ANTIBODIES DIRECTED TO ANGIOPOIETIN-2 AND USES THEREOF

[0001] This application claims benefit to U.S. Provisional Application Serial No. 60/638,354, filed December 21, 2004, and U.S. Provisional Application Serial No. 60/711,289, filed August 25, 2005, which are incorporated herein by reference.

FIELD

[0002] The invention relates to monoclonal antibodies against Angiopoietin-2 (Ang-2) and uses of such antibodies. More specifically, the invention relates to fully human monoclonal antibodies directed to Ang-2 and uses of these antibodies. Aspects of the invention also relate to hybridomas or other cell lines expressing such antibodies. The described antibodies are useful as diagnostics and for the treatment of diseases associated with the activity and/or overproduction of Ang-2.

BACKGROUND

[0003] Angiogenesis is the process of forming new capillaries from preexisting blood vessels and is an essential component of embryogenesis, normal physiological growth, repair, and tumor expansion. Although a variety of factors can modulate endothelial cell (EC) responses *in vitro* and blood vessel growth *in vivo*, only vascular endothelial growth factor (VEGF) family members and the angiopoietins are believed to act almost exclusively on vascular ECs. Yancopoulos *et al.*, *Nature* **407**:242-48 (2000).

[0004] The angiopoietins were discovered as ligands for the Ties, a family of tyrosine kinases that is selectively expressed within the vascular endothelium. Yancopoulos *et al.*, *Nature* **407**:242-48 (2000). There are now four definitive members of the angiopoietin family. Angiopoietin-3 and -4 (Ang-3 and Ang-4) may represent widely diverged counterparts of the same gene locus in mouse and man. Kim *et al.*, *FEBS Let*, **443**:353-56 (1999); Kim *et al.*, *J Biol Chem* **274**:26523-28 (1999). Ang-1 and Ang-2 were originally identified in tissue culture experiments as agonist and antagonist, respectively. Davis *et al.*, *Cell* **87**:1161-69 (1996); Maisonpierre *et al.*, *Science* **277**:55-60 (1997). All of the known angiopoietins bind primarily to Tie2, and both Ang-1 and -2 bind to Tie2 with an affinity of 3 nM (Kd). Maisonpierre *et al.*, *Science* **277**:55-60

(1997). Ang-1 was shown to support EC survival and to promote endothelium integrity, Davis *et al.*, *Cell* **87**:1161-69 (1996); Kwak *et al.*, *FEBS Lett* **448**:249-53 (1999); Suri *et al.*, *Science* 282:468-71 (1998); Thurston *et al.*, *Science* **286**: 2511-14 (1999); Thurston *et al.*, *Nat. Med.* **6**:460-63 (2000), whereas Ang-2 had the opposite effect and promoted blood vessel destabilization and regression in the absence of the survival factors VEGF or basic fibroblast growth factor. Maisonpierre *et al.*, *Science* **277**:55-60 (1997). However, many studies of Ang-2 function have suggested a more complex situation. Ang-2 might be a complex regulator of vascular remodeling that plays a role in both vessel sprouting and vessel regression. Supporting such roles for Ang-2, expression analyses reveal that Ang-2 is rapidly induced, together with VEGF, in adult settings of angiogenic sprouting, whereas Ang-2 is induced in the absence of VEGF in settings of vascular regression. Holash *et al.*, *Science* **284**:1994-98 (1999); Holash *et al.*, *Oncogene* **18**:5356-62 (1999). Consistent with a context-dependent role, Ang-2 binds to the same endothelial-specific receptor, Tie-2, which is activated by Ang-1, but has context-dependent effects on its activation. Maisonpierre *et al.*, *Science* **277**:55-60 (1997).

[0005] Corneal angiogenesis assays have shown that both Ang-1 and Ang-2 had similar effects, acting synergistically with VEGF to promote growth of new blood vessels. Asahara *et al.*, *Circ. Res.* **83**:233-40 (1998). The possibility that there was a dose-dependent endothelial response was raised by the observation that *in vitro* at high concentration, Ang-2 can also be pro-angiogenic. Kim *et al.*, *Oncogene* **19**:4549-52 (2000). At high concentration, Ang-2 acts as an apoptosis survival factor for endothelial cells during serum deprivation apoptosis through activation of Tie2 via PI-3 kinase and Akt pathway. Kim *et al.*, *Oncogene* **19**:4549-52 (2000).

[0006] Other *in vitro* experiments suggested that during sustained exposure, the effects of Ang-2 may progressively shift from that of an antagonist to an agonist of Tie2, and at later time points, it may contribute directly to vascular tube formation and neovessel stabilization. Teichert-Kuliszewska *et al.*, *Cardiovasc. Res.* **49**:659-70 (2001). Furthermore, if ECs were cultivated on fibrin gel, activation of Tie2 with Ang-2 was also observed, perhaps suggesting that the action of Ang-2 could depend on EC differentiation state. Teichert-Kuliszewska *et al.*, *Cardiovasc. Res.* **49**:659-70 (2001). In microvascular EC cultured in a three-dimensional collagen gel, Ang-2 can also induce Tie2 activation and promote formation of capillary-like structures. Mochizuki *et al.*, *J. Cell. Sci.* **115**:175-83 (2002). Use of a 3-D spheroidal coculture as an *in vitro* model of vessel maturation demonstrated that direct contact between ECs and mesenchymal cells

abrogates responsiveness to VEGF, whereas the presence of VEGF and Ang-2 induced sprouting. Korff *et al.*, *Faseb J.* **15**:447-57 (2001). Etoh *et al.* demonstrated that ECs that constitutively express Tie2, the expression of MMP-1, -9 and u-PA were strongly up-regulated by Ang-2 in the presence of VEGF. Etoh, *et al.*, *Cancer Res.* **61**:2145-53 (2001). With an *in vivo* pupillary membrane model, Lobov *et al.* showed that Ang-2 in the presence of endogenous VEGF promotes a rapid increase in capillary diameter, remodeling of the basal lamina, proliferation and migration of endothelial cells, and stimulates sprouting of new blood vessels. Lobov *et al.*, *Proc. Natl. Acad. Sci. USA* **99**:11205-10 (2002). By contrast, Ang-2 promotes endothelial cell death and vessel regression without endogenous VEGF. Lobov *et al.*, *Proc. Natl. Acad. Sci. USA* **99**:11205-10 (2002). Similarly, with an *in vivo* tumor model, Vajkoczy *et al.* demonstrated that multicellular aggregates initiate vascular growth by angiogenic sprouting via the simultaneous expression of VEGFR-2 and Ang-2 by host and tumor endothelium. Vajkoczy *et al.*, *J. Clin. Invest.* **109**:777-85 (2002). This model illustrated that the established microvasculature of growing tumors is characterized by a continuous remodeling, putatively mediated by the expression of VEGF and Ang-2. Vajkoczy *et al.*, *J. Clin. Invest.* **109**:777-85 (2002).

[0007] Knock-out mouse studies of Tie-2 and Angiopoietin-1 show similar phenotypes and suggest that Angiopoietin-1 stimulated Tie-2 phosphorylation mediates remodeling and stabilization of developing vessel, promoting blood vessel maturation during angiogenesis and maintenance of endothelial cell-support cell adhesion (Dumont *et al.*, *Genes & Development,* **8**:1897-1909 (1994); Sato, *Nature*, **376**:70-74 (1995); (Thurston, G. et al., 2000 Nature Medicine: 6, 460-463)). The role of Angiopoietin-1 is thought to be conserved in the adult, where it is expressed widely and constitutively (Hanahan, *Science*, **277**:48-50 (1997); Zagzag, *et al.*, *Exp Neurology*, **159**:391-400 (1999)). In contrast, Angiopoietin-2 expression is primarily limited to sites of vascular remodeling where it is thought to block the constitutive stabilizing or maturing function of Angiopoietin-1, allowing vessels to revert to, and remain in, a plastic state which may be more responsive to sprouting signals (Hanahan, 1997; Holash *et al.*, *Oncogene* **18**:5356-62 (1999); Maisonpierre, 1997). Studies of Angiopoietin-2 expression in pathological angiogenesis have found many tumor types to show vascular Angiopoietin-2 expression (Maisonpierre *et al.*, *Science* **277**:55-60 (1997)). Functional studies suggest Angiopoietin-2 is involved in tumor angiogenesis and associate Angiopoietin-2 overexpression with increased tumor growth in a mouse xenograft model (Ahmad, *et al.*,

Cancer Res., **61**:1255-1259 (2001)). Other studies have associated Angiopoietin-2 overexpression with tumor hypervascularity (Etoh, *et al.*, *Cancer Res.* **61**:2145-53 (2001); Tanaka *et al.*, *Cancer Res.* **62**:7124-29 (2002)).

[0008] In recent years Angiopoietin-1, Angiopoietin-2 and/or Tie-2 have been proposed as possible anti-cancer therapeutic targets. For example US6166185, US5650490 and US5814464 each disclose anti-Tie-2 ligand and receptor antibodies. Studies using soluble Tie-2 were reported to decrease the number and size of tumors in rodents (Lin, 1997; Lin 1998). Siemester et al. (1999) generated human melanoma cell lines expressing the extracellular domain of Tie-2, injected these into nude mice and reported soluble Tie-2 to result in significant inhibition of tumor growth and tumor angiogenesis. Given both Angiopoietin-1 and Angiopoietin-2 bind to Tie-2, it is unclear from these studies whether Angiopoietin-1, Angiopoietin-2 or Tie-2 would be an attractive target for anti-cancer therapy. However, effective anti-Angiopoietin-2 therapy is thought to be of benefit in treating diseases such as cancer, in which progression is dependant on aberrant angiogenesis where blocking the process can lead to prevention of disease advancement (Folkman, J., *Nature Medicine.* **1**: 27-31 (1995). In addition some groups have reported the use of antibodies that bind to Angiopoietin-2, See, for example, U.S. Patent No. 6,166,185 and U.S. Patent Application Publication No. 2003/0124129 A1. Study of the effect of focal expression of Angiopoietin-2 has shown that antagonizing the Angiopoietin-1/Tie-2 signal loosens the tight vascular structure thereby exposing ECs to activating signals from angiogenesis inducers, e.g. VEGF (Hanahan, 1997). This pro-angiogenic effect resulting from inhibition of Angiopoietin-1 indicates that anti-Angiopoietin-1 therapy would not be an effective anti-cancer treatment.

[0009] Ang-2 is expressed during development at sites where blood vessel remodeling is occurring. Maisonpierre *et al.*, *Science* **277**:55-60 (1997). In adult individuals, Ang-2 expression is restricted to sites of vascular remodeling as well as in highly vascularized tumors, including glioma, Osada *et al.*, *Int. J. Oncol.* **18**:305-09 (2001); Koga *et al.*, *Cancer Res.* **61**:6248-54 (2001), hepatocellular carcinoma, Tanaka et al, *J. Clin. Invest.* **103**:341-45 (1999), gastric carcinoma, Etoh, *et al.*, *Cancer Res.* **61**:2145-53 (2001); Lee et al, *Int. J. Oncol.* **18**:355-61 (2001), thyroid tumor, Bunone *et al.*, *Am J Pathol* **155**:1967-76 (1999), non-small cell lung cancer, Wong *et al.*, *Lung Cancer* **29**:11-22 (2000), and cancer of colon, Ahmad *et al.*, *Cancer* **92**:1138-43 (2001), and prostate Wurmbach *et al.*, *Anticancer Res.* **20**:5217-20 (2000). Some tumor cells are found to express Ang-2. For example, Tanaka *et al.*, *J. Clin. Invest.* **103**:341-45 (1999)

detected Ang-2 mRNA in 10 out of 12 specimens of human hepatocellular carcinoma (HCC). Ellis' group reported that Ang-2 is expressed ubiquitously in tumor epithelium. Ahmad *et al.*, *Cancer* **92**:1138-43 (2001). Other investigators reported similar findings. Chen *et al.*, *J. Tongji Med. Univ.* **21**:228-30, 235 (2001). By detecting Ang-2 mRNA levels in archived human breast cancer specimens, Sfilogoi *et al.*, *Int. J. Cancer* **103**:466-74 (2003) reported that Ang-2 mRNA is significantly associated with auxiliary lymph node invasion, short disease-free time and poor overall survival. Tanaka *et al.*, *Cancer Res.* **62**:7124-29 (2002) reviewed a total of 236 patients of non-small cell lung cancer (NSCLC) with pathological stage-I to –IIIA, respectively. Using immunohistochemistry, they found that 16.9% of the NSCLC patients were Ang-2 positive. The microvessel density for Ang-2 positive tumor is significantly higher than that of Ang-2 negative. Such an angiogenic effect of Ang-2 was seen only when VEGF expression was high. Moreover, positive expression of Ang-2 was a significant factor to predict a poor postoperative survival. Tanaka *et al.*, *Cancer Res.* **62**:7124-29 (2002). However, they found no significant correlation between Ang-1 expression and the microvessel density. Tanaka *et al.*, *Cancer Res.* **62**:7124-29 (2002). These results suggest that Ang-2 is an indicator of poor prognosis patients with several types of cancer.

[0010] Recently, using an Ang-2 knockout mouse model, Yancopoulos' group reported that Ang-2 is required for postnatal angiogenesis. Gale *et al.*, *Dev. Cell* **3**:411-23 (2002). They showed that the developmentally programmed regression of the hyaloid vasculature in the eye does not occur in the Ang-2-/- mice and their retinal blood vessels fail to sprout out from the central retinal artery. Gale *et al.*, *Dev. Cell* **3**:411-23 (2002). They also found that deletion of Ang-2 results in profound defects in the patterning and function of the lymphatic vasculature. Gale *et al.*, *Dev. Cell* **3**:411-23 (2002). Genetic rescue with Ang-1 corrects the lymphatic, but not the angiogenesis defects. Gale *et al.*, *Dev. Cell* **3**:411-23 (2002).

[0011] Peters and his colleagues reported that soluble Tie2, when delivered either as recombinant protein or in a viral expression vector, inhibited *in vivo* growth of murine mammary carcinoma and melanoma in mouse models. Lin *et al.*, *Proc. Natl. Acad. Sci. USA* **95**:8829-34 (1998); Lin *et al.*, *J. Clin. Invest.* **100**:2072-78 (1997). Vascular densities in the tumor tissues so treated were greatly reduced. In addition, soluble Tie2 blocked angiogenesis in the rat corneal stimulated by tumor cell conditioned media. Lin *et al.*, *J. Clin. Invest.* **100**:2072-78 (1997). Furthermore, Isner and his team demonstrated that addition of Ang-2 to VEGF promoted significantly longer and more

circumferential neovascularity than VEGF alone. Asahara *et al.*, *Circ. Res.* **83**:233-40 (1998). Excess soluble Tie2 receptor precluded modulation of VEGF-induced neovascularization by Ang-2. Asahara *et al.*, *Circ. Res.* **83**:233-40 (1998). Siemeister *et al.*, *Cancer Res.* **59**:3185-91 (1999) showed with nude mouse xenografts that overexpression of the extracellular ligand-binding domains of either Flt-1 or Tie2 in the xenografts results in significant inhibition of pathway could not be compensated by the other one, suggesting that the VEGF receptor pathway and the Tie2 pathway should be considered as two independent mediators essential for the process of *in vivo* angiogenesis. Siemeister *et al.*, *Cancer Res.* **59**:3185-91 (1999). This is proven by a more recent publication by White *et al.*, *Proc. Natl. Acad. Sci. USA* **100**:5028-33 (2003). In their study, it was demonstrated that a nuclease-resistant RNA aptamer that specifically binds and inhibits Ang-2 significantly inhibited neovascularization induced by bFGF in the rat corneal micropocket angiogenesis model.

SUMMARY

[0012] Embodiments of the invention relate to targeted binding agents that specifically bind to Angiopoietin-2 and therein inhibit tumor angiogenesis and reduce tumor growth. Mechanisms by which this can be achieved can include and are not limited to either inhibition of binding of Ang-2 to its receptor Tie2, inhibition of Ang-2 induced Tie2 signaling, or increased clearance of Ang-2, therein reducing the effective concentration of Ang-2.

[0013] One embodiment of the invention, the targeted binding agent is a fully human antibody that binds to Ang-2 and prevents Ang-2 binding to Tie2. Yet another embodiment of the invention is a fully human monoclonal antibody that binds to Ang-2 and and Ang-1, and also inhibits Ang-2 induced Tie2 phosphorylation. The antibody may bind Ang-2 with a K_d of less than 100 pM, 30 pM, 20 pM, 10pM or 5 pM.

[0014] The antibody may comprise a heavy chain amino acid sequence having a complementarity determining region (CDR) with one of the sequences shown in Table 11. It is noted that those of ordinary skill in the art can readily accomplish CDR determinations. See for example, Kabat *et al.*, *Sequences of Proteins of Immunological Interest,* Fifth Edition, NIH Publication 91-3242, Bethesda MD (1991), vols. 1-3.

[0015] One embodiment of the invention comprises fully human monoclonal antibodies 3.3.2 (ATCC Accession Number PTA-7258), 3.19.3 (ATCC Accession

Number PTA-7260) and 5.88.3 (ATCC Accession Number PTA-7259) which specifically bind to Ang-2, as discussed in more detail below.

[0016] Yet another embodiment is an antibody that binds to Ang-2 and comprises a light chain amino acid sequence having a CDR comprising one of the sequences shown in Table 12. In certain embodiments the antibody is a fully human monoclonal antibody.

[0017] A further embodiment is an antibody that binds to Ang-2 and comprises a heavy chain amino acid sequence having one of the CDR sequences shown in Table 11 and a light chain amino acid sequence having one of the CDR sequences shown in Table 12. In certain embodiments the antibody is a fully human monoclonal antibody. A further embodiment of the invention is an antibody that cross-competes for binding to Ang-2 with the fully human antibodies of the invention, preferably an antibody comprising a heavy chain amino acid sequence having one of the CDR sequences shown in Table 11 and a light chain amino acid sequence having one of the CDR sequences shown in Table 12. A further embodiment of the invention is an antibody that binds to the same epitope on Ang-2 as a fully human antibodies of the invention, preferably an antibody comprising a heavy chain amino acid sequence having one of the CDR sequences shown in Table 11 and a light chain amino acid sequence having one of the CDR sequences shown in Table 12.

[0018] Further embodiments of the invention include human monoclonal antibodies that specifically bind to Angiopoietin-2, wherein the antibodies comprise a heavy chain complementarity determining region 1 (CDR1) corresponding to canonical class 1. The antibodies provided herein can also include a heavy chain complementarity determining region 2 (CDR2) corresponding to canonical class 3, a light chain complementarity determining region 1 (CDR1) corresponding to canonical class 2, a light chain complementarity determining region 2 (CDR2) corresponding to canonical class 1, and a light chain complementarity determining region 3 (CDR3) corresponding to canonical class 1.

[0019] The invention further provides methods for assaying the level of Angiopoietin-2 (Ang-2) in a patient sample, comprising contacting an anti-Ang-2 antibody with a biological sample from a patient, and detecting the level of binding between said antibody and Ang-2 in said sample. In more specific embodiments, the biological sample is blood.

[0020] In other embodiments the invention provides compositions, including an antibody or functional fragment thereof, and a pharmaceutically acceptable carrier.

[0021] Still further embodiments of the invention include methods of effectively treating an animal suffering from an angiogenesis-related disease, including selecting an animal in need of treatment for a neoplastic or non-neoplastic disease, and administering to said animal a therapeutically effective dose of a fully human monoclonal antibody that specifically binds to Angiopoietin-2 (Ang-2).

[0022] Treatable angiogenesis-related diseases can include neoplastic diseases, such as, melanoma, small cell lung cancer, non-small cell lung cancer, glioma, hepatocellular (liver) carcinoma, thyroid tumor, gastric (stomach) cancer, prostate cancer, breast cancer, ovarian cancer, bladder cancer, lung cancer, glioblastoma, endometrial cancer, kidney cancer, colon cancer, pancreatic cancer, esophageal carcinoma, head and neck cancers, mesothelioma, sarcomas, biliary (cholangiocarcinoma), small bowel adenocarcinoma, pediatric malignancies and epidermoid carcinoma.

[0023] Additional embodiments of the invention include methods of inhibiting Angiopoietin-2 (Ang-2) induced angiogenesis in an animal. These methods include selecting an animal in need of treatment for Ang-2 induced angiogenesis, and administering to said animal a therapeutically effective dose of a fully human monoclonal antibody wherein said antibody specifically binds to Ang-2.

[0024] Further embodiments of the invention include the use of an antibody of in the preparation of medicament for the treatment of angiogenesis-related diseases in an animal, wherein said monoclonal antibody specifically binds to Angiopoietin-2 (Ang-2). Treatable angiogenesis-related diseases can include neoplastic diseases, such as, melanoma, small cell lung cancer, non-small cell lung cancer, glioma, hepatocellular (liver) carcinoma, thyroid tumor, gastric (stomach) cancer, prostate cancer, breast cancer, ovarian cancer, bladder cancer, lung cancer, glioblastoma, endometrial cancer, kidney cancer, colon cancer, pancreatic cancer, esophageal carcinoma, head and neck cancers, mesothelioma, sarcomas, cholangiocarcinoma, small bowel adenocarcinoma, pediatric malignancies and epidermoid carcinoma.

[0025] In still further embodiments, the antibodies described herein can be used for the preparation of a medicament for the effective treatment of Angiopoietin-2 induced angiogenesis in an animal, wherein said monoclonal antibody specifically binds to Angiopoietin-2 (Ang-2).

[0026] Embodiments of the invention described herein relate to monoclonal antibodies that bind Ang-2 and affect Ang-2 function. Other embodiments relate to fully human anti-Ang-2 antibodies and anti-Ang-2 antibody preparations with desirable properties from a therapeutic perspective, including high binding affinity for Ang-2, the ability to neutralize Ang-2 *in vitro* and *in vivo*, and the ability to inhibit Ang-2 induced angiogenesis.

[0027] In a preferred embodiment, antibodies described herein bind to Ang-2 with very high affinities (Kd). For example a human, rabbit, mouse, chimeric or humanized antibody that is capable of binding Ang-2 with a Kd less than, but not limited to, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9}, 10^{-10}, 10^{-11}, 10^{-12}, 10^{-13} or 10^{-14} M, or any range or value therein. Affinity and/or avidity measurements can be measured by KinExA® and/or BIACORE®, as described herein.

[0028] Accordingly, one embodiment described herein includes isolated antibodies, or fragments of those antibodies, that bind to Ang-2. As known in the art, the antibodies can advantageously be, for example, polyclonal, oligoclonal, monoclonal, chimeric, humanized, and/or fully human antibodies. Embodiments of the invention described herein also provide cells for producing these antibodies.

[0029] Another embodiment of the invention is a fully human antibody that binds to other Angiopoietin-2 family members including, but not limited to, Angiopoietin-1, Angiopoietin-3, and Angiopoietin-4. A further embodiment herein is an antibody that cross-competes for binding to Tie2 with Ang-2 with the fully human antibodies of the invention. In one embodiment of the invention, the antibody binds to and neutralizes Angiopoietin-2, and also binds to and neutralizes, Angiopoietin-1.

[0030] It will be appreciated that embodiments of the invention are not limited to any particular form of an antibody or method of generation or production. For example, the anti-Ang-2 antibody may be a full-length antibody (*e.g.,* having an intact human Fc region) or an antibody fragment (*e.g.,* a Fab, Fab' or $F(ab')_2$). In addition, the antibody may be manufactured from a hybridoma that secretes the antibody, or from a recombinantly produced cell that has been transformed or transfected with a gene or genes encoding the antibody.

[0031] Other embodiments of the invention include isolated nucleic acid molecules encoding any of the antibodies described herein, vectors having isolated nucleic acid molecules encoding anti-Ang-2 antibodies or a host cell transformed with any of such nucleic acid molecules. In addition, one embodiment of the invention is a

method of producing an anti-Ang-2 antibody by culturing host cells under conditions wherein a nucleic acid molecule is expressed to produce the antibody followed by recovering the antibody. It should be realized that embodiments of the invention also include any nucleic acid molecule which encodes an antibody or fragment of an antibody of the invention including nucleic acid sequences optimized for increasing yields of antibodies or fragments thereof when transfected into host cells for antibody production.

[0032] A further embodiment herein includes a method of producing high affinity antibodies to Ang-2 by immunizing a mammal with human Ang-2, or a fragment thereof, and one or more orthologous sequences or fragments thereof.

[0033] Other embodiments are based upon the generation and identification of isolated antibodies that bind specifically to Ang-2. Ang-2 is expressed at elevated levels in angiogenesis-related diseases, such as neoplastic diseases. Inhibition of the biological activity of Ang-2 can prevent Ang-2 induced angiogenesis and other desired effects.

[0034] Another embodiment of the invention includes a method of diagnosing diseases or conditions in which an antibody prepared as described herein is utilized to detect the level of Ang-2 in a patient sample. In one embodiment, the patient sample is blood or blood serum. In further embodiments, methods for the identification of risk factors, diagnosis of disease, and staging of disease is presented which involves the identification of the overexpression of Ang-2 using anti-Ang-2 antibodies.

[0035] Another embodiment of the invention includes a method for diagnosing a condition associated with the expression of Ang-2 in a cell by contacting the serum or a cell with an anti-Ang-2 antibody, and thereafter detecting the presence of Ang-2. Preferred conditions include angiogenesis-related diseases including, but not limited to, neoplastic diseases, such as, melanoma, small cell lung cancer, non-small cell lung cancer, glioma, hepatocellular (liver) carcinoma, glioblastoma, and carcinoma of the thyroid, stomach, prostate, breast, ovary, bladder, lung, uterus, kidney, colon, and pancreas, salivary gland, and colorectum.

[0036] In another embodiment, the invention includes an assay kit for detecting Angiopoietin-2 and Angiopoietin family members in mammalian tissues, cells, or body fluids to screen for angiogenesis-related diseases. The kit includes an antibody that binds to Angiopoietin-2 and a means for indicating the reaction of the antibody with Angiopoietin-2, if present. Preferably the antibody is a monoclonal antibody. In one embodiment, the antibody that binds Ang-2 is labeled. In another embodiment the antibody is an unlabeled primary antibody and the kit further includes a means for

detecting the primary antibody. In one embodiment, the means includes a labeled second antibody that is an anti-immunoglobulin. Preferably the antibody is labeled with a marker selected from the group consisting of a fluorochrome, an enzyme, a radionuclide and a radiopaque material.

[0037] Yet another embodiment includes methods for treating diseases or conditions associated with the expression of Ang-2 in a patient, by administering to the patient an effective amount of an anti-Ang-2 antibody. The anti-Ang-2 antibody can be administered alone, or can be administered in combination with additional antibodies or chemotherapeutic drug or radiation therapy. For example, a monoclonal, oligoclonal or polyclonal mixture of Ang-2 antibodies that block angiogenesis can be administered in combination with a drug shown to inhibit tumor cell proliferation directly. The method can be performed *in vivo* and the patient is preferably a human patient. In a preferred embodiment, the method concerns the treatment of angiogenesis-related diseases including, but not limited to, neoplastic diseases, such as, melanoma, small cell lung cancer, non-small cell lung cancer, glioma, hepatocellular (liver) carcinoma, glioblastoma, and carcinoma of the thyroid, stomach, prostate, breast, ovary, bladder, lung, uterus, kidney, colon, and pancreas, salivary gland, and colorectum.

[0038] In another embodiment, the invention provides an article of manufacture including a container. The container includes a composition containing an anti-Ang-2 antibody, and a package insert or label indicating that the composition can be used to treat angiogenesis-related diseases characterized by the overexpression of Ang-2.

[0039] In some embodiments, the anti-Ang-2 antibody is administered to a patient, followed by administration of a clearing agent to remove excess circulating antibody from the blood.

[0040] Yet another embodiment is the use of an anti-Ang-2 antibody in the preparation of a medicament for the treatment of diseases such as angiogenesis-related diseases. In one embodiment, the angiogenesis-related diseases include carcinoma, such as breast, ovarian, stomach, endometrial, salivary gland, lung, kidney, colon, colorectum, esophageal, thyroid, pancreatic, prostate and bladder cancer. In another embodiment, the angiogenesis-related diseases include, but are not limited to, neoplastic diseases, such as, melanoma, small cell lung cancer, non-small cell lung cancer, glioma, hepatocellular (liver) carcinoma, sarcoma, head and neck cancers, mesothelioma, biliary (cholangiocarcinoma), small bowel adenocarcinoma, pediatric malignancies and glioblastoma.

[0041] Ang-2 is an important "on-switch" of angiogenesis. Accordingly, antagonizing this molecule is expected to inhibit pathophysiological procedures, and thereby act as a potent therapy for various angiogenesis-dependent diseases. Besides solid tumors and their metastases, haematologic malignancies, such as leukemias, lymphomas and multiple myeloma, are also angiogenesis-dependent. Excessive vascular growth contributes to numerous non-neoplastic disorders. These non-neoplastic angiogenesis-dependent diseases include: atherosclerosis, haemangioma, haemangioendothelioma, angiofibroma, vascular malformations (e.g. Hereditary Hemorrhagic Teleangiectasia (HHT), or Osler-Weber syndrome), warts, pyogenic granulomas, excessive hair growth, Kaposis' sarcoma, scar keloids, allergic oedema, psoriasis, dysfunctional uterine bleeding, follicular cysts, ovarian hyperstimulation, endometriosis, respiratory distress, ascites, peritoneal sclerosis in dialysis patients, adhesion formation result from abdominal surgery, obesity, rheumatoid arthritis, synovitis, osteomyelitis, pannus growth, osteophyte, hemophilic joints, inflammatory and infectious processes (e.g. hepatitis, pneumonia, glomerulonephritis), asthma, nasal polyps, liver regeneration, pulmonary hypertension, retinopathy of prematurity, diabetic retinopathy, age-related macular degeneration., leukomalacia, neovascular glaucoma, corneal graft neovascularization, trachoma, thyroiditis, thyroid enlargement, and lymphoproliferative disorders.

BRIEF DESCRIPTION OF THE DRAWINGS

[0042] Figure 1 is a Western Blot showing that Ang-2 mAbs inhibit Ang-2-induced phosphorylation of Tie2 ectopically expressed in HEK293F cells.

[0043] Figure 2 is a line graph of the dose-response relationship of anti-Ang-2 monoclonal antibodies on the inhibition of Ang-2 induced Tie2 phosphorylation.

[0046] Figure 3 is a line graph showing inhibition of both Ang-1 (top graph) and Ang-2 (bottom graph) binding to Tie2 in a dose-dependent manner using mAb 3.19.3 or Tie2/Fc.

[0047] Figure 4 is a Western blot showing inhibition of Angiopoietin-1 stimulated phosphorylation of Tie-2 on Eahy 926 endothelial cells by mAb 3.19.3. Inhibition of Angiopoietin-1 induced Tie-2 phosphorylation is observed in this system. The antibody concentrations are shown in nM.

[0048] Figure 5 is a line graph showing inhibition of Angiopoietin-1 stimulated phosphorylation of Tie-2 on Eahy 926 endothelial cells by mAb 3.19.3. The

IC50 = 99nM. The x axis is the concentration of mAb 3.19.3 and the y axis indicates the response.

[0049] Figure 6 is a schematic diagram of the protein structure of human Ang-2 and Ang-2_{443}. The upper numbers denote amino acid sequences (diagram taken from Injune et al., (2000) *JBC* 275: 18550).

[0050] Figure 7 displays the amino acid sequence of a Mouse/Human chimeric molecule (SEQ ID NO: 1). The human residues (cloned as *StuI-TfiI* fragment) 310-400 are underlined.

[0051] Figure 8 is an amino acid sequence comparison of human Ang-1 (SEQ ID NO: 2), human Ang-2 (SEQ ID NO: 3), and mouse Ang-2 (SEQ ID NO: 4) proteins. The fusion points of Ang-2 chimeric molecules and point mutations are noted in bold.

[0052] Figure 9 is an amino acid sequence comparison of mouse Ang-1 (SEQ ID NO: 5), human Ang-1 (SEQ ID NO: 2), mouse Ang-2 (SEQ ID NO: 4), and human Ang-2 (SEQ ID NO: 3). The arrowhead shows the cleavage site for hydrophobic leader sequences. The arrows define the limits of the coiled-coil and fibrinogen like domains. The solid circles show the conserved cysteine residues (image taken from Maisonpierre et al., 1997, *Science* 277:55).

[0053] Figure 10 is a line graph demonstrating mouse cross-reactivity in a dose-response relationship. Monoclonal antibody clones 5.2.1, 5.28.1, 3.19.3, and 3.31.2 are shown.

[0054] Figure 11 is a line graph showing inhibition of both human (black triangles) and mouse (black squares) Ang-2 binding to human Tie2 in a dose-dependent manner using mAb 3.19.3.

[0055] Figure 12 is a bar graph analysis of the effect of antibodies on MCF-7 cell-induced angiogenesis. Figure 12A shows the effect of anti-Ang-2 antibodies on the number of blood vessels ends where the x axis indicates the experimental groups and the y axis indicates the mean number of vessel ends (+/- SD). Figure 12B demonstrates the effect of anti-Ang-2 antibodies on blood vessel length where the x axis indicates the experimental groups and the y axis indicates the mean vessel length (+/- SD).

[0056] Figure 13 is a line graph showing the anti-tumor effect of the anti-Ang-2 monoclonal antibody clone 3.19.3, as tested in a mouse xenograft model of human skin epidermoid carcinoma using the A431 cell line. The x axis indicates the number of days post tumor cell implantation and the y axis indicates the mean tumor volume +/- SEM in cm^3. The Solid black triangles represent the post implantation tumor volume

measurements of mice injected with anti-Ang-2 monoclonal antibody clone 3.19.3; the solid black circles represent the post implantation tumor volume measurements of mice injected with an isotype control antibody PK16.3.1.

[0058] Figure 14A is a line graph showing prevention of tumor growth in the human colon adenocarcinoma LoVo xenograft model with tumor size indicated for mice treated with 0.5, 2, and 10 mg/kg or isotype control indicated. The x axis indicates the number of days post tumor cell implantation and the y axis indicates the mean tumor volume +/- SEM in cm^3. Figure 14B is a line graph showing tumor growth inhibitory effect of the mAb in a human colon adenocarcinoma SW480 xenograft model.

[0059] Figure 15A is a line graph showing prevention of tumor growth in the HT29 xenograft model. The x axis indicates the number of days post tumor cell implantation, and the y axis indicates the mean tumor volume+/- SEM in cm3. Figure 15B is a line graph showing prevention of tumor growth in the Calu-6 xenograft model with tumor size indicated for mice treated with 10mg/kg of mAb clone 3.3.2 or 3.19.3 or with isotype control antibody. Figure 15C is a bar graph indicating the density of CD31+ staining in tumors from MDA-MB-231 tumor-bearing mice treated with either IgG control or 10mg/kg 3.19.3 mAb. Results from both threshold and manual grid counting methods are shown.

DETAILED DESCRIPTION

[0060] Embodiments of the invention described herein relate to monoclonal antibodies that bind to Ang-2. In some embodiments, the antibodies bind to Ang-2 and inhibit the binding of Ang-2 to its receptor, Tie2. Other embodiments of the invention include fully human anti-Ang-2 antibodies, and antibody preparations that are therapeutically useful. Such anti-Ang-2 antibody preparations preferably have desirable therapeutic properties, including strong binding affinity for Ang-2, the ability to neutralize Ang-2 *in vitro*, and the ability to inhibit Ang-2-induced angiogenesis *in vivo*.

[0061] One embodiment of the invention includes an antibody that binds to and neutralizes Ang-2, but does not bind to Ang-1. In another embodiment, the antibody binds to both Ang-2 and Ang-1, but only neutralizes Ang-2. In another embodiment, the antibody binds to both Ang-2 and Ang-1, and neutralizes binding of both Ang-1 and Ang-2 to Tie2.

[0062] Embodiments of the invention also include isolated binding fragments of anti-Ang-2 antibodies. Preferably, the binding fragments are derived from fully human anti-Ang-2 antibodies. Exemplary fragments include Fv, Fab' or other well know antibody fragments, as described in more detail below. Embodiments of the invention also include cells that express fully human antibodies against Ang-2. Examples of cells include hybridomas, or recombinantly created cells, such as Chinese hamster ovary (CHO) cells, variants of CHO cells (for example DG44) and NS0 cells that produce antibodies against Ang-2. Additional information about variants of CHO cells can be found in Andersen and Reilly (2004) Current Opinion in Biotechnology 15, 456-462 which is incorporated herein in its entirety by reference.

[0063] In addition, embodiments of the invention include methods of using these antibodies for treating diseases. Anti-Ang-2 antibodies are useful for preventing Ang-2 mediated Tie2 signal transduction, thereby inhibiting angiogenesis. The mechanism of action of this inhibition may include inhibition of Ang-2 from binding to its receptor, Tie2, inhibition of Ang-2 induced Tie2 signaling, or enhanced clearance of Ang-2 therein lowering the effective concentration of Ang-2 for binding to Tie-2. Diseases that are treatable through this inhibition mechanism include, but are not limited to, neoplastic diseases, such as, melanoma, small cell lung cancer, non-small cell lung cancer, glioma, hepatocellular (liver) carcinoma, glioblastoma, and cancers and tumors of the thyroid, stomach, prostate, breast, ovary, bladder, lung, uterus, kidney, colon, and pancreas, salivary gland, and colorectal.

[0064] Other embodiments of the invention include diagnostic assays for specifically determining the quantity of Ang-2 in a biological sample. The assay kit can include anti-Ang-2 antibodies along with the necessary labels for detecting such antibodies. These diagnostic assays are useful to screen for angiogenesis-related diseases including, but not limited to, neoplastic diseases, such as, melanoma, small cell lung cancer, non-small cell lung cancer, glioma, hepatocellular (liver) carcinoma, glioblastoma, and carcinoma of the thyroid, stomach, prostate, breast, ovary, bladder, lung, uterus, kidney, colon, and pancreas, salivary gland, and colorectum.

[0065] According to one aspect of the invention there is provided an antagonist of the biological activity of Angiopoietin-1 and Angiopoietin-2 wherein the antagonist does not bind to the ATP-binding site of Tie-2.

[0066] According to another aspect of the invention there is provided an antagonist of the biological activity of Angiopoietin-1 and Angiopoietin-2 wherein the antagonist binds to Angiopoietin-1 and Angiopoietin-2.

[0067] According to another aspect of the invention there is provided an antagonist of the biological activity of Angiopoietin-1 and Angiopoietin-2 wherein the antagonist is not a compound.

[0068] In one embodiment there is provided an antagonist of the biological activity of Angiopoietin-1 and Angiopoietin-2 wherein the Angiopoietin-1 antagonist activity and the Angiopoietin–2 antagonist activity is comprised within one molecule. In an alternative embodiment there is provided an antagonist wherein the Angiopoietin-1 antagonist activity and the Angiopoietin–2 antagonist activity is comprised within more than one molecule.

[0069] In one embodiment there is provided an antagonist of the biological activity of Angiopoietin-1 and Angiopoietin-2 wherein the antagonist may bind to:

i) **[0070]** the Tie-2 receptor;

ii) **[0071]** Angiopoietin-1 and/or Angiopoietin-2;

iii) **[0072]** Tie-2 receptor-Angiopoietin-1 complex; or

iv) **[0073]** Tie-2 receptor-Angiopoietin-2 complex,

[0074] or any combination of these.

[0075] In one embodiment the antagonist of the biological activity of Angiopoietin-1 and Angiopoietin-2 may bind to Angiopoietin-1 and/or Angiopoietin-2 and/or Tie-2 and thereby prevent Angiopoietin-1 and Angiopoietin-2 mediated Tie-2 signal transduction, thereby inhibiting angiogenesis. The mechanism of action of this inhibition may include;

i) **[0076]** binding of the antagonist to Angiopoietin-1 and inhibiting the binding of Angiopoietin-1 to its receptor, Tie-2, and/or

ii) **[0077]** binding of the antagonist to Angiopoietin-2 and inhibit the binding of Angiopoietin-2 to its receptor, Tie-2, and/or

iii) **[0078]** enhancing the clearance of Angiopoietin-1 and/or Angiopoietin-2 therein lowering the effective concentration of Angiopoietin-1 and/or Angiopoietin-2 available for binding to Tie-2,

[0079] or any combination of these, sufficient to antagonize the biological activity of Angiopoietin-1 and Angiopoietin-2.

[0080] Without wishing to be bound by theoretical considerations mechanisms by which antagonism of the biological activity of Angiopoietin-1 and Angiopoietin-2 can be achieved include, but are not limited to, inhibition of binding of Angiopoietin-1 and Angiopoietin-2 to the receptor Tie-2, inhibition of Angiopoietin-1 and Angiopoietin-2 induced Tie-2 signaling, or increased clearance of Angiopoietin-1 and Angiopoietin-2, therein reducing the effective concentration of Angiopoietin-1 and Angiopoietin-2.

[0081] In one embodiment there is provided an antagonist of the biological activity of Angiopoietin-1 and Angiopoietin-2 wherein the antagonist is an antibody. Preferably the antibody is able to antagonize the biological activity of Angiopoietin-1 and/or Angiopoietin-2 *in vitro* and *in vivo*. Preferably the antibody is a polyclonal or monoclonal antibody. More preferably the antibody is a monoclonal antibody and more preferably the antibody is a fully human monoclonal antibody. Most preferably the antibody is the fully human monoclonal antibody 3.19.3.

[0082] In one embodiment there is provided an antibody which binds to the same epitope or epitopes as fully human monoclonal antibody 3.19.3.

[0083] In one embodiment of the invention there is provided a fully human antibody that binds to Angiopoietin-1 and prevents Angiopoietin-1 binding to Tie-2. Yet another embodiment of the invention is a fully human monoclonal antibody that binds to Angiopoietin-1 and inhibits Angiopoietin-1 induced Tie-2 phosphorylation. In one embodiment, the antibody binds Angiopoietin-1 with a K_d of less than 1 nanomolar (nM). More preferably, the antibody binds with a Kd less than 500 picomolar (pM). More preferably, the antibody binds with a Kd less than 100 picomolar (pM). More preferably, the antibody binds with a Kd less than 30 picomolar (pM). More preferably, the antibody binds with a K_d of less than 20 pM. Even more preferably, the antibody binds with a K_d of less than 10 or 5 pM.

[0084] In one embodiment of the invention there is provided a fully human antibody that binds to Angiopoietin-2 and prevents Angiopoietin-2 binding to Tie-2. Yet another embodiment of the invention is a fully human monoclonal antibody that binds to Angiopoietin-2 and inhibits Angiopoietin-2 induced Tie-2 phosphorylation. In one embodiment, the antibody binds Angiopoietin-2 with a K_d of less than 1 nanomolar (nM). More preferably, the antibody binds with a Kd less than 500 picomolar (pM). More preferably, the antibody binds with a Kd less than 100 picomolar (pM). More preferably, the antibody binds with a Kd less than 30 picomolar (pM). More preferably, the antibody

binds with a K_d of less than 20 pM. Even more preferably, the antibody binds with a K_d of less than 10 or 5 pM.

[0085] In one embodiment there is provided a hybridoma that produces the light chain and/or the heavy chain of antibody as described hereinabove. Preferably the hybridoma produces the light chain and/or the heavy chain of a fully human monoclonal antibody. More preferably the hybridoma produces the light chain and/or the heavy chain of the fully human monoclonal antibody 3.19.3, 3.3.2 or 5.88.3. Alternatively the hybridoma produces an antibody which binds to the same epitope or epitopes as fully human monoclonal antibody 3.19.3, 3.3.2 or 5.88.3.

[0086] In one embodiment there is provided a nucleic acid molecule encoding the light chain or the heavy chain of the antibody as described hereinabove. Preferably there is provided a nucleic acid molecule encoding the light chain or the heavy chain of a fully human monoclonal antibody. More preferably there is provided a nucleic acid molecule encoding the light chain or the heavy chain of the fully human monoclonal antibody 3.19.3.

[0087] In one embodiment of the invention there is provided a vector comprising a nucleic acid molecule or molecules as described hereinabove, wherein the vector encodes a light chain and/or a heavy chain of an antibody as defined hereinabove.

[0088] In one embodiment of the invention there is provided a host cell comprising a vector as described hereinabove. Alternatively the host cell may comprise more than one vector.

[0089] In addition, one embodiment of the invention is a method of producing an antibody by culturing host cells under conditions wherein a nucleic acid molecule is expressed to produce the antibody, followed by recovery of the antibody.

[0090] In one embodiment of the invention there is provided a method of making an antibody comprising transfecting at least one host cell with at least one nucleic acid molecule encoding the antibody as described hereinabove, expressing the nucleic acid molecule in said host cell and isolating said antibody.

[0091] According to another aspect of the invention there is provided a method of antagonising the biological activity of Angiopoietin-1 and Angiopoietin–2 comprising administering an antagonist as described hereinabove. The method may include selecting an animal in need of treatment for disease-related angiogenesis, and administering to said animal a therapeutically effective dose of an antagonist of the biological activity of Angiopoietin-1 and Angiopoietin-2.

[0092] According to another aspect of the invention there is provided a method of antagonising the biological activity of Angiopoietin-1 and Angiopoietin–2 comprising administering an antibody as described hereinabove. The method may include selecting an animal in need of treatment for disease-related angiogenesis, and administering to said animal a therapeutically effective dose of an antibody which antagonises the biological activity of Angiopoietin-1 and Angiopoietin-2.

[0093] According to another aspect there is provided a method of treating disease-related angiogenesis in a mammal comprising administering a therapeutically effective amount of an antagonist of the biological activity of Angiopoietin-1 and Angiopoietin-2. The method may include selecting an animal in need of treatment for disease-related angiogenesis, and administering to said animal a therapeutically effective dose of an antagonist of the biological activity of Angiopoietin-1 and Angiopoietin-2.

[0094] According to another aspect there is provided a method of treating disease-related angiogenesis in a mammal comprising administering a therapeutically effective amount of an antibody which antagonizes the biological activity of Angiopoietin-1 and Angiopoietin-2. The method may include selecting an animal in need of treatment for disease-related angiogenesis, and administering to said animal a therapeutically effective dose of an antibody which antagonises the biological activity of Angiopoietin-1 and Angiopoietin-2. The antibody can be administered alone, or can be administered in combination with additional antibodies or chemotherapeutic drug or radiation therapy.

[0095] According to another aspect there is provided a method of treating cancer in a mammal comprising administering a therapeutically effective amount of an antagonist of the biological activity of Angiopoietin-1 and Angiopoietin-2. The method may include selecting an animal in need of treatment for cancer, and administering to said animal a therapeutically effective dose of an antagonist which antagonises the biological activity of Angiopoietin-1 and Angiopoietin-2. The antagonist can be administered alone, or can be administered in combination with additional antibodies or chemotherapeutic drug or radiation therapy.

[0096] According to another aspect there is provided a method of treating cancer in a mammal comprising administering a therapeutically effective amount of an antibody which antagonizes the biological activity of Angiopoietin-1 and Angiopoietin-2. The method may include selecting an animal in need of treatment for cancer, and administering to said animal a therapeutically effective dose of an antibody which

antagonises the biological activity of Angiopoietin-1 and Angiopoietin-2. The antibody can be administered alone, or can be administered in combination with additional antibodies or chemotherapeutic drug or radiation therapy.

[0097] According to another aspect of the invention there is provided the use of an antagonist of the biological activity of Angiopoietin-1 and Angiopoietin-2 for the manufacture of a medicament for the treatment of disease-related angiogenesis.

[0098] According to another aspect of the invention there is provided the use of an antibody which antagonizes the biological activity of Angiopoietin-1 and Angiopoietin-2 for the manufacture of a medicament for the treatment of disease-related angiogenesis.

[0099] In a preferred embodiment the present invention is particularly suitable for use in antagonizing Angiopoietin-1 or Angiopoietin-2, in patients with a tumour which is dependent alone, or in part, on a Tie-2 receptor.

[0100] Another embodiment of the invention includes an assay kit for detecting Angiopoietin-1 and/or Angiopoietin-2 in mammalian tissues, cells, or body fluids to screen for angiogenesis-related diseases. The kit includes an antibody that binds to Angiopoietin-1 and/or Angiopoietin-1 and a means for indicating the reaction of the antibody with Angiopoietin-1 and/or Angiopoietin-2, if present. The antibody may be a monoclonal antibody. In one embodiment, the antibody that binds Angiopoietin-2 is labeled. In another embodiment the antibody is an unlabeled primary antibody and the kit further includes a means for detecting the primary antibody. In one embodiment, the means includes a labeled second antibody that is an anti-immunoglobulin. Preferably the antibody is labeled with a marker selected from the group consisting of a fluorochrome, an enzyme, a radionuclide and a radio-opaque material.

[0101] Further embodiments, features, and the like regarding anti-Ang-2 antibodies are provided in additional detail below.

Sequence Listing

[0102] Embodiments of the invention include the specific anti-Ang-2 antibodies listed below in Table 1. This table reports the identification number of each anti-Ang-2 antibody, along with the SEQ ID number of the corresponding heavy chain and light chain genes.

[0103] Each antibody has been given an identification number that includes either two or three numbers separated by one or two decimal points. For most of the antibodies, only two identification numbers, separated by one decimal point, are listed.

[0104] However, in some cases, several clones of one antibody were prepared. Although the clones have the identical nucleic acid and amino acid sequences as the parent sequence, they may also be listed separately, with the clone number indicated by the number to the right of a second decimal point. Thus, for example, the nucleic acid and amino acid sequences of antibody 5.35 are identical to the sequences of antibody 5.35.1, 5.35.2, and 5.35.3.

Table 1

mAb ID No.:	Sequence	SEQ ID NO:
3.1	Nucleotide sequence encoding the variable region of the heavy chain	6
	Amino acid sequence encoding the variable region of the heavy chain	7
	Nucleotide sequence encoding the variable region of the light chain	8
	Amino acid sequence encoding the variable region of the light chain	9
3.2	Nucleotide sequence encoding the variable region of the heavy chain	10
	Amino acid sequence encoding the variable region of the heavy chain	11
	Nucleotide sequence encoding the variable region of the light chain	12
	Amino acid sequence encoding the variable region of the light chain	13
3.3	Nucleotide sequence encoding the variable region of the heavy chain	14
	Amino acid sequence encoding the variable region of the heavy chain	15
	Nucleotide sequence encoding the variable region of the light chain	16
	Amino acid sequence encoding the variable region of the light chain	17
3.3.1	Nucleotide sequence encoding the variable region of the heavy chain	18
	Amino acid sequence encoding the variable region of the heavy chain	19
	Nucleotide sequence encoding the variable region of the light chain	20
	Amino acid sequence encoding the variable region of the light chain	21
3.3.2	Nucleotide sequence encoding the variable region of the heavy chain	22
	Amino acid sequence encoding the variable region of the heavy chain	23
	Nucleotide sequence encoding the variable region of the light chain	24
	Amino acid sequence encoding the variable region of the light chain	25
3.6	Nucleotide sequence encoding the variable region of the heavy chain	26
	Amino acid sequence encoding the variable region of the heavy chain	27
	Nucleotide sequence encoding the variable region of the light chain	28
	Amino acid sequence encoding the variable region of the light chain	29
3.7	Nucleotide sequence encoding the variable region of the heavy chain	30
	Amino acid sequence encoding the variable region of the heavy chain	31
	Nucleotide sequence encoding the variable region of the light chain	32
	Amino acid sequence encoding the variable region of the light chain	33

3.8	Nucleotide sequence encoding the variable region of the heavy chain	34
	Amino acid sequence encoding the variable region of the heavy chain	35
	Nucleotide sequence encoding the variable region of the light chain	36
	Amino acid sequence encoding the variable region of the light chain	37
3.9	Nucleotide sequence encoding the variable region of the heavy chain	38
	Amino acid sequence encoding the variable region of the heavy chain	39
	Nucleotide sequence encoding the variable region of the light chain	40
	Amino acid sequence encoding the variable region of the light chain	41
3.10	Nucleotide sequence encoding the variable region of the heavy chain	42
	Amino acid sequence encoding the variable region of the heavy chain	43
	Nucleotide sequence encoding the variable region of the light chain	44
	Amino acid sequence encoding the variable region of the light chain	45
3.11	Nucleotide sequence encoding the variable region of the heavy chain	46
	Amino acid sequence encoding the variable region of the heavy chain	47
	Nucleotide sequence encoding the variable region of the light chain	48
	Amino acid sequence encoding the variable region of the light chain	49
3.12	Nucleotide sequence encoding the variable region of the heavy chain	50
	Amino acid sequence encoding the variable region of the heavy chain	51
	Nucleotide sequence encoding the variable region of the light chain	52
	Amino acid sequence encoding the variable region of the light chain	53
3.13	Nucleotide sequence encoding the variable region of the heavy chain	54
	Amino acid sequence encoding the variable region of the heavy chain	55
	Nucleotide sequence encoding the variable region of the light chain	56
	Amino acid sequence encoding the variable region of the light chain	57
3.14	Nucleotide sequence encoding the variable region of the heavy chain	58
	Amino acid sequence encoding the variable region of the heavy chain	59
	Nucleotide sequence encoding the variable region of the light chain	60
	Amino acid sequence encoding the variable region of the light chain	61
3.15	Nucleotide sequence encoding the variable region of the light chain	62
	Amino acid sequence encoding the variable region of the light chain	63
3.16	Nucleotide sequence encoding the variable region of the light chain	64
	Amino acid sequence encoding the variable region of the light chain	65
3.17	Nucleotide sequence encoding the variable region of the heavy chain	66
	Amino acid sequence encoding the variable region of the heavy chain	67
	Nucleotide sequence encoding the variable region of the light chain	68
	Amino acid sequence encoding the variable region of the light chain	69
3.18	Nucleotide sequence encoding the variable region of the heavy chain	70
	Amino acid sequence encoding the variable region of the heavy chain	71
	Nucleotide sequence encoding the variable region of the light chain	72
	Amino acid sequence encoding the variable region of the light chain	73
3.19.1	Nucleotide sequence encoding the variable region of the heavy chain	74
	Amino acid sequence encoding the variable region of the heavy chain	75
	Nucleotide sequence encoding the variable region of the light chain	76
	Amino acid sequence encoding the variable region of the light chain	77
3.19.3	Nucleotide sequence encoding the variable region of the heavy chain	78
	Amino acid sequence encoding the variable region of the heavy chain	79
	Nucleotide sequence encoding the variable region of the light chain	80

	Amino acid sequence encoding the variable region of the light chain	81
3.20	Nucleotide sequence encoding the variable region of the light chain	82
	Amino acid sequence encoding the variable region of the light chain	83
3.21	Nucleotide sequence encoding the variable region of the heavy chain	84
	Amino acid sequence encoding the variable region of the heavy chain	85
	Nucleotide sequence encoding the variable region of the light chain	86
	Amino acid sequence encoding the variable region of the light chain	87
3.22	Nucleotide sequence encoding the variable region of the heavy chain	88
	Amino acid sequence encoding the variable region of the heavy chain	89
	Nucleotide sequence encoding the variable region of the light chain	90
	Amino acid sequence encoding the variable region of the light chain	91
3.26	Nucleotide sequence encoding the variable region of the heavy chain	92
	Amino acid sequence encoding the variable region of the heavy chain	93
	Nucleotide sequence encoding the variable region of the light chain	94
	Amino acid sequence encoding the variable region of the light chain	95
3.28.1	Nucleotide sequence encoding the variable region of the heavy chain	96
	Amino acid sequence encoding the variable region of the heavy chain	97
3.31.1	Nucleotide sequence encoding the variable region of the heavy chain	98
	Amino acid sequence encoding the variable region of the heavy chain	99
	Nucleotide sequence encoding the variable region of the light chain	100
	Amino acid sequence encoding the variable region of the light chain	101
3.31.2	Nucleotide sequence encoding the variable region of the heavy chain	102
	Amino acid sequence encoding the variable region of the heavy chain	103
	Nucleotide sequence encoding the variable region of the light chain	104
	Amino acid sequence encoding the variable region of the light chain	105
3.32	Nucleotide sequence encoding the variable region of the heavy chain	106
	Amino acid sequence encoding the variable region of the heavy chain	107
	Nucleotide sequence encoding the variable region of the light chain	108
	Amino acid sequence encoding the variable region of the light chain	109
3.33	Nucleotide sequence encoding the variable region of the heavy chain	110
	Amino acid sequence encoding the variable region of the heavy chain	111
	Nucleotide sequence encoding the variable region of the light chain	112
	Amino acid sequence encoding the variable region of the light chain	113
3.34	Nucleotide sequence encoding the variable region of the heavy chain	114
	Amino acid sequence encoding the variable region of the heavy chain	115
	Nucleotide sequence encoding the variable region of the light chain	116
	Amino acid sequence encoding the variable region of the light chain	117
3.35	Nucleotide sequence encoding the variable region of the heavy chain	118
	Amino acid sequence encoding the variable region of the heavy chain	119
3.37	Nucleotide sequence encoding the variable region of the heavy chain	120
	Amino acid sequence encoding the variable region of the heavy chain	121
	Nucleotide sequence encoding the variable region of the light chain	122
	Amino acid sequence encoding the variable region of the light chain	123
3.39	Nucleotide sequence encoding the variable region of the heavy chain	124
	Amino acid sequence encoding the variable region of the heavy chain	125
	Nucleotide sequence encoding the variable region of the light chain	126
	Amino acid sequence encoding the variable region of the light chain	127

3.40	Nucleotide sequence encoding the variable region of the heavy chain	128
	Amino acid sequence encoding the variable region of the heavy chain	129
	Nucleotide sequence encoding the variable region of the light chain	130
	Amino acid sequence encoding the variable region of the light chain	131
3.41	Nucleotide sequence encoding the variable region of the heavy chain	132
	Amino acid sequence encoding the variable region of the heavy chain	133
	Nucleotide sequence encoding the variable region of the light chain	134
	Amino acid sequence encoding the variable region of the light chain	135
3.42	Nucleotide sequence encoding the variable region of the heavy chain	136
	Amino acid sequence encoding the variable region of the heavy chain	137
	Nucleotide sequence encoding the variable region of the light chain	138
	Amino acid sequence encoding the variable region of the light chain	139
4.2	Nucleotide sequence encoding the variable region of the heavy chain	140
	Amino acid sequence encoding the variable region of the heavy chain	141
	Nucleotide sequence encoding the variable region of the light chain	142
	Amino acid sequence encoding the variable region of the light chain	143
4.3	Nucleotide sequence encoding the variable region of the heavy chain	144
	Amino acid sequence encoding the variable region of the heavy chain	145
	Nucleotide sequence encoding the variable region of the light chain	146
	Amino acid sequence encoding the variable region of the light chain	147
4.4	Nucleotide sequence encoding the variable region of the light chain	148
	Amino acid sequence encoding the variable region of the light chain	149
4.5	Nucleotide sequence encoding the variable region of the heavy chain	150
	Amino acid sequence encoding the variable region of the heavy chain	151
	Nucleotide sequence encoding the variable region of the light chain	152
	Amino acid sequence encoding the variable region of the light chain	153
4.6	Nucleotide sequence encoding the variable region of the heavy chain	154
	Amino acid sequence encoding the variable region of the heavy chain	155
4.7	Nucleotide sequence encoding the variable region of the heavy chain	156
	Amino acid sequence encoding the variable region of the heavy chain	157
4.8	Nucleotide sequence encoding the variable region of the heavy chain	158
	Amino acid sequence encoding the variable region of the heavy chain	159
	Nucleotide sequence encoding the variable region of the light chain	160
	Amino acid sequence encoding the variable region of the light chain	161
4.9	Nucleotide sequence encoding the variable region of the heavy chain	162
	Amino acid sequence encoding the variable region of the heavy chain	163
	Nucleotide sequence encoding the variable region of the light chain	164
	Amino acid sequence encoding the variable region of the light chain	165
4.11	Nucleotide sequence encoding the variable region of the heavy chain	166
	Amino acid sequence encoding the variable region of the heavy chain	167
	Nucleotide sequence encoding the variable region of the light chain	168
	Amino acid sequence encoding the variable region of the light chain	169
4.13	Nucleotide sequence encoding the variable region of the heavy chain	170
	Amino acid sequence encoding the variable region of the heavy chain	171
	Nucleotide sequence encoding the variable region of the light chain	172
	Amino acid sequence encoding the variable region of the light chain	173
4.14	Nucleotide sequence encoding the variable region of the heavy chain	174

	Amino acid sequence encoding the variable region of the heavy chain	175
	Nucleotide sequence encoding the variable region of the light chain	176
	Amino acid sequence encoding the variable region of the light chain	177
4.15	Nucleotide sequence encoding the variable region of the heavy chain	178
	Amino acid sequence encoding the variable region of the heavy chain	179
	Nucleotide sequence encoding the variable region of the light chain	180
	Amino acid sequence encoding the variable region of the light chain	181
4.16	Nucleotide sequence encoding the variable region of the heavy chain	182
	Amino acid sequence encoding the variable region of the heavy chain	183
	Nucleotide sequence encoding the variable region of the light chain	184
	Amino acid sequence encoding the variable region of the light chain	185
4.18	Nucleotide sequence encoding the variable region of the heavy chain	186
	Amino acid sequence encoding the variable region of the heavy chain	187
	Nucleotide sequence encoding the variable region of the light chain	188
	Amino acid sequence encoding the variable region of the light chain	189
5.1	Nucleotide sequence encoding the variable region of the heavy chain	190
	Amino acid sequence encoding the variable region of the heavy chain	191
	Nucleotide sequence encoding the variable region of the light chain	192
	Amino acid sequence encoding the variable region of the light chain	193
5.2	Nucleotide sequence encoding the variable region of the heavy chain	194
	Amino acid sequence encoding the variable region of the heavy chain	195
5.2.1	Nucleotide sequence encoding the variable region of the heavy chain	196
	Amino acid sequence encoding the variable region of the heavy chain	197
5.3	Nucleotide sequence encoding the variable region of the light chain	198
	Amino acid sequence encoding the variable region of the light chain	199
5.4	Nucleotide sequence encoding the variable region of the heavy chain	200
	Amino acid sequence encoding the variable region of the heavy chain	201
5.5	Nucleotide sequence encoding the variable region of the heavy chain	202
	Amino acid sequence encoding the variable region of the heavy chain	203
5.6	Nucleotide sequence encoding the variable region of the heavy chain	204
	Amino acid sequence encoding the variable region of the heavy chain	205
	Nucleotide sequence encoding the variable region of the light chain	206
	Amino acid sequence encoding the variable region of the light chain	207
5.7	Nucleotide sequence encoding the variable region of the heavy chain	208
	Amino acid sequence encoding the variable region of the heavy chain	209
5.8	Nucleotide sequence encoding the variable region of the heavy chain	210
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Definitions

[0105] Unless otherwise defined, scientific and technical terms used herein 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. Generally, nomenclatures utilized in connection with, and techniques of, cell and tissue culture, molecular biology, and protein and oligo- or polynucleotide chemistry and hybridization described herein are those well known and commonly used in the art.

[0106] Standard techniques are used for recombinant DNA, oligonucleotide synthesis, and tissue culture and transformation (e.g., electroporation, lipofection). Enzymatic reactions and purification techniques are performed according to manufacturer's specifications or as commonly accomplished in the art or as described herein. The foregoing techniques and procedures 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. *See e.g.,* Sambrook *et al. Molecular Cloning: A Laboratory Manual* (3rd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (2001)), which is incorporated herein by reference. The nomenclatures utilized 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 are used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients.

[0107] As utilized in accordance with the present disclosure, the following terms, unless otherwise indicated, shall be understood to have the following meanings:

[0108] An antagonist may be a polypeptide, nucleic acid, carbohydrate, lipid, small molecular weight compound, an oligonucleotide, an oligopeptide, RNA interference (RNAi), antisense, a recombinant protein, an antibody, or conjugates or fusion proteins thereof. For a review of RNAi see Milhavet O, Gary DS, Mattson MP. (Pharmacol Rev. 2003 Dec;55(4):629-48. Review.) and antisense see Opalinska JB, Gewirtz AM. (Sci STKE. 2003 Oct 28;2003(206):pe47.)

[0109] Disease-related angiogenesis may be any abnormal, undesirable or pathological angiogenesis, for example tumor-related angiogenesis. Angiogenesis-related diseases include, but are not limited to, non-solid tumors such as leukaemia, multiple myeloma or lymphoma, and also solid tumors such as melanoma, small cell lung cancer, non-small cell lung cancer, glioma, hepatocellular (liver) carcinoma, glioblastoma, carcinoma of the thyroid, bile duct, bone, gastric, brain/CNS, head and neck, hepatic, stomach, prostate, breast, renal, testicular, ovarian, skin, cervical, lung, muscle, neuronal, oesophageal, bladder, lung, uterine, vulval, endometrial, kidney, colorectal, pancreatic, pleural/peritoneal membranes, salivary gland, and epidermoid tumors.

[0110] A compound refers to any small molecular weight compound with a molecular weight of less than about 2000 Daltons.

[0111] The term "Ang-2" refers to the molecule Angiopoietin-2.

[0112] The term "neutralizing" when referring to an antibody relates to the ability of an antibody to eliminate, or significantly reduce, the activity of a target antigen. Accordingly, a "neutralizing" anti-Ang-2 antibody is capable of eliminating or significantly reducing the activity of Ang-2. A neutralizing Ang-2 antibody may, for example, act by blocking the binding of Ang-2 to its receptor Tie2. By blocking this binding, the Tie2 mediated signal transduction is significantly, or completely, eliminated. Ideally, a neutralizing antibody against Ang-2 inhibits angiogenesis.

[0113] The term "isolated polynucleotide" as used herein shall mean a polynucleotide that has been isolated from its naturally occurring environment. Such polynucleotides may be genomic, cDNA, or synthetic. Isolated polynucleotides preferably are not associated with all or a portion of the polynucleotides they associate with in nature. The isolated polynucleotides may be operably linked to another polynucleotide that it is not linked to in nature. In addition, isolated polynucleotides preferably do not occur in nature as part of a larger sequence.

[0114] The term "isolated protein" referred to herein means a protein that has been isolated from its naturally occurring environment. Such proteins may be derived

from genomic DNA, cDNA, recombinant DNA, recombinant RNA, or synthetic origin or some combination thereof, which by virtue of its origin, or source of derivation, the "isolated protein" (1) is not associated with proteins found in nature, (2) is free of other proteins from the same source, e.g. free of murine proteins, (3) is expressed by a cell from a different species, or (4) does not occur in nature.

[0115] The term "polypeptide" is used herein as a generic term to refer to native protein, fragments, or analogs of a polypeptide sequence. Hence, native protein, fragments, and analogs are species of the polypeptide genus. Preferred polypeptides in accordance with the invention comprise the human heavy chain immunoglobulin molecules and the human kappa light chain immunoglobulin molecules, as well as antibody molecules formed by combinations comprising the heavy chain immunoglobulin molecules with light chain immunoglobulin molecules, such as the kappa or lambda light chain immunoglobulin molecules, and vice versa, as well as fragments and analogs thereof. Preferred polypeptides in accordance with the invention may also comprise solely the human heavy chain immunoglobulin molecules or fragments thereof.

[0116] The term "naturally-occurring" as used herein as applied to an object refers to the fact that an object can be found in nature. For example, a polypeptide or polynucleotide sequence that is present in an organism (including viruses) that can be isolated from a source in nature and which has not been intentionally modified by man in the laboratory or otherwise is naturally-occurring.

[0117] The term "operably linked" as used herein refers to positions of components so described that are in a relationship permitting them to function in their intended manner. For example, a control sequence "operably linked" to a coding sequence is connected in such a way that expression of the coding sequence is achieved under conditions compatible with the control sequences.

[0118] The term "control sequence" as used herein refers to polynucleotide sequences that are necessary either to effect or to affect the expression and processing of coding sequences to which they are connected. The nature of such control sequences differs depending upon the host organism; in prokaryotes, such control sequences generally include promoter, ribosomal binding site, and transcription termination sequence; in eukaryotes, generally, such control sequences may include promoters, enhancers, introns, transcription termination sequences, polyadenylation signal sequences, and 5' and '3 untranslated regions. The term "control sequences" 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.

[0119] The term "polynucleotide" 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, or RNA-DNA hetero-duplexes. The term includes single and double stranded forms of DNA.

[0120] The term "oligonucleotide" referred to herein includes naturally occurring, and modified nucleotides linked together by naturally occurring, and non-naturally occurring linkages. Oligonucleotides are a polynucleotide subset generally comprising a length of 200 bases or fewer. Preferably, oligonucleotides are 10 to 60 bases in length and most preferably 12, 13, 14, 15, 16, 17, 18, 19, or 20 to 40 bases in length. Oligonucleotides are usually single stranded, e.g. for probes; although oligonucleotides may be double stranded, e.g. for use in the construction of a gene mutant. Oligonucleotides can be either sense or antisense oligonucleotides.

[0121] The term "naturally occurring nucleotides" referred to herein includes deoxyribonucleotides and ribonucleotides. The term "modified nucleotides" referred to herein includes nucleotides with modified or substituted sugar groups and the like. The term "oligonucleotide linkages" referred to herein includes oligonucleotides linkages such as phosphorothioate, phosphorodithioate, phosphoroselenoate, phosphorodiselenoate, phosphoroanilothioate, phosphoraniladate, phosphoroamidate, and the like. *See e.g.,* LaPlanche *et al. Nucl. Acids Res.* 14:9081 (1986); Stec *et al. J. Am. Chem. Soc.* 106:6077 (1984); Stein *et al. Nucl. Acids Res.* 16:3209 (1988); Zon *et al. Anti-Cancer Drug Design* 6:539 (1991); Zon *et al. Oligonucleotides and Analogues: A Practical Approach*, pp. 87-108 (F. Eckstein, Ed., Oxford University Press, Oxford England (1991)); Stec *et al.* U.S. Patent No. 5,151,510; Uhlmann and Peyman *Chemical Reviews* 90:543 (1990), the disclosures of which are hereby incorporated by reference. An oligonucleotide can include a label for detection, if desired.

[0122] The term "selectively hybridize" referred to herein means to detectably and specifically bind. Polynucleotides, oligonucleotides and fragments thereof selectively hybridize to nucleic acid strands under hybridization and wash conditions that minimize appreciable amounts of detectable binding to nonspecific nucleic acids. High stringency conditions can be used to achieve selective hybridization conditions as known in the art and discussed herein. Generally, the nucleic acid sequence homology between the polynucleotides, oligonucleotides, or antibody fragments and a nucleic acid sequence

of interest will be at least 80%, and more typically with preferably increasing homologies of at least 85%, 90%, 95%, 99%, and 100%.

[0123] Two amino acid sequences are "homologous" if there is a partial or complete identity between their sequences. For example, 85% homology means that 85% of the amino acids are identical when the two sequences are aligned for maximum matching. Gaps (in either of the two sequences being matched) are allowed in maximizing matching; gap lengths of 5 or less are preferred with 2 or less being more preferred. Alternatively and preferably, two protein sequences (or polypeptide sequences derived from them of at least about 30 amino acids in length) are homologous, as this term is used herein, if they have an alignment score of at more than 5 (in standard deviation units) using the program ALIGN with the mutation data matrix and a gap penalty of 6 or greater. *See* Dayhoff, M.O., in *Atlas of Protein Sequence and Structure*, pp. 101-110 (Volume 5, National Biomedical Research Foundation (1972)) and Supplement 2 to this volume, pp. 1-10. The two sequences or parts thereof are more preferably homologous if their amino acids are greater than or equal to 50% identical when optimally aligned using the ALIGN program. It should be appreciated that there can be differing regions of homology within two orthologous sequences. For example, the functional sites of mouse and human orthologues may have a higher degree of homology than non-functional regions.

[0124] The term "corresponds to" is used herein to mean that a polynucleotide sequence is homologous (i.e., is identical, not strictly evolutionarily related) to all or a portion of a reference polynucleotide sequence, or that a polypeptide sequence is identical to a reference polypeptide sequence.

[0125] In contradistinction, the term "complementary to" is used herein to mean that the complementary sequence is homologous to all or a portion of a reference polynucleotide sequence. For illustration, the nucleotide sequence "TATAC" corresponds to a reference sequence "TATAC" and is complementary to a reference sequence "GTATA".

[0126] The following terms are used to describe the sequence relationships between two or more polynucleotide or amino acid sequences: "reference sequence", "comparison window", "sequence identity", "percentage of sequence identity", and "substantial identity". A "reference sequence" is a defined sequence used as a basis for a sequence comparison. A reference sequence may be a subset of a larger sequence, for example, as a segment of a full-length cDNA or gene sequence given in a sequence listing

or may comprise a complete cDNA or gene sequence. Generally, a reference sequence is at least 18 nucleotides or 6 amino acids in length, frequently at least 24 nucleotides or 8 amino acids in length, and often at least 48 nucleotides or 16 amino acids in length. Since two polynucleotides or amino acid sequences may each (1) comprise a sequence (i.e., a portion of the complete polynucleotide or amino acid sequence) that is similar between the two molecules, and (2) may further comprise a sequence that is divergent between the two polynucleotides or amino acid sequences, sequence comparisons between two (or more) molecules are typically performed by comparing sequences of the two molecules over a "comparison window" to identify and compare local regions of sequence similarity. A "comparison window", as used herein, refers to a conceptual segment of at least about 18 contiguous nucleotide positions or about 6 amino acids wherein the polynucleotide sequence or amino acid sequence is compared to a reference sequence of at least 18 contiguous nucleotides or 6 amino acid sequences and wherein the portion of the polynucleotide sequence in the comparison window may include additions, deletions, substitutions, and the like (i.e., gaps) of 20 percent or less as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. Optimal alignment of sequences for aligning a comparison window may be conducted by the local homology algorithm of Smith and Waterman *Adv. Appl. Math.* 2:482 (1981), by the homology alignment algorithm of Needleman and Wunsch *J. Mol. Biol.* 48:443 (1970), by the search for similarity method of Pearson and Lipman *Proc. Natl. Acad. Sci. (U.S.A.)* 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package Release 7.0, (Genetics Computer Group, 575 Science Dr., Madison, Wis.), GENEWORKS™, or MACVECTOR® software packages), or by inspection, and the best alignment (i.e., resulting in the highest percentage of homology over the comparison window) generated by the various methods is selected.

[0127] The term "sequence identity" means that two polynucleotide or amino acid sequences are identical (i.e., on a nucleotide-by-nucleotide or residue-by-residue basis) over the comparison window. The term "percentage of sequence identity" is calculated by comparing two optimally aligned sequences over the window of comparison, determining the number of positions at which the identical nucleic acid base (e.g., A, T, C, G, U, or I) or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the comparison window (i.e., the window size), and multiplying

the result by 100 to yield the percentage of sequence identity. The terms "substantial identity" as used herein denotes a characteristic of a polynucleotide or amino acid sequence, wherein the polynucleotide or amino acid comprises a sequence that has at least 85 percent sequence identity, preferably at least 90 to 95 percent sequence identity, more preferably at least 99 percent sequence identity, as compared to a reference sequence over a comparison window of at least 18 nucleotide (6 amino acid) positions, frequently over a window of at least 24-48 nucleotide (8-16 amino acid) positions, wherein the percentage of sequence identity is calculated by comparing the reference sequence to the sequence which may include deletions or additions which total 20 percent or less of the reference sequence over the comparison window. The reference sequence may be a subset of a larger sequence.

[0128] As used herein, the twenty conventional amino acids and their abbreviations follow conventional usage. *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. 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 may also be suitable components for polypeptides of the present invention. Examples of unconventional amino acids 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). In the polypeptide notation used herein, the left-hand direction is the amino terminal direction and the right-hand direction is the carboxy-terminal direction, in accordance with standard usage and convention.

[0129] Similarly, unless specified otherwise, the left-hand end of single-stranded polynucleotide sequences is the 5' end; the left-hand direction of double-stranded polynucleotide sequences is referred to as the 5' direction. The direction of 5' to 3' addition of nascent RNA transcripts is referred to as the transcription direction; sequence regions on the DNA strand having the same sequence as the RNA and which are 5' to the 5' end of the RNA transcript are referred to as "upstream sequences"; sequence regions on the DNA strand having the same sequence as the RNA and which are 3' to the 3' end of the RNA transcript are referred to as "downstream sequences".

[0130] As applied to polypeptides, the term "substantial identity" means that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 80 percent sequence identity, preferably at least 90 percent sequence identity, more preferably at least 95 percent sequence identity, and most preferably at least 99 percent sequence identity. Preferably, residue positions that are not identical differ by conservative amino acid substitutions. Conservative amino acid substitutions refer to the interchangeability of residues having similar side chains. For example, a group of amino acids having aliphatic side chains is glycine, alanine, valine, leucine, and isoleucine; a group of amino acids having aliphatic-hydroxyl side chains is serine and threonine; a group of amino acids having amide-containing side chains is asparagine and glutamine; a group of amino acids having aromatic side chains is phenylalanine, tyrosine, and tryptophan; a group of amino acids having basic side chains is lysine, arginine, and histidine; and a group of amino acids having sulfur-containing side chains is cysteine and methionine. Preferred conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, glutamic-aspartic, and asparagine-glutamine.

[0131] As discussed herein, minor variations in the amino acid sequences of antibodies or immunoglobulin molecules are contemplated as being encompassed by the present invention, providing that the variations in the amino acid sequence maintain at least 75%, more preferably at least 80%, 90%, 95%, and most preferably 99% sequence identity to the antibodies or immunoglobulin molecules described herein. In particular, conservative amino acid replacements are contemplated. Conservative replacements are those that take place within a family of amino acids that have related side chains. Genetically encoded amino acids are generally divided into families: (1) acidic=aspartate, glutamate; (2) basic=lysine, arginine, histidine; (3) non-polar=alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan; and (4) uncharged polar=glycine, asparagine, glutamine, cysteine, serine, threonine, tyrosine. More preferred families are: serine and threonine are an aliphatic-hydroxy family; asparagine and glutamine are an amide-containing family; alanine, valine, leucine and isoleucine are an aliphatic family; and phenylalanine, tryptophan, and tyrosine are an aromatic family. 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 function or properties of the resulting molecule, especially if the

replacement does not involve an amino acid within a framework site. Whether an amino acid change results in a functional peptide can readily be determined by assaying the specific activity of the polypeptide derivative. Assays are described in detail herein. Fragments or analogs of antibodies or immunoglobulin molecules can be readily prepared by those of ordinary skill in the art. Preferred amino- and carboxy-termini of fragments or analogs 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. Preferably, computerized comparison methods are used to identify sequence motifs or predicted protein conformation domains that occur in other proteins of known structure and/or function. Methods to identify protein sequences that fold into a known three-dimensional structure are known. Bowie *et al. Science* 253:164 (1991). Thus, the foregoing examples demonstrate that those of skill in the art can recognize sequence motifs and structural conformations that may be used to define structural and functional domains in accordance with the antibodies described herein.

[0132] Preferred amino acid substitutions are those which: (1) reduce susceptibility to proteolysis, (2) reduce susceptibility to oxidation, (3) alter binding affinity for forming protein complexes, (4) alter binding affinities, and (4) confer or modify other physicochemical or functional properties of such analogs. Analogs can include various muteins of a sequence other than the naturally-occurring peptide sequence. For example, single or multiple amino acid substitutions (preferably conservative amino acid substitutions) may be made in the naturally-occurring sequence (preferably in the portion of the polypeptide outside the domain(s) forming intermolecular contacts. A conservative amino acid substitution should not substantially change the structural characteristics of the parent sequence (e.g., a replacement amino acid should not tend to break a helix that occurs in the parent sequence, or disrupt other types of secondary structure that characterizes the parent sequence). Examples of art-recognized polypeptide secondary and tertiary structures are described in *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 at. *Nature* 354:105 (1991), which are each incorporated herein by reference.

[0133] The term "polypeptide fragment" as used herein refers to a polypeptide that has an amino-terminal and/or carboxy-terminal deletion, but where the remaining

amino acid sequence is identical to the corresponding positions in the naturally-occurring sequence deduced, for example, from a full-length cDNA sequence. Fragments typically are at least 5, 6, 8 or 10 amino acids long, preferably at least 14 amino acids long, more preferably at least 20 amino acids long, usually at least 50 amino acids long, and even more preferably at least 70 amino acids long. The term "analog" as used herein refers to polypeptides which are comprised of a segment of at least 25 amino acids that has substantial identity to a portion of a deduced amino acid sequence and which has at least one of the following properties: (1) specific binding to a Ang-2, under suitable binding conditions, (2) ability to block appropriate Ang-2 binding, or (3) ability to inhibit Ang-2 activity. Typically, polypeptide analogs comprise a conservative amino acid substitution (or addition or deletion) with respect to the naturally-occurring sequence. Analogs typically are at least 20 amino acids long, preferably at least 50 amino acids long or longer, and can often be as long as a full-length naturally-occurring polypeptide.

[0134] Peptide analogs are commonly used in the pharmaceutical industry as non-peptide drugs with properties analogous to those of the template peptide. These types of non-peptide compound are termed "peptide mimetics" or "peptidomimetics". Fauchere, *J. Adv. Drug Res.* 15:29 (1986); Veber and Freidinger *TINS* p.392 (1985); and Evans *et al. J. Med. Chem.* 30:1229 (1987), which are incorporated herein by reference. Such compounds are often developed with the aid of computerized molecular modeling. Peptide mimetics that are structurally similar to therapeutically useful peptides may be used to produce an equivalent therapeutic or prophylactic effect. Generally, peptidomimetics are structurally similar to a paradigm polypeptide (i.e., a polypeptide that has a biochemical property or pharmacological activity), such as human antibody, but have one or more peptide linkages optionally replaced by a linkage selected from the group consisting of: --CH_2NH--, --CH_2S--, --CH_2-CH_2--, --CH=CH--(cis and trans), --$COCH_2$--, --$CH(OH)CH_2$--, and –CH_2SO--, by methods well known in the art. Systematic substitution of one or more amino acids of a consensus sequence with a D-amino acid of the same type (e.g., D-lysine in place of L-lysine) may be used to generate more stable peptides. In addition, constrained peptides comprising a consensus sequence or a substantially identical consensus sequence variation may be generated by methods known in the art (Rizo and Gierasch *Ann. Rev. Biochem.* 61:387 (1992), incorporated herein by reference); for example, by adding internal cysteine residues capable of forming intramolecular disulfide bridges which cyclize the peptide.

[0135] As used herein, the term "antibody" refers to a polypeptide or group of polypeptides that are comprised of at least one binding domain that is formed from the folding of polypeptide chains having three-dimensional binding spaces with internal surface shapes and charge distributions complementary to the features of an antigenic determinant of an antigen. An antibody typically has a tetrameric form, comprising two identical pairs of polypeptide chains, each pair having one "light" and one "heavy" chain. The variable regions of each light/heavy chain pair form an antibody binding site.

[0136] As used herein, a "targeted binding agent" is an antibody, or binding fragment thereof, that preferentially binds to a target site. In one embodiment, the targeted binding agent is specific for only one target site. In other embodiments, the targeted binding agent is specific for more than one target site. In one embodiment, the targeted binding agent may be a monoclonal antibody and the target site may be an epitope.

[0137] "Binding fragments" of an antibody are produced by recombinant DNA techniques, or by enzymatic or chemical cleavage of intact antibodies. Binding fragments include Fab, Fab', $F(ab')_2$, Fv, and single-chain antibodies. An antibody other than a "bispecific" or "bifunctional" antibody is understood to have each of its binding sites identical. An antibody substantially inhibits adhesion of a receptor to a counter-receptor when an excess of antibody reduces the quantity of receptor bound to counter-receptor by at least about 20%, 40%, 60% or 80%, and more usually greater than about 85% (as measured in an *in vitro* competitive binding assay).

[0138] An antibody may be oligoclonal, a polyclonal antibody, a monoclonal antibody, a chimeric antibody, a CDR-grafted antibody, a multi-specific antibody, a bi-specific antibody, a catalytic antibody, a chimeric antibody, a humanized antibody, a fully human antibody, an anti-idiotypic antibody and antibodies that can be labeled in soluble or bound form as well as fragments, variants or derivatives thereof, either alone or in combination with other amino acid sequences provided by known techniques. An antibody may be from any species. The term antibody also includes binding fragments of the antibodies of the invention; exemplary fragments include Fv, Fab , Fab', single stranded antibody (svFC), dimeric variable region (Diabody) and disulphide stabilized variable region (dsFv).

[0139] The term "epitope" includes any protein determinant capable of specific binding to an immunoglobulin or T-cell receptor. Epitopic determinants usually consist of chemically active surface groupings of molecules such as amino acids or sugar

side chains and may, but not always, have specific three-dimensional structural characteristics, as well as specific charge characteristics. An antibody is said to specifically bind an antigen when the dissociation constant is ≤1 μM, preferably ≤ 100 nM and most preferably ≤ 10 nM.

[0140] The term "agent" is used herein to denote a chemical compound, a mixture of chemical compounds, a biological macromolecule, or an extract made from biological materials.

[0141] "Active" or "activity" in regard to an Ang-2 polypeptide refers to a portion of an Ang-2 polypeptide that has a biological or an immunological activity of a native Ang-2 polypeptide. "Biological" when used herein refers to a biological function that results from the activity of the native Ang-2 polypeptide. A preferred Ang-2 biological activity includes, for example, Ang-2 induced angiogenesis.

[0142] "Mammal" when used herein refers to any animal that is considered a mammal. Preferably, the mammal is human.

[0143] Digestion of antibodies with the enzyme, papain, results in two identical antigen-binding fragments, known also as "Fab" fragments, and a "Fc" fragment, having no antigen-binding activity but having the ability to crystallize. Digestion of antibodies with the enzyme, pepsin, results in the a $F(ab')_2$ fragment in which the two arms of the antibody molecule remain linked and comprise two-antigen binding sites. The $F(ab')_2$ fragment has the ability to crosslink antigen.

[0144] "Fv" when used herein refers to the minimum fragment of an antibody that retains both antigen-recognition and antigen-binding sites.

[0145] "Fab" when used herein refers to a fragment of an antibody that comprises the constant domain of the light chain and the CH1 domain of the heavy chain.

[0146] The term "mAb" refers to monoclonal antibody.

[0147] "Liposome" when used herein refers to a small vesicle that may be useful for delivery of drugs that may include the Ang-2 polypeptide of the invention or antibodies to such an Ang-2 polypeptide to a mammal.

[0148] "Label" or "labeled" as used herein refers to the addition of a detectable moiety to a polypeptide, for example, a radiolabel, fluorescent label, enzymatic label chemiluminescent labeled or a biotinyl group. Radioisotopes or radionuclides may include ^{3}H, ^{14}C, ^{15}N, ^{35}S, ^{90}Y, ^{99}Tc, ^{111}In, ^{125}I, ^{131}I, fluorescent labels may include

rhodamine, lanthanide phosphors or FITC and enzymatic labels may include horseradish peroxidase, β-galactosidase, luciferase, alkaline phosphatase.

[0149] The term "pharmaceutical agent or drug" as used herein refers to a chemical compound or composition capable of inducing a desired therapeutic effect when properly administered to a patient. Other chemistry terms herein are used according to conventional usage in the art, as exemplified by *The McGraw-Hill Dictionary of Chemical Terms* (Parker, S., Ed., McGraw-Hill, San Francisco (1985)), (incorporated herein by reference).

[0150] As used herein, "substantially pure" means an object species is the predominant species present (i.e., on a molar basis it is more abundant than any other individual species in the composition), and preferably a substantially purified fraction is a composition wherein the object species comprises at least about 50 percent (on a molar basis) of all macromolecular species present. Generally, a substantially pure composition will comprise more than about 80 percent of all macromolecular species present in the composition, more preferably more than about 85%, 90%, 95%, and 99%. Most preferably, the object species is purified to essential homogeneity (contaminant species cannot be detected in the composition by conventional detection methods) wherein the composition consists essentially of a single macromolecular species.

[0151] The term "patient" includes human and veterinary subjects.

Human Antibodies and Humanization of Antibodies

[0152] Human antibodies avoid some 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 can be generated through the introduction of functional human antibody loci into a rodent, other mammal or animal so that the rodent, other mammal or animal produces fully human antibodies.

[0153] 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. Nature Genetics* 15:146-156 (1997) and Green and

Jakobovits *J. Exp. Med.* 188:483-495 (1998). The XenoMouse® strains are available from Abgenix, Inc. (Fremont, CA).

[0154] The production of the XenoMouse® strains of mice is further 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.

[0155] 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. *See further* Taylor *et al.*, 1992, Chen *et al.*, 1993, Tuaillon *et al.*, 1993, Choi *et al.*, 1993, Lonberg *et al.*, (1994), Taylor *et al.*, (1994), and Tuaillon *et al.*, (1995), Fishwild *et al.*, (1996), the disclosures of which are hereby incorporated by reference in their entirety.

[0156] 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.*, Cloning Stem Cells, (2002) 4:91-102).

[0157] 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.

Preparation of Antibodies

[0158] Antibodies, as described herein, were prepared through the utilization of the XenoMouse® technology, as described below. Such mice, then, are capable of producing human immunoglobulin molecules and antibodies and are deficient in the production of murine immunoglobulin molecules and antibodies. Technologies utilized for achieving the same are disclosed in the patents, applications, and references disclosed in the background section herein. In particular, however, a preferred embodiment of transgenic production of mice and antibodies therefrom is 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. Nature Genetics* **15**:146-156 (1997), the disclosure of which is hereby incorporated by reference.

[0159] 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. Ang-2), 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. Provided herein are methods for the production of multiple hybridoma cell lines that produce antibodies specific to Ang-1 and Ang-2. 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.

[0160] Alternatively, instead of being fused to myeloma cells to generate hybridomas, B cells can be directly assayed. For example, CD19+ 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 Ang-2 immunogen. The supernatants might also be screened for immunoreactivity against fragments of Ang-2 to further map the different antibodies for binding to domains of functional interest on Ang-2. The antibodies may also be screened against Ang-1, Ang-3 or Ang-4, other related human chemokines and against the rat, the mouse, and non-human primate, such as Cynomolgus monkey, orthologues of Ang-2, 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 Ang-1 or Ang-2-specific hemolytic plaque assay (see for example Babcook *et al.*, *Proc. Natl. Acad. Sci. USA* 93:7843-48 (1996)). Cells targeted for lysis are preferably sheep red blood cells (SRBCs) coated with the Ang-2

antigen. In screening for an antibody also able to antagonize Angiopoietin-1 the above methods can equally be used substituting Angiopoietin-2 with Angiopoietin-1.

[0161] In the presence of a B-cell culture containing plasma cells secreting the immunoglobulin of interest and complement, the formation of a plaque indicates specific Ang-1/Ang-2-mediated lysis of the sheep red blood cells surrounding the plasma cell of interest. The single antigen-specific plasma cell in the center of the plaque can be isolated and the genetic information that encodes the specificity of the antibody is isolated from the single plasma cell. Using reverse-transcription followed by PCR (RT-PCR), the DNA encoding the heavy and light chain variable regions of the antibody can be cloned. Such cloned DNA can then be further inserted into a suitable expression vector, preferably a vector cassette such as a pcDNA, more preferably such a pcDNA vector containing the constant domains of immunglobulin heavy and light chain. The generated vector can then be transfected into host cells, e.g., HEK293 cells, CHO cells, and cultured in conventional nutrient media modified as appropriate for inducing transcription, selecting transformants, or amplifying the genes encoding the desired sequences.

[0162] In general, antibodies produced by the fused hybridomas were human IgG2 heavy chains with fully human kappa or lambda light chains. Antibodies described herein possess human IgG4 heavy chains as well as IgG2 heavy chains. Antibodies can also be of other human isotypes, including IgG1. The antibodies possessed high affinities, typically possessing a Kd of from about 10^{-6} through about 10^{-12} M or below, when measured by solid phase and solution phase techniques. Antibodies possessing a KD of at least 10^{-11} M are preferred to inhibit the activity of Ang-1 and/or Ang-2.

[0163] As will be appreciated, antibodies can be expressed in cell lines other than hybridoma cell lines. Sequences encoding particular antibodies can be used to transform a suitable mammalian host cell. Transformation 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.

[0164] 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 antibodies with constitutive Ang-2 binding properties.

[0165] Based on the ability of mAbs to significantly neutralize Angiopoietin-1 and Angiopoietin-2 activity (as demonstrated in the Examples below), these antibodies will have therapeutic effects in treating symptoms and conditions resulting from Angiopoietin-1 and/or Angiopoietin-2 expression. In specific embodiments, the antibodies and methods herein relate to the treatment of symptoms resulting from Angiopoietin-1 and/or Angiopoietin-2 induced angiogenesis.

[0166] According to another aspect of the invention there is provided a pharmaceutical composition comprising an antagonist of the biological activity of Angiopoietin-1 and Angiopoietin-2, and a pharmaceutically acceptable carrier. In one embodiment the antagonist comprises an antibody. According to another aspect of the invention there is provided a pharmaceutical composition comprising an antagonist of the biological activity of Angiopoietin-2, and a pharmaceutically acceptable carrier. In one embodiment the antagonist comprises an antibody.

[0167] Anti-Ang-2 antibodies are useful in the detection of Ang-2 in patient samples and accordingly are useful as diagnostics for disease states as described herein. In addition, based on their ability to significantly neutralize Ang-2 activity (as demonstrated in the Examples below), anti-Ang-2 antibodies have therapeutic effects in treating symptoms and conditions resulting from Ang-2 expression. In specific embodiments, the antibodies and methods herein relate to the treatment of symptoms resulting from Ang-2 induced angiogenesis. Further embodiments involve using the antibodies and methods described herein to treat angiogenesis-related diseases including neoplastic diseases, such as, melanoma, small cell lung cancer, non-small cell lung cancer, glioma, hepatocellular (liver) carcinoma, thyroid tumor, gastric (stomach) cancer,

prostate cancer, breast cancer, ovarian cancer, bladder cancer, lung cancer, glioblastoma, endometrial cancer, kidney cancer, colon cancer, and pancreatic cancer.

Therapeutic Administration and Formulations

[0168] Embodiments of the invention include sterile pharmaceutical formulations of anti-Ang-2 antibodies or antibodies which bind to both Ang-1 and Ang-2 that are useful as treatments for diseases. Such formulations would inhibit the binding of Ang-2 or Ang-1 and Ang-2 to its receptor Tie2, thereby effectively treating pathological conditions where, for example, serum or tissue Ang-1 and/or Ang-2 is abnormally elevated. Anti-Ang-2 antibodies preferably possess adequate affinity to potently neutralize Ang-2, and preferably have an adequate duration of action to allow for infrequent dosing in humans. Anti-Ang-1/Ang-2 antibodies preferably possess adequate affinity to potently neutralize Ang-1 and Ang-2, and preferably have an adequate duration of action to allow for infrequent dosing in humans. A prolonged duration of action will allow for less frequent and more convenient dosing schedules by alternate parenteral routes such as subcutaneous or intramuscular injection.

[0169] Sterile formulations can be created, for example, by filtration through sterile filtration membranes, prior to or following lyophilization and reconstitution of the antibody. The antibody ordinarily will be 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.

[0170] The route of antibody administration is in accord with known methods, e.g., injection or infusion by intravenous, intraperitoneal, intracerebral, intramuscular, intraocular, intraarterial, intrathecal, inhalation or intralesional routes, or by sustained release systems as noted below. The antibody is preferably administered continuously by infusion or by bolus injection.

[0171] An effective amount of antibody 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 that the therapist titer the dosage and modify the route of administration as required to obtain the optimal therapeutic effect. Typically, the clinician will administer antibody until a dosage is reached that achieves

the desired effect. The progress of this therapy is easily monitored by conventional assays or by the assays described herein.

[0172] Antibodies, 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 having due regard for pH, 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.

[0173] 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.

[0174] 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.*, *J. Biomed Mater. Res.*, (1981) 15:167-277

and Langer, *Chem. Tech.*, (1982) 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.*, *Biopolymers*, (1983) 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).

[0175] 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.

[0176] Sustained-released compositions also include preparations of crystals of the antibody suspended in suitable formulations capable of maintaining crystals in suspension. These preparations when injected subcutaneously or intraperitonealy 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.*, *Proc. Natl. Acad. Sci. USA*, (1985) 82:3688-3692; Hwang *et al.*, *Proc. Natl. Acad. Sci. USA*, (1980) 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.

[0177] The dosage of the antibody 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.

[0178] An effective amount of the antibodies, 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 0.001mg/kg to up to 100mg/kg or more, depending on the factors mentioned above. Typically, the clinician will administer the therapeutic antibody 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.

[0179] 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 in accordance with the present invention, 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." *Regul. Toxicol. Pharmacol.* 32(2):210-8 (2000), Wang W. "Lyophilization and development of solid protein pharmaceuticals." *Int. J. Pharm.* 203(1-2):1-60 (2000), Charman WN "Lipids, lipophilic drugs, and oral drug delivery-some emerging concepts." *J Pharm Sci* .89(8):967-78 (2000), Powell *et al.* "Compendium of excipients for parenteral formulations" *PDA J Pharm Sci Technol.* 52:238-311 (1998) and the citations therein for additional information related to formulations, excipients and carriers well known to pharmaceutical chemists.

Combinations

[0180] The anti-angiogenic treatment defined herein may be applied as a sole therapy or may involve, in addition to the compounds of the invention, conventional surgery or radiotherapy or chemotherapy. Such chemotherapy may include one or more of the following categories of anti tumor agents:

(i) cytostatic agents such as antioestrogens (for example tamoxifen, toremifene, raloxifene, droloxifene and iodoxyfene), oestrogen receptor down regulators (for example fulvestrant), antiandrogens (for example bicalutamide, flutamide, nilutamide and cyproterone acetate), LHRH antagonists or LHRH agonists (for example goserelin, leuprorelin and buserelin), progestogens (for example megestrol acetate), aromatase inhibitors (for example as anastrozole, letrozole, vorazole and exemestane) and inhibitors of 5* reductase such as finasteride;

(ii) agents which inhibit cancer cell invasion (for example metalloproteinase inhibitors like marimastat and inhibitors of urokinase plasminogen activator receptor function);

(iii) inhibitors of growth factor function, for example such inhibitors include growth factor antibodies, growth factor receptor antibodies (for example the anti erbb2 antibody trastuzumab [Herceptin™] and the anti erbb1 antibody cetuximab [C225]) , farnesyl transferase inhibitors, tyrosine kinase inhibitors and serine/threonine kinase inhibitors, for example inhibitors of the epidermal growth factor family (for example EGFR family tyrosine kinase inhibitors such as N (3 chloro 4 fluorophenyl) 7 methoxy 6 (3 morpholinopropoxy)quinazolin 4 amine (gefitinib, AZD1839), N (3 ethynylphenyl) 6,7 bis(2 methoxyethoxy)quinazolin 4 amine (erlotinib, OSI 774) and 6 acrylamido N (3 chloro 4 fluorophenyl) 7 (3 morpholinopropoxy)quinazolin 4 amine (CI 1033)), for example inhibitors of the platelet derived growth factor family and for example inhibitors of the hepatocyte growth factor family;

(iv) antiangiogenic agents such as those which inhibit the effects of vascular endothelial growth factor, (for example the anti vascular endothelial cell growth factor antibody bevacizumab [Avastin™], anti-vascular endothelial growth factor receptor antibodies such anti-KDR antibodies and anti-flt1 antibodies, compounds such as those disclosed in International Patent Applications WO 97/22596, WO 97/30035, WO 97/3285, WO 98/13354, WO00/47212 and WO01/32651) and compounds that work by other mechanisms (for example linomide, inhibitors of integrin avb3 function and angiostatin);

(v) vascular damaging agents such as Combretastatin A4 and compounds disclosed in International Patent Applications WO 99/02166, WO 00/40529, WO 00/41669, WO 01/92224, WO 02/04434 and WO 02/08213;

(vi) antisense therapies, for example those which are directed to the targets listed above, such as ISIS 2503, an anti ras antisense;

(vii) gene therapy approaches, including for example approaches to replace aberrant genes such as aberrant p53 or aberrant BRCA1 or BRCA2, GDEPT (gene directed enzyme pro drug therapy) approaches such as those using cytosine deaminase, thymidine kinase or a bacterial nitroreductase enzyme and approaches to increase patient tolerance to chemotherapy or radiotherapy such as multi drug resistance gene therapy; and

(viii) immunotherapy approaches, including for example ex vivo and in vivo approaches to increase the immunogenicity of patient tumour cells, such as transfection with cytokines such as interleukin 2, interleukin 4 or granulocyte macrophage colony stimulating factor, approaches to decrease T cell anergy, approaches using transfected immune cells such as cytokine transfected dendritic cells, approaches using cytokine transfected tumour cell lines and approaches using anti idiotypic antibodies.

[0181] In one embodiment of the invention the anti-angiogenic treatments of the invention are combined with agents which inhibit the effects of vascular endothelial growth factor (VEGF), (for example the anti-vascular endothelial cell growth factor antibody bevacizumab (Avastin®), anti-vascular endothelial growth factor receptor antibodies such anti-KDR antibodies and anti-flt1 antibodies, compounds such as those disclosed in International Patent Applications WO 97/22596, WO 97/30035, WO 97/3285, WO 98/13354, WO00/47212 and WO01/32651) and compounds that work by other mechanisms (for example linomide, inhibitors of integrin avb3 function and angiostatin); In another embodiment of the invention the anti-angiogenic treatments of the invention are combined agents which inhibit the tyrosine kinase activity of the vascular endothelial growth factor receptor, KDR (for example AZD2171 or AZD6474). Additional details on AZD2171 may be found in Wedge et al (2005) Cancer Research. 65(10):4389-400. Additional details on AZD6474 may be found in Ryan & Wedge (2005) British Journal of Cancer. 92 Suppl 1:S6-13. Both publications are herein incorporated by reference in their entireties. In another embodiment of the invention the fully human antibodies 3.19.3, 3.3.2 or 5.88.3 are combined alone or in combination with Avastin™, AZD2171 or AZD6474.

[0182] Such conjoint treatment may be achieved by way of the simultaneous, sequential or separate dosing of the individual components of the treatment. Such combination products employ the compounds of this invention, or pharmaceutically acceptable salts thereof, within the dosage range described hereinbefore and the other pharmaceutically active agent within its approved dosage range.

EXAMPLES

[0183] 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.

EXAMPLE 1

IMMUNIZATION AND TITERING

Immunization

[0184] Recombinant human Ang-2 obtained from R&D Systems, Inc. (Minneapolis, MN Cat. No. 623-AM/CF) was used as an antigen. Monoclonal antibodies against Ang-2 were developed by sequentially immunizing XenoMouse® mice (XenoMouse strains XMG2 and XMG4 (3C-1 strain), Abgenix, Inc. Fremont, CA). XenoMouse animals were immunized via footpad route for all injections. The total volume of each injection was 50 μl per mouse, 25 μl per footpad. The first injection was with 2.35 μg recombinant human Ang-2 (rhAng-2, cat#623-AM/CF; lot #BN023202A) in pyrogen-free Dulbecco's PBS (DPBS) and admixed 1:1 v/v with 10 μg CpG (15 μl of ImmunEasy Mouse Adjuvant, catalog # 303101; lot #11553042; Qiagen) per mouse. The next 6 boosts were with 2.35 μg rhANG-2 in pyrogen-free DPBS, admixed with 25 μg of Adju-Phos (aluminum phosphate gel, Catalog # 1452-250, batch #8937, HCI Biosector) and 10 μg CpG per mouse, followed by a final boost of 2.35 μg rhAng-2 in pyrogen-free DPBS, without adjuvant. The XenoMouse mice were immunized on days 0, 3, 6, 10, 13, 17, 20, and 24 for this protocol and fusions were performed on day 29.

Selection of Animals for Harvest by Titer

[0185] Anti-Ang-2 antibody titers in the serum from immunized XenoMouse mice were determined by ELISA. Briefly, recombinant Ang-2 (1 μg/ml) was coated onto Costar Labcoat Universal Binding Polystyrene 96-well plates (Corning, Acton, MA) overnight at four degrees in Antigen Coating Buffer (0.1 M Carbonate Buffer, pH 9.6 $NaHCO_3$ 8.4 g/L). The next day, the plates were washed 3 times 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 h. After the one-hour blocking, the plates

were washed 3 times with washing buffer using a Biotek plate washer. Sera from either Ang-2 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 hr, and the plates were then washed 3 times with washing buffer using a Biotek plate washer. A goat anti-human IgG Fc-specific horseradish peroxidase (HRP, Pierce, Rockford, IL) conjugated antibody was added at a final concentration of 1 μg/ml and incubated for 1 hour at room temperature. Then the plates were washed 3 times with washing buffer using a Biotek plate washer.

[0186] After washing, the plates were developed with the addition of TMB chromogenic substrate (BioFx BSTP-0100-01) for 10-20 min 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 Tables 2 and 3 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 Ang-2.

Table 2. Group 1: 10 mice (XMG2 strain)

	After 4 injections	**After 6 injections**
Mouse ID	Reactivity to rhAng-2	
	Titers via hIgG	
O825-1	92,000	231,000
O825-2	56,000	212,000
O825-3	73,000	331,000
O825-4	16,000	175,000
O825-5	95,000	236,000
O825-6	27,000	119,000
O825-7	100,000	239,000
O825-8	25,000	165,000
O825-9	68,000	136,000
O825-10	120,000	264,000
NC	35	65
PC	Sensitivity: 10 ng/ml	Sensitivity: 9 ng/ml

* NC = xmg2 strain, ova gp2, fp
* PC = goat AB, anti-huAng-2 (R&D Systems, Catalog No. AF623) 1 mg/ml

Table 3. Group 2: 10 mice (XMG4 strain)

	After 4 injections	After 6 injections
Mouse ID	Reactivity to rhAng-2	
	Titers via hIgG	
O824-1	750	4,600
O824-2	200	5,800
O824-3	500	7,400
O824-4	225	4,700
O824-5	300	5,800
O824-6	550	7,400
O824-7	1,600	11,000
O824-8	45	2,400
O824-9	600	6,900
O824-10	225	2,300
NC	<100	35
PC	Sensitivity: 12 ng/ml	Sensitivity: 8 ng/ml

* NC = 3c-1 N128-3
* PC = goat AB, anti-huAng-2 (R&D Systems, Catalog No. AF623) 1 mg/ml

EXAMPLE 2

RECOVERY OF LYMPHOCYTES, B-CELL ISOLATIONS, FUSIONS AND GENERATION OF HYBRIDOMAS

[0187] Immunized mice were sacrificed by cervical dislocation, and the draining lymph nodes 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 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).

[0188] 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, J. Immunol. 123, 1979, 1548-1550) at a ratio of 1:1. The cell mixture was gently pelleted by centrifugation at 800 x g. After complete removal of the supernatant, the cells were 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, 0.3M Sucrose, Sigma, Cat# S7903, 0.1mM Magnesium Acetate, Sigma, Cat# M2545, 0.1mM 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.

[0189] 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:

[0190] Alignment condition: voltage: 50 V, time: 50 sec.

[0191] Membrane breaking at: voltage: 3000 V, time: 30 μsec

[0192] Post-fusion holding time: 3 sec

[0193] After ECF, 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 x g (1000 rpm) 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 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 re-fed with Hybridoma Selection Medium.

EXAMPLE 3

SELECTION OF CANDIDATE ANTIBODIES BY ELISA

[0194] After 14 days of culture, hybridoma supernatants were screened for Ang-2-specific monoclonal antibodies. The ELISA plates (Fisher, Cat. No. 12-565-136) were coated with 50 μl/well of human Ang-2 (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) 3 times. 200 µl/well Blocking Buffer (0.5% BSA, 0.1% Tween 20, 0.01% Thimerosal in 1x PBS) were added and the plates incubated at room temperature for 1 hour. After incubation, the plates were washed with Washing Buffer three times. 50 µl/well of hybridoma supernatants, and positive and negative controls were added and the plates incubated at room temperature for 2 hours. The positive control used throughout was serum from the Ang-2 immunized XenoMouse mouse, XMG2 Ang-2 Group 1, footpad (fp) N160-7, and the negative control was serum from the KLH-immunized XenoMouse mouse, XMG2 KLH Group 1, footpad (fp) L627-6.

[0195] After incubation, the plates were washed three times with Washing Buffer. 100 µl/well of detection antibody goat anti-huIgGFc-HRP (Caltag, Cat. No. H10507), was added and the plates incubated at room temperature for 1 hour. In the secondary screen, the positives in first screening were screened in two sets, one for hIgG detection and the other for human Ig kappa light chain detection (goat anti-hIg kappa-HRP (Southern Biotechnology, Cat. No. 2060-05) in order to demonstrate fully human composition for both IgG and Ig kappa. After incubation, the plates were washed three times with Washing Buffer. 100 µl/well of TMB (BioFX Lab. Cat. No. TMSK-0100-01) were added and the plates 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 read on an ELISA plate reader at 450nm. There were 185 fully human IgG kappa antibodies against Ang-2.

[0196] All antibodies that bound in the ELISA assay were counter screened for binding to Ang-1 by ELISA in order to exclude those that cross-reacted with Ang-1. The ELISA plates (Fisher, Cat. No. 12-565-136) were coated with 50 µl/well of recombinant Ang-1 (2 µg/ml, obtained from R&D Systems, Cat. # 293-AN-025/CF) in Coating Buffer (0.1 M Carbonate Buffer, pH 9.6, $NaHCO_3$ 8.4 g/L), then incubated at 4°C overnight. Under the experimental conditions described here, when the recombinant Ang-1 molecule was immobilized on the ELISA plate, no antibodies were found to bind to Ang-1. However, the counter screening described here has technical limitations. First, the antibodies derive from line materials, but not a cloned hybridoma. Binding signals from a particular clone, which only account for a minor percentage of the line, may fall below the detection sensitivity threshold. Second, certain epitopes in the antigen may be concealed from the antibodies in this experiment due to slight conformational changes

induced by immobilization of the antigen. For all of these reasons, the cross-reactivity of each antibody to Ang-1 was further examined using cloned antibodies and a Biacore system (see Example 8). As described in Example 8, one clone (mAb 3.19.3) was in fact found to have strong cross-reactivity to human recombinant Ang-1 (Examples 8, 9 and 12).

EXAMPLE 4

INHIBITION OF ANG-2 BINDING TO TIE2

[0197] As discussed above, Ang-2 exerts its biological effect by binding to the Tie2 receptor. Monoclonal antibodies that inhibited Ang-2/Tie2 binding were identified by a competitive binding assay using a modified ELISA. The mAbs used were products of micro-purification from 50 ml of exhaustive supernatants of the hybridoma pools that were specific for Ang-2 (see Example 3). 96-well Nunc Immplates™ were coated with 100 µl of recombinant human Tie2/Fc fusion protein (R&D Systems, Inc., Cat. No. 313-TI-100) at 4 µg/ml by incubating overnight at 4°C. The plates were washed four times using Phosphate Buffer Saline (PBS) with a Skan™ Washer 300 station (SKATRON). The wells were blocked by 100 µl of ABX-blocking buffer (0.5% BSA, 0.1%Tween, 0.01% Thimerosal in PBS) for 1 hour.

[0198] Biotinylated recombinant human Ang-2 (R&D Systems, Inc. Cat. No. BT623) at 100 ng/ml was added in each well with or without the anti Ang-2 mAbs at 100 µg/ml. The plates were incubated at room temperature for two hours before the unbound molecules were washed off. Bound biotinylated Ang-2 was then detected using 100 µl/well of Streptavidin-HRP conjugate at 1:200 by incubating at room temperature for half an hour. After washing twice, the bound Streptavidin was detected by HRP substrate (R&D Systems, Cat. No. DY998). The plates were incubated for 30 minutes before 450 stop solution (100 µl/well, BioFX, Cat# BSTP-0100-01) was added to terminate the reaction. The light absorbance at 450 nm was determined by a Spectramax Plus reader.

[0199] Soluble recombinant Tie2/Fc fusion protein at 10-fold molar excess to Ang-2 was used as a positive control. At this concentration, Tie2/Fc inhibited binding of Ang-2 to immobilized Tie2 by 80%. With this as an arbitrary criterion, 74 out of 175 Ang-2 binding mAbs showed inhibitory activity. For the convenience of operation, the top 27 neutralizers were selected for subsequent hybridoma cloning.

[0200] Each hybridoma was cloned using a limited dilution method by following standard procedures. Three sister clones were collected from each hybridoma. For each clone, the supernatant was tested using ELISA binding to human Ang-2 and counter binding to Ang-1, as described above, to ensure that each antibody was only specific for Ang-2. Concentrations of IgG in the exhaustive supernatants were determined, and one clone with the highest yield among the three sister clones from each hybridoma was selected for IgG purification. 0.5 to 1 mg of IgG was purified from each supernatant for further characterization.

[0201] To quantitate the inhibitory activities of the mAbs on Ang-2 binding to Tie2, the titer was determined for purified mAbs from the top 27 candidates using a competitive binding assay. Each concentration of the mAb was tested in duplicate. The concentration-response relationship was found by curve fitting using Graphpad Prism™ graphic software (non-linear, Sigmoid curve). The maximal inhibition (efficacy) and IC_{50} (potency) were calculated by the software. Ten monoclonal antibodies that exhibited both relative high efficacy and potency were selected for further investigation. The efficacy and potency of these 10 mAbs are listed in Table 4.

Table 4. Efficacy and potency of top 10 mAbs

Clone	**Efficacy***	**EC50 (μg/ml)**
3.31.2	0.3751	0.04169
5.16.3	0.3279	0.08532
5.86.1	0.3844	0.1331
5.88.3	0.4032	0.1557
3.3.2	0.3881	0.1684
5.103.1	0.2317	0.3643
5.101.1	0.3639	0.3762
3.19.3	0.3945	0.7976
5.28.1	0.3892	2.698
5.78.3	0.2621	5.969

* Efficacy is expressed as the ratio of bound Ang-2 with mAbs (30 μg/ml) versus without mAbs.

EXAMPLE 5

BINNING OF ANTIBODIES

[0202] Epitope binning was performed to determine which of the anti-Ang-2 antibodies would cross compete with one another, and thus were likely to bind to the

same epitope on Ang-2. The binning process is described in U.S. Patent Application 20030175760, also described in Jia et al., J. Immunol. Methods, (2004) 288:91-98, both of which are incorporated by reference in entirety. Briefly, Luminex beads were coupled with mouse anti-huIgG (Pharmingen# 555784) following the protein coupling protocol provided on the Luminex website. Pre-coupled beads were prepared for coupling to primary unknown antibody using the following procedure, protecting the beads from light. Individual tubes were used for each unknown supernatant. The volume of supernatant needed was calculated using the following formula: (nX2+10) x 50 µl (where n = total number of samples). A concentration of 0.1 µg/ml was used in this assay. The bead stock was gently vortexed, and diluted in supernatant to a concentration of 2500 of each bead in 50 µl per well or $0.5X10^5$ beads/ml.

[0203] Samples were incubated on a shaker in the dark at room temperature overnight.

[0204] The filter plate was pre-wetted by adding 200 µl wash buffer per well, which was then aspirated. 50 µl of each bead was added to each well of the filter plate. Samples were washed once by adding 100 µl/well wash buffer and aspirating. Antigen and controls were added to the filter plate at 50 µl/well. The plate was covered, incubated in the dark for 1 hour on a shaker, and then samples were washed 3 times. A secondary unknown antibody was then added at 50 µl/well. A concentration of 0.1 µg/ml was used for the primary antibody. The plate was then incubated in the dark for 2 hours at room temperature on a shaker, and then samples were washed 3 times. 50 µl/well of biotinylated mouse anti-human IgG (Pharmingen #555785) diluted at 1:500 was added, and samples were incubated in the dark for 1 hour with shaking at room temperature.

[0205] Samples were washed 3 times. 50 µl/well Streptavidin-PE at a 1:1000 dilution was added, and samples were incubated in the dark for 15 minutes with shaking at room temperature. After running two wash cycles on the Luminex100, samples were washed 3 times. Contents in each well were resuspended in 80 µl blocking buffer. Samples were carefully mixed with pipetting several times to resuspend the beads. Samples were then analyzed on the Luminex100. Results are presented below in Table 5.

Table 5. Bins for top 24 of Ang-2 antibodies positive in functional assay

Bin 1	Bin 2	Bin 3	Bin 4	Bin 5	Bin 6	Bin 7	Bin 8
3.3	3.38	5.56*	5.28	5.78	3.19	6.3	5.35
3.28	5.103						5.40
3.31							
5.14	*5.2*						
5.16							
5.39							
5.41							
5.49							
5.54							
5.62							
5.83							
5.86							
5.88							
5.101							
5.108							

*Note: mAb 5.56 had a similar binding pattern as that of 3.38 and 5.103 with minor differences and much lower signal.

EXAMPLE 6

DETERMINATION OF ANTI-ANG-2 ANTIBODY AFFINITY USING BIACORE ANALYSIS

Low Resolution Screen of 27 Purified Monoclonal Antibodies

[0206] The label-free surface plasmon resonance (SPR), or Biacore, was utilized to measure the antibody affinity to the antigen. For this purpose, a high-density goat anti- human antibody surface over a CM5 Biacore chip was prepared using routine amine coupling. All the purified mAbs were diluted to approximately 8 μg/ml in HBS-P running buffer containing 100 μg/ml BSA and 10 mg/mL carboxymethyldextran. Each mAb was captured on a separate surface using a 42-second contact time, and a 5-minute wash for stabilization of the mAb baseline.

[0207] Ang-2 was injected at 90.9 nM over all surfaces for one minute, followed by a 10-minute dissociation. Double-referenced binding data was prepared by subtracting the signal from a control flow cell and subtracting the baseline drift of a buffer injected just prior to the Ang-2 injection. Ang-2 binding data for each mAb were normalized for the amount of mAb captured on each surface, and the normalized, drift-corrected responses for the 27 mAbs were determined. Data were fit globally to a 1:1 interaction model to determine the binding kinetics. The kinetic analysis results of Ang-2

binding at 25°C are listed in the table below. The mAbs are ranked from highest to lowest affinity.

Table 6. Ang-2 low resolution Biacore screen of 27 purified monoclonal antibodies

Sample	Amt. Captured (RU)	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_d (pM)
5.16	157	3.6×10^5	1.0×10^{-5} *	27
5.41	152	3.6×10^5	1.0×10^{-5} *	28
5.35	138	3.4×10^5	1.0×10^{-5} *	29
3.38	143	3.4×10^5	1.0×10^{-5} *	30
5.108	66	3.2×10^5	1.0×10^{-5} *	31
3.3	125	3.0×10^5	1.0×10^{-5} *	33
5.49	260	3.0×10^5	1.0×10^{-5} *	33
3.28	280	2.7×10^5	1.0×10^{-5} *	37
5.88	65	2.7×10^5	1.0×10^{-5} *	37
5.28	136	2.5×10^5	1.0×10^{-5} *	40
5.78	222	2.4×10^5	1.0×10^{-5} *	42
5.39	166	2.3×10^5	1.0×10^{-5} *	43
5.103	127	2.2×10^5	1.0×10^{-5} *	45
5.13	78	2.1×10^5	1.0×10^{-5} *	47
5.14	471	2.0×10^5	1.0×10^{-5} *	49
3.31	196	1.9×10^5	1.0×10^{-5} *	51
5.56	144	1.9×10^5	1.0×10^{-5} *	52
5.2	111	1.6×10^5	1.0×10^{-5} *	62
5.62	126	1.5×10^5	1.0×10^{-5} *	65
5.54	131	1.5×10^5	1.0×10^{-5} *	66
6.3	221	1.4×10^5	1.0×10^{-5} *	73
3.19	252	9.0×10^4	1.0×10^{-5} *	111
5.40	130	7.8×10^4	1.0×10^{-5} *	129
5.83	157	6.8×10^4	1.0×10^{-5} *	147
5.101	217	1.5×10^5	8.7×10^{-5}	581
5.86	126	1.5×10^5	1.1×10^{-4}	744
5.52	114	1.3×10^5	1.0×10^{-5} *	750

[0208] The asterisks next to the k_d results for all mAbs except for mAbs 5.101 and 5.86 indicate that these k_d's were held constant as a best estimate for the order of magnitude characteristic of slow off-rate data. The fitting model for these samples detected no measurable change in the off-rate over the relatively brief dissociation time and therefore required the k_d to be constant in order to fit the on-rate data. The data for those indicated k_d's also fit well in a simulation with the k_d on the order of 10^{-6} s^{-1}, therefore the interactions may be 10-fold or more stronger than reported above.

[0209] Dissociation data is normally measured 4-6 hours for high-resolution kinetic experiments with mAbs having sub-100 pM affinities. The maximum dissociation time that can be measured without drift artifacts from a captured mAb surface is 20 minutes. Almost negligible signal decay is measured over such a relatively short time with high affinity mAbs so the k_d estimate may vary by as much as two orders of magnitude.

EXAMPLE 7

DETERMINATION OF ANTI-ANG-2 ANTIBODY AFFINITY USING BIACORE ANALYSIS

Ang-2 Medium/High Resolution Screen with Three Purified Monoclonal Antibodies

[0210] Purified mAbs 5.16, 5.35, and 5.41 were diluted to approximately 8 μg/ml in 10 mM sodium acetate, pH 5.0. Each diluted mAB was then immobilized on a different flow cell surface (CM5 Biacore chip) using routine amine coupling.

[0211] For on-rate data acquisition, eight concentrations (2-fold dilutions) ranging from 90.9 – 0.71 nM of Ang-2 were randomly injected for 90 seconds in triplicate with several buffer injections interspersed for double referencing, followed by a four minute dissociation. The antibody surfaces were regenerated with two 9-second pulses of 10 mM glycine-HCl, pH 1.5 after each injection cycle.

[0212] For off-rate data acquisition, three 90.9 nM Ang-2 samples in HBS-P running buffer containing 100 μg/ml BSA were injected as described above and dissociation data was recorded over eight hours. The sample injections alternated with three blank injection cycles. Regeneration was performed as described above.

[0213] The data were globally fit to a 1:1 interaction model with mass transport using CLAMP (David G. Myszka and Thomas Morton (1998) "CLAMP©: a biosensor kinetic data analysis program," TIBS **23**, 149-150). The resulting binding constants are shown in Table 7 below.

Table 7. Ang-2 medium resolution Biacore screen of 3 purified monoclonal antibodies

Sample	R_{max}	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_d (pM)
5.16	36	3.41×10^5	2.77×10^{-6}	8.13
5.35	54	5.64×10^5	1.87×10^{-6}	3.31
5.41	44	4.69×10^5	8.31×10^{-6}*	17.7*

[0214] Significant signal decay was measured over the 8-hour dissociation time. With the eight hour dissociation data, CLAMP was able to more reasonably estimate the k_d for each mAb. Here the k_d's for both 5.16 and 5.35 are on the order of 10^{-6} s^{-1}.

[0215] The cross-reactivity of antibodies to Ang-1 was then investigated by measuring the affinity of the mAbs to Ang-1, as described below in Example 8.

EXAMPLE 8

DETERMINATION OF ANTI-ANG-1 ANTIBODY AFFINITY USING BIACORE ANALYSIS

[0216] The cross-reactivity of antibodies to Ang-1 was further investigated by measuring the affinity of the mAbs to Ang-1. Instead of immobilizing Ang-1, as described in ELISA-based counter-binding (Example 3), Ang-2 mAbs were immobilized to the CM5 Biacore chips, and Ang-1 in solution was injected for the determination of the on-rate and off-rate. Six mAbs, including 3.3.2, 3.31.2, 5.16.3, 5.86.1, 5.88.3, and 3.19.3, were tested in this experiment as described below to determine how strongly they cross-reacted with Ang-1.

Medium Resolution Screen of Six Purified Monoclonal Antibodies

[0217] Label-free surface plasmon resonance (SPR), or a Biacore 2000 instrumentation, was utilized to measure antibody affinity to Ang-1. For this purpose, a high-density goat α-human antibody surface over a CM5 Biacore chip was prepared using routine amine coupling. For developmental experiments, purified mAbs (clones 3.19.3, 3.3.2, 5.88.3, 5.86.1, 3.31.2, 5.16.3) were diluted to approximately 2.5-3.5 μg/ml in HBS-P running buffer containing 100 μg/ml BSA. The capture level for each mAb was approximately 150 RU. A 5-minute wash followed each capture cycle to stabilize the mAb baseline.

[0218] A single Ang-1 sample diluted to 87.4 nM in the running buffer was injected for one minute over all capture surfaces. No binding was evident for five mAbs, although Ang-1 was found to bind to mAb 3.19.3. This experiment was repeated by increasing the mAb capture levels to well over 500-600 RU and injecting 380 nM Ang-1 for one minute. The mAb 3.19.3 was again found to bind Ang-1.

[0219] Because Ang-1 only showed binding activity towards mAb 3.19.3, the affinity of this mAb to both Ang-1 and Ang-2 was determined. Since Ang-1 displayed a slow off-rate during the above developmental experiments, a medium resolution capture experiment would not have recorded sufficient off-rate data to accurately estimate kd. As a result, the binding of Ang-1 and Ang-2 to mAb 3.19.3 was measured under high-resolution Biacore conditions.

EXAMPLE 9

DETERMINATION OF MAB 3.19.3 AFFINITY FOR ANG-1 AND ANG-2 USING HIGH RESOLUTION BIACORE ANALYSIS

[0217] Purified mAb 3.19.3 was diluted to 12.5 μg/ml in 10 mM sodium acetate, pH 4.0. The mAb was then immobilized on flow cells 1-3 (CM5 Biacore chip) using routine amine coupling, leaving flow cell 4 as the reference flow cell.

[0220] For on-rate data acquisition, eight concentrations (2-fold dilutions) ranging from 39.8 – 0.31 nM of Ang-1 (in HBS-P running buffer containing 100 μg/ml BSA) were randomly injected for 90 seconds (100 μL/min. flow rate) in triplicate with several buffer injections interspersed for double referencing, followed by a four minute dissociation. The antibody surfaces were regenerated with a 6-second pulse of 10 mM NaOH after each injection cycle.

[0221] For off-rate data acquisition, three 19.9 nM Ang-1 samples were injected as described above and dissociation data was recorded over six hours. The sample injections alternated with three blank injection cycles. Regeneration was performed as described above.

[0222] The data were globally fit to a 1:1 interaction model with a term for mass transport included using CLAMP (David G. Myszka and Thomas Morton (1998) "CLAMP©: a biosensor kinetic data analysis program," TIBS **23**, 149-150).

ANG-2 High Resolution Biacore Study with Purified MAb 3.19.3

[0223] Purified mAb 3.19.3 was diluted to 12.5 μg/ml in 10 mM sodium acetate, pH 4.0. The mAb was then immobilized on flow cells 1-3 (CM5 Biacore chip) using routine amine coupling, leaving flow cell 4 as the reference flow cell.

[0224] For on-rate data acquisition, eight concentrations (2-fold dilutions) ranging from 30.0 – 0.23 nM of Ang-2 (in HBS-P running buffer containing 100 μg/ml

BSA) were randomly injected for 90 seconds (100 μL/min. flow rate) in triplicate with several buffer injections interspersed for double referencing, followed by a four minute dissociation. The antibody surfaces were regenerated with a 6-second pulse of 15 mM NaOH after each injection cycle.

[0225] For off-rate data acquisition, three 15.0 nM Ang-2 samples were injected as described above and dissociation data were recorded over six hours. The sample injections alternated with three blank injection cycles. Each surface was regenerated with a 6-second pulse of 15 mM NaOH after each long off-rate injection cycle.

[0226] The data were globally fit to a 1:1 interaction model with a term for mass transport included using CLAMP (David G. Myszka and Thomas Morton (1998) "CLAMP©: a biosensor kinetic data analysis program," TIBS **23**, 149-150).

Results And Discussion: ANG-1 and ANG-2 High Resolution Biacore Study With MAb 3.19.3

[0227] Two independent experiments were run with each antigen. The results are shown in Table 8 below:

Table 8. High resolution Biacore results of Ang-1 and Ang-2 binding to purified mAb 3.19.3

Antigen	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (pM)
Ang-1 (1st)	1.33×10^5	4.05×10^{-6}	30.4
Ang-1 (2nd)	1.82×10^5	5.51×10^{-6}	30.2
Ang-2 (1st)	1.89×10^5	1.00×10^{-6}*	5.3
Ang-2 (2nd)	1.78×10^5	1.00×10^{-6}*	5.6

[0228] The k_d's for Ang-2 in the above table are asterisked because these values were held constant during the 1:1 interaction model fit in CLAMP software. There was no significant dissociation signal recorded for the Ang-2 experiments, so the best off-rate estimate was to hold k_d constant at 1×10^{-6} sec^{-1}. The long off-rate data for Ang-2 actually displayed an upward trend over the six hours of data acquisition. This trend was repeated on two different sensor chips with two different instruments after both instruments had been subjected to a "super clean" maintenance protocol. In order to more precisely measure the binding affinity of mAb 3.19.3 for Ang-1 and Ang-2, a further experiment (see Example 10) was run to determine the Kd of mAb 3.19.3 towards these antigens.

[0229] Interestingly, mAb 3.19.3 did not bind to Ang-1 in the ELISA-based binding assay (Example 3), when Ang-1 was immobilized on the ELISA plate. The likely explanation for this discrepancy is that when Ang-1 was immobilized on the surface of plastics, a subtle epitope critical for the binding of mAb 3.19.3 was not exposed appropriately. However, when Ang-1 was in the liquid phase, such as in the Biacore experimental conditions, this epitope became accessible to mAb 3.19.3 and binding occurred.

EXAMPLE 10

DETERMINATION OF AFFINITY OF MAB 3.19.3 TO HUMAN ANG-2 USING HIGH RESOLUTION KINEXA (KINETIC EXCLUSION ASSAY)

[0230] When affinity of mAb 3.19.3 to human Ang-2 was measured using high resolution Biacore (Example 9), it was found that there was no significant dissociation signal. The long off-rate data for Ang-2 shows an upward trend over the six-hour data acquisition. Because of this, the K_D of mAb 3.19.3 binding to human Ang-2 was determined using KinExA technology, with the goal of obtaining a more reliable Kd value. For this purpose, a KinExA 3000 instrument was utilized. Firstly, 1 mL (~271 μg) of stock Ang-2 (R&D Systems, Inc., Lot# BNO32510A) was buffer exchanged into 1xPBS, pH 7.0 using a 10 mL desalting column (Pierce D-Salt™ polyacrylamide column, 6000 molecular weight cut-off, Lot# GF97965). The concentration of the pooled fractions was determined to be 1.7 μM using the protein concentration determination method described by C. Nick Pace (Pace, et al., *Protein Science*, Vol. 4: 2411-2423, 1995). Secondly, 200 mg of polymethyl methacrylate (PMMA, Lot# 206-01) beads were coupled with 450μL (~122 μg) of stock Ang-2 overnight at 24 °C. The beads were then centrifuged and washed once with blocking buffer (1xPBS, 10 mg/ml BSA), centrifuged again, and then incubated in blocking buffer for one hour at 24 °C. After blocking, the beads were diluted in approximately 30 mL of HBS buffer (0.01 M Hepes, 0.15 M NaCl, pH 7.4) in a standard KinExA bead reservoir vial and placed on the instrument.

K_D-controlled titration

[0231] Twelve solutions containing a mAb 3.19.3 binding site concentration of 25.3 pM were titrated with increasing concentrations of Ang-2. The buffer-exchanged Ang-2 was used for the sample preparations. Each solution had a total volume of 25 mL

and was allowed to equilibrate for 5 days at ~24 °C. The titration solutions were prepared using volumetric glassware and the Ang-2 concentrations varied from 5.09 nM to 99.3 fM. The KinExA instrument method used for the analysis of these solutions consisted of a bead packing step in which the PMMA beads were packed into a glass capillary, and the equilibrated solutions were flowed through the bead column at 0.25 mL/min for 6 min (1.5 mL) in duplicate. Subsequently, a fluorescently labeled Cy-5 goat anti-human (Fc specific) polyclonal antibody at 3.4 nM was flowed through the bead pack for 1 min at 0.5 mL/min to label the mAb with free binding sites 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, Sapidyne, Inc.). The resulting K_D-controlled titration curve was fit with the KinExA software to a 1:1 equilibrium isotherm with a drift correction factor included. The value of the K_D that fit the data optimally was 86.4 pM with low and high 95% confidence limits at 64.3 pM and 98.7 pM, respectively. A mAb-controlled titration curve was not performed.

EXAMPLE 11

BLOCKAGE OF ANG-2-INDUCED TIE2 PHOSPHORYLATION ECTOPICALLY EXPRESSED IN HEK293 CELLS

[0232] As discussed above, Tie2 is an endothelial cell specific receptor tyrosine kinase. *In vitro* experiments with vascular endothelial cells show that Ang-1 induces Tie2 phosphorylation, whereas, Ang-2 inhibits the receptor phosphorylation induced by Ang-1. However, when Tie2 is expressed ectopically, such as in fibroblasts, Ang-2 is also able to induce Tie2 phosphorylation under certain conditions, including but not limited to, prolonged exposure to Angiopoietin-2 or exposure to high concentrations of Angiopoietin-2.

[0233] Ang-2 induced Tie2 phosphorylation also occurs when the receptor is expressed in HEK293F cells. The ability of anti-Ang-2 mAbs to block Ang-2 induced Tie2 phosphorylation was examined using HEK293F cells transfected with human Tie2 receptor. Plasmid vector pORK/pBS-SK having a Tie2 cDNA was obtained from the ATCC (Cat. No. 69003, Genbank sequence BC033514). The accuracy of the cDNA was confirmed by nucleotide sequencing. A 3.9 kb fragment containing a 3375 bp cDNA that encodes human Tie2 was removed from the vector by EcoRI digestion. This fragment

was subcloned in the proper orientation into a pCR3.1 vector digested with EcoRI following standard procedures. The selected plasmid was amplified and purified by standard protocols.

[0234] The Tie2 containing construct obtained by the above procedures was transfected into HEK293F cells by the calcium phosphate transfection method. $1X10^6$ HEK293F cells were grown in 100 mm tissue culture dishes coated with 1% gelatin at 37°C with 5% CO_2. The cells were fed with fresh media for 2-3 hours before transfection. 10 μg of the plasmid DNA was dissolved in 248 mM calcium phosphate solution. Transfection was performed using standard procedures. Stable transfectants were selected by incubation in 0.5 mg/ml G418. Stable transfectants expressing Tie2 were identified by FACS analysis using mouse anti-Tie2 mAb (R&D Cat. No. MAB313) and a goat anti-mouse IgG-PE conjugate antibody (Caltag, Cat. No. M30004-4) for detection.

[0235] To perform the Tie2 phosphorylation assay, HEK 293F/Tie-2 transfectants were grown in 60 mm cell culture dishes at a density of $2x10^6$ cells/plate with complete medium at 37°C with 5% CO_2 until they reached sub-confluency. The complete medium in each plate was replaced with 2 ml of serum free medium. The cells were incubated for an additional 16 hours. Subsequently, the medium was replaced again with 2 ml of serum-free medium. After an additional 2-hr incubation, the cells were treated with 0.1 mM sodium orthovanadate (Sigma, Cat. No. S 6508) for 20 minutes. The cells were treated with Ang-2 (2 μg/ml) in the presence or absence of mAbs at 100 μg/ml. Treatments were performed in duplicate. A negative control without Ang-2 treatment was included. Cells were rinsed with ice-cold TBS containing vanadate and lysed with 300 μl/plate of cooled NP-40 lysis buffer (50mM Hepes, pH7.2, supplemented with 0.15M NaCl, 10% glycerol, 10mM pyrophosphate, 50mM NaF, 1% NP40, 100U/ml aprotinin, 1mM PMSF, 0.1mM orthovanadate, 10 μM leupeptin and 10 μM pepstatin A), while putting the plates on ice for 10 minutes. The treated cells were scraped from the plates into a microtube pre-chilled on ice.

[0236] The cell lysates were sonicated briefly and centrifuged at 12,000 x g for 10 minutes at 4°C in a tabletop microfuge. Supernatants were collected into fresh microtubes, and 1-5 μg of anti-Tie2 mAb (R&D Systems, Inc.) were added to the supernatant, followed by gentle rocking for 2 hours at 4°C. 50 μl of ImmunoPure Immobilized Protein A (PIERCE Cat. No. 20333) was added to the mixture, and

incubated for at least 3 hours at 4°C on a rocking platform. Complexes were collected by centrifugation at 12,000 x g for 10 minutes. After carefully removing the supernatant, the complexes were washed twice with the lysis buffer by centrifuging (12,000 x g, 4°C) for 4 minutes. The pellets were re-suspended in 50 µl of 2X electrophoresis sample buffer (Invitrogen, Cat. No. LC-2676) with 1 mM of β-mercaptoethanol or DTT, and boiled for 5 minutes before being centrifuged (12,000 x g, 4°C) for 5 minutes. The supernatants were transferred to fresh tubes.

[0237] The samples were loaded into the wells of an SDS-PAGE gel (e.g., 4-20% Tris-Glycine gel, Invitrogen, Cat. No. EC 6025). Electrophoreses was performed in Tris-Glycine buffer system. After electrophoresis, the gel was blotted onto a PVDF membrane (Invitrogen, Cat. No. LC 2005) following a standard protocol. Tyrosine phosphorylation was probed with 4G10 anti-phosphotyrosine antibody at 1 µg/ml (Upstate, Cat. No. 05-321) by incubation for 1 hour at room temperature with shaking, and followed by washing with 1x TBST (TBS with 0.1% of Tween-20) three times. The bound antibodies were detected by incubation with horseradish peroxidase-conjugated goat anti-mouse IgG (Santa Cruz, Cat. No. sc-2302) at 1:10,000 dilution for 1 hour at room temperature, followed by the enhance chemiluminescence reaction using SuperSignal West Dura Extended Duration Substrate system (PIERCE Cat. No. 34075). Subsequently, the blot was stripped with Restore Western Blot Stripping Buffer (PIERCE, Cat. No. 21059) and re-probed with specific antibodies against RTK to verify the quality of sample loading.

[0238] It was discovered that when human Tie2 was ectopically expressed in HEK293F cells, autophosphorylation of Tie2 was not detectable. In response to Ang-2 (2 µg/ml) treatment, a significant level of tyrosine phosphorylation was detected by a mAb to phosphorylated tyrosine (4G10) from Tie2 immunoprecipitated by the specific mAb. At a concentration of 100 µg/ml, all the anti-Ang-2 mAbs tested showed obvious inhibition of Tie2 phosphorylation, whereas, the isotype control mAb did not have inhibitory effect (Figure 1). Monoclonal antibody 5.103.1, which is not shown in Figure 1, had a similar inhibitory effect.

[0239] To rank the potency of the Ang-2 mAbs to inhibit Ang-2-induced Tie2 phosphorylation *in vitro*, an ELISA-based quantifiable method to detect Tie2 phosphorylation was established. Briefly, cell lysates were made from HEK293F/Tie2 transfectants that were treated with Ang-2 with the mAbs at various concentrations. Total

Tie2 from the lysate was captured in the 96-well ELISA plate that was coated with mouse anti-hTie-2 mAb. The phosphorylated Tie2 was detected using primary antibody 4G10-HRP (purchased from Upstate) and HRP substrate solution. OD at 650 nm was determined by a SpectraMax reader. The concentration-response relationship was found by curve fitting using Graphpad Prism™ graphic software (non-linear, Sigmoid curve). The maximal inhibition (efficacy) and IC_{50} (potency) were calculated as shown in Figure 2. The EC50 was calculated as shown in below in Table 9.

Table 9

mAb	EC50 (μg/ml)	95% CI
3.19.3	0.006	0.004 to 0.009
5.86.1	0.008	0.007 to 0.011
5.88.3	0.016	0.011 to 0.024
3.31.2	0.043	0.029 to 0.064
3.3.2	0.046	0.020 to 0.105
5.16.3	0.089	0.046 to 0.174
5.103.3	0.095	0.046 to 0.199
5.101.1	0.733	0.487 to 1.105

[0240] As reported above, it was found that mAb 3.19.3 cross-reacted with Ang-1. However, the results of initial experiments did not find inhibition of Angiopoietin-1 induced Tie-2 phosphorylation by mAb 3.19.3. It is worthwhile to note that ectopic expression of Tie2 may affect its susceptibility to activation by different ligands, as evidenced by the fact that Ang-2 does not induce Tie2 phosphorylation in HUVECs, whereas, it does induce robust Tie2 phosphorylation when the receptor is ecotopically expressed in HEK293 cells.

[0241] In view of these results further experiments were performed to more fully investigate whether mAb 3.19.3 was capable of inhibiting binding of both Ang-1 and Ang-2 to cell-bound Tie2. In addition, inhibition of Angiopoietin-1 induced Tie-2 phosphorylation by mAb 3.19.3 was investigated in more detail as described in Example 12 below.

EXAMPLE 12

MAB 3.19.3 INHIBITS ANGIOPOIETIN-1 BINDING TO TIE-2 AND ANG-1 INDUCED TIE2 PHOSPHORYLATION

[0242] The mAb 3.19.3 cross-reacts with human Ang-1 (Examples 8 and 9). However, initial experiments indicated that mAb 3.19.3 did not inhibit Tie2

phosphorylation induced by Ang-1. The discrepancy may be explained by the following: (1) high concentration of Ang-1, which is far above physiological concentration, may be required to generate robust Tie2 phosphorylation signal; or (2) Ecotopic expression of Tie2 in HEK293 may alter the conformation of Tie2, and thus change its susceptibility to different ligands. To test there hypotheses mAb 3.19.3 was tested in a binding assay where low concentration of Ang-1 or Ang-2 (3 nM) bound to cell surface Tie2. It was found that mAb 3.19.3 inhibited binding of both Ang-1 and Ang-2 in this experiment. Secondly, immortalized endothelial cells (EA.hy926/B3) were used to investigate Ang-1 induced Tie2 phosphorylation. The results of this experiment, as described in more detail below, demonstrate that mAb 3.19.3 inhibits Ang-1 induced Tie2 phosphorylation in a dose-dependent manner.

[0243] HEK293F/Tie2 transfectants were allowed to grow until 95% confluent in culture flasks before being harvested. Cell suspension of 4 million cells/mL in FACS Buffer were prepared, and then aliquoted to a 96-well polypropylene plate with 50 ul per well. The mAb 3.19.3 with indicated concentrations were added into the cell suspension. Subsequently, solutions of recombinant human Ang-1 and Ang-2 were added into the cell suspension, followed by incubation at room temperature for 2 hours. Cells were washed by centrifuging the plate at 1,200rpm for 5 minutes, removing the supernatant by aspirating, and resuspending the cells with 200ul per well of FACS Buffer. Washing procedures were repeated twice. The cells were then suspended with 100ul of mouse Anti-6X-Histidine antibody diluted to 2ug/ml in FACS Buffer, and incubated at room temperature for 30 minutes. After washing, the cells were suspended in 100ul of PE-conjugated goat anti-mouse-IgG, which was diluted 1:100 in FACS Buffer, for incubation at room temperature for 30 minutes. The volume of the samples were brought to 300ul with FACS Buffer, and measured with FACS Calibur.

[0244] The results are illustrated in Figure 3 and summarized in Table 10. As shown, soluble Tie2/Fc dose-dependently inhibited binding of both Ang-1 and Ang-2 by blocking the ligands, whereas, the isotype control mAb, PK16.3.1 had no effect in the binding of either ligand. The mAb 3.19.3 showed concentration dependent inhibition of both Ang-1 and Ang-2. Interestingly, with the potency of Tie2/Fc as a reference, the potency of mAb 3.19.3 to the binding of Ang-2 was higher than that to Ang-1.

Table 10. Inhibition of Ang-1 and Ang-2 binding to Tie2

	EC50 (nM)	
	Ang-1	**Ang-2**
Tie2/Fc	18.73	25.70
3.19.3	218.5	0.7310

[0245] These results indicated that mAb 3.19.3 not only bound to human Ang-1, but also blocked its binding to the receptor Tie2. This was further confirmed by the inhibition of Ang-1 induced Tie2 phosphorylation in immortalized endothelial cells as described below.

[0246] The inhibitory activity of the mAb 3.19.3 on Ang-1 induced Tie2 phosphorylation was quantified as follows. The mAb showed an obvious increasing inhibition of Tie-2 phosphorylation with increasing antibody concentration, as shown in Figures 4 and 5. A plot of the dose response curve led to the calculation of an IC50 of 99nM.

Angiopoietin-1 ligand stimulated Tie-2 receptor phosphorylation assay

[0247] EA.hy926/B3 cells were seeded into 6 well plates using 2.5 x 105 EA.hy 926 cells /well in 2 ml volume DMEM; HAT; 10 % FCS and incubated for 3 days under standard mammalian cell growth conditions.

[0248] The growth medium was replaced with 2 mls of DMEM (with no FCS) and the cells serum-starved for a total of 2 hours. Test compounds were diluted in DMEM; 1% FCS to twice the desired final concentration. After 1 hour 40 minutes of the serum starvation, the medium was removed from the cells and replaced with 1ml of test compound dilutions. Similarly, non-compound treated controls were also progressed to provide samples that represent 100% ligand stimulation standards.

[0249] Incubation was continued for a further 10 minutes after which 100 µl of an 10mM orthovanadate in DMEM solution was added to each well to obtain a final concentration of 1 mM orthovanadate in each well. The cells were then incubated for the last 10 minutes of the 2 hour serum starvation period.

[0250] Once the 2 hour serum starvation period was complete, 1ml of Angiopoietin-1 (diluted to the appropriate concentration in DMEM and containing 1 mM orthovanadate) was added to each well and incubated at 37°C for a further 10 minutes.

[0251] The 6 well plate(s) were then cooled by placing on an ice cold metal plate (itself kept on ice). The cell medium was removed and the cell layer washed with 5 ml of cold PBS; 1 mM orthovanadate. One ml of ice cold lysis buffer (20 mM Tris pH 7.6, 150 mM NaCl, 50 mM NaF, 0.1 % SDS, 1% NP40, 0.5 % DOC, 1 mM orthovanadate, 1mM EDTA, 1 mM PMSF, 30 µl / ml Aprotinin, 10 µg/ml Pepstatin, 10 µg/ml Leupeptin) was added to each well and left on ice for 10 - 20 minutes. The cells were scraped off the plate using a cell lifter and the whole lysate solution transfer to a 1.5 ml Eppendorf tube and kept on ice. The samples were then spun for 3 minutes at 13000 rpm at 4°C and all subsequent steps carried out at 4°C.

[0252] 50 µl of each lysate were kept for the BCA Protein assay (Pierce, Cat. No. 23225) (in low protein binding polypropylene microtiter plates from Greiner). The protein concentration was determined using the standard assay conditions supplied with the kit. A further 800 µl of each sample lysate was transferred to a fresh 2 ml Eppendorf tube in preparation for the immunoprecipitation (IP). 15 µl (3 mg) of anti P-Y (Santa Cruz Cat. No. E2203) were added to the lysates and left to incubate for 2 hours at 4°C before adding 600 µl of the Magnabind beads (goat anti mouse IgG, Pierce Cat. No.21354). The Magnabind beads were prepared as follows: The required volume was transferred into 15 ml conical tubes. Tubes were then placed in the presence of a magnetic field and the liquid was removed. Fresh PBS was added using the original volume, and the beads were re-suspended. This process was repeated twice. The lysate-containing solution was then mixed with the beads and the tubes left to rotate overnight at 4°C on a rotor mixer.

[0253] The samples were exposed for about 1 min to the magnet and the liquid carefully removed. 1 ml lysis buffer was added and the tubes rotated for 5 min to wash. The wash steps were repeated twice. The liquid was completely removed and the beads re-suspended in 12 µl of hot (94°C) 2x Laemmli loading buffer + bME, then left to stand for 15 min at room temperature. The tubes were exposed for 1 min in the magnet, and the liquid which separated from the beads was analyzed on PAGE/SDS gels.

[0254] The samples were analyzed using PAGE/SDS gels using 4-12 % BisTris NuPAGE / MOPS gels with 15 wells (Novex). The total 12 µl of each immunoprecipitate was loaded per slot. The gels were run at 200 V / 120 mA / 25 Watts for 55 minutes and then the samples western blotted onto nitrocellulose membrane for 1

hr 30 min at 50 V / 250 mA. All blots were then treated with 5% Marvel in PBS-Tween for 1 hour at room temperature and then washed with PBS-Tween

[0255] Rabbit anti Tie-2 antibody (Santa Cruz Cat. No. C1303) was diluted 1:500 in 0.5 % Marvel / PBS-Tween and 12.5 mls added to each blot and left at 4°C overnight. The blots were then washed with PBS-Tween and goat anti rabbit -POD (Dako Cat. No. P 0448) (1:5000 dilution in 0.5 % Marvel / PBS-Tween) added to each blot and left for 1 hour at room temperature. The blots were washed with PBS-Tween and each blot developed for 10 minutes using 12.5 mLs (equal volumes of solution A and B) of Supersignal (PIERCE Cat. No. 34080). The blots were transfer to X-Ray cassette and expose to film (5 sec/ 15 sec / 30 sec/ 60 sec /150 second exposures). Figure 4 is a Western blot showing the results of this assay. In this system, inhibition of Angiopoietin-1 stimulated phosphorylation of Tie-2 by mAb 3.19.3 was observed.

[0256] The images seen on the film for each sample were then evaluated using the FluorS BioRad image analyzer system. The pixel density was measured as OD / mm2 and expressed in percentage volume. The percentage volume results were normalized to 1 mg protein/immunoprecipitation using the protein concentration determined using the BCA assay and the lysate volume of each sample used in the immunoprecipitation. The percentage phosphorylation of each sample was calculated against the 100 % phosphorylation value of the untreated control sample on each gel with percentage inhibition of each sample being calculated against the 100 % phosphorylation value, which itself represents 0 % inhibition). Figure 5 is a graphical representation of these values, and shows that the IC50 for inhibition of Angiopoietin-1 stimulated Tie-2 phosphorylation is 99nM.

Taken together, these data show that, in this system, the mAb inhibits Angiopoietin-1 induced phosphorylation of Tie-2.

EXAMPLE 13

STRUCTURAL ANALYSIS OF ANG-2 ANTIBODIES

[0257] The variable heavy chains and the variable light chains of the antibodies were sequenced to determine their DNA sequences. The complete sequence information for the anti-Ang-2 antibodies is provided in the sequence listing with nucleotide and amino acid sequences for each gamma and kappa chain combination. The variable heavy sequences were analyzed to determine the VH family, the D-region sequence and the J-region sequence. The sequences were then translated to determine the

primary amino acid sequence and compared to the germline VH, D and J-region sequences to assess somatic hypermutations.

[0258] Table 11 is a table comparing the antibody heavy chain regions to their cognate germ line heavy chain region. Table 12 is a table comparing the antibody kappa light chain regions to their cognate germ line light chain region.

[0259] The variable (V) regions of immunoglobulin chains are encoded by multiple germ line DNA segments, which are joined into functional variable regions (V_HDJ_H or V_KJ_K) during B-cell ontogeny. The molecular and genetic diversity of the antibody response to Ang-2 was studied in detail. These assays revealed several points specific to anti-Ang-2 antibodies.

[0260] Analysis of 152 individual antibodies specific to Ang-2resulted in the determination that the antibodies were derived from 21 different germline VH genes, 112 of them from the VH3 family, with 46 antibodies being derived from the VH3-33 gene segment. Tables 11 and 12 show the results of this analysis.

[0261] It should be appreciated that amino acid sequences among the sister clones collected from each hybridoma are identical. For example, the heavy chain and light chain sequences for mAb 3.19.3 are identical to the sequences shown in Tables 11 and 12 for mAbs 3.19 and 3.19.1.

Table 11. Heavy chain analysis

Chain Name	SEQ ID NO:	V	D	J	FR1	CDR1	FR2	CDR2	FR3	CDR3	J
	558	Germline			EVQLVESGGGLVK PGGSLRLSCAAS	GFTFS NAWMS	WVRQAPG KGLEWVG	RIKSKTDGGT TDYAAPVKG	RFTISRDDSKNTLYLQM NSLKTEDTAVYYCTT	DYGDYGM DV	WGQGTT VTVSSA
4.2	141	VH3-15	D4-17	JH6B	EVQLVESGGGLVK PGGSLRLSCAAS	GFTFS NAWMT	WVRQAPG KGLEWVG	RIKSKTVGGT TDYAAPVKG	RFTISRDDSKNTLYLQM NSLKTEDTAVYYCTT	DYGDYYN SGYGMDV	WGQGTT VTVSSA
	559	Germline			QVQLVQSGAEVKK PGASVKVSCKAS	GYTFT GYYMH	WVRQAPG QGLEWMG	WINPNSGGTN YAQKFQG	RVTMTRDTSISTAYMEL SRLRSDDTAVYYCAR	IAVAGFD Y	WGQGTL VTVSSA
5.103.1	503	VH1-2	D6-19	JH4A	QVQLVQSGAEVKK PGASVKVSCKAS	GYTFT GYYLY	WVpQAPG QGLEWMG	WIsPNSGGTN YAQKFQG	RVTMTRDTSISTAYMEL SRLRSDDTAVYYCAR	DQVIAVA GPFDY	WAQGTL VTVSSA
	560	Germline			QVQLVESGGGVVQ PGRSLRLSCAAS	GFTFS SYGMH	WVRQAPG KGLEWVA	VIWYDGSNKY YADSVKG	RFTISRDNSKNTLYLQM NSLRAEDTAVYYCAR	DYGGNFD Y	WGQGTL VTVSSA
3.42	137	VH3-33	D4-23	JH4B	QVQLVESGGGVVQ PGRSLRLSCAAS	GFTFS SYGIH	WVRQAPG KGLEWVA	VIWYDGSSKY YADSVKG	RFTISRDNSKNTLYLQM NSLRAEDTAVYYCAR	ANDYGGN GLFDY	WGQGTL VTVSSA
	561	Germline			QVQLVESGGGVVQ PGRSLRLSCAAS	GFTFS SYGMH	WVRQAPG KGLEWVA	VIWYDGSNKY YADSVKG	RFTISRDNSKNTLYLQM NSLRAEDTAVYYCAR	AFDI	WGQGTM VTVSSA
5.11	221	VH3-33	-NA-	JH3B	QVQLVESGGGVVQ PGRSLRLPCAAS	GFTFS SYGMH	WVRQAPG KGLEWVA	VIWYDGSNKY YADSVKG	RFTISRDNSKNTLYLQM NSLRAEDTAVYYCAR	DKALAFD I	WGQGTM VTVSSA
	562	Germline			QVQLVESGGGVVQ PGRSLRLSCAAS	GFTFS SYGMH	WVRQAPG KGLEWVA	VIWYDGSNKY YADSVKG	RFTISRDNSKNTLYLQM NSLRAEDTAVYYCAR	EL	WGQGTL VTVSSA
5.108	513	VH3-33	D1-7	JH5B	QVQLVESGGGVVQ PGRSLRLSCAAS	GFTFS SYGMH	WVRQAPG KGLEWVA	VIWYDGSNKY YADSVKG	RFTISRDNSKNTLYLQM NSLRAEDTAMYYCAR	ELAL	WGQGTL VTVSSA
5.108.1	513	"	"	"	QVQLVESGGGVVQ PGRSLRLSCAAS	GFTFS SYGMH	WVRQAPG KGLEWVA	VIWYDGSNKY YADSVKG	RFTISRDNSKNTLYLQM NSLRAEDTAMYYCAR	ELAL	WGQGTL VTVSSA
	563	Germline			EVQLVESGGGVVR PGGSLRLSCAAS	GFTFD DYGMS	WVRQAPG KGLEWVS	GINWNGGSTG YADSVKG	RFTISRDNAKNSLYLQM NSLRAEDTALYHCAR	QWLWYFD L	WGRGTL VTVSSA
5.19	251	VH3-20	D6-19	JH2	EVQLVESGGGVVR PGGSLRLSCAAS	GFSFD DYGMS	WVRQAPG KGLEWVS	GINWNGGRTV YADSVKG	RFTISRDSAKNSLYLQM NSLRAEDTALYHCAR	NKQWLWY FDL	WGRGTL VTVSSA
5.6	205	"	"	"	EVQLVESGGGVVR PGGSLRLSCAAS	GFSFD DYGMS	WVRQAPG KGLEWVS	GINWNGGRTV YADSVKG	RFTISRDSAKNSLYLQM NSLRAEDTALYHCAR	NKQWLWY FDL	WGRGTL VTVSSA
5.8	211	"	"	"	EVQLVESGGGVVR PGGSLRLSCAAS	GFTFD DYGMS	WVRQAPG KGLEWVS	GINWNGGGTG YADSMKG	RFTISRDDAKNSLYLQM NSLRAEDTALYHCAR	NKQWLWY FDL	WGRGTL VTVSSA
5.35	297	"	"	"	EVQLVESGGGVVR PGGSLRLSCTTS	GFTFD DYGMS	WVRQAPG KGLEWVS	GINWNGGSTV YADSVKG	RFTISRDSAKNSLYLQM NSLRAEDTALYHCAR	NKQWLWY FDL	WGRGTL VTVSSA
5.38	309	"	"	"	EVKLVESGGGMVR PGGSLRLSCAAS	GFTFD DYGMS	WVRQAPG KGLEWVS	GINWNGGGTA YADSVKG	RFTISRDNAKNSLYLQL NSLRAEDTALYHCAR	NKQWLWY FDL	WGRGTL VTVSSA

5.44	329	"	"	"	EVQLVESGGGVVR TGGSLRLSCAAS	GFSFD DYGMS	WVRQAPG KGLEWVS	GINWNGGRTV YADSVKG	RFTISRDSAKNSLYLQM NSLRAEDTALYHCAR	NKQWLWY FDL	WGRGTL VTVSSA
5.35.1	297	"	"	"	EVQLVESGGGVVR PGGSLRLSCTTS	GFTFD DYGMS	WVRQAPG KGLEWVS	GINWNGGSTV YADSVKG	RFTISRDSAKNSLYLQM NSLRAEDTALYHCAR	NKQWLWY FDL	WGRGTL VTVSSA
	564	Germline			QVQLVESGGGVVQ PGRSLRLSCAAS	GFTFS SYGMH	WVRQAPG KGLEWVA	VIWYDGSNKY YADSVKG	RFTISRDNSKNTLYLQM NSLRAEDTAVYYCA	YGGNSYY YYYGMDV	WGQGTT VTVSSA
3.1	7	VH3-33	D4-23	JH6B	QVQLVESGGGVVQ PGRSLRLSCAAS	GFTFS SYGMH	WVRQAPG KGLEWVA	VIWYDGSNKY YADSVKG	RFTISRDNSKNTLYLQM NSLRAEDTAVYYCAG	DYGEYFY YGMDV	WGQGTT VTVSSA
3.18	71	"	"	"	QVQLVESGGGVVQ PGRSLRLSCAAS	GFTFS SFGMH	WVRQAPG KGLEWVA	VIWFDGSNKY YADSVKG	RFTISRDNSKNTLYLQM NSLRAEDTAVYYCAS	DYGDYFY YGMDV	WGQGTT VTVSSA
3.39	125	"	"	"	QVQLVESGGGVVQ PGRSLRLSCAAS	GFTFS SYGMH	WVRQAPG KGLEWVA	VIWYDGSNKY YADSVKG	RFTISRDNSKNTLYLQM NSLRAEDTAVYYCA	DRSYGGN SFYYYYY GMDV	WGQGTT VTVSSA
3.11	47	"	"	"	QVQLVESGGGVVQ PGRSLRLSCAAS	GFTFS SYGMH	WVRQAPG KGLEWVA	VIWYDGSNKY YADSVKG	RFTISRDNSKNTLYLQM NSLRAEDTAVYYCAG	DYGDYFY YGMDV	WGQGTT VTVSSA
3.26	93	"	"	"	QVQLVESGGGVVQ PGRSLRLSCAAS	GFTFS SYGMH	WVRQAPG KGLEWVA	VIWYDGSNKY YADSVKG	RFTISRDNSKNTLYLQM NSLRAEDTAVYYCAG	DYGEYFY YGMDV	WGQGTT VTVSSA
	565	Germline			EVQLVESGGVVVQ PGGSLRLSCAAS	GFTFD DYTMH	WVRQAPG KGLEWVS	LISWDGGSTY YADSVKG	RFTISRDNSKNSLYLQM NSLRTEDTALYYCAK	DIAVAGF DY	WGQGTL VTVSSA
5.28	277	VH3-43	D6-19	JH4B	EVQLVESGGIVVQ PGGSLRLSCAAS	GFTFD DYTMH	WVRQTPG KGLEWVS	LISWDGGSTY YADSVKG	RFTISRDNSKNSLYLQM NSLRTEDTALYYCAK	DIDIAVA GTGFDH	WGQGTL VTVSSA
5.28.1	277	"	"	"	EVQLVESGGIVVQ PGGSLRLSCAAS	GFTFD DYTMH	WVRQTPG KGLEWVS	LISWDGGSTY YADSVKG	RFTISRDNSKNSLYLQM NSLRTEDTALYYCAK	DIDIAVA GTGFDH	WGQGTL VTVSSA
	566	Germline			QVQLVESGGGVVQ PGRSLRLSCAAS	GFTFS SYGMH	WVRQAPG KGLEWVA	VIWYDGSNKY YADSVKG	RFTISRDNSKNTLYLQM NSLRAEDTAVYYCAR	NWNYFDY	WGQGTL VTVSSA
5.56.1	363	VH3-33	D1-7	JH4B	QVQLVESGGGVVQ PGRSLRLSCAAS	GFTFS SYGMH	WVRQAPG KGLEWVA	VIWYDGSNKY YADSVKG	RFTISRDNSKNTLYLQM NSLRAEDTAVYYCAR	EDNWNFY FDY	WGQGTL VTVSSA
5.56	363	"	"	"	QVQLVESGGGVVQ PGRSLRLSCAAS	GFTFS SYGMH	WVRQAPG KGLEWVA	VIWYDGSNKY YADSVKG	RFTISRDNSKNTLYLQM NSLRAEDTAVYYCAR	EDNWNFY FDY	WGQGTL VTVSSA
	567	Germline			QVQLQQWGAGLLK PSETLSLTCAVY	GGSFS GYYWS	WIRQPPG KGLEWIG	EINHSGSTNY NPSLKS	RVTISVDTSKNQFSLKL SSVTAADTAVYYCAR	DYGDFDY	WGQGTL VTVSSA
3.37	121	VH4-34	D4-17	JH4B	QVQLQQWGAGLLK PSETLSLTCAVY	GGSFS GYYWS	WIRQPPG KGLEWIG	EIYHSGSTNY NPSLKS	RVTISVDTSKNQFSLKL SSVTAADTAVYSCAR	NDYGDHE GFDY	WGQGTL VTVSSA
	568	Germline			QVQLQESGPGLVK PSETLSLTCTVS	GGSVS SGGYY WS	WIRQPPG KGLEWIG	YIYYSGSTNY NPSLKS	RVTISVDTSKNQFSLKL SSVTAADTAVYYCAR	GYSYGYY FDY	WGQGTL VTVSSA
5.36	301	VH4-61	D5-5	JH4B	QVQLQESGPGLVK PSETLSLTCTVS	GGSVS SGGYY WS	WIRQPPG KGLEWIG	YINYSRSTNH NPSLKS	RVTISVDTSKNQFSLKL SSVTAADTAVYYCAR	EGRGDSY GYYFDY	WGQGTL VTVSSA

6.7	551	"	"	"	QVQLQESGPGLVK PSETLSLTCTVS	GGSVS SGGYY WS	WIRQPPG KGLEWIG	YIYYSRSTNY NPSLKS	RVTISVDTSKNQFSLKL SSVTAADTAVYYCAR	EGRGYSY GYYFDY	WGQGTL VTVSSA
5.107	511	"	"	"	QVQLQESGPGLVK PSETLSLTCTVS	GGSVS SGGYY WS	WIRQPPG KGLEWIG	YIYYSRSTNY NPSLKS	RVTISVDTSKNQFSLKL SSVTAADTAVYYCAR	EGRGNSY GYYFDY	WGQGTL VTVSSA
	569	Germline			QVQLVESGGGVVQ PGRSLRLSCAAS	GFTFS SYGMH	WVRQAPG KGLEWVA	VISYDGSNKY YADSVKG	RFTISRDNSKNTLYLQM NSLRAEDTAVYYCAR	WLRYYYY GMDV	WGQGTT VTVSSA
5.111	521	VH3-30	D5-12	JH6B	QVQLVESGGDVVQ PGRSLRLSCAAS	GFTFS SYGMH	WVRQAPG KGLEWVA	VISYDGSNKY YADSVKG	RFTISRDNSKNTLYLQM HLRAEDTAVYYCAR	DGGWLRL DYYYYGM DV	WGQGTT VTVSSA
	570	Germline			EVQLVESGGGLVK PGGSLRLSCAAS	GFTFS NAWMS	WVRQAPG KGLEWVG	RIKSKTDGGT TDYAAPVKG	RFTISRDDSKNTLYLQM NSLKTEDTAVYYCTT	YSSGWYW YFDL	WGRGTL VTVSSA
5.45	333	VH3-15	D6-19	JH2	EVQLVESGGGLVK PGGSLRLSCAAS	GFTFS NAWMS	WVRQAPG KGLEWVG	RIKSKTDGGT TDYAAPVKG	RFTISRDDSKNTLYLQM NSLKTEDTAVYYCTT	SYSSGWF YWYFDI	WGRGTP VTVSSA
	571	Germline			EVQLVESGGGLVQ PGGSLRLSCAAS	GFTFS SYSMN	WVRQAPG KGLEWVS	YISSSSSTIY YADSVKG	RFTISRDNAKNSLYLQM NSLRDEDTAVYYCAR	AAAGFDY	WGQGTL VTVSSA
5.43	325	VH3-48	D6-13	JH4B	EVQLVESGGGLVQ PGGSLRLSCAAS	GFTFS TYSMN	WVRQAPG KGLEWIS	YISRSSRTIN HADSVKG	RFTVSRDNAKNSLYLQM ISLRDEDTAVYYCAR	KAAAGPF DY	WGQGTL VTVASA
	572	Germline			EVQLLESGGGLVQ PGGSLRLSCAAS	GFTFS SYAMS	WVRQAPG KGLEWVS	AISGSGGSTY YADSVKG	RFTISRDNSKNTLYLQM NSLRAEDTAVYYCAK	DYGGNFD Y	WGQGTL VTVSSA
5.97	491	VH3-23	D4-23	JH4B	EVQLLESGGGLVQ PGGSLRLSCAAS	GFTFS SYAMS	WVRQAPG KGLEWVS	GISGSGGNTY HADSVKG	RFTISRDNSKNTLYLQM NSLRAEDTAVYYCAK	DEDYGGN YSDFDY	WGQGTL VTVSSA
6.8	553	"	"	"	EVQLLESGGGLVQ PGGSLRLSCAAS	GFTFS SYAMS	WVRQAPG KGLEWVS	AISGSGGSTY YADSVKG	RFTISRDNSKNTLYLQM NSLRAEDTAVYYCAK	DEDYGGN SDFDY	WGQGTL VTVSSA
	573	Germline			QVQLQQSGPGLVK PSQTLSLTCAIS	GDSVS SNSAA WN	WIRQSPS RGLEWLG	RTYYRSKWYN DYAVSVKS	RITINPDTSKNQFSLQL NSVTPEDTAVYYCA	WFYWYFD L	WGRGTL VTVSSA
5.61	377	VH6-1	D3-9	JH2	QVQLQQSGPGLVK PSQTLSLTCAIS	GDSVS SNSAA WN	WIRQSPS RGLEWLG	MTYYRSKWSN DYAVSLKS	RITINPDTSKNQFSLQL NSVTPEDTAVYYCAR	GNWFYWY FDL	WGRGTL VTVSSA
	574	Germline			QVQLVESGGGVVQ PGRSLRLSCAAS	GFTFS SYGMH	WVRQAPG KGLEWVA	VIWYDGSNKY YADSVKG	RFTISRDNSKNTLYLQM NSLRAEDTAVYYCAR	TGDY	WGQGTL VTVSSA
5.52	347	VH3-33	D1-1	JH4B	QVQLVESGGGVVQ PGRSLRLSCAAS	GFTFS SYGMH	WVRQAPG KGLEWVA	VRWYDESNKY YADSVKG	RFTISRDNSKNTLYLQM NSLRAEDTAVYYCAR	DPFETGT TFDY	WGQGTL VTVSSA
5.52.1	351	"	"	"	QVQLVESGGGVVQ PGRSLRLSCAAS	GFTFS SYGMH	WVRQAPG KGLEWVA	VRWYDESNKY YADSVKG	RFTISRDNSKNTLYLQM NSLRAEDTAVYYCAR	DPFETGT TFDY	WGQGTL VTVSSA
5.53	355	"	"	"	QVQLVESGGGVVQ PGRSLRLSCAAS	GFTFS DYGMH	WVRQAPG KGLEWMA	VLWYDESNKY YADSVKG	RFTISRDSSKNTLYLQM NSLRAEDTAVYYCAR	DPFETGT TFDY	WGQGTL VTVSSA

5.26	273	"	"	"	QVQLVESGGGVVQ PGRSLRLSCAAS	GFTFS SYGMH	WVRQAPG KGLEWVA	VIWYDESNKY YADSVKG	RFTISRDNSKNTLYLQM NSLRAEDTAVYYCAR	DPFETGT TFDY	WGQGTL VTVSSA
	575	Germline			QVQLVESGGGVVQ PGRSLRLSCAAS	GFTFS SYGMH	WVRQAPG KGLEWVA	VISYDGSNKY YADSVKG	RFTISRDNSKNTLYLQM NSLRAEDTAVYYCAR	GYSSGWF DY	WGQGTL VTVSSA
5.20	253	VH3-30	D6-19	JH4B	QVQLVESGGGVVQ PGRSLRLSCAAS	GFTFS TYGMH	WVRQAPG KGLEWVA	VISYDGSKKY YADSVKG	RFTISRDNSKNTLYLQM NSLRAEDTALYYCAR	GGYSTGW GPDFDY	WGQGTL VTVSSA
	576	Germline			QVQLVESGGGVVQ PGRSLRLSCAAS	GFTFS SYGMH	WVRQAPG KGLEWVA	VIWYDGSNKY YADSVKG	RFTISRDNSKNTLYLQM NSLRAEDTAVYYCAR	CGGDCYY YYYYGMD V	WGQGTT VTVSSA
3.22	89	VH3-33	D2-21	JH6B	QVQLVESGGGVVQ PGRSLRLSCAAS	GFTFS NYGMH	WVRQAPG KGLEWVA	VIWYDGSNKY YADSVKG	RFTISRDNSRNTLYLQM NSLRAEDTAVYYCAR	EGGYCGG DCWVYGM DV	WGQGTT VTVSSA
5.51	345	"	"	"	QVQLVESGGGVVQ PGRSLRLSCAAS	GFTFS SYGMH	WVRQAPG KGLEWVA	VIWYDGSNKY YADSVKG	RFTISRDNSKNTLYLQM NSLRAEDTAVYYCAR	ENCGGDC YQLNYYY YYGMDV	WGQGTT VTVSSA
5.47	339	"	"	"	QVQLVESGGGVVQ PGRSLRLSCAAS	GFTFS SYGMH	WVRQAPG KGLEWVA	VIWYDGSNKY YADSVKG	RFTISRDNSKNTLYLQM NSLRAEDTAVYYCAR	ENCGGDC YQLNYYY YYGMDV	WGQGTT VTVSSA
	577	Germline			QVQLQESGPGLVK PSQTLSLTCTVS	GGSIS SGGYY WS	WIRQHPG KGLEWIG	YIYYSGSTYY NPSLKS	RVTISVDTSKNQFSLKL SSVTAADTAVYYCAR	WDFDY	WGQGTL VTVSSA
3.21	85	VH4-31	D7-27	JH4B	QVQLQESGPGLVK PSQTLSLTCTVS	GGSIS SGGYF WS	WIRQHPG KGLEWIG	YIYYSGRTYY NPSLKS	RVTISVDASKNQFSLKL SSVTAADTAVYHCAR	EGSYWDF DY	WGQGTL VTVSSA
	578	Germline			QVQLQESGPGLVK PSETLSLTCTVS	GGSIS SYYWS	WIRQPPG KGLEWIG	YIYYSGSTNY NPSLKS	RVTISVDTSKNQFSLKL SSVTAADTAVYYCAR	P	WGQGTL VTVSSA
5.66	389	VH4-59	-NA-	JH5B	QVQLQESGPGLVK PSETLSLTCTVS	GGSIS SYYWS	WIRQPPG KGLEWIG	FIYYSGTTNY NPSLKS	RVTISVDTSKNQFSLKL SSVTAADTAVYYCAR	AYDP	WGQGTL VTVSSA
	579	Germline			QVQLVESGGGVVQ PGRSLRLSCAAS	GFTFS SYGMH	WVRQAPG KGLEWVA	VIWYDGSNKY YADSVKG	RFTISRDNSKNTLYLQM NSLRAEDTAVYYCAR	NWNYYYY GMDV	WGQGTT VTVSSA
5.73	409	VH3-33	D1-20	JH6B	QVQLVESGGGVVQ PGRSLRLSCAAS	GFTFS SYGMH	WVRQAPG KGLEWVA	VIWYDGSNKY YADSVKG	RFTISRDNSKNTLYLQM NSLRAEDTAVYYCAR	GDNWNYE GDGMDV	WGQGTT VTVSSA
5.29	281	"	"	"	QVQLVESGGGVVQ PGRSLRLSCAAS	GFTFS SYGMH	WVRQAPG KGLEWVA	VIWYDGSNKY YADSVKG	RFTISRDNSKNTLYLQM NSLRAEDTAVYYCAR	APYDWNS YYGLDV	WGQGTT VTVSSA
5.104	507	"	"	"	QVQLVESGGGVVQ PGRSLRLSCAAS	GFTFS SYGMH	WVRQAPG KGLEWVA	VIWYDGSNKY YADSVKD	RFTISRDNSKNTLYLQM NSLRAEDTAVYYCAR	APYDWNS YYGLDV	WGQGTT VTVSSA
	580	Germline			QVQLVESGGGVVQ PGRSLRLSCAAS	GFTFS SYGMH	WVRQAPG KGLEWVA	VISYDGSNKY YADSVKG	RFTISRDNSKNTLYLQM NSLRAEDTAVYYCAR	YYGSGSY GMDV	WGQGTT VTVSSA

5.21	255	VH3-30	D3-10	JH6B	QVQLVESAGGVVQ PGRSLRLSCAAS	GFTFS IYGMH	WVRQAPG KGLEWVA	VISYDGSNKY YADSVKG	RFTISRDDSKNTLYLQM NSLRAEDTAVYYCAR	NYYGSGS PYGMDV	WGQGTT VTVSSA
	581	Germline			QVQLQESGPGLVK PSETLSLTCTVS	GGSVS SGGYY WS	WIRQPPG KGLEWIG	YIYYSGSTNY NPSLKS	RVTISVDTSKNQFSLKL SSVTAADTAVYYCAR	YYGSGYY YYGMDV	WGQGTT VTVSSA
3.2	11	VH4-61	D3-10	JH6B	QVQLQESGPGLVK PSETLSLTCTVS	GGSVS SGGYY WN	WIRQPPG KGLEWIG	YIYYSGSTNY NPSLKS	RVTISVDTSKNQFSLKL SSVTAADTAVYYCAR	DQDYYGS GRGYYYY GMDV	WGQGTT VTVSS
	582	Germline			QVQLVQSGAEVKK PGASVKVSCKAS	GYTFT GYYMH	WVRQAPG QGLEWMG	WINPNSGGTN YAQKFQG	RVTMTRDTSISTAYMEL SRLRSDDTAVYYCAR	NWNYFDY	WGQGTL VTVSSA
6.3.1	539	VH1-2	D1-7	JH4B	QVQLVQSGAEVKK PGASVKVSCKAS	GYTFT GYFMH	WVPQAPG QGLEWMG	WINPNSGGTN YAQNFQG	RVTMTRDTSISTAYMEL SRLRSDDTPVYYCAR	DPWQNWN SYFDY	WGQGTL VTVSSA
	583	Germline			EVQLVESGGGLVK PGGSLRLSCAAS	GFTFS NAWMS	WVRQAPG KGLEWVG	RFTISRDDSKNTLYLQM TDYAAPVKG	RFTISRDDSKNTLYLQM NSLKTEDTAVYYCT	YGWYFDL	WGRGTL VTVSSA
5.46	337	VH3-15	D4-17	JH2	EVQLVESGGGLVK PGGSLRLSCAAS	GFTFS NAWMS	WVRQAPG KGLEWVG	RIKSKTDGGT TDYAAPVKG	RFTMSRDDSKNTLYLQM NSLKTEDTAVYYCTI	LYGDFWY FDL	WGRGTL VTVSSA
3.9	39	"	"	"	EVQLVESGGGLVK PGGSLRLSCAAS	GFTFS NAWMS	WVRQAPG KGLEWVG	RIKSKTDGGT TDYAAPVKG	RFTISRDDSKNTLYLQM NSLKTEDTAVYYCTT	EYGDFWY FDF	WGRGTL VTVSSA
	584	Germline			QVQLQESGPGLVK PSETLSLTCTVS	GGSIS SYYWS	WIRQPAG KGLEWIG	RIYTSGSTNY NPSLKS	RVTMSVDTSKNQFSLKL SSVTAADTAVYYCAR	WWYFDL	WGRGTL VTVSSA
5.68	397	VH4-4	D2-21	JH2	QVQLQESGPGLVK PSETLSLTCTVS	GGSIS SHYWI	WIRQPAG KGLEWIG	RIYSSGSTNY NPSLKS	RVTMSGDTSKNQFSLKL SSVTAADTAVYYCAR	GRWGSWY FDL	WGRGTL VTVSSA
	585	Germline			QVQLVESGGGVVQ PGRSLRLSCAAS	GFTFS SYGMH	WVRQAPG KGLEWVA	VIWYDGSNKY YADSVKG	RFTISRDNSKNTLYLQM NSLRAEDTAVYYCAR	Y	WGQGTL VTVSSA
5.67	393	VH3-33	-NA-	JH4B	QVQLVESGGGVVQ PGRSLRLSCAAS	GFTFS SYGMH	WVRQAPG KGLEWVA	VIWYDGSNKY YADSVKG	RFTISRDNSKNTLYLQM NSLRAEDTAVYYCAR	ELAY	WGQGTL VTVSSA
	586	Germline			QVQLVQSGAEVKK PGASVKVSCKAS	GYTFT GYYMH	WVRQAPG QGLEWMG	WINPNSGGTN YAQKFQG	RVTMTRDTSISTAYMEL SRLRSDDTAVYYCAR	WYYYYYY GMDV	WGQGTT VTVSSA
5.78.1	429	VH1-2	D2-2	JH6B	QVQLVQSGAEVKK PGASVKVSCKAS	GYTFT GYYMH	WVRQAPG QGLEWMG	WINPNSGGTN YAQKFQG	RVTMTRDTSISTAYMEL SRLRSDDTAVYYCAR	DRGWNYA DYYYYGM DV	WGQGTT VTVSSA
	587	Germline			EVQLVESGGGLVK PGGSLRLSCAAS	.GFTFS SYSMN	WVRQAPG KGLEWVS	SISSSSSYIY YADSVKG	RFTISRDNAKNSLYLQM NSLRAEDTAVYYCAR	VGAFDY	WGQGTL VTVSSA
4.18	187	VH3-21	D1-26	JH4B	EVQLVESGGGLVK PGGSLRLSCAAS	GFTFS SYRMN	WVRQAPG KGLEWVS	SISSSSSHIY YVDSVKG	RFTISRDNAKNSLYLQM NSLRAEDTAVYYCAR	DRGVGAP FDY	WGQGTL VTVSSA
3.40	129	"	"	"	EVQLVESGGGLVK PGGSLRLSCAAS	GFTFS SYRMN	WVRQAPG KGLEWVS	SISSSSSHIY YVDSVKG	RFTISRDNAKNSLYLQM NSLRAEDTAVYYCAR	DRGVGAP FDY	WGQGTL VTVSSA
5.80	435	"	"	"	EVQLVESGGGLVK PGGSLRLSCAAS	GFTFS SYRMN	WVRQAPG KGLEWVS	SISSSGSYIY YADSVKG	RFTISRDNAKNSLYLQM NSLRAEDTAVYYFAR	DRGVGAA FDY	WGQGTL VTVSSA

3.41	133	"	"	"	EVQLVESGGGLVK PGGSLRLSCAAS	GFTFS SYRMN	WVRQAPG KGLEWVS	SISSSSSHIY YVDSVKG	RFTISRDNAKNSLYLQM NSLRAEDTAVYYCAR	DRGVGAP FDY	WGQGTL VTVSSA
	588	Germline			QVQLVESGGGVVQ PGRSLRLSCAAS	GFTFS SYGMH	WVRQAPG KGLEWVA	VIWYDGSNKY YADSVKG	RFTISRDNSKNTLYLQM NSLRAEDTAVYYCAR	YSYFDY	WGQGTL VTVSSA
5.22	259	VH3-33	D6-6	JH4B	QVQLVESGGGVVQ PGRSLRLSCAAS	GFTFS SYGMH	WVRQAPG KGLEWVA	VIWYDGSNKY YADSVKG	RFTISRDNSKNTLYLQM NSLRAEDTAVYYCAR	GGPLYSN SFYYFDY	WGQGTL VTVSSA
5.31	289	"	"	"	QVQLVESGGGVVQ PGRSLRLSCAAS	GFTFS SYGMH	WVRQAPG KGLEWVA	IIWFDGSNEY YADSVKG	RFTISRDNSKNTLYLQM NSLRAEDTAVYFCAR	GGPLYSN SFYYFDY	WGQGTL VTVSSA
5.37	305	"	"	"	QVQLVESGGGVVQ PGRSLRLSCAAS	GFTFS NYGMH	WVRQAPG KGLEWVA	VIWYDGSHKY YADSVKG	RFTISRDNSKNSLYLQM NSLRAEDTAVYYCAR	GGPLYSN SFYYFDY	WGQGTL VTVSSA
5.112	525	"	"	"	QVQLVESGGGVVQ PGRSLRLSCAAS	GFTFS SYGMH	WVRQAPG KGLEWVA	ILWYDGSNKY YADSVKG	RFTISRDNSKNTLYLQM NSLRAEDTAVYYCAR	GGPLYTN SFYYFDY	WGQGTL VTVSSA
5.10	217	"	"	"	QVQLVESGGGVVQ PGRSLRLSCAAS	GFTFS SYGMH	WVRQAPD KGLEWVA	VIWYDGSYKY YADSVKG	RFTISRDNSKNTLYLQM NSLRAEDTAVYYCAR	GGPLYSN SFYYFDY	WGQGTL VTVSSA
5.76	419	"	"	"	QVQLVESGGGVVQ PGRSLRLSCVAS	GFTFS SYGMH	WVRQAPG KGLEWVA	VIWYDGSNKY YADSVKG	RFTISRDNSKNTLYLQM NSLRAEDTAVYYCAR	GGPLYSN SFYYFDY	WGQGTL VTVSSA
5.17	243	"	"	"	QVQLVESGGGVVQ PGRSLRLSCAAS	GFTFS SYGMH	WVRQAPG KGLEWVT	VIWYDGSNKY YADSVKG	RFTISRDSSKNTLYLQM NSLRAEDTAVYYCAR	GGPLYSN SFYYFDY	WGQGTL VTVSSA
	589	Germline			QVQLQESGPGLVK PSETLSLTCTVS	GGSIS SYYWS	WIRQPAG KGLEWIG	RIYTSGSTNY NPSLKS	RVTMSVDTSKNQFSLKL SSVTAADTAVYYCAR	DDYSYYY YYGMDV	WGQGTT VTVSSA
5.81	439	VH4-4	D4-11	JH6B	QVQLQESGPGLVK PSETLSLTCTVS	GGSIS SYYWS	WIRQPAG KGLEWIG	RIYTSGSTNY NPSLKS	RVTMSVDTSKNQFSLKL SSVTAADTAVYYCAR	DDYSHSY YYYYGMD V	WGQGTT VTVSSA
	590	Germline			EVQLVESGGGLVK PGGSLRLSCAAS	GFTFS NAWMS	WVRQAPG KGLEWVG	RIKSKTDGGT TDYAAPVKG	RFTISRDDSKNTLYLQM NSLKTEDTAVYYCTT	YGGNSYG MDV	WGQGTT VTVSSA
4.5	151	VH3-15	D4-23	JH6B	EVQLVESGGGLVK PGGSLRLSCAAS	GFTFS NAWMN	WVRQAPG KGLEWVG	RIKSKTDGGT TDYAAPVKG	RFTISRDDSKNTLYLQM NSLKTEDTAVYYCTT	AYGGNSD QEDYGMD V	WGQGTT VTVSSA
	591	Germline			EVQLVESGGGLVQ PGGSLRLSCAAS	GFTFS SYWMS	WVRQAPG KGLEWVA	NIKQDGSEKY YVDSVKG	RFTISRDNAKNSLYLQM NSLRAEDTAVYYCAR	GIAVAFD Y	WGQGTL VTVSSA
3.31.1	99	VH3-7	D6-19	JH4B	EVQLVESGGGLVQ PGGSLRLSCAAS	GFTFS SYWMS	WVRQAPG KGLEWVA	NIKQDGSDKY YVDSVKG	RFTISRDNAKNSLYLRM NSLRAEