturally occurring vegetable oil like sesame, peanut, or cottonseed oil or a synthetic fatty vehicle like ethyl oleate or the like may be desired. Buffers, preservatives, antioxidants and the like can be incorporated according to accepted pharmaceutical practice.

[000267]Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the polypeptide, which matrices are in the form of shaped articles, films or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (*e.g.*, poly(2-hydroxyethyl-methacrylate) as described by Langer *et al.*,

1981, *J. Biomed Mater. Res.* 15:167-277 and Langer, 1982, *Chem. Tech.* 12:98-105, or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma ethyl-L-glutamate (Sidman *et al.*, 1983, *Biopolymers* 22:547-556), non-degradable ethylene-vinyl acetate (Langer *et al.*, *supra*), degradable lactic acid-glycolic acid copolymers such as the LUPRON Depot™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid (EP 133,988).

[000268]While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods. When encapsulated proteins remain in the body for a long time, they may denature or aggregate as a result of exposure to moisture at 37°C, resulting in a loss of biological activity and possible changes in immunogenicity. Rational strategies can be devised for protein stabilization depending on the mechanism involved. For example, if the aggregation mechanism is discovered to be intermolecular S-S bond formation through disulfide interchange, stabilization may be achieved by modifying sulfhydryl residues, lyophilizing from acidic solutions, controlling moisture content, using appropriate additives, and developing specific polymer matrix compositions.

[000269]Sustained-released compositions also include preparations of crystals of the antigen binding protein suspended in suitable formulations capable of maintaining crystals in suspension. These preparations when injected subcutaneously or intraperitoneally can produce a sustained release effect. Other compositions also include liposomally entrapped antibodies. Liposomes containing such antibodies are prepared by methods known per se: U.S. Pat. No. DE 3,218,121; Epstein *et al.*, 1985, *Proc. Natl. Acad. Sci. USA* 82:3688-3692; Hwang *et al.*, 1980, *Proc. Natl. Acad. Sci. USA* 77:4030-4034; EP 52,322; EP 36,676; EP 88,046; EP 143,949; 142,641; Japanese patent application 83-118008; U.S. Pat. Nos. 4,485,045 and 4,544,545; and EP 102,324.

[000270]The dosage of the antigen binding protein formulation for a given patient will be determined by the attending physician taking into consideration various factors known to modify the action of drugs including severity and type of disease, body weight, sex, diet, time and route of administration, other medications and other relevant clinical factors. Therapeutically effective dosages may be determined by either *in vitro* or *in vivo* methods.

[000271]An effective amount of the antigen binding proteins, described herein, to be employed therapeutically will depend, for example, upon the therapeutic objectives, the route of administration, and the condition of the patient. Accordingly, it is preferred for the therapist to titer the dosage and modify the route of administration as required to obtain the optimal therapeutic effect. A typical daily dosage might range from about 0.001 mg/kg to up to 100

mg/kg or more, depending on the factors mentioned above. Typically, the clinician will administer the therapeutic antigen binding protein until a dosage is reached that achieves the desired effect. The progress of this therapy is easily monitored by conventional assays or as described herein.

[000272]It will be appreciated that administration of therapeutic entities in accordance with the compositions and methods herein will be administered with suitable carriers, excipients, and other agents that are incorporated into formulations to provide improved transfer, delivery, tolerance, and the like. These formulations include, for example, powders, pastes, ointments, jellies, waxes, oils, lipids, lipid (cationic or anionic) containing vesicles (such as Lipofectin™), DNA conjugates, anhydrous absorption pastes, oil-in-water and water-in-oil emulsions, emulsions carbowax (polyethylene glycols of various molecular weights), semi-solid gels, and semi-solid mixtures containing carbowax. Any of the foregoing mixtures may be appropriate in treatments and therapies involving the HB-EGF antigen binding protein as described herein, provided that the active ingredient in the formulation is not inactivated by the formulation and the formulation is physiologically compatible and tolerable with the route of administration. *See,* also Baldrick P. "Pharmaceutical excipient development: the need for preclinical guidance," 2000, *Regul. Toxicol. Pharmacol.* 32:210-218; Wang, "Lyophilization and development of solid protein pharmaceuticals," 2000, *Int. J. Pharm.* 203:1-60; Charman WN "Lipids, lipophilic drugs, and oral drug delivery-some emerging concepts," *J. Pharm. Sci* .89:967-978; Powell *et al.,* 1998, "Compendium of excipients for parenteral formulations," *PDA J. Pharm. Sci. Technol.* 52:238-311 and the citations therein for additional information related to formulations, excipients and carriers well known to pharmaceutical chemists.

[000273]The following examples, including the experiments conducted and results achieved are provided for illustrative purposes only and are not to be construed as limiting upon the teachings herein.

EXAMPLES

K. Example 1: Generation Of Immunogen

[000274]HB-EGF including EGF-like domain (aa 1-149) was amplified from a pcDNA3-VSV-HB-EGF expression construct (Prenzel *et al.*, 1999, *supra*) and cloned into an expression vector that provides an in-frame 6His tag at the carboxyl-terminus (pcDNA 3.1 myc-his, InVitrogen). This HB-EGF immunogen with C-terminal myc(HIS)6 tag was expressed in HEK293 cells and purified by a two step purification on Ni-NTA sepharose (Amersham Pharmacia) and heparin sepharose (Sigma).

[000275]The HB-EGF portion of the immunogen had the following sequence (SEQ ID NO:1077).

1 MKLLPSVVLK LFLAAVLSAL VTGESLERLR RGLAAGTSNP

41 DPPTVSTDQL LPLGGGRDRK VRDLQEADLD LLRVTLSSKP
81 QALATPNKEE HGKRKKKGKG LGKKRDPCLR KYKDFCIHGE
121 CKYVKELRAP SCICHPGYHG ERCHGLSLP

B. Example 2: Immunization Of Xenomouse Mice And Titers Observed

[000276]Monoclonal antibodies against HB-EGF were developed by sequentially immunizing XenoMouse® mice (XenoMouse strains: XMG2 (human IgG2-producing) and XM3C-1 (human IgG4-producing), Abgenix, Inc. Fremont, CA).

1. Immunization

[000277]XenoMouse animals were immunized *via* the footpad for all injections. The total volume of each injection was 50 µl per mouse, 25 µl per footpad.

[000278]For both cohort 1 (10 XMG2 mice) and Cohort 2 (10 XM3C-1), the initial immunization was with 10 µg of HB-EGF protein admixed 1:1 (v/v) with TITERMAX GOLD® (Sigma, Oakville, ON) per mouse. The subsequent four boosts were made with 10 µg of HB-EGF protein admixed 1:1 (v/v) with 100 µg alum gel (Sigma, Oakville, ON) in pyrogen-free D-PBS. The fifth boost consisted of 10 µg of HB-EGF protein admixed 1:1 (v/v) with TITERMAX GOLD®. The sixth and seventh injection consisted of 10 µg of HB-EGF protein admixed 1:1 v/v with 100 µg alum gel. A final boost was made with 10 µg of HB-EGF protein in pyrogen-free DPBS, without adjuvant. The XenoMouse mice were immunized on days 0, 4, 8, 14, 18, 21, 25, and 28 for this protocol and fusions were performed on day 32. The two bleeds were made through Retro-Orbital Bleed procedure on day 16 after the fourth boost, on day 23 after the sixth boost.

2. Selection Of Animals For Harvest By Titer

[000279]Anti-HB-EGF antibody titers in the serum from immunized XenoMouse mice were determined by ELISA. Briefly, the HB-EGF protein (2 µg/ml) was coated onto Costar Labcoat Universal Binding Polystyrene 96-well plates (Corning, Acton, MA) overnight at 4°C in Antigen Coating Buffer (0.1 M Carbonate Buffer, pH 9.6 $NaHCO_3$ (MW 84) 8.4 g/L). The next day, the plates were washed one time with washing buffer (0.05% Tween 20 in 1x PBS) using a Biotek plate washer. The plates were then blocked with 200 µl/well blocking buffer (0.5% BSA, 0.1% Tween 20, 0.01% Thimerosal in 1x PBS) and incubated at room temperature for 1 hour. After the one-hour blocking, the plates were washed one time with washing buffer using a Biotek plate washer. Sera from either the HB-EGF protein immunized XenoMouse mice or naïve XenoMouse animals were titrated in 0.5% BSA/PBS buffer at 1:3 dilutions in duplicate from a 1:100 initial dilution. The last well was left blank. These plates were incubated at room temperature for 2 hours, and the plates were then washed three times with washing buffer using

a Biotek plate washer. A goat anti-human IgG Fc-specific horseradish peroxidase (CALTAG, Cat NO, H10507) conjugated antibody was added at a final concentration of 1:2000 in blocking buffer and incubated for 1 hour at room temperature. The plates were washed three times with washing buffer using a Biotek plate washer. After washing, the plates were developed with the addition of TMB chromogenic substrate (BioFx BSTP-0100-01) for 10-20 minutes or until negative control wells start to show color. Then the ELISA was stopped by the addition of Stop Solution (650 nM Stop reagent for TMB (BioFx BSTP-0100-01), reconstituted with 100 ml H_2O per bottle). The specific titer of each XenoMouse animal was determined from the optical density at 650 nm and is shown in TABLE 1 below. The titer value is the reciprocal of the greatest dilution of sera with an OD reading two-fold that of background. Therefore, the higher the number, the greater was the humoral immune response to HB-EGF.

[000280]

[000281]TABLE 1

[000282]

Group 1, fp, XMG2,10 mice,		
	After 4 inj.	After 6 inj.
Mouse ID	Reactivity to HB-EGF	
P4721	3,800	59,000
P4722	6,500	68,000
P4723	2,500	43,000
P4724	2,400	22,000
P4725	2,400	71,000
P4726	450	58,000
P4727	1,600	20,000
P4728	2,700	61,000
P4729	800	61,000
P47210	5,700	78,000
NC	<100	<100
PC	<100	<100

Group 2, fp, XM3C-1,10 mice,		
	After 4 inj.	After 6 inj.
Mouse ID	Reactivity to HB-EGF	
P2664	35	2,300
P2666	20	750
P2669	30	850
P26610	75	1,900
P2892	60	550
P2894	60	1,400
P2895	95	2,600
P2896	45	2,500
P3672	50	2,000
P3678	60	1,800
NC	<100	<100
PC	35	20

C. Example 3: Hybridoma Generation And Primary Screen For Binders

[000283]Immunized mice were sacrificed and the lymph nodes were harvested and pooled from each cohort. The lymphoid cells were dissociated by grinding in DMEM to release the cells from the tissues, and the cells were suspended in DMEM. The cells were counted, and 0.9 ml DMEM per 100 million lymphocytes was added to the cell pellet to resuspend the cells gently but completely. Using 100 µl of CD90+ magnetic beads per 100 million cells, the cells were labeled by incubating the cells with the magnetic beads at 4°C for 15 minutes. The magnetically-labeled cell suspension containing up to 10^8 positive cells (or up to $2x10^9$ total cells) was loaded onto a LS+ column and the column washed with DMEM. The total effluent was collected as the CD90-negative fraction (most of these cells were expected to be B cells).

[000284]The fusion was performed by mixing washed enriched B cells from above and nonsecretory myeloma P3X63Ag8.653 cells purchased from ATCC, cat.# CRL 1580 (Kearney *et al*, 1979, *J. Immunol.* 123:1548-1550) at a ratio of 1:1. The cell mixture was gently centrifuged at 800 g. After complete removal of the supernatant, the cellular pellet was treated with 2-4 ml of Pronase solution (CalBiochem, cat. # 53702; 0.5 mg/ml in PBS) for no more than 2 minutes. Then, 3-5 ml of FBS was added to stop the enzyme activity and the suspension was adjusted to

40 ml total volume using electro cell fusion solution, ECFS (0.3 M Sucrose, Sigma, Cat# S7903, 0.1 mM Magnesium Acetate, Sigma, Cat# M2545, 0.1 mM Calcium Acetate, Sigma, Cat# C4705). The supernatant was removed after centrifugation and the cells were resuspended in 40 ml ECFS. This wash step was repeated and the cells again were resuspended in ECFS to a concentration of $2x10^6$ cells/ml.

[000285]Electro-cell fusion was performed using a fusion generator, model ECM2001, Genetronic, Inc., San Diego, CA. The fusion chamber size used was 2.0 ml, using the following instrument settings: Alignment condition: voltage: 50 V, time: 50 seconds; membrane breaking at: voltage: 3000 V, time: 30 µsec; post-fusion holding time: 3 seconds.

[000286]After electro-cell fusion, the cell suspensions were carefully removed from the fusion chamber under sterile conditions and transferred into a sterile tube containing the same volume of Hybridoma Culture Medium (DMEM (JRH Biosciences), 15% FBS (Hyclone), supplemented with L-glutamine, pen/strep, OPI (oxaloacetate, pyruvate, bovine insulin) (all from Sigma) and IL-6 (Boehringer Mannheim)). The cells were incubated for 15-30 minutes at 37°C, and then centrifuged at 400 g for five minutes. The cells were gently resuspended in a small volume of Hybridoma Selection Medium (Hybridoma Culture Medium supplemented with 0.5x HA (Sigma, cat. # A9666)), and the volume was adjusted appropriately with more Hybridoma Selection Medium, based on a final plating of $5x10^6$ B cells total per 96-well plate and 200 µL per well. The cells were mixed gently and pipetted into 96-well plates and allowed to grow. On day 7 or 10, one-half the medium was removed, and the cells were re-fed with Hybridoma Selection Medium.

[000287]After 14 days of culture, hybridoma supernatants from the cohort #1 and cohort #2 were screened for HB-EGF-specific monoclonal antibodies by ELISA. In the Primary screen, the ELISA plates (Fisher, Cat. No. 12-565-136) were coated with 50 µL/well of HB-EGF protein (2 µg/ml) in Coating Buffer (0.1 M Carbonate Buffer, pH 9.6, $NaHCO_3$ 8.4 g/L), then incubated at 4°C overnight. After incubation, the plates were washed with Washing Buffer (0.05% Tween 20 in PBS) one time. 200 µL/well Blocking Buffer (0.5% BSA, 0.1% Tween 20, 0.01% Thimerosal in 1x PBS) were added and the plates were incubated at room temperature for 1 hour. After incubation, the plates were washed with Washing Buffer one time. Aliquots (50 µL/well) of hybridoma supernatants and positive and negative controls were added, and the plates were incubated at room temperature for 2 h. The positive control used throughout was serum from the relevant HB-EGF protein-immunized XenoMouse mouse and the negative control was serum from a KLH-immunized relevant strain of XenoMouse mouse. After incubation, the plates were washed three times with Washing Buffer. 100 µL/well of detection antibody goat anti-huIgGFc-HRP (Caltag Inc., Cat. No. H10507, using concentration was 1:2000 dilution) was added and the plates were incubated at room temperature for 1 hour. After incubation, the plates were

washed three times with Washing Buffer and 100 µl/well of TMB (BioFX Lab. Cat. No. TMSK-0100-01) was added. Plates were then allowed to develop for about 10 minutes (until negative control wells barely started to show color). 50 µl/well stop solution (TMB Stop Solution (BioFX Lab. Cat. No. STPR-0100-01) was then added and the plates were read on an ELISA plate reader at a wavelength of 450 nm.

[000288]The old culture supernatants from the positive hybridoma cells growth wells based on primary screen were removed and the HB-EGF positive hybridoma cells were suspended with fresh hybridoma culture medium and were transferred to 24-well plates. After 2 days in culture, these supernatants were ready for a secondary confirmation screen. In the secondary confirmation screen, the positives in the first screening were screened by ELISA (described as above) with two sets of detective antibodies: goat anti-huIgGFc-HRP (Caltag Inc., Cat. No. H10507, using concentration was 1:2000 dilution) for human gamma chain detection and goat anti-hIg kappa-HRP (Southern Biotechnology, Cat. No. 2060-05) for human kappa light chain detection in order to demonstrate that the antibody preparation was HB-EGF-specific and fully human in its composition. The two sets of ELISA procedures were identical to the descriptions above except the two different detection antibodies were used separately.

[000289]In parallel with the secondary confirmation screen, the counter ELISA screen was performed to exclude those antibodies that respond to myc(his)6 tag. The ELISA procedures were identical to the descriptions above except the coated with irrelevant myc(his)6 tag protein (ML-myc(his)6 protein) instead of coating with HB-EGF myc(his)6 protein.

[000290]After the secondary confirmation and the counter ELISA screen, 49 fully human IgG/kappa HB-EGF specific monoclonal antibodies were identified from cohorts 1 and 2.

D. Example 4: Scale-Up And Testing Of Antibodies In Functional Assays

[000291]This Example describes the identification of hybridoma cell lines that produce anti-HB-EGF antibodies with affinity for HB-EGF.

1. FACS Detection Of Hybridoma-Produced Anti-HB-EGF Antibodies Bound To HB-EGF Expressing Cell-Lines

[000292]HB-EGF expression on cell-lines was determined by FACS analysis. To perform this analysis $2x10^5$ selected HB-EGF-expressing cells were harvested with 10 mM EDTA in PBS, resuspended in FACS-buffer (PBS, 3% FCS, 0.4% azide) and seeded on a 96-well round bottom plate. After centrifugation for 3 minutes at 1000 rpm to remove supernatant, the cells were resuspended in hybridoma-derived anti-HB-EGF antibody dilution (100 µl/well) and incubated at 4°C for 45 min. The cells were washed twice with FACS buffer and resuspended with secondary antibody (100 µl/well) donkey-anti-human-PE (Jackson) diluted 1:100 in FACS buffer. The cell

suspensions were incubated at 4 °C in the dark for 30 min, washed twice with FACS buffer and analyzed (FACS, Beckman Coulter).

[000293]The results of these assays are provided in TABLES 2 and 3 below, which provide the fluorescence mean values for each FACS assay. As illustrated in TABLES 3 and 4, substantially no HB-EGF expression was detected in CHO control cells that do not express HB-EGF. However, when HB-EGF is recombinantly overexpressed in CHO cells, several monoclonal antibody preparations exhibit significant binding to those HB-EGF-expressing cells. Binding to MDA-MB231, SCC-9 and COS-7 cells was somewhat variable but an antibody preparation that bound to one HB-EGF-expressing cell type typically bound to another. As shown in TABLE 2, several antibody preparations, including, for example, the U2-24, U2-5, U2-19, U2-21, U2-15 and U2-42 antibody preparations exhibited strong binding to HB-EGF-expressing CHO cells. Similarly, antibody preparations U2-39, U2-26, U2-44, U2-45 and U2-48 also exhibited good binding to HB-EGF-expressing CHO cells.

[000294]

[000295]

TABLE 2
FACS Analysis Of Anti-HB-EGF Antibody Supernatants (Cohort 1)

	CHO control cells vs. HB-EGF-expressing CHO cells		Cell Lines Endogenously Expressing HB-EGF		
Antibody	CHO-K1	CHO-HB-EGF	MDA-MB231	SCC-9	COS-7
KLH	0.2	0.3	0.4	0.2	0.4
U2-18	0.3	154	3.1	1.2	1.6
U2-68	0.2	238	1.7	0.6	1.1
U2-24	0.2	360	2	0.6	6.7
U2-14	0.3	132	2.8	0.8	1.1
U2-1	0.2	64	1.8	0.7	3.1
U2-32	0.2	76	1.7	1.2	1.8
U2-40	0.2	137	2.1	1.3	1.1
U2-5	0.3	371	6.9	1.7	2.2
U2-8	0.3	148	3.7	1.4	1.4
U2-13	0.2	136	0.4(2.6)	0.5	0.8
U2-17	0.2	170	1.6	0.6	1.4
U2-19	0.4	347	5.5	1.8	6.1
U2-38	0.3	30	3.9	0.9	1.4
U2-21	0.2	344	3.9	3.4	2.1
U2-15	0.2	370	3	0.6	0.9
U2-16	0.2	197	1.5	0.4	0.9
U2-30	0.3	273	3.5	1	3.4
U2-44	0.3	5.6	0.8	0.2	5.2
U2-42	1.2	277	3.3	0.5	6.7
U2-36	0.9	112	1.6	0.3	4.9
U2-22	0.3	221	1.1	0.2	4.9
U2-56	0.2	38	0.5	0.2	1.8
Neg	0.2	0.2	0.3		
Pos (goat aHB)	0.2	80	3		

[000296]

[000297]

TABLE 3
FACS Analysis Of Anti-HB-EGF Antibody Supernatants (Cohort 2)

	CHO control cells vs. HB-EGF-expressing CHO cells		Cell Lines Endogenously Expressing HB-EGF		
Antibody	CHO-K1	CHO-HB-EGF	MDA-MB231	SCC-9	COS-7
U2-63	0.2	36(0.4)	0.5	0.2	0.6
U2-54	0.3	49	0.6	0.2	2.4
U2-65	0.2	1.9	0.4	0.2	0.3
U2-10	0.2	2.3	0.5	0.2	1.2
U2-53	0.2	144	0.5	0.2	0.6
U2-66	0.2	149	0.5	0.2	0.5
U2-61	0.4	28	0.4	0.2	1.4
U2-67	0.2	82	0.5	0.2	0.6
U2-28	0.3	198	1	0.2	3.2
U2-2	0.2	127	0.5	0.2	0.5
U2-62	0.2	47	0.5	0.2	0.6
U2-39	0.2	205	2.6	0.6	8.3
U2-3	0.2	168	0.9	0.2	2.5
U2-43	0.4	185	0.8	0.2	1.7
U2-34	0.7	125	1.2	0.3	6.4
U2-26	0.2	242	1.5	0.3	4.7
U2-41	0.3	186	1.2	0.2	3.3
U2-44	0.4	248	1.2	0.2	2.2
U2-45	0.6	248	1.4	0.2	6.4
U2-57	0.2	201	0.7	0.2	0.5
U2-12	0.6	1.2	1.2	0.5	1.6
U2-46	0.4	129	1	0.2	2.5
U2-48	0.4	273	1.3	0.3	2.1
U2-6	0.5	108	3.9	1.5	5.9
U2-58	0.2	277	0.9	0.2	0.4
U2-51	0.5	176	1.1	0.2	3.6
U2-60	0.2	3.6	0.4	0.2	0.3
Neg	0.2	0.2	0.3		
Pos (goat aHB)	0.2	80	3		

2. Inhibition of HB-EGF-Induced EGFR tyrosine phosphorylation

[000298]The following protocol was used to identify which anti-HB-EGF antibody preparations inhibit HB-EGF-induced epidermal growth factor receptor tyrosine phosphorylation. Such experiments further characterize the antibodies and help identify which hybridoma cell lines produce antibody that inhibit HB-EGF activity and then should be cloned and expanded.

[000299]40000 SCC9 human squamous cancer cells were seeded on a 96-well plate in 100 µl medium. Cells were starved in 60 µl serum free medium for 24 hr. A black Maxisorp 96-well plate was coated with 100 µl anti-EGFR antibody (2 µg/ml) overnight at 4°C. The coating solution was replaced by 300 µl blocking solution (PBS + 0.5% BSA) without washing and left to incubate 2 hours at room temperature. 10 µg/ml of IgG2-control (Sigma) or anti-HB-EGF hybridoma-derived antibodies were added to 20 ng/ml HB-EGF (R&D Systems) and preincubated for 30 minutes at 37 °C (volume: 40 µl). Cells were treated with medium alone or with the antibody/ligand solution for 3 minutes at 37°C. The medium was removed and cells were lysed on ice with 100 µl Triton-X-100-based lysis buffer containing 1 mM PMSF, 10 µg/ml Aprotinin, 10 mM NaF and 2 mM Na-Orthovanadate. The blocked Maxisorp plate was washed 6 times with PBS + 0.05% Tween-20. 80 µl of the cell lysate was transferred directly to the washed Maxisorp plate and incubated overnight at 4 °C with gentle agitation. The plate was washed 6 times with PBS + 0.05% Tween-20, then 100 µl 4G10-biotin (UBI) diluted 1:4000 in dilution buffer (PBS+0.5% BSA+0,05% Tween-20+5 mM EDTA) was added to each well and incubated for 2 hours at room temperature. The plate was washed 6 times with PBS + 0.05% Tween20 and 100 µl AP-conjugated streptavidin (UBI) diluted 1:20000 in dilution buffer (PBS+0.5% BSA+0,05% Tween20+5 mM EDTA) was added to each well for 30 minutes at room temperature. The plate was washed 6 times with PBS + 0.05% Tween-20 and 100 µl AttoPhos substrate was added to each well. The plate was incubated for up to 3 hours at room temperature in the dark and the developing fluorescence was monitored at 30, 90 and 180 min (Excitation: 430 nm, emission: 580 nm).

[000300]The percent inhibition of HB-EGF-induced EGFR tyrosine phosphorylation was calculated by reduction in the amount of phosphorylation observed with IgG2-control (Sigma) by each hybridoma-derived anti-HB-EGF antibody preparation.

[000301]Results for the different anti-HB-EGF antibody preparations are provided in FIGURES 23A and 23B. As illustrated, monoclonal antibody preparations U2-18, U2-24, U2-19, U2-42, U2-39, U2-34, U2-45 and U2-6 strongly inhibit EGFR tyrosine phosphorylation.

3. Anti-HB-EGF Antibody Preparations Inhibit LPA-Induced EGFR Phosphorylation

[000302]EGFR-dependent signaling pathways can be activated upon stimulation of G-protein-coupled receptors (GPCR). Ligand activation of heterotrimeric G proteins by interaction with a GPCR results in an intracellular signal that induces the extracellular activity of a transmembrane metalloproteinase. Ligands that can activate the GPCR pathway include LPA (lysophosphatidic acid), thrombin, carbachol, bombesin, and endothelin. Such activation leads to extracellular processing of a transmembrane growth factor precursor and release of the mature factor which, directly or through the proteoglycan matrix, interacts with the ectodomain of EGFR and activates it through phosphorylation. *See*, Prenzel *et al.*,1999, *Nature* 402:884-888.

[000303]The anti-HB-EGF antibody preparations provided herein were tested to ascertain whether they could inhibit EGFR tyrosine phosphorylation induced by the GPCR ligand LPA in COS-7 cells, using the following procedure.

[000304]250.000 COS-7 cells were seeded on a 6-well plate, in 2 ml medium and cultured over night. Cells were starved in 1 ml serum free medium for 24 hours. Following preincubation with antibodies (37°C, 1h), cells were stimulated with 10 µM LPA (37°C, 3 min) and lysed on ice with 400 µl Triton-X-100-based lysis buffer containing 1 mM PMSF, 10 µg/ml Aprotinin, 10 mM NaF and 2 mM Na-Orthovanadate. Following immunoprecipitation of the EGFR (340 µl lysate, 340 µl HNTG, 30 µl Prot. A Sepharose, 1.5µl αEGFR (108.1, Prenzel *et al.*, 1999, *supra*)) for 4 hours in the coldroom precipitates were washed 3 times with 500 µl HNTG buffer and run on a 7.5% SDS PAGE. Following transfer on a nitrocellulose membrane (Schleicher & Schuell) the blot was probed with an antibody recognizing phosphotyrosine residues (primary ab 4G10 (1:2000, Upstate biotechnology); secondary anti-mouse Ab 1:10000, Jackson laboratories). Reblot with sheep anti EGFR (Upstate technology) after stripping showed that equal amounts of the receptor were precipitated in each lane.

[000305]FIGURE 24 provides a western blot illustrating which anti-HB-EGF antibody preparations inhibit LPA-induced EGFR tyrosine phosphorylation. As shown in FIGURE 24, the U2-19 and U2-42 anti-HB-EGF antibody preparations strongly inhibited LPA-induced EGFR phosphorylation. The U2-24 anti-HB-EGF antibody preparation inhibited LPA-induced EGFR phosphorylation to a somewhat lesser extent.

E. Example 5: Hybridoma Cloning To Generate Monoclonal Antibodies

[000306]Based on the test results observed from experiments described in the preceding Examples, of the 49 original isolates, 43 hybridoma cell lines were selected for cloning by limiting dilution and further characterization of their monoclonal antibody. The lines selected for cloning bound to HB-EGF-expressing cell lines as exhibited by FACS analysis and inhibited HB-EGF-stimulation of EGF receptor tyrosine phosphorylation. For some hybridomas, insufficient

antibody was generated to run all the primary screening assays. This subset of hybridomas was advanced to hybridoma cloning as well.

F. Example 6: Further Characterization Of Antibody-Related Inhibition Of GPCR Induced Tyrosine Phosphorylation Of EGFR

[000307]This Example provides further data showing that several anti-HB-EGF antibody preparations provided herein exhibit dose-dependent inhibition of GPCR-induced EGF receptor tyrosine phosphorylation.

[000308]Inhibition by candidate antibody preparations U2-42, U2-39 and U2-45 was examined using different concentrations of these antibody preparations and the following procedure.

[000309]150,000 cells (MDA-MB231, PPC1) were seeded on a 12-well plate in 1 ml medium. Cells were starved in 500 µl serum free medium for 24 hr. A black Maxisorp 96-well plate was coated with 100 µl anti-EGFR antibody (2 µg/ml) overnight at 4°C. The coating solution was replaced by 300 µl blocking solution (PBS + 0.5% BSA) without washing and left to incubate 2 hours at room temperature.Cells were pre-incubated with 10 µg/ml anti-HB-EGF Abs for 30 minutes at 37°C and then treated with the GPCR ligands LPA (10 µM, PPC1 cells) or Thrombin (1U/ml, MDA-MB231 cells) for 3 minutes at 37 °C. The medium was removed and cells were lysed on ice with 200µl Triton-X-100-based lysis buffer containing 1 mM PMSF, 10 µg/ml Aprotinin, 10 mM NaF and 2 mM Na-Orthovanadate. The blocked Maxisorp plate was washed 6x with PBS + 0.05% Tween-20. Cell lysate was transferred directly to a washed Maxisorp plate and incubated overnight at 4 °C with gentle agitation.

[000310]The plate was washed 6 times with PBS + 0.05% Tween-20, then 100 µl 4G10-biotin (UBI) diluted 1:4000 in dilution buffer (PBS+0.5%BSA+0.05% Tween-20+5mM EDTA) was added to each well and incubated for 2 hours at room temperature. The plate was washed 6 times with PBS + 0.05% Tween-20 and 100µl AP-conjugated streptavidin (UBI) diluted 1:20000 in dilution buffer (PBS+0.5% BSA+0,05% Tween-20+5mM EDTA) was added to each well for 30 minutes at room temperature. The plate was washed 6 times with PBS + 0.05% Tween-20 and 100 µl Attophos substrate was added to each well. The plate was incubated for 3 hours at room temperature in the dark and the developing fluorescence was monitored at 30, 90 and 180 min (Excitation: 430 nm, emission: 580 nm).

[000311]As shown in FIGURE 26, inhibition of LPA-induced EGFR tyrosine phosphorylation was dose dependent – greater inhibition of EGFR tyrosine phosphorylation was observed as the amount of anti-HB-EGF antibody was increased.

[000312]FIGURE 25 illustrates that candidate antibody preparations U2-42, U2-39 and U2-45 also effectively inhibit thrombin-induced EGFR phosphorylation in MDA-MB231 cells. A dosage dependent inhibition is observed, with increased inhibition as more anti-HB-EGF antibody is added. FIGURE 26 illustrates that inhibition of LPA-induced EGFR tyrosine phosphorylation in

PPC-1 cells by anti-HB-EGF antibody preparations provided herein is dose dependent. As shown, greater inhibition of EGFR tyrosine phosphorylation was detected as the amount of anti-HB-EGF antibody was increased.

G. Example 7: Inhibition Of GPCR-Induced MDA-MB231 Cell Migration By Human Anti-HB-EGF Antibodies

[000313]Transmigration experiments were performed in order to investigate whether the antibodies provided herein block cell migration induced by the GPCR ligand Sphingosine-1-phosphate.

[000314]Serum-starved human breast cancer MDA-MB231 cells were preincubated with the indicated antibody to the cell suspension for 45 min at 37°C. Thereafter, 500 ml cell suspension (50,000 cells) was placed in the top chamber of collagen I-coated transwells (BD Falcon, 8 µm pores). 750 ml medium (MEM, amino acids, Na-Pyruvate, Pen.-Strept., 0.1% BSA) alone or containing the GPCR ligand Sphingosine-1-phosphate (R&D Systems) was used in the bottom chamber. After migration for 8 hours at 37°C cells were fixed, stained with DAPI and cell nuclei were counted for statistical evaluation.

[000315]The results for these MDA-MB231 cell migration assays using candidate anti-HB-EGF antibody preparations U2-42, U2-39 and U2-45 are provided in FIGURE 27. As shown, anti-HB-EGF antibody preparation U2-42 inhibited MDA-MB231 cell migration by about 70%; anti-HB-EGF antibody preparation U2-45 inhibited MDA-MB231 cell migration by about 100%; and anti-HB-EGF antibody preparation U2-39 inhibited MDA-MB231 cell migration by about 100%. Hence, the ability of these anti-HB-EGF antibodies to inhibit MDA-MB231 cell migration is substantial.

[000316]

H. Example 8: Inhibition Of HB-EGF-Induced Migration Of MCF-7 Cells By Human Anti-HB-EGF Antibodies

[000317]Transmigration experiments were performed in order to investigate whether the antibodies provided herein block cell migration that would otherwise be directly induced by HB-EGF. The results of these tests highlight which antibody preparations may be use for development as anti-metastatic cancer agents.

[000318]A 500 ml cell suspension of serum-starved human breast cancer MCF7 cells (50,000 cells) was placed in the top chamber of collagen I-coated transwells (BD Falcon, 8 µm pores). Aliquots of 750 ml medium (MEM, amino acids, Na-pyruvate, Pen.-Strept., 0,1% BSA) alone or containing 20 ng/ml HB-EGF (R&D Systems) in the presence or absence of 10 µg/ml HB-EGF antibodies were placed in the bottom chamber. After incubation and migration for 8 hours at 37°C, cells were fixed, stained with DAPI and cell nuclei were counted for statistical evaluation.

[000319]The results for these migration assays using candidate anti-HB-EGF antibody preparations U2-42, U2-39 and U2-45 are provided in FIGURE 28. As shown, anti-HB-EGF antibody preparation U2-42 inhibited MCF-7 cell migration by about 55%; anti-HB-EGF antibody preparation U2-45 inhibited MCF-7 cell migration by about 93%; and anti-HB-EGF antibody preparation U2-39 inhibited MCF-7 cell migration by about 98%. Hence, the ability of these anti-HB-EGF antibodies to inhibit MCF-7 cell migration is substantial.

I. Example 9: Characteristics Of Top 10 Anti-HB-EGF Antibodies

[000320]A summary of results is provided in TABLE 4, *infra*, for top 10 hybridoma-derived antibody preparations in GPCR-induced triple membrane-passing signal (TMPS) experiments. The TMPS experiments involved LPA-stimulation in SCC9 cells, thrombin stimulation in MDA-MB231 cells, LPA stimulation of SkOV-8 cells and Sphingosine-1-phosphate migration of MDA-MB231 cells. The data provided represent the percent inhibition of the TMPS transactivation signal that was observed when antibody preparations were used in the assays compared to the same assay when no antibody was present. The top three antibodies of each experiment are highlighted in bold letters.

[000321]

TABLE 4
Percent Inhibition of TMPS Transactivation Signal

	TMPS					Migration
Ab	**SCC9.3**	**MDA-MB231**	**MDA-MB231**	**SkoV-8**	**SkoV-8**	**MDA-MB231**
U2-39	**56.9**	**126.6**	**131.1**	**130.7**	65.5	**115**
U2-45	**58.5**	**136.8**	**95.7**	99.4	**75.1**	**104**
U2-42	**62.8**	123.2	**125.6**	**110.7**	**73.1**	69.9
U2-34	50.6	109.5	93.6	95.1	73	**99.6**
U2-46	36.6	88.1	77.5	80.5	**76.1**	n.d.
U2-19	n.d.	n.d.	n.d.	n.d.	n.d.	85
U2-26	n.d.	**143**	91.8	**106.1**	31.4	55.9
U2-51	n.d.	n.d.	n.d.	n.d.	n.d.	85.3
U2-15	n.d.	121.7	92.6	87	17.6	45.5
U2-22	n.d.	74.2	94.3	64.4	18.2	n.d.

J. Example 10: Inhibition Of HB-EGF-Induced HER4 Tyrosine Phosphorylationby Human Anti-HB-EGF Antibodies

[000322]This Example shows that anti-HB-EGF antibody preparations inhibit HB-EGF induced tyrosine phosphorylation of HER4. The following procedures were employed to assess the effects of anti-HB-EGF antibodies on HER4 tyrosine phosphorylation.

[000323]125,000 cells T47D human breast cancer cells were seeded on a 24-well plate in 500 µl medium. Cells were starved in 200 µl serum free medium for 24 hr. An R&D Systems Human Phospho-ErbB4 ELISA-Kit was used for detection of HER4 tyrosine phosphorylation. A clear Maxisorp 96-well plate was coated with 100 µl mouse anti-human-ErbB4 antibody (Capture Antibody 1 µg/ml) overnight at room temperature. The coated Maxisorp plate was washed 6 times with PBS+0.05%Tween-20, the washing solution was replaced by 300 µl blocking solution (PBS + 0.5% BSA) and incubated 2 hours at room temperature. 50 µl serum-free medium with 5x-concentration of anti-HB-EGF Abs (10 µg/ml) was incubated with 5x-concentration HB-EGF (20 ng/ml) for 30min at 37 °C, then added to each well (in duplicate). The medium was removed and cells were lysed on ice with 200 µl Triton-X-100-based lysis buffer containing 1 mM PMSF, 10 µg/ml Aprotinin, 10 mM NaF and 2 mM Na-Orthovanadate. The blocked Maxisorp plate was washed 3 times with PBS + 0.05% Tween-20. Cell lysate was transferred directly to a washed Maxisorp plate and incubated overnight at 4 °C with gentle agitation. The plate was washed 6 times with PBS+0.05% Tween-20, then 100 µl anti-Phospho-tyrosine-HRP (Detection Antibody 600ng/ml) diluted in dilution buffer (PBS+0.5%BSA+0,05% Tween-20+5 mM EDTA) was added to each well and incubated for 2 hours at room temperature. The plate was washed 6 times with PBS + 0.05% Tween20 and 100 µl Tetramethylbenzidine (TMB, Calbiochem) was added to each well for 20 minutes at room temperature. The reaction was stopped by addition of 50 µl 1 M H_2SO_4 and the absorbance was read at 450 nm (Thermo Lab Systems plate reader).

[000324]The results are shown in FIGURE 29. As illustrated, increasing concentrations of U2-42.1 or U2-39.1 anti-HB-EGF antibody preparations led to increased inhibition of HB-EGF-induced HER4 phosphorylation.

K. Example 11: Monoclonal Antibody Cross-Reactivity

[000325]This Example provides further data showing cross reactivity of anti-HB-EGF antibody preparations for the cyno HB-EGF, mouse HB-EGF and the related EGF-like growth factor Amphiregulin. Following cloning of the monkey and mouse form of HB-EGF, each expression construct was transfected in HEK293 cells and anti-HB-EGF antibodies were tested on their ability to bind these proteins in a FACS experiment. Amphiregulin cross reactivity was tested by ELISA format assay.

1. Cloning Cyno HB-EGF

[000326]In the present study, cyno HB-EGF plasmids were prepared. The cyno HB-EGF cDNA was cloned by polymerase chain reaction (PCR) from cyno kidney cDNA with primers based on the sequence of cyno HB-EGF.

[000327]The primers used for the amplification of cyno HB-EGF were as follows:

[000328]Forward primer: 5'-GGG TTA ACG CCA CCA TGA AGC TGC TGC CGT CG-3' (SEQ ID NO:1078)

[000329]Reverse primer: 5'-CCG CTC GAG GTG GGA ATT AGT CAT GCC C -3' (SEQ ID NO:1079)

[000330]The PCR product was digested with Hpa1 and Xho1 and ligated into pCDNA3.1 digested with Hind3. After purification, cyno HB-EGF plasmids were transformed into DH5α bacterial cells and multiplied under ampicilin selection. The plasmid was then highly expressed in ampicilin selection media using a single transformed colony. After purifying using a commercially available DNA-purification kit, cyno HB-EGF plasmids were transiently transfected in HEK293T cells.

2. Cloning Mouse HB-EGF

[000331]In the present study, mouse HB-EGF plasmids were prepared. The mouse HB-EGF cDNA was cloned by polymerase chain reaction (PCR) from mouse lung cDNA with primers based on the sequence of mouse HB-EGF.

[000332]The primers used for the amplification of mouse HB-EGF were as follows:

[000333]Forward primer: 5'-GGA ATT CGC CAC CAT GAA GCT GCT GCC GTC G-3' (SEQ ID NO:1080)

[000334]Reverse primer: 5'-CCG CTC GAG GTG GGA GCT AGC AGC CAC GCC-3' (SEQ ID NO:1081)

[000335]The PCR product and pCDNA3.1 vector DNA were digested with EcoR1 and Xho1 and ligated. After purification, mouse HB-EGF plasmids were transformed into DH5α bacterial cells and multiplied under ampicillin selection. The plasmid was then highly expressed in ampicillin selection media using a single transformed colony. After purifying using a commercially available DNA-purification kit, mouse HB-EGF plasmids were transiently transfected in HEK293T cells.

3. Transfection And Expression Of Cyno And Mouse HB-EGF

[000336]To screen for cross-reactivity of antibodies provided herein HEK293T cells were transiently transfected with either cyno or mouse HB-EGF plasmids using a Ca-phosphate method and subsequently analysed by FACS analysis.

[000337]Therefore, 30 hours before transfection, 3x106 HEK293T-cells were seeded in 16ml on a 15cm- cell culture plate and incubated at 7% CO_2 and 37°C. 32 µg DNA of either cyno or mouse HB-EGF DNA or empty vector in 720 µl ddH_2O were mixed with 2.5 M $CaCl_2$ and 2 x BBS (pH6.96) and incubated at room temperature for 10 minutes. After incubation, the solution was added drop wise onto the cells and incubated at 3% CO_2 and 37°C for 8 hours. After soaking the media, the cells were incubated with fresh growing media at 7% CO_2 and 37°C for 24 hours.

4. FACS Analysis Was Performed To Screen For Cross-Reactivity Of The Antibodies

[000338]Therefore, $2x10^5$ transfected cells were harvested with 10 mM EDTA in PBS, resuspended in FACS-buffer (PBS, 3% FCS, 0.4% azide) and seeded on a 96-well round bottom plate. After centrifugation for 3 min at 1000 rpm to remove supernatant, the cells were resuspended in anti-HB-EGF antibody dilution (100 µl/well) and incubated at 4°C for 45 min. The cells were washed twice with FACS buffer and resuspended with secondary antibody (100 µl/well) donkey-anti-human-PE (Jackson) diluted 1:100 in FACS buffer. The cell suspensions were incubated at 4 °C in the dark for 30 min, washed twice with FACS buffer and analyzed (FACS, Beckman Coulter).

[000339]FIGURE 30A shows that three mAb preparations cross-react with pro-HB-EGF from Cynomolgus monkeys.

[000340]FIGURE 30B shows that the U2-45 mAb preparation cross-reacts with mouse HB-EGF as detected by FACS analysis. Further testing showed that antibodies U2-46 and U2-51 were also detecting mouse HB-EGF. However, the antibody U2-45.1 was only very weakly (5-10%) neutralizing mouse HB-EGF induced tyrosine phosphorylation of the EGFR.

5. Protocol For Amphiregulin Cross Reactivity ELISA Assay

[000341]Different concentrations of Amphiregulin (R & D systems, conc. 1 ng/ml, 10ng/ml, 100ng/ml in PBS) were coated overnight at 4°C in a 96-well plate (Nunc, Maxisorp). Following plate wash (6-times) with washing buffer (PBS + 0,05% Tween 20; 150 µl per well), the plate was incubated with blocking buffer (PBS + 0,5% BSA, 100 µl / well) for 4 hours at room temperature. The plate was washed 6-times with washing buffer. As primary antibody 5 µg/ml purified human anti-HB-EGF antibodies in Ab dilution buffer (PBS containing 0.5% BSA, 0.05% Tween 20, 5 mM EDTA) were used and incubated for 90 minutes at room temperature. The plate was washed 6-times with washing buffer and a secondary anti human-POD (Dianova) antibody was added (1:10 000 in PBS, 0.5% BSA, 0.05% Tween 20, 5 mM EDTA) and incubated for 60 minutes at room temperature.

[000342]The plate was washed 6-times with washing buffer and the TMB substrate (Merck Biosciences) was added for 15 minutes at room temperature. After stopping the development of the blue color by adding 100 µl of 250 mM HCl the absorbance was measured at 450nm with a plate reader (Thermo labsystems).

[000343]FIGURE 30C shows that antibody U2-45 and weakly antibody U2-46 bind to Amphiregulin as determined by an ELISA format assay. The U2-45 mAb binds Amphiregulin but the U2-45.1 K_D for Amphiregulin was only 8 nM (versus 0.043 nM for HB-EGF). The U2-45.1 mAb was also non-neutralizing for AR.

L. Example 12: Kinetic Exclusion Assay Analysis Of K_d Values For Anti-HB-EGF Mabs U2-42.2, U2-39.1, U2-45.3, U2-26.2, And U2-34.1

[000344]The K_Ds of mAbs U2-42.2, U2-39.1, U2-45.3, and U2-26.2, and U2-34.1 binding to human HB-EGF, were determined using KinExA technology. For this purpose, a KinExA 3000 instrument was utilized. For all mAb titrations, 50 mg of azlactone beads were coupled with HB-EGF (~29 µg) in 50 mM sodium carbonate buffer, pH 9.0 overnight at 4 °C. After conjugation of HB-EGF to the beads, the beads were centrifuged and washed once with blocking buffer (1 M Tris buffer, pH 8.3, 10 mg/ml BSA) and centrifuged again, and then incubated in blocking buffer for one to two hours at ~23 °C in order to block any remaining reactive azlactone groups present on the surface of the beads. After blocking, the beads were transferred to a standard KinExA bead vial and placed on the instrument.

[000345]MAb U2-42.2: A dual curve analysis was performed to determine the K_D. Twelve solutions containing a nominal mAb binding site concentration of 37 pM were titrated with increasing concentrations of HB-EGF for the K_D-controlled titration, and 1110 pM binding site was titrated in the mAb-controlled titration curve. Each solution had a total volume of 10 ml (K_D-controlled) or 2 ml (mAb-controlled) and was allowed to equilibrate for 30-36 hours (K_D-controlled) or 6 hours (mAb-controlled) at ~23 °C. All solutions for the titration were prepared using volumetric glassware and the HB-EGF concentrations varied from 10.5 nM to 205 fM. The instrument method used for the analysis of these solutions consisted of a bead packing step in which the beads were packed into a glass capillary, and the equilibrated solutions were flowed through the bead column at 0.25 ml/min for 10 min (2.5 ml, K_D-controlled) or 1 min (0.25 ml, mAb-controlled) in triplicate. Subsequently, a fluorescently labeled cy-5 goat anti-human (Fc specific) polyclonal antibody at 3.4 nM (K_D-controlled) or 1.1 nM (mAb-controlled) was flowed through the bead pack for 2 min at 0.5 ml/min to label the free mAb binding site captured on the beads. The fluorescence emission from the bead pack was measured at 670 nm with excitation at 620 nm.

[000346]The resulting fluorescence measurements were converted into %-free mAb binding site versus total antigen concentration as standardly done with the accompanying KinExA software package (version 1.0.3). The dual titration curves were fit with the KinExA software to a 1:1 equilibrium isotherm with drift correction factors included.

[000347]The value of the K_D that fit the data optimally was 53 pM with low and high 95% confidence limits at 34 pM and 81 pM, respectively.

[000348]MAb U2-39.1: A K_D-controlled titration curve was performed to determine the K_D. Twelve solutions containing a nominal mAb binding site concentration of 55 pM were titrated with increasing concentrations of HB-EGF. Each solution had a total volume of 10 ml and was allowed to equilibrate for 30-36 hours at ~23 °C. All solutions for the titration were prepared using volumetric glassware and the HB-EGF concentrations varied from 10.5 nM to 205 fM. The instrument method used for the analysis of these solutions consisted of a bead packing step in which the beads were packed into a glass capillary, and the equilibrated solutions were flowed through the bead column at 0.25 ml/min for 10 min (2.5 ml) in triplicate. Subsequently, a fluorescently labeled cy-5 goat anti-human (Fc specific) polyclonal antibody at 3.4 nM was flowed through the bead pack for 2 min at 0.5 ml/min to label the free mAb binding site captured on the beads. The fluorescence emission from the bead pack was measured at 670 nm with excitation at 620 nm. The resulting fluorescence measurements were converted into %free mAb binding site versus total antigen concentration using the accompanying KinExA software package (version 1.0.3). Owing to ligand nonspecific binding to the bead pack, the titration curve was fit with the KinExA software to a 1:1 equilibrium isotherm with a term for ligand nonspecific binding included.

[000349]The value of the K_D that fit the data optimally was 7.8 pM with low and high 95% confidence limits at 5.6 pM and 11 pM, respectively.

[000350]MAb U2-45.3: A dual curve analysis was performed to determine the K_D. Twelve solutions containing a nominal mAb binding site concentration of 40 pM were titrated with increasing concentrations of HB-EGF for the K_D-controlled titration, and 1060 pM binding was titrated in the mAb-controlled titration curve. Each solution had a total volume of 10 ml (K_D-controlled) or 2 ml (mAb-controlled) and was allowed to equilibrate for 30-36 hours (K_D-controlled) or 6 hours (mAb-controlled) at ~23 °C. All solutions for the titration were prepared using volumetric glassware and the HB-EGF concentrations varied from 5.25 nM to 102 fM. The instrument method used for the analysis of these solutions consisted of a bead packing step in which the beads were packed into a glass capillary, and the equilibrated solutions were flowed through the bead column at 0.25 ml/min for 10 min (2.5 ml, K_D-controlled) or 1 min (0.25 ml, mAb-controlled) in triplicate. Subsequently, a fluorescently labeled cy-5 goat anti-human (Fc specific) polyclonal antibody at 3.4 nM (K_D-controlled) or 1.1 nM (mAb-controlled) was flowed

through the bead pack for 2 min at 0.5 ml/min to label the free mAb binding site captured on the beads. The fluorescence emission from the bead pack was measured at 670 nm with excitation at 620 nm. The resulting fluorescence measurements were converted into %free mAb binding site versus total antigen concentration as standardly done with the accompanying KinExA software package (version 1.0.3). The dual titration curves were fit with the KinExA software to a 1:1 equilibrium isotherm with drift correction factors included.

[000351]The value of the K_D that fit the data optimally was 43 pM with low and high 95% confidence limits at 27 pM and 65 pM, respectively.

[000352]MAb U2-26.2: A dual curve analysis was performed to determine the K_D. Twelve solutions containing a nominal mAb binding site concentration of 41 pM were titrated with increasing concentrations of HB-EGF for the K_D-controlled titration, and 1060 pM binding site was titrated in the mAb-controlled titration curve. Each solution had a total volume of 10 ml (K_D-controlled) or 2 ml (mAb-controlled) and was allowed to equilibrate for 30-36 hours (K_D-controlled) or 6 hours (mAb-controlled) at ~23 °C. All solutions for the titration were prepared using volumetric glassware and the HB-EGF concentrations varied from 2.63 nM-51.4 fM (K_D-controlled) and 5.26 nM-103 fM (mAb-controlled). The instrument method used for the analysis of these solutions consisted of a bead packing step in which the beads were packed into a glass capillary, and the equilibrated solutions were flowed through the bead column at 0.25 ml/min for 10 min (2.5 ml, K_D-controlled) or 1 min (0.25 ml, mAb-controlled) in triplicate. Subsequently, a fluorescently labeled cy-5 goat anti-human (Fc specific) polyclonal antibody at 3.4 nM (K_D-controlled) or 1.1 nM (mAb-controlled) was flowed through the bead pack for 2 min at 0.5 ml/min to label the free mAb binding site captured on the beads. The fluorescence emission from the bead pack was measured at 670 nm with excitation at 620 nm. The resulting fluorescence measurements were converted into %free mAb binding site versus total antigen concentration as standardly done with the accompanying KinExA software package (version 1.0.3). The dual titration curves were fit with the KinExA software to a 1:1 equilibrium isotherm with drift correction factors included.

[000353]The value of the K_D that fit the data optimally was 61 pM with low and high 95% confidence limits at 37 pM and 100 pM, respectively.

[000354]MAb U2-34.1: A K_D-controlled titration curve was performed to determine the K_D. Twelve solutions containing a nominal mAb binding site concentration of 40 pM were titrated with increasing concentrations of HB-EGF. Each solution had a total volume of 10 ml and was allowed to equilibrate for 30-36 hours at ~23 °C. All solutions for the titration were prepared using volumetric glassware and the HB-EGF concentrations varied from 5.26 nM-103 fM. The instrument method used for the analysis of these solutions consisted of a bead packing step in which the beads were packed into a glass capillary, and the equilibrated solutions were flowed

through the bead column at 0.25 ml/min for 10 minutes (2.5 ml) in triplicate. Subsequently, a fluorescently labeled cy-5 goat anti-human (Fc specific) polyclonal antibody at 5.1 nM was flowed through the bead pack for 2 minutes at 0.5 ml/min to label the free mAb binding site captured on the beads. The fluorescence emission from the bead pack was measured at 670 nm with excitation at 620 nm. The resulting fluorescence measurements were converted into %free mAb binding site versus total antigen concentration as standardly done with the accompanying KinExA software package (version 1.0.3). Owing to ligand nonspecific binding to the bead pack, the titration curve was fit with the KinExA software to a 1:1 equilibrium isotherm with a term for ligand nonspecific binding included.

[000355]The value of the K_D that fit the data optimally was 59 pM with low and high 95% confidence limits at 32 pM and 87 pM, respectively.

M. Example 13: Determination Of Antibody Affinity Scatchard Analysis

[000356]Affinity measurements of antibodies provided herein were performed by indirect FACS Scatchard analysis. To perform this analysis $2x10^5$ cells of interest were harvested with 10 mM EDTA in PBS, resuspended in FACS-buffer (PBS, 3% FCS, 0.4% azide) and seeded on a 96-well round bottom plate. After centrifugation for 3 min at 1000 rpm to remove supernatant, the cells were resuspended in anti-HB-EGF antibody dilution (100 µl/well) starting with 20 µg/ml, diluted in 1:2 dilution steps. Cell suspensions were incubated at 4 °C for 45 min, washed twice with FACS buffer and resuspended with secondary antibody (100 µl/well) donkey-anti-human-PE (Jackson) diluted 1:100 in FACS buffer. The cell suspensions were incubated at 4 °C in the dark for 30 min, washed twice with FACS buffer and analyzed (FACS, Beckman Coulter).

[000357]According to the FACS Scatchard analysis, the fluorescence mean was calculated for each measurement. Background fluorescence of cells without HB-EGF antibodies was subtracted from each fluorescence mean. Scatchard plot with x-value = fluorescence mean and y-value = fluorescence mean/concentration of mAb (nM) was generated. The K_D was taken as the absolute value of 1/m of linear equation.

TABLE 5
FACS Scatchard Determined Affinities Of Antibodies U2-42 And U2-39 On Three Different Human Cancer Cell Lines

Cell line / Antibody	DLD-1	NCI-ADR	MDA-MB231
U2-42	4,88	5,98	0,41
U2-39	9,05	7,63	6,21

N. Example 14: Kinetic Exclusion Assay Analysis Of K_d Values For Anti-HB-EGF Mab U2-45.3 Binding To Amphiregulin

[000358]A K_D-controlled titration curve was performed to determine the K_D. Twelve solutions containing a nominal mAb binding site concentration of 4.4 nM were titrated with increasing concentrations of Amphiregulin. Each solution had a total volume of 10 ml and was allowed to equilibrate for 30-36 hours at ~23 °C. All solutions for the titration were prepared using volumetric glassware and the Amphiregulin concentrations varied from 1.23 µM to 24 pM. The instrument method used for the analysis of these solutions consisted of a bead packing step in which the beads were packed into a glass capillary, and the equilibrated solutions were flowed through the bead column at 0.25 ml/min for 1 minute (0.25 ml) in triplicate. Subsequently, a fluorescently labeled cy-5 goat anti-human (Fc specific) polyclonal antibody at 684 pM was flowed through the bead pack for 2 minutes at 0.5 ml/min to label the free mAb binding site captured on the beads. The fluorescence emission from the bead pack was measured at 670 nm with excitation at 620 nm. The resulting fluorescence measurements were converted into %free mAb binding site versus total antigen concentration as standardly done with the accompanying KinExA software package (version 1.0.3). Owing to ligand nonspecific binding to the bead pack, only one replicate out of the three collected at each concentration (the highest three 2-fold Amphiregulin concentrations were excluded from the analysis) could be analyzed and fit with the KinExA software to a 1:1 equilibrium isotherm.

[000359]The value of the K_D that fit the data optimally was 5.0 nM with low and high 95% confidence limits at 3.1 nM and 7.7 nM, respectively.

O. Example 15: Selection Criterion For Top Antibody Preparations

[000360]The following criteria were used to identify the top antibody preparations: potency in inhibiting TMPS, potency in directly inhibiting HB-EGF as measured by observing the degree to which the antibodies inhibited tyrosine phosphorylation of EGF receptor and HER4, the affinity of the antibodies for HB-EGF, the cross-reactivity of the antibodies for other molecules and the characteristics of the epitopes.

P. Example 16: Anti-Hb-Egf Antibodies Inhibit HB-EGF Stimulation Of Huvec Cellular Proliferation And Tube Formation

[000361]This Example illustrates that while HB-EGF stimulates human vascular endothelial cell (HUVEC) proliferation, anti-HB-EGF antibodies provided herein inhibit basal HUVEC cell proliferation. Also, as shown by this Example, anti-HB-EGF antibodies inhibit HUVEC tube formation, which is an *in vitro* model for neo-angiogenesis. Antibody preparations that inhibit

HUVEC proliferation and/or angiogenesis are useful not only for treating cancer but also for treating non-cancerous conditions involving undesired angiogenesis (*e.g.*,diabetic retinopathy).

1. Procedures: Determination of HB-EGF Expression on HUVEC Cells by Flow Cytometry

[000362]HB-EGF expression on human endothelial cells was determined by FACS analysis. Therefore, $2x10^5$ cells of interest were harvested with 10 mM EDTA in PBS, resuspended in FACS-buffer (PBS, 3% FCS, 0.4% azide) and plated on a 96-well round bottom plate. After centrifugation for 3 min at 1000 rpm, supernatant was removed, the cells were resuspended in anti-HB-EGF antibody dilution (100 µl/well, 10 µg/ml anti-HB-EGF antibody) and incubated at 4°C for 45 min. The cells were washed twice with FACS buffer and resuspended with secondary antibody (100 µl/well) anti-human-PE (Jackson) diluted 1:100 in FACS buffer. The cell suspensions were incubated at 4 °C in the dark for 30 min, washed twice with FACS buffer and analyzed (FACS, Beckman Coulter).

[000363]To test for the effects of anti-HB-EGF antibodies on HUVEC proliferation, approximately 5000 HUVEC cells were seeded into each of 48 wells containing media with EGM-2, hydrocortisone, ascorbic acid, gentamycin-amphothericin and 2% FCS containing bFGF, VEGF, EGF and IGF-1 (Cambrex). After incubating the cells overnight at 37 C, the cells were washed twice with PBS containing 0.5% FCS. The cells were then starved 8h in EGM-2, 0.5% FCS without supplementation of growth factors. HB-EGF or anti-HB-EGF antibody preparations were added in 500 µl starvation media.

[000364]Cells were then cultured for an additional 60 hours, trypsinized and counted.

[000365]To test for the effects of anti-HB-EGF antibodies on HUVEC tube formation, 200 µl growth factor reduced matrigel (BD biosciences) was plated on 48 wells. 250 µl HUVEC medium was added per 48 well (EBM-2 + hydrocortisone + ascorbic acid gentamycin-amphothericin + 0,25% FCS from Cambrex). Following preincubation for 20 min, 20,000 HUVEC cells in 50 µl medium + 0.25% FCS containing HB-EGF (10 ng/ml) or U2-42, U2-39 or U2-45 anti-HB-EGF antibodies (10 µg/ml) were added. Tube formation was monitored by obtaining photomicrographs of representative areas of the culture wells. For a quantitative analysis closed areas of HUVEC tubes were counted.

2. Results

[000366]As shown by the FACS analysis in FIGURE 30, HB-EGF is expressed on HUVECs. The results of the cellular proliferation tests are provided in FIGURES 32A-B. As shown in FIGURE 32A, HB-EGF stimulates HUVEC cellular proliferation by about 38%. However, upon addition of anti-HB-EGF antibody preparations U2-42, U2-39 or U2-45, such stimulation of

cellular proliferation is inhibited by about 8% to 14% (FIGURE 31B). In this assay, the U2-39 anti-HB-EGF antibody preparation provided the highest level of inhibition.

[000367]The results of the tube formation tests are provided in FIGURES 33A-M. Control assays, without anti-HB-EGF antibodies shown in FIGURES 34A-C, show that HUVEC cells join to form circular structures or "tubes." However, upon addition of anti-HB-EGF antibody preparations U2-42, U2-39 or U2-45, such tube formation is inhibited. A summary of the number of tubes observed is provided in FIGURE 33M. As shown in FIGURE 33M, the U2-42 anti-HB-EGF antibody preparation provided the highest acceleration of tube regression, followed by the U2-39 anti-HB-EGF antibody preparation.

Q. Example 17: Anti-HB-EGF Antibodies Inhibit HB-EGF-Stimulated And Basal Colony Formation

[000368]Soft agar assays were conducted in order to investigate the ability of the antibodies provided herein to inhibit anchorage independent cell growth. The soft agar colony formation assay is a standard *in vitro* assay to test for transformed cells, as only such transformed cells can grow in soft agar.

[000369]To perform this assay, OVCAR-8, BM-1640 and NCI-H226 cells were incubated with 10 ng/ml HB-EGF and with anti-HB-EGF antibodies or IgG2 (SIGMA) as negative control, at 20 µg/ml in IMDM medium (Gibco) and resuspended in 0.2% Difco noble agar. The cell suspension was plated on a 0.4% agar-underlayer in quadruplicate in a 96-well plate and overlaid with IMDM medium. Colonies were allowed to form for approximately 14 days and were then stained with 40µl MTT (Sigma, 1 mg/ml in PBS) for 4 hours. Stimulation of HB-EGF and inhibitory effects of anti-HB-EGF antibodies were quantified by HTSBonit (LemnaTec) colony formation software.

[000370]In another assay, 750 or 1000 cells (depending on SkOV-3 clone 71 or 74, FIGURE 34D and E) were preincubated with anti-HB-EGF antibodies or IgG2 (SIGMA) as negative control, at 20 µg/ml in IMDM medium (Gibco) for 30 min at 37 °C and resuspended in 0.4% Difco noble agar (or 0.2% for clone 74). The cell suspension was plated on a 0.75% agar-underlayer (0.4% for clone 74) in quadruplicate in a 96-well plate and overlaid with IMDM medium. In a similar assay, 2000 BxPC-3 cells (FIGURE 34F) were preincubated with 20 µg/ml anti-HB-EGF antibodies or 20 µg/ml IgG2 (SIGMA) as negative control, in IMDM medium (Gibco) containing 20% FCS for 30 min at 37 °C. Cells were resuspended in 0.4% Difco noble agar and the cell suspension was plated on a 0.75% agar-underlayer in quadruplicate in a 96-well plate. The wells were overlaid with IMDM medium. Both layers contained 20% FCS.

[000371]Colonies were allowed to form for approximately 14 days and were then stained with 40µl MTT (Sigma, 1 mg/ml in PBS) for 4 hours. Results are shown in FIGURES 34A-F, which illustrate that HB-EGF stimulated colony formation (FIGURE 34A-C) and basal colony formation

(FIGURES 34D-F) is significantly reduced by the anti-HB-EGF antibodies. As shown, for example, in FIGURE 34A, HB-EGF stimulated OVCAR-8 cells to form a significantly larger mean colony size than control OVCER-8 cells cultured without HB-EGF. However, when OVCAR-8 cells were cultured with anti-HB-EGF U2-39 antibodies in the presence of HB-EGF, mean colony size was reduced to a size similar to that observed for control cells without HB-EGF treatment (FIGURE 34A). Similar results were observed for BM1604 cells (derived from prostate cancer tissue) (*see*, FIGURE 34B). Anti-HB-EGF U2-45 and U2-42 antibodies also inhibited BM1604 colony formation.

[000372]Anti-HB-EGF antibodies also inhibit HB-EGF-stimulated colony formation of NCI-H226 lung carcinoma cells (FIGURE 34C). As shown in FIGURE 36C, when NCI-H226 cells were cultured with anti-HB-EGF U2-39 antibodies in the presence of HB-EGF, mean colony size was reduced to a size similar to that observed for control cells without HB-EGF treatment.

[000373]The numbers of colonies, as well as the colony size, are reduced by the treatment with the present anti-HB-EGF antibodies (FIGURES 34D-F). Thus, FIGURE 34D illustrates that anti-HB-EGF antibodies reduce the number of basal colonies formed by SkOV-3 HB-EGF clone 71 cells (derived from SkOV-3 ovarian cancer cells stably transfected with a proHB-EGF expression construct). As shown, control SkOV-3 cells overexpressing HB-EGF formed large numbers of colonies. However, when SkOV-3 HB-EGF cl. 71 cells were cultured with either anti-HB-EGF U2-42 or U2-39 antibodies in the presence of HB-EGF, the number of colonies was dramatically reduced. Similarly, anti-HB-EGF antibodies inhibit colony formation of SkOV-3 (clone 74) cells, derived from ovarian cancer tissue, and BxPC3 cells, derived from pancreatic adenocarcinoma tissue (FIGURES 34E-F).

[000374]These data indicate that colony formation and tumors by a large variety of cancer cell types can be inhibited by the present anti-HB-EGF antibodies, including the U2-42, U2-39 and U2-45 antibody preparations provided herein.

R. Example 18: Anti HB-EGF Antibodies Inhibit Tumor Growth *In Vivo*

[000375]FIGURES 37 illustrates the mean volume of pancreatic BxPC3 tumors formed in xenograft experiments with SCID mice. As shown, established tumor growth was significantly inhibited in the presence of antibody preparations U2-42 and/or U2-39 when compared to the vehicle control. In FIGURE 38A it is shown that anti-HB-EGF antibodies U2-42, U2-39 and U2-45 inhibit the established growth of EFO-27 HB-EGF clone 58 cells *in vivo*. The effect of tumor growth inhibition could be shown to be dose-dependent, with 25 mg/kg as a highly effective treatment while lower doses such as 1 or 5 mg/kg were less efficient (FIGURE 38B).

S. Example 19: Anti-HB-EGF Antibodies In Combination Therapy With The Antiegfr Antibody Erbitux

[000376]FIGURE 35 shows that single agent inhibition of EFO-27 HB-EGF cl. 58 cells with anti-HB-EGF antibodies is moderate to strong. However, in a dose-controlled combination of anti-HB-EGF and anti-EGFR antibodies the inhibition of colony formation is extremely effective. Moreover, *in vivo* xenograft growth is strongly inhibited by the anti-HB-EGF and anti-EGFR antibody combination leading to a complete regression of ovarian cancer tumor growth (FIGURE 38C).

T. Example 20: HB-EGF Expression On A Variety Of Cancer Cell Types

[000377]HB-EGF expression on human cancer cell-lines was determined by FACS analysis. To perform this analysis $2x10^5$ cells were harvested with 10 mM EDTA in PBS, resuspended in FACS-buffer (PBS, 3% FCS, 0.4% azide) and transferred to a 96-well round bottom plate. After centrifugation for 3 min at 1000 rpm to remove supernatant, the cells were resuspended in anti-HB-EGF antibody dilution (100 µl/well) and incubated at 4°C for 45 min. The cells were washed twice with FACS buffer and resuspended with secondary antibody (100 µl/well) donkey-anti-human-PE (Jackson) diluted 1:100 in FACS buffer. The cell suspensions were incubated at 4 °C in the dark for 30 minutes, washed twice with FACS buffer and analyzed (FACS, Beckman Coulter).

[000378]The results of these assays are shown in TABLE 6, below.

[000379]

TABLE 6
HB-EGF Expression in Cancer Cells

Cell line	Tissue	Expression Level
MDA-MB231	Breast	++
NCI-ADR	Breast	+++
ZR75-1	Breast	-/+
MKN-1	Gastric	+
MKN-28	Gastric	+++
PPC1	Prostate	++
PC3	Prostate	++
HT144	Melanoma	-/+
MelGerlach	Melanoma	+++
IGROV-1	Ovarian	+
ES2	Ovarian	++
SkOV-3	Ovarian	+
SkOV-8	Ovarian	+
TOV21G	Ovarian	++
OVCAR-8	Ovarian	+++
Calu-6	Lung	+
NCI-H460	Lung	++
MS-751	Cervix	++
SIHA	Cervix	+
HelaS3	Cervix	+
U266	Myeloma	-
SCABER	Bladder	++
HCT-116	Colon	++
HCT-15	Colon	+
SW620	Colon	++

U. Example 21: Anti-HB-EGF Antibodies For The Detection Of HB-EGF In Tissue And Body Fluids By Immunohistochemistry And Elisa

[000380]Anti-HB-EGF antibodies U2-42 and U2-39 were tested for their ability to stain HB-EGF expressed in human fixed samples. As shown in FIGURE 39A both antibodies show a prominent membrane and cytoplasmic staining of human kidney tubular cells while the control does not show any staining. In addition to the immunohistochemical detection of HB-EGF in

patient tissue samples HB-EGF is released as a growth factor into various body fluids. Based on human anti-HB-EGF antibodies as coating reagents an ELISA was established which detects HB-EGF in liquid samples down to levels below 40 pg/ml (FIGURE 39B).

V. Example 22: Canonical Classes Of Antibodies

[000381]The genes encoding top antibodies were sequenced as described in the last Example. This sequence data was used to assign the antibodies to canonical classes.

[000382]Chothia *et al.* have described antibody structure in terms of "canonical classes" for the hypervariable regions of each immunoglobulin chain (Chothia, *et al.,* 1987, *J. Mol. Biol.,* 196(4) :901-17). The atomic structures of the Fab and VL fragments of a variety of immunoglobulins were analyzed to determine the relationship between their amino acid sequences and the three-dimensional structures of their antigen binding sites. Chothia *et al.* found that there were relatively few residues that, through their packing, hydrogen bonding or the ability to assume unusual phi, psi or omega conformations, were primarily responsible for the main-chain conformations of the hypervariable regions. These residues were found to occur at sites within the hypervariable regions and in the conserved α-sheet framework. By examining sequences of immunoglobulins having unknown structure, Chothia, *et al.* show that many immunoglobulins have hypervariable regions that are similar in size to one of the known structures and additionally contained identical residues at the sites responsible for the observed conformation.

[000383]Their discovery indicated that these hypervariable regions have conformations close to those in the known structures. For five of the hypervariable regions, the repertoire of conformations appeared to be limited to a relatively small number of discrete structural classes. These commonly occurring main-chain conformations of the hypervariable regions were termed "canonical structures." Further work by Chothia, *et al.,* 1989; *Nature* 342:877-83) and others (Martin *et al.,* 1996; J. *Mol. Biol.* 263:800-15) confirmed that there is a small repertoire of main-chain conformations for at least five of the six hypervariable regions of antibodies.

[000384]The complementarity determining regions (CDRs) of each antibody preparation were analyzed to determine their canonical class. As is known, canonical classes have only been assigned for CDR1 and CDR2 of the antibody heavy chain, along with CDR1, CDR2 and CDR3 of the antibody light chain. The TABLES below summarize the results of the analysis. The Canonical Class data is in the form of HCDR1-HCDR2-LCDR1-LCDR2-LCDR3 (H1-H2-L1-L2-L3), wherein "HCDR" refers to the heavy chain CDR and "LCDR" refers to the light chain CDR. Thus, for example, a canonical class of 1-3-2-1-1 refers to an antibody that has a HCDR1 that falls into canonical class 1, a HCDR2 that falls into canonical class 3, a LCDR1 that falls into canonical class 2, a LCDR2 that falls into canonical class 1, and a LCDR3 that falls into canonical class 1.

[000385]Assignments were made to a particular canonical class where the amino acids in the antibody match with the amino acids defined for each canonical class. The amino acids defined for each canonical class can be found, for example, in the articles by Chothia, *et al.* referred to above. TABLE 7 and TABLE 8 report the canonical class assignments for each of the HB-EGF antibodies. Where there was no matching canonical class, the canonical class assignment is marked with a letter s and a number, such as "s18", meaning the CDR is of size 18.

[000386]

TABLE 7

Antibody (sorted)	H1-H2-L1-L2-L3	H3length
U2-18.1	3-1-2-1-1	17
U2-13.1	1-3-4-1-1	14
U2-19.1	3-1-3-1-1	13
U2-38.1	3-1-2-1-1	23
U2-21.1	3-1-3-1-s9	8
U2-15.1	1-3-4-1-1	14
U2-16.1	1-2-8-1-1	11
U2-30.1	1-2-3-1-1	13
U2-42.1	1-3-2-1-s9	8
U2-36.1	3-s18-2-1-s10	13
U2-22.1	1-3-3-1-1	11
U2-56.1	3-1-2-1-1	8
U2-24.1	3-s16-3-1-s9	12
U2-24.2.1	3-s16-3-1-s9	12
U2-14.1	1-3-4-1-1	14
U2-1.1	1-3-4-1-1	5
U2-32.1	1-1-3-1-1	15
U2-40.1	1-3-2-1-3	8
U2-5.1	1-3-4-1-1	6
U2-8.1	3-1-4-1-1	17
U2-39.1	1-3-2-1-1	6
U2-3.1	3-1-4-1-1	16
U2-43.1	1-1-2-1-1	12
U2-34.1	1-3-2-1-1	16
U2-26.1	3-1-3-1-s9	9

U2-41.1	1-3-2-1-1	12
U2-45.1	1-3-2-1-1	19
U2-54.1	3-1-2-1-1	15
U2-57.1	3-1-2-1-1	8
U2-12.1	1-3-2-1-s9	9
U2-46.1	1-3-2-1-1	16
U2-48.2	1-1-2-1-1	12
U2-6.1.1	1-3-4-1-1	11
U2-6.1.2	1-3-2-1-1	16
U2-58.1	3-s16-2-1-1	8
U2-51.1	1-3-2-1-1	16
U2-65.2	3-1-2-1-1	8
U2-53.1	1-1-2-1-1	12
U2-61.1	1-3-2-1-1	9
U2-28.1	3-1-3-1-s9	12

TABLE 8

Antibody	H1-H2-L1-L2-L3 (sorted)	H3 Length
U2-43.1	1-1-2-1-1	12
U2-48.2	1-1-2-1-1	12
U2-53.1	1-1-2-1-1	12
U2-32.1	1-1-3-1-1	15
U2-30.1	1-2-3-1-1	13
U2-16.1	1-2-8-1-1	11
U2-39.1	1-3-2-1-1	6
U2-61.1	1-3-2-1-1	9
U2-41.1	1-3-2-1-1	12
U2-34.1	1-3-2-1-1	16
U2-46.1	1-3-2-1-1	16
U2-6.1.2	1-3-2-1-1	16
U2-51.1	1-3-2-1-1	16
U2-45.1	1-3-2-1-1	19
U2-40.1	1-3-2-1-3	8
U2-42.1	1-3-2-1-s9	8
U2-12.1	1-3-2-1-s9	9
U2-22.1	1-3-3-1-1	11
U2-1.1	1-3-4-1-1	5
U2-5.1	1-3-4-1-1	6
U2-6.1.1	1-3-4-1-1	11
U2-13.1	1-3-4-1-1	14
U2-15.1	1-3-4-1-1	14
U2-14.1	1-3-4-1-1	14
U2-56.1	3-1-2-1-1	8
U2-57.1	3-1-2-1-1	8
U2-65.2	3-1-2-1-1	8
U2-54.1	3-1-2-1-1	15
U2-18.1	3-1-2-1-1	17
U2-19.1	3-1-3-1-1	13
U2-21.1	3-1-3-1-s9	8

U2-26.1	3-1-3-1-s9	9
U2-28.1	3-1-3-1-s9	12
U2-3.1	3-1-4-1-1	16
U2-8.1	3-1-4-1-1	17
U2-58.1	3-s16-2-1-1	8
U2-24.1	3-s16-3-1-s9	12
U2-24.2.1	3-s16-3-1-s9	12
U2-36.1	3-s18-2-1-s10	13
U2-38.1	3-1-2-1-1	23

[000387]TABLE 9 is an analysis of the number of antibodies per class. The number of antibodies having the particular canonical class designated in the left column is shown in the right column.

[000388]The most commonly seen structure is 1-3-2-1-1. Eight out of a total of 40 mAbs had this combination.

[000389]

TABLE 9
Number of Anti-HB-EGF Antibodies in Each Canonical Class Combination

H1-H2-L1-L2-L3	Count
1-1-2-1-1	3
1-1-3-1-1	1
1-2-3-1-1	1
1-2-8-1-1	1
1-3-2-1-1	8
1-3-2-1-3	1
1-3-2-1-s9	2
1-3-3-1-1	1
1-3-4-1-1	6
3-1-2-1-1	5
3-1-3-1-1	1
3-1-3-1-s9	3
3-1-4-1-1	2
3-s16-2-1-1	1
3-s16-3-1-s9	2
3-s18-2-1-s10	1
3-1-2-1-1	1

W. Example 23: Epitope Mapping Of Anti HB-EGF Antibodies

[000390]This Example describes the mapping of epitopes recognized by antibody preparations.

[000391]Antibodies tested: Five XenoMouse derived human monoclonal antibody preparations capable of neutralizing the activity of HB-EGF were analyzed: U2-39; U2-42; U2-45; U2-26 and U2-19. Four of these monoclonal antibody preparations were shown to be specific for human HB-EGF while antibody preparation U2-45 exhibited some cross-reactivity with mouse HB-EGF and human Amphiregulin. All of these neutralizing antibody preparations map to MCAB Bins 7 & 8 (*see*, below).

1. Epitope Mapping

[000392]The human HB-EGF cDNA was isolated from HeLa mRNA by PCR amplification and the mature HB-EGF sequence was cloned into a pSecTAg vector as a myc-His fusion protein,

using the Ig kappa signal peptide sequence. The mature HB-EGF polypeptide was expressed in 293T cells, with secretion into the media.

[000393]The diphtheria toxin binding site of HB-EGF was mutated as well as the EGF receptor binding site by site-directed mutagenesis.

[000394]The short form of HB-EGF (Loukianov *et al*; *Gene* 195:81-86), missing the third disulphide bond of the EGF-like domain, was also cloned.

[000395]The EGF-like domain of HB-EGF having SEQ ID NO:1082 (DPCLRKYKD FCIHGECKYVKELRAPSCICHPGYHGERCHGLSLP) was cloned into pSecTag and expressed and secreted as a Myc-His fusion protein.

2. Results

[000396]All of the U2-39; U2-42; U2-45; U2-26 and U2-19 antibody preparations recognize a discontinuous epitope. None of the antibodies recognize the Short form of the HB-EGF.

[000397]The binding site for all five antibody preparations is within the EGF-like domain, which included residues 44-86 of the mature protein having the following sequence: DPCLRKYKDFCIHGECKYVKELRAPSCICHPGYHGER CHGLSLP (SEQ ID NO:1082). The third disulfide bond in the EGF-like domain is required for binding of all of the U2-39; U2-42; U2-45; U2-26 and U2-19 antibody preparations.

3. Structure-Function Analysis of Antibody Binding Site by Site Directed Mutagenesis

[000398]Twelve independent mutations were created in HB-EGF by replacing one to four residues within the EGF-like domain of human pro-HB-EGF with the corresponding amino acid residue normally found in the mouse pro-HB-EGF.

[000399]Given that many of the present antibody preparations were species selective, site-directed mutagenesis for the purpose of identifying HB-EGF epitopes was done at known difference between the human HB-EGF protein and related proteins from different species. In particular, the amino acid differences between human and mouse HB-EGF are as follows: K122R; V124L; K125Q; I133K; and H135L.

[000400]A complete list of HB-EGF mutant polypeptide sequences for epitope mapping is provided in TABLE 10. Mutant HB-EGF nucleic acids encoding the desired mutant protein were transiently expressed in 293T cells, and monoclonal antibody binding was measured by ELISA.

[000401]Thus, HB-EGF mutant polypeptides were made with these mutations and such mutant polypeptides were tested to ascertain if the present antibody preparations still bound to the HB-EGF mutant polypeptide. If not, then the mutated amino acid was likely important for antibody binding, and hence formed an important part of an epitope.

[000402]TABLE 11 summarizes the binding results obtained, where "Yes" indicates that binding took place despite the indicated mutation, "No" indicates that binding was substantially eliminated by the indicated mutation, and "Reduced" indicates that reduced binding occurred when the mutation was present.

[000403]

[000404]TABLE 10

Wild type	LGKKRDPCLRKYKDFCIHGE-CKYVKELRAPSCICHPGYHGERCHGLSLP	SEQ ID NO:
F115Y	----------Y----------	1083
L127F	----------F----------	1084
E141H	----------H-------	1085
K122R; V124L; K125Q	----------R-LQ----------	1086
F115Y; K122R; V124L; K125Q	----------Y------R-LQ----------	1087
K122R; V124L; K125Q; E141H	----------R-LQ----------H-------	1088
I133K; H135L	----------K-L----------	1089
F115Y; I133K; H135L	----------Y----------K-L----------	1090
L127F; I133K; H135L	----------F-----K-L----------	1091
L148T	----------T-	1092
E141H; L148T	----------H-----T-	1093
117-120 IHGE changed to LHDGV	----------LHDGV----------	1094

TABLE 11
Binding Results

	Construct/ Mab	U2-42.3	U2-39.1	U2-19.3	U2-26.1	U2-45
1	Human proHB-EGF (WT)	Yes	Yes	Yes	Yes	Yes
2	F115Y	**No**	**No**	Yes	Yes	**Reduced**
3	L127F	Yes	**No**	Yes	Yes	Yes
4	E141H	**No**	Yes	Yes	Yes	Yes
5	K122R, V124L, K125Q	Yes	Yes	Yes	Yes	Yes
6	F115Y,K122R, V124L, K125Q	**No**	**No**	Yes	Yes	**Reduced**
7	K122R, V124L, K125Q, E141H	**No**	Yes	Yes	Yes	Yes
8	I133K, H135L	Yes	Yes	**No**	**No**	Yes
9	F115Y, I133K, H135L	**No**	**No**	**No**	**No**	**Reduced**
10	L127F,I133K, H135L,	Yes	**No**	**No**	**No**	Yes
11	S147T	Yes	Yes	Yes	Yes	Yes
12	E141H, S147T	**No**	Yes	Yes	Yes	Yes
13	IHGE (117-120) to LHDGV	Yes	Yes	**No**	**No**	**No**
	Critical residues	F115 & E141	F115 & L127	I133 or H135	I133 or H135	F115?

[000405]These binding studies indicate that the U2-42 and U2-39 antibody preparations recognize the diphtheria toxin binding domain and the F115, L127 and E141 residues are important for diphtheria toxin binding.

[000406]Furthermore, when the F115Y or E141H mutations are present in HB-EGF, binding is substantially eliminated for the U2-42 antibody preparations. Thus, Phe-115 and Glu-141 are important for U2-42 antibody binding. The U2-45 antibody preparation also appears to require Phe-115 because binding by this antibody preparation is reduced when Phe-115 is mutated.

[000407]The U2-39 antibody preparation requires Phe-115 and Leu-127 for binding HB-EGF because mutation of either of those residues substantially eliminates antibody binding.

[000408]The U2-19, U2-26 and U2-45 antibody preparations bind the conserved region between residues 117-120 (IHGE). As shown in TABLE 11, antibody preparations U2-19 and U2-26 also recognize an epitope at Ile-133 and/or His-135, because at least one of these residues is critical for their binding.

[000409]Based on their binding properties, of the antibodies were placed in the relationship "bins" listed in TABLE 12.

[000410]Binning is a method to group antibodies based on their competition for binding to the antigen (*see,* Jia *et al.,* 2004, *J. Immunol. Methods* 288:91-98).

[000411]The assignment of bins depended on how different the observed binding patterns for all the antibodies tested are. Therefore, bins do not always correlate with epitopes determined by other means and can be used to only roughly define epitopes.

[000412]

TABLE 12
Antibody Relationship Bins

Bin#1	Bin#2	Bin#3	Bin#4	Bin#5	Bin#6	Bin#7	Bin#8
U2-24.2	U2-1.3	U2-15.3	U2-13.3	U2-16.3	U2-18.3	U2-19.2	U2-3.2
U2-32.3		U2-30.2	U2-14.3		U2-38.1	U2-21.3	
U2-5.3			U2-2.1		1.19.2	U2-34.1	
U2-17.1			U2-57.1		U2-36.3	U2-26.2	
U2-56.3			U2-58.3		U2-40.3	U2-41.3	
			U2-61.1		U2-8.3	U2-45.3	
					U2-22.3	U2-46.3	
						U2-	
					U2-39.1	48.2.1	
					U2-54.2	U2-6.1	
						U2-51.2	
						U2-53.3	
						U2-28.2	

[000413]In general the epitope mapping of U2-45 antibody preparation indicates that the Bin 7 antibody preparations cross-react with mouse HB-EGF and human Amphiregulin.

[000414]Mutations of F115 to Ala or Tyr affect the binding affinity of U2-45 antibodies. However, U2-45 antibody binding was not affected by mutations of I133 and H135, which did affect some other Bin 7 antibody preparations. When the IHGE human HB-EGF sequence was changed to LHDGV, which is present in mouse HB-EGF and human Aphiregulin, all Bin7 antibody preparations failed to bind. Therefore, the IHGE residues (117-120) likely form the epitope for bin 7 antibody preparations.

[000415]Further site-directed mutagenesis studies involving binding of HB-EGF mutants to EGFR, indicate that residues including Asp-106 and Pro-107 are both necessary for optimal binding of HB-EGF to the EGF receptor. Moreover, Leu-148, which is necessary for HB-EGF

binding to the EGF receptor, did not appear to be involved in the binding of any of the anti-HB-EGF antibody preparations.

[000416]

HH. Example 24: Sequences Of Key Elements Of Anti HB-EGF Antibodies

[000417]This Example provides the sequences of antibody preparations in Figures 1 – 21.

[000418]Example 25A: Scratch assay - Inhibition of HB-EGF-induced migration of CLS354 epithelial squamous carcinoma cells (mouth)

Scratch experiments were performed in order to investigate whether the antibodies of the invention block cell migration that would otherwise be directly induced by HB-EGF.

[000419]1 x 10^6 CLS354 cells were seeded in medium (RPMI medium with 10% FCS) in 1 ml on a 12-well plate and serum starved (medium with 0.5% FCS) over night. After cells have reached a confluent layer, a scratch was performed in the middle of the well using a sterile plasic tip. Cells were washed with PBS and scratched CLS354 cells were treated alone or containing 20 ng/ml HB-EGF in the presence or absence of 10 µg/ml U2-39, Erbitux or human IgG. The experiment was stopped after 12 hour incubation at 37°C. Medium was withdrawn, cells were washed with PBS and fixed with 100% ice-cold methanol at -20°C, stained with crysal-violet, washed and dried over night. Photographes were taken for documentation.

[000420]Figure 40A shows that HB-EGF treatment stimulates the closure of the scratch and that the antibody of invention, U2-39, inhibits HB-EGF-mediated migration of CLS354 epithelial squamous carcinoma cells into the scratch.

[000421]Example 25B: Transmigration assay - Inhibition of HB-EGF-induced migration of Detroit 562 epithelial carcinoma cells (pharynx)

[000422]Transmigration experiments were performed in order to investigate whether the antibodies of the invention block cell migration that would otherwise be directly induced by HB-EGF.

[000423]A 500 ml cell suspension of serum-starved human epithelial carcinoma cells (50,000 cells) was placed in the top chamber of fibronectin-coated transwells (BD Falcon, 8 µm pores). Aliquots of 750 ml medium (Minimum essential medium (Eagle) in Earle's BSS with non-essential amino acids, sodium pyruvate (1 mM) and lactalbumin hydrolysate (0.1%), 90%; fetal bovine serum 10%, Pen.-Strept., 0,1% BSA) alone or containing 20 ng/ml HB-EGF (R&D Systems) in the presence or absence of 10 µg/ml human IgG, U2-39 or Erbitux antibodies were placed in the bottom chamber after 30 min pre-incubation at 37°C. After incubation and migration for 6hours at 37°C, cells were fixed, stained with DAPI and transwells were photographed for evaluation.

[000424]The result demonstrates that-HB-EGF antibody U2-39 effectively inhibits HB-EGF-induced Detroit 562 epithelial carcinoma cell migration comparable to the inhibition of HB-EGF-mediated cell migration by Erbitux treatment.

[000425]Example 26: Spheroid-based cellular angiogenesis assay – Inhibition of VEGF-stimulated endothelial cell sprouting

[000426]Spheroid-based cellular angiogenesis assays were performed in order to investigate whether the antibodies of the invention are able to inhibit VEGF-induced endothelial cell (EC) sprouting in a collagen matrix. Primary human umbilical vein endothelial cells (HUVEC) were seeded out at 500 cells in a hanging drop on plastic dishes to allow overnight spheroid aggregation. 50 EC spheroids were seeded in 0.9 ml of collagen solution (2 mg/ml) and pipetted into individual wells of a 24 well plate to allow polymerization The antibody of invention U2-39 was directly mixed in the collagen solution before polymerization (different concentrations) and the growth factor VEGF-A (25ng/ml) was added after 30 min by pipetting 100µl of a 10-fold concentrated working dilution on top of the polymerized gel. Plates were incubated at 37°C for 24 hours and fixed by adding 4% paraformaldehyde. Sprouting intensity of EC spheroids was quantitated by an image analysis system determining the cumulative sprout length per spheroid using an inverted microscope and the digital imaging software Analysis 3.2.

[000427]Figure 41A depicts the mean of the cumulative sprout length of 10 randomly selected spheroids per data point. Figure 41B shows the relative inhibition of the cumulative sprout length of 10 randomly selected spheroids per data point by U2-39. The fitting of IC50 curves and calculation of IC50 values was performed with GraphPad Prism 4.03.

[000428]The results of Example 26 demonstrate that the antibody of the invention U2-39 inhibits VEGF-A-stimulated human umbilical vein endothelial cell sprouting in a dose-dependent manner in the spheroid-based assay using a collagen matrix. HUVEC sprouting was inhibited with an IC50 value of 5.2×10^{-8} Molar.

[000429]Example 27: Immunohistochemistry (IHC) analysis of human tumor xenograft samples – Inhibition of CD31 staining of tumor *in vivo*.

[000430]In order to investigate the efficacy of the antibody of invention, U2-39, on inhibition of angiogenesis *in vivo*, human tumor xenografts treated with U2-39 or Erbitux were analyzed by immunohistochemistry analysis.

[000431]The human ovarian adenocarcinoma cell line EFO27 was genetically engineered to overexpress HB-EGF and the clone EFO27-Cl58 was chosen for xenograft studies in SCID mice. 3 x 10^6 EFO27-Cl58 cells in 100µl PBS/Matrigel (1:1) were injected subcutaneously into the left flank of 7 week old female C.B-17 SCID mice. Tumor-bearing mice with mean tumor volumes of 250 mm^3 were randomized into groups containing 10 animals. Animals were treated intraperitoneally with weekly doses of 25 mg/kg U2-39 or 25 mg/kg Erbitux or control vehicle, PBS, for 3 weeks. After 28 days mice were sacrificed, primary tumor tissues were collected and one half of the tumor was snap-frozen in liquid nitrogen and stored at -80°C.

[000432]5 to 8 µm sections of the tumor prepared on glass chamber slides were fixed in 100% acetone for 10 min at 4°C and dried completely. To block unspecific binding sites slides with fixed tumor sections were treated with Avidin D block (15 minutes), Biotin block (15 minutes) and a 1.25% BSA solution (1 hour). Between each treatment step slides were washed twice with PBS. For immunohistochemical examination of the tumor vasculature the expression of the classical endothelial cell marker CD31, also known as PECAM-1 (Platelet Endothelial Cell Adhesion Molecule-1) was analyzed by treatment of the slides with 2µg/ml anti-CD31 antibody (diluted in 1.25% BSA solution and incubated for 2h at room temperature in a humidified chamber). Detection was performed by applying a biotinylated goat ant-rat IgG antibody (30 min at room temperature) and Alexa 546 Streptavidin (15 min in the dark). PBS washing steps were performed between each treatment step. Sections were mounted with VECTASHIELD mounting medium with DAPI in the dark, photographed (fluorescence microscope) for documentation and stored at 4°C.

[000433]Figure 42 demonstrates that human tumor xenografts treated with U2-39 show a reduced endothelial cell marker staining (CD31 staining) compared to Erbitux-treated or control treated tumor xenografts. This result demonstrates the anti-angiogenic efficacy of the antibody of invention in vivo.

[000434]Example 28: In vivo ovarian tumor xenograft model – combination treatment of U2-39 with Cisplatin and Avastin

[000435]In order to evaluate the anti-tumor efficacy of the antibody of invention administered as a monotherapy or in combination with Cisplatin or Avastin, an ovarian cancer xenograft study was conducted.

[000436]The human ovarian adenocarcinoma cell line EFO27 was genetically engineered to overexpress HB-EGF. The clone EFO27-Cl58 was chosen for xenograft studies in SCID mice. 3 x 10^6 EFO27-Cl58 cells in 100µl PBS/Matrigel (1:1) were injected subcutaneously into the left flank of 7 week old female C.B-17 SCID mice. Tumor-bearing mice with mean tumor volumes between 75 and 175 mm^3 were randomized into groups containing 10 animals. Animals were treated intraperitoneally with weekly doses of 25 mg/kg U2-39, 25 mg/kg Avastin or 5 mg/kg Cisplatin or control vehicle, PBS. Combination of U2-39 with Avastin was given at 12.5 mg/kg each and combination of U2-39 with Cisplatin was given at 25 mg/kg antibody with 5 mg/kg Cisplatin. Primary tumor sizes were determined 3 times a week. Following calliper measurement, tumor size was calculated according to the formula W2xL/2 with L=length and W= the perpendicular width of the tumor. Kaplan-Meier log-rank method was used to define time to progression to 500 mm^3 (defined as "event" for statistical reasons).

[000437]Figure 43A demonstrates that combination of U2-39 with Cisplatin led to a stronger tumor reduction during the administration period than treatment with Cisplatin alone. In addition, a combination of U2-39 and Cisplatin delayed the time to progression of the median tumor size to 500 mm^3 compared to U2-39 monotherapy.

[000438]The result in Figure 43B shows that combination of U2-39 with Avastin significantly delayed the time to progression to 500 mm^3 tumor volumes compared to the treatment with Avastin as monotherapy although only half of the single agent dose was administered.

[000439]All patents and publications referenced or mentioned herein are indicative of the levels of skill of those skilled in the art to which the invention pertains, and each such referenced patent or publication is hereby incorporated by reference to the same extent as if it had been incorporated by reference in its entirety individually or set forth herein in its entirety. Applicants reserve the right to physically incorporate into this specification any and all materials and information from any such cited patents or publications.

[000440]The specific methods and compositions described herein are representative of preferred embodiments and are exemplary and not intended as limitations on the scope of the invention. Other objects, aspects, and embodiments will occur to those skilled in the art upon consideration of this specification, and are encompassed within the spirit of the invention as defined by the scope of the claims. It will be readily apparent to one skilled in the art that varying substitutions and modifications may be made to the invention disclosed herein without departing from the scope and spirit of the invention. The invention illustratively described herein suitably may be practiced in the absence of any element or elements, or limitation or limitations, which is not specifically disclosed herein as essential. The methods and processes illustratively described herein suitably may be practiced in differing orders of steps, and they are not

necessarily restricted to the orders of steps indicated herein or in the claims. As used herein and in the appended claims, the singular forms "a," "an," and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, a reference to "an antibody" includes a plurality (for example, a solution of antibodies or a series of antibody preparations) of such antibodies, and so forth. Under no circumstances may the patent be interpreted to be limited to the specific examples or embodiments or methods specifically disclosed herein. Under no circumstances may the patent be interpreted to be limited by any statement made by any Examiner or any other official or employee of the Patent and Trademark Office unless such statement is specifically and without qualification or reservation expressly adopted in a responsive writing by Applicants.

[000441]The terms and expressions that have been employed are used as terms of description and not of limitation, and there is no intent in the use of such terms and expressions to exclude any equivalent of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention as claimed. Thus, it will be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and such modifications and variations are considered to be within the scope of this invention as defined by the appended claims.

[000442]The invention has been described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form part of the invention. This includes the generic description of the invention with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

Claims

1. An isolated antigen binding protein that binds HB-EGF, comprising:

A) one or more light chain complementary determining regions (CDRLs) selected from the group consisting of:

(i) a CDRL1 selected from the group consisting of SEQ ID NOs:189-217;

(ii) a CDRL2 selected from the group consisting of SEQ ID NOs:218-233;

(iii) a CDRL3 selected from the group consisting of SEQ ID NOs:234-274; and

(iv) a CDRL of (i), (ii) or (iii) that contains one or more amino acid substitutions, deletions or insertions of no more than four amino acids; or

B) one or more heavy chain complementary determining regions (CDRHs) selected from the group consisting of:

(i) a CDRH1 selected from the group consisting of SEQ ID NOs:275-299;

(ii) a CDRH2 selected from the group consisting of SEQ ID NOs:300-331;

(iii) a CDRH3 selected from the group consisting of SEQ ID NOs:332-372; and

(iv) a CDRH of (i), (ii) or (iii) that contains one or more amino acid substitutions, deletions or insertions of no more than four amino acids.

2. The isolated antigen binding protein of Claim 1, comprising one or more light chain CDRLs of A), and one or more heavy chain CDRHs of B).

3. The isolated antigen binding protein of Claim 1 that comprises at least two CDRLs of A) and at least two CDRHs of B).

4. The isolated antigen binding protein of Claim 1 that comprises said CDRH1, CDRH2, CDRH3, CDRL1, CDRL2 and CDRL3.

5. The isolated antigen binding protein of Claim 1, wherein

said CDRL of A) is selected from the group consisting of:

(i) a CDRL1 selected from the group consisting of SEQ ID NOs:189-217;

(ii) a CDRL2 selected from the group consisting of SEQ ID NOs:218-233;

(iii) a CDRL3 selected from the group consisting of SEQ ID NOs:234-274; and

(iv) a CDRL of (i), (ii) or (iii) that contains one or more amino acid substitutions, deletions or insertions of no more than two amino acids;

said CDRH of B) is selected from the group consisting of:

(i) a CDRH1 selected from the group consisting of SEQ ID NOs:275-299;

(ii) a CDRH2 selected from the group consisting of SEQ ID NOs:300-331;

(iii) a CDRH3 selected from the group consisting of SEQ ID NOs:332-372; and

(iv) a CDRH of (i), (ii) or (iii) that contains one or more amino acid substitutions, deletions or insertions of no more than two amino acids;

or

C) one or more light chain CDRLs of A) and one or more heavy chain CDRHs of B).

6. The isolated antigen binding protein of Claims 1, wherein said antigen binding protein comprises

A) a CDRL selected from the group consisting of

(i) a CDRL1 selected from the group consisting of SEQ ID NOs:189-217;

(ii) a CDRL2 selected from the group consisting of SEQ ID NOs:218-233; and

(iii) a CDRL3 selected from the group consisting of SEQ ID NOs:234-274;

B) a CDRH selected from the group consisting of

(i) a CDRH1 selected from the group consisting of SEQ ID NOs:275-299;

(ii) a CDRH2 selected from the group consisting of SEQ ID NOs:300-331;and

(iii) a CDRH3 selected from the group consisting of SEQ ID NOs:332-372;

or

C) one or more light chain CDRLs of A) and one or more heavy chain CDRHs of B).

7. The isolated antigen binding protein of Claims 6, wherein said antigen binding protein comprises

A) a CDRL1 of SEQ ID NOs:189-217, a CDRL2 of SEQ ID NOs:218-233, and a CDRL3 of SEQ ID NOs:234-274, and/or

B) a CDRH1 of SEQ ID NOs:275-299, a CDRH2 of SEQ ID NOs:300-331, and a CDRH3 of SEQ ID NOs:332-372.

8. The isolated antigen binding protein of Claim 1, wherein said antigen binding protein comprises a light chain variable region (V_L) having at least 80% sequence identity with an amino acid sequence selected from the group consisting of SEQ ID NOs:94-141, and/or a heavy chain variable region (V_H) having at least 80% sequence identity with an amino acid sequence selected from the group consisting of SEQ ID NOs:142-186.

9. The isolated antigen binding protein of Claim 8, wherein the V_L has at least 90% sequence identity with an amino acid sequence selected from the group consisting of SEQ ID NOs:94-141, and/or the V_H has at least 90% sequence identity with an amino acid sequence selected from the group consisting of SEQ ID NOs:142-186.

10. The isolated antigen binding protein of Claim 8, wherein the V_L is selected from the group consisting of SEQ ID NOs:94-141, and/or the V_H is selected from the group consisting of SEQ ID NOs:142-186.

11. An isolated antigen binding protein that specifically recognizes at least an IHGE-containing epitope and/or an EGF-like domain of HB-EGF.

12. An isolated antigen binding protein that competes for binding with the antigen binding protein of Claims 1.

13. An isolated antigen binding protein that binds HB-EGF, wherein said antigen binding protein comprises:

A) one or more light chain CDRs (CDRLs) selected from the group consisting of:

(i) a CDRL1 with at least 80% sequence identity to SEQ ID NOs:189-217;

(ii) a CDRL2 with at least 80% sequence identity to SEQ ID NOs:218-233; and

(iii) a CDRL3 with at least 80% sequence identity to SEQ ID NOs:234-274;

B) one or more heavy chain CDRs (CDRHs) selected from the group consisting of:

(i) a CDRH1 with at least 80% sequence identity to SEQ ID NOs:275-299;

(ii) a CDRH2 with at least 80% sequence identity to SEQ ID NOs:300-331; and

(iii) a CDRH3 with at least 80% sequence identity to SEQ ID NOs:332-372;

or

C) one or more light chain CDRLs of A) and one or more heavy chain CDRHs of B).

14. The isolated antigen binding protein of Claims 13, wherein said antigen binding protein comprises:

A) one or more CDRLs selected from the group consisting of:

(i) a CDRL1 with at least 90% sequence identity to SEQ ID NOs:189-217;

(ii) a CDRL2 with at least 90% sequence identity to SEQ ID NOs:218-233; and

(iii) a CDRL3 with at least 90% sequence identity to SEQ ID NOs:234-274;

B) one or more CDRHs selected from the group consisting of:

(i) a CDRH1 with at least 90% sequence identity to SEQ ID NOs:275-299;

(ii) a CDRH2 with at least 90% sequence identity to SEQ ID NOs:300-331; and

(iii) a CDRH3 with at least 90% sequence identity to SEQ ID NOs:332-372;

or

C) one or more light chain CDRLs of A) and one or more heavy chain CDRHs of B).

15. An isolated antigen binding protein that binds HB-EGF, the antigen binding protein comprising:

A) a light chain complementary determining region (CDRL) selected from the group consisting of

(i) a CDRL3 selected from the group consisting of SEQ ID NOs:234-274,

(ii) a CDRL3 that differs in amino acid sequence from the CDRL3 of (i) by an amino acid addition, deletion or substitution of not more than two amino acids; and

(iii) a CDRL3 amino acid sequence selected from the group consisting of

$X_1QX_2X_3X_4X_5PX_6X_7$ (SEQ ID NO:1046), wherein

X_1 is selected from the group consisting of I and M,

X_2 is selected from the group consisting of A, G and S,

X_3 is selected from the group consisting of I and T,

X_4 is selected from the group consisting of H and Q,

X_5 is selected from the group consisting of F, L and W,

$7X_6$ is selected from the group consisting of C, I, H, L and T,

X_7 is selected from the group consisting of S and T;

$QQX_1X_2X_3X_4X_5IT$ (SEQ ID NO:1047), wherein

X_1 is selected from the group consisting of I and S,

X_2 is selected from the group consisting of F and Y,

X_3 is selected from the group consisting of F, I, S and Y,

X_4 is selected from the group consisting of A, S and T,

X_5 is selected from the group consisting of P and S;

$X_1X_2X_3X_4X_5X_6X_7X_8T$ (SEQ ID NO:1048), wherein

X_1 is selected from the group consisting of L and Q,

X_2 is selected from the group consisting of K, N and Q,

X_3 is selected from the group consisting of A, H, S and Y,

X_4 is selected from the group consisting of H, N and Y,

X_5 is selected from the group consisting of N, S and T,

X_6 is selected from the group consisting of A, F, I, T, V and Y,

X_7 is selected from the group consisting of P and no amino acid,

X_8 is selected from the group consisting of F, L and P;

$QX_1X_2DX_3LPX_4X_5$ (SEQ ID NO:1049), wherein

X_1 is selected from the group consisting of H and Q,

X_2 is selected from the group consisting of C and Y,

X_3 is selected from the group consisting of D, I, N, S and Y,

X_4 is selected from the group consisting of F, I and L,

X_5 is selected from the group consisting of A, S and T;

$QQX_1X_2X_3X_4PX_5X_6X_7$ (SEQ ID NO:1050), wherein

X_1 is selected from the group consisting of H and Y,

X_2 is selected from the group consisting of G and N,

X_3 is selected from the group consisting of N and S,

X_4 is selected from the group consisting of S and W,

X_5 is selected from the group consisting of P and no amino acid,

X_6 is selected from the group consisting of R and W,

X_7 is selected from the group consisting of S and T; and

$X_1QYX_2X_3X_4X_5X_6X_7F$ (SEQ ID NO:1051), wherein

X_1 is selected from the group consisting of H and Q,

X_2 is selected from the group consisting of F and Y,

X_3 is selected from the group consisting of G, I and S,

X_4 is selected from the group consisting of F, I and T,

X_5 is selected from the group consisting of M, P, S and T,

X_6 is selected from the group consisting of F, L, R and W,

X_7 is selected from the group consisting of S and T; and/or

B) a heavy chain complementary determining region (CDRH) selected from the group consisting of

(i) a CDRH3 selected from the group consisting of SEQ ID NOs:332-372,

(ii) a CDRH3 that differs in amino acid sequence from the CDRH3 of (i) by an amino acid addition, deletion or substitution of not more than two amino acids; and

(iii) a CDRH3 amino acid sequence selected from the group consisting of

$X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}DX_{12}$ (SEQ ID NO:1065), wherein

X_1 is selected from the group consisting of E and S,

X_2 is selected from the group consisting of D, G and no amino acid,

X_3 is selected from the group consisting of D, N and no amino acid,

X_4 is selected from the group consisting of G and no amino acid,

X_5 is selected from the group consisting of G and no amino acid,

X_6 is selected from the group consisting of W, Y and no amino acid,

X_7 is selected from the group consisting of I, N and Y,

X_8 is selected from the group consisting of A and Y,

X_9 is selected from the group consisting of G, V and Y,

X_{10} is selected from the group consisting of A, F and G,

X_{11} is selected from the group consisting of F, L and M,

X_{12} is selected from the group consisting of V and Y;

$QX_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}YX_{12}X_{13}X_{14}DX_{15}$ (SEQ ID NO:1066), wherein

X_1 is selected from the group consisting of G and no amino acid,

X_2 is selected from the group consisting of K, L and Y,

X_3 is selected from the group consisting of A, G and S,

X_4 is selected from the group consisting of S, V and Y,

X_5 is selected from the group consisting of A and G,

X_6 is selected from the group consisting of G and no amino acid,

X_7 is selected from the group consisting of T and no amino acid,

X_8 is selected from the group consisting of S and no amino acid,

X_9 is selected from the group consisting of Y and no amino acid,

X_{10} is selected from the group consisting of W and Y,

X_{11} is selected from the group consisting of G, S and Y,

X_{12} is selected from the group consisting of F and Y,

X_{13} is selected from the group consisting of G and no amino acid,

X_{14} is selected from the group consisting of M and no amino acid,

X_{15} is selected from the group consisting of V and Y;

$X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}X_{14}$ (SEQ ID NO:1067), wherein

X_1 is selected from the group consisting of D, G, L, S and no amino acid,

X_2 is selected from the group consisting of G, H, W, Y and no amino acid,

X_3 is selected from the group consisting of A, F, W, Y and no amino acid,

X_4 is selected from the group consisting of D, G, Q, T and no amino acid,

X_5 is selected from the group consisting of G, I, Q, S and no amino acid,

X_6 is selected from the group consisting of A, D, N, Q, S and no amino acid,

X_7 is selected from the group consisting of G, Y and no amino acid,

X_8 is selected from the group consisting of D, Y and no amino acid,

X_9 is selected from the group consisting of Y and no amino acid,

X_{10} is selected from the group consisting of A, E, N and Y,

X_{11} is selected from the group consisting of G, P, T, V and Y,

X_{12} is selected from the group consisting of F and I,

X_{13} is selected from the group consisting of D and Q,

X_{14} is selected from the group consisting of C, H, V and Y;

$X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}X_{14}X_{15}X_{16}X_{17}DX_{18}$ (SEQ ID NO:1068), wherein

X_1 is selected from the group consisting of E, D and no amino acid,

X_2 is selected from the group consisting of G, R and no amino acid,

X_3 is selected from the group consisting of I, V, Y and no amino acid,

X_4 is selected from the group consisting of A, G, L and N,

X_5 is selected from the group consisting of A, G, V and W,

X_6 is selected from the group consisting of A, N, R and T,

X_7 is selected from the group consisting of G, N, P and no amino acid,

X_8 is selected from the group consisting of G, T and no amino acid,

X_9 is selected from the group consisting of A and no amino acid,

X_{10} is selected from the group consisting of D, E and no amino acid,

X_{11} is selected from the group consisting of S, Y and no amino acid,

X_{12} is selected from the group consisting of G, Y and no amino acid,

X_{13} is selected from the group consisting of N, Y and no amino acid,

X_{14} is selected from the group consisting of Y and no amino acid,

X_{15} is selected from the group consisting of D, Y and no amino acid,

X_{16} is selected from the group consisting of A, G and no amino acid,

X_{17} is selected from the group consisting of F and M,

X_{18} is selected from the group consisting of I, V and Y;

$X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}X_{12}X_{13}X_{14}X_{15}X_{16}X_{17}X_{18}X_{19}X_{20}X_{21}X_{22}X_{23}$ (SEQ ID NO:1069), wherein

X_1 is selected from the group consisting of A, D, G, S and T,

X_2 is selected from the group consisting of A, E, G, L, N, R, Y and no amino acid,

X_3 is selected from the group consisting of A, G, L, N, R, T, Y and no amino acid,

X_4 is selected from the group consisting of D, G, R, S, V, Y and no amino acid,

X_5 is selected from the group consisting of A, G, I, S, V, Y and no amino acid,

X_6 is selected from the group consisting of F, G, L, R, V and no amino acid,

X_7 is selected from the group consisting of L, T, Y and no amino acid,

X_8 is selected from the group consisting of Y and no amino acid,

X_9 is selected from the group consisting of Y and no amino acid,

X_{10} is selected from the group consisting of D and no amino acid,

X_{11} is selected from the group consisting of S and no amino acid,

X_{12} is selected from the group consisting of S and no amino acid,

X_{13} is selected from the group consisting of G and no amino acid,

X_{14} is selected from the group consisting of D, L, M, S, Y and no amino acid,

X_{15} is selected from the group consisting of H, I, P, V, W and no amino acid,

X_{16} is selected from the group consisting of F, G, L, R, S, Y and no amino acid,

X_{17} is selected from the group consisting of D, F, V, W, Y and no amino acid,

X_{18} is selected from the group consisting of C, F, L, P, S and Y,

X_{19} is selected from the group consisting of D, F, G and Y,

X_{20} is selected from the group consisting of A, C, G, P, R, V and Y,

X_{21} is selected from the group consisting of F, L, M, S and no amino acid,

X_{22} is selected from the group consisting of A, D and no amino acid,

X_{23} is selected from the group consisting of I, L, V, Y and no amino acid;

$X_1YSSGWX_2X_3YGX_4X_5DX_6$ (SEQ ID NO:1070), wherein

X_1 is selected from the group consisting of M and V,

X_2 is selected from the group consisting of S and no amino acid,

X_3 is selected from the group consisting of F and no amino acid,

X_4 is selected from the group consisting of V and no amino acid,

X_5 is selected from the group consisting of F and M,

X_6 is selected from the group consisting of V and Y; and

$RX_1X_2X_3PFX_4Y$ (SEQ ID NO:1071), wherein

X_1 is selected from the group consisting of G, H, L, N and R,

X_2 is selected from the group consisting of E, T and W,

X_3 is selected from the group consisting of L, N, T and V,

X_4 is selected from the group consisting of D and E.

16. The isolated antigen binding protein of Claim 15, said antigen binding protein further comprising:

A) a CDRL selected from the group consisting of:

(i) a CDRL1 selected from the group consisting of SEQ ID NOs:189-217;

(ii) a CDRL1 that differs in amino acid sequence from the CDRL1 of (i) by an amino acid addition, deletion or substitution of not more than two amino acids;

(iii) a CDRL1 amino acid sequence selected from the group consisting of

$X_1SSQSLX_2X_3SDGX_4TYLX_5$ (SEQ ID NO:1035), wherein

X_1 is selected from the group consisting of K and R,

X_2 is selected from the group consisting of L and V,

X_3 is selected from the group consisting of H and Y,

X_4 is selected from the group consisting of K and N,

X_5 is selected from the group consisting of N, S and Y;

$RASQX_1ISX_2YLN$ (SEQ ID NO:1036), wherein

X_1 is selected from the group consisting of R, S and T,

X_2 is selected from the group consisting of R and S;

$RASQX_1IX_2X_3X_4LX_5$ (SEQ ID NO:1037), wherein

X_1 is selected from the group consisting of D, G, S and T,

X_2 is selected from the group consisting of A, R and S,

X_3 is selected from the group consisting of H, I, N, R, S and T,

X_4 is selected from the group consisting of D, W and Y,

X_5 is selected from the group consisting of A, G and N;

$QASQDIX_1X_2X_3LN$ (SEQ ID NO:1038), wherein

X_1 is selected from the group consisting of S and T,

X_2 is selected from the group consisting of D and N,

X_3 is selected from the group consisting of S and Y;

$RASQX_1VX_2X_3X_4X_5LA$ (SEQ ID NO:1039), wherein

X_1 is selected from the group consisting of S and T,

X_2 is selected from the group consisting of I and S,

X_3 is selected from the group consisting of R and S,

X_4 is selected from the group consisting of S, N and no amino acid,

X_5 is selected from the group consisting of Y and no amino acid; and

$KSSQX_1X_2LX_3X_4SNNKNYLX_5$ (SEQ ID NO:1040), wherein

X_1 is selected from the group consisting of N and S,

X_2 is selected from the group consisting of I and V,

X_3 is selected from the group consisting of D and Y,

X_4 is selected from the group consisting of N, R and S,

X_5 is selected from the group consisting of A and V;

(iv) a CDRL2 selected from the group consisting of SEQ ID NOs:218-233;

(v) a CDRL2 that differs in amino acid sequence from the CDRL2 of (iv) by an amino acid addition, deletion or substitution of not more than two amino acids; and

(vi) a CDRL2 amino acid sequence selected from the group consisting of

$X_1X_2SNX_3X_4S$ (SEQ ID NO:1041), wherein

X_1 is selected from the group consisting of E and K,

X_2 is selected from the group consisting of I and V,

X_3 is selected from the group consisting of R and W,

X_4 is selected from the group consisting of D and F;

$X_1X_2SX_3LQS$ (SEQ ID NO:1042), wherein

X_1 is selected from the group consisting of A and T,

X_2 is selected from the group consisting of A, E and V,

X_3 is selected from the group consisting of S and T;

X_1ASX_2LQS (SEQ ID NO:1043), wherein

X_1 is selected from the group consisting of A and V,

X_2 is selected from the group consisting of S and T;

$DASX_1LET$ (SEQ ID NO:1044), wherein

X_1 is selected from the group consisting of I and N;

GASSRAT (SEQ ID NO:223); and

$WASX_1RES$ (SEQ ID NO:1045), wherein

X_1 is selected from the group consisting of A and T; or

B) a CDRH selected from the group consisting of:

(i) a CDRH1 selected from the group consisting of SEQ ID NOs:275-299;

(ii) a CDRH1 that differs in amino acid sequence from the CDRH1 of (i) by an amino acid addition, deletion or substitution of not more than two amino acids;

(iii) a CDRH1 amino acid sequence selected from the group consisting of

$GYTX_1TX_2X_3X_4X_5X_6$ (SEQ ID NO:1052), wherein

X_1 is selected from the group consisting of F and L,

X_2 is selected from the group consisting of E, G and S,

X_3 is selected from the group consisting of H, L and Y,

X_4 is selected from the group consisting of G, S and Y,

X_5 is selected from the group consisting of I and M,

X_6 is selected from the group consisting of H and S;

$GYX_1FTSYWIG$ (SEQ ID NO:1053), wherein

X_1 is selected from the group consisting of R and S;

$GFTFX_1SX_2X_3MH$ (SEQ ID NO:1054), wherein

X_1 is selected from the group consisting of R and S,

X_2 is selected from the group consisting of H and Y,

X_3 is selected from the group consisting of D and G;

$GFX_1FSX_2YX_3MX_4$ (SEQ ID NO:1055), wherein

X_1 is selected from the group consisting of P and T,

X_2 is selected from the group consisting of A, R and S,

X_3 is selected from the group consisting of A and S,

X_4 is selected from the group consisting of N and S;

$GX_1SX_2SX_3X_4X_5X_6X_7WX_8$ (SEQ ID NO:1056), wherein

X_1 is selected from the group consisting of D and G,

X_2 is selected from the group consisting of F, I and V,

X_3 is selected from the group consisting of R, S and no amino acid,

X_4 is selected from the group consisting of G, Y and no amino acid,

X_5 is selected from the group consisting of D, G, S and no amino acid,

X_6 is selected from the group consisting of A, S and Y,

X_7 is selected from the group consisting of A and Y,

X_8 is selected from the group consisting of N and S;

GFSLSNARMGVS (SEQ ID NO:279); and

$GFSLX_1TGGVGVG$ (SEQ ID NO:1057), wherein

X_1 is selected from the group consisting of S and N;

(iv) a CDRH2 selected from the group consisting of SEQ ID NOs:300-331;

(v) a CDRH2 that differs in amino acid sequence from the CDRH2 of (iv) by an amino acid addition, deletion or substitution of not more than two amino acids; and

(vi) a CDRH2 amino acid sequence selected from the group consisting of

$X_1X_2X_3X_4X_5X_6GX_7TX_8X_9X_{10}QKX_{11}X_{12}$ (SEQ ID NO:1058), wherein

X_1 is selected from the group consisting of S and W,

X_2 is selected from the group consisting of F and I,

X_3 is selected from the group consisting of D, N and S,

X_4 is selected from the group consisting of A and P,

X_5 is selected from the group consisting of E, N and S,

X_6 is selected from the group consisting of D, N and S,

X_7 is selected from the group consisting of E, G and N,

X_8 is selected from the group consisting of I and N,

X_9 is selected from the group consisting of C, H and Y,

X_{10} is selected from the group consisting of A and T,

X_{11} is selected from the group consisting of F and L,

X_{12} is selected from the group consisting of D and G;

IIYPX_1DSDX_2RYSPSFQG (SEQ ID NO:1059), wherein

X_1 is selected from the group consisting of D and G,

X_2 is selected from the group consisting of A, I and T;

X_1IX_2X_3DGS$X_4X_5X_6$YX_7DSVX_8G (SEQ ID NO:1060), wherein

X_1 is selected from the group consisting of F and V,

X_2 is selected from the group consisting of S and W,

X_3 is selected from the group consisting of D, S and Y,

X_4 is selected from the group consisting of I, N and T,

X_5 is selected from the group consisting of K and Q,

X_6 is selected from the group consisting of N, R and Y,

X_7 is selected from the group consisting of A, T and V,

X_8 is selected from the group consisting of K and R;

X_1ISX_2S$X_3X_4X_5X_6$YYADSVKG (SEQ ID NO:1061), wherein

X_1 is selected from the group consisting of A, H and Y,

X_2 is selected from the group consisting of G, R and S,

X_3 is selected from the group consisting of G and S,

X_4 is selected from the group consisting of G, R and S,

X_5 is selected from the group consisting of S, T and Y,

X_6 is selected from the group consisting of I and T;

$X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}YX_{12}X_{13}SX_{14}$KS (SEQ ID NO:1062), wherein

X_1 is selected from the group consisting of E, R and Y,

X_2 is selected from the group consisting of I and T,
X_3 is selected from the group consisting of H, N and Y,
X_4 is selected from the group consisting of C, H, S, T and Y,
X_5 is selected from the group consisting of S and R,
X_6 is selected from the group consisting of G and S,
X_7 is selected from the group consisting of G, K, S and T,
X_8 is selected from the group consisting of T and W,
X_9 is selected from the group consisting of N and Y,
X_{10} is selected from the group consisting of N and no amino acid,
X_{11} is selected from the group consisting of D and no amino acid,
X_{12} is selected from the group consisting of A and N,
X_{13} is selected from the group consisting of P and V,
X_{14} is selected from the group consisting of L and V;

X_1IFSNDEKSYSTSLKS (SEQ ID NO:1063), wherein
X_1 is selected from the group consisting of H and LI; and

LIYWNX_1X_2KRYSPSLX_3S (SEQ ID NO:1064), wherein
X_1 is selected from the group consisting of D and V,
X_2 is selected from the group consisting of D and E,
X_3 is selected from the group consisting of K and R.

17. The isolated antigen binding protein of Claim 16, wherein said antigen binding protein comprises said first amino acid sequence and said second amino acid sequence.

18. The isolated antigen binding protein of Claim 17, wherein said first amino acid sequence is covalently bonded to said second amino acid sequence.

19. The isolated antigen binding protein of Claim 17, wherein said first amino acid sequence comprises said CDRL3 of SEQ ID NOs:234-274, CDRL2 of SEQ ID NOs:218-233, and CDRL1 of SEQ ID NOs: 189-217, and said second amino acid sequence comprises said CDRH3 of SEQ ID NOs:332-372, CDRH2 of SEQ ID NOs:300-331, and CDRH1 of SEQ ID NOs:275-299.

20. The isolated antigen binding protein of any of Claim 1-19, wherein said antigen binding protein is a monoclonal antibody, a polyclonal antibody, a recombinant antibody, a human antibody, a humanized antibody, a chimeric antibody, a multispecific antibody, or an antibody fragment thereof.

21. The isolated antigen binding protein of Claim 20, wherein said antibody fragment is a Fab fragment, a Fab' fragment, a $F(ab')_2$ fragment, a Fv fragment, a diabody, or a single chain antibody molecule.

22. The isolated antigen binding protein of Claim 20, wherein said antigen binding protein is a human antibody.

23. The isolated antigen binding protein of Claim 20, wherein said antigen binding protein is a monoclonal antibody.

24. The isolated antigen binding protein of any of Claims 1-19 wherein said antigen binding protein is of the IgG1-, IgG2- IgG3- or IgG4-type.

25. The isolated antigen binding protein of Claim 24, wherein said antigen binding protein is of the IgG2- or IgG4-type.

26. The isolated antigen binding protein of any of Claims 1-19, wherein said antigen binding protein is coupled to a labeling group.

27. The isolate antigen binding protein of Claim 26, wherein the labeling group is a radioisotope, radionuclide, a fluorescent group, an enzymatic group, a chemiluminescent group, a biotinyl group, or a predetermined polypeptide group.

28. The isolated antigen binding protein of any of Claims 1-19, wherein said antigen binding protein is coupled to an effector group.

29. The isolated antigen binding protein of Claim 28, wherein said effector group is a radioisotope, a radionuclide, a toxin, a therapeutic group, or a chemotherapeutic group.

30. The isolated antigen binding protein of Claim 29, wherein said therapeutic group or chemotherapeutic group is calicheamicin, auristatin-PE, geldanamycin, maytanasine, or derivatives thereof.

31. An isolated antigen binding protein that competes for binding to human HB-EGF with an antigen binding protein of one of Claims 1-19.

32. The isolated antigen binding protein of Claim 31, wherein said antigen binding protein is a monoclonal antibody, a polyclonal antibody, a recombinant antibody, a human antibody, a humanized antibody, a chimeric antibody, a multispecific antibody, or an antibody fragment thereof.

33. The isolated antigen binding protein of Claim 32, wherein said antibody fragment is a Fab fragment, a Fab' fragment, a $F(ab')_2$ fragment, a Fv fragment, a diabody, or a single chain antibody molecule.

34. The isolated antigen binding protein of Claim 32, wherein said antigen binding protein is a human antibody.

35. The isolated antigen binding protein of Claim 32, wherein said antigen binding protein is a monoclonal antibody.

36. The isolated antigen binding protein of any of Claim 31, wherein said antigen binding protein is of the IgG1-, IgG2- IgG3- or IgG4-type.

37. The isolated antigen binding protein of Claim 36, wherein said antigen binding protein is of the IgG2- or the IgG4-type.

38. The isolated antigen binding protein of any of Claim 31, wherein said antigen binding protein is coupled to a labeling group.

39. The isolate antigen binding protein of Claim 38, wherein the labeling group is a radioisotope, radionuclide, a fluorescent group, an enzymatic group, a chemiluminescent group, a biotinyl group, or a predetermined polypeptide group.

40. The isolated antigen binding protein of Claim 31, wherein said antigen binding protein is coupled to an effector group.

41. The isolated antigen binding protein of Claim 40, wherein said effector group is a radioisotope, a radionuclide, a toxin, a therapeutic group, or a chemotherapeutic group.

42. The isolated antigen binding protein of Claim 41, wherein said therapeutic group or chemotherapeutic group is calicheamicin, auristatin-PE, geldanamycin, maytanasine, or derivatives thereof.

43. The isolated antigen binding protein of one of Claims 1-19, wherein said antigen binding protein reduces at least partially HB-EGF-mediated signal transduction..

44. A nucleic acid molecule encoding the antigen binding protein according to any one of Claims 1-19.

45. The nucleic acid molecule according to Claim 44, wherein said nucleic acid molecule is operably linked to a control sequence.

46. A vector comprising a nucleic acid molecule according to Claim 44.

47. A vector comprising a nucleic acid molecule according to Claim 45.

48. A host cell comprising the nucleic acid molecule according to Claim 45.

49. A host cell comprising the vector according to one of Claim 46 or 47.

50. A method of making the antigen binding protein according to any one of Claims 1-19, comprising the step of preparing said antigen binding protein from a host cell that secretes said antigen binding protein.

51. A pharmaceutical composition comprising at least one antigen binding protein according to any one of Claims 1-19, and pharmaceutically acceptable carrier, diluents and/or adjuvants.

52. The pharmaceutical composition of Claim 51, further comprises an additional active agent.

53. The pharmaceutical composition according to Claim 52, wherein the at least one further active agent is an anti-neoplastic agent.

54. The pharmaceutical composition of Claim 53, wherein the anti-neoplastic agent is an anti-tumor antibody.

55. The pharmaceutical composition of Claim 54, wherein the anti-tumor antibody is an antibody directed against a receptor tyrosine kinase.

56. The pharmaceutical composition of Claim 53, wherein the anti-tumor antibody is directed against EGFR.

57. The pharmaceutical composition of Claim 51, for the diagnosis, prevention or treatment of a hyperproliferative disease.

58. The pharmaceutical composition to Claim 57, wherein said hyperproliferative disease is associated with HB-EGF expression.

59. The pharmaceutical composition according to Claim 57, wherein said hyperproliferative disease is associated with or accompanied by a disturbed, *e.g.*,pathologically enhanced growth factor receptor activation.

60. The pharmaceutical composition of Claim 59, wherein said pathologically enhanced growth factor receptor activation is associated with or caused by a pathological increase in the activity of a G protein and/or a G protein coupled receptor.

61. The pharmaceutical composition of Claim 51 for the diagnosis, prevention or treatment of cancer.

62. The pharmaceutical composition Claim 61, wherein said cancer is selected from the group consisting of breast cancer, gastrointestinal cancer, pancreas cancer, prostate cancer, ovarian cancer, stomach cancer, endometrial cancer, salivary gland cancer, lung cancer, kidney cancer, colon cancer, colorectal cancer, thyroid cancer, bladder cancer, glioma, melanoma, carcinoma, in particular epithelial or squamous carcinoma, other HB-EGF expressing or overexpressing cancers, and formation of tumor metastases.

63. Use of at least one antigen binding protein of Claims 1-19, for the manufacture of a pharmaceutical composition for the diagnosis, prevention or treatment of a hyperproliferative disease.

64. The use according to Claim 63, wherein said hyperproliferative disease is a hyperproliferative disease as defined in Claim 58.

65. A method for diagnosing a condition associated with the expression of HB-EGF, comprising contacting a sample with an antigen binding protein of Claims 1-19, and determining the presence of HB-EGF in said sample.

66. The method according to Claim 65, wherein the condition is a hyperproliferative disease as defined in Claim 58.

67. A method for preventing or treating a condition associated with the expression of HB-EGF in a patient, comprising administering to a patient in need thereof an effective amount of at least one antigen binding protein of Claims 1-19.

68. The method according to Claim 62, wherein the condition is a hyperproliferative disease as defined in any one of Claims 57-60.

69. The method of Claim 57, wherein the patient is a mammalian patient.

70. A kit comprising a antigen binding protein of Claims 1-19, a nucleic acid molecule of Claim 44 or 45 or a vector according to Claim 46 or 47.

71. The kit of Claim 70 comprising at least one further active agent.

72. The kit of Claim 71, wherein the further active agent is an anti-neoplastic agent.

73. The pharmaceutical composition according to claim 53, wherein the the anti-neoplastic agent id Cisplatin or Avastin.

74. The pharmaceutical composition according to any of the claims 51 to 62 which is to be administered as a monotherapy or in combination with a further pharmaceutical composition preferably comprising an anti-neoplastic agent such as cisplatin or Avastin.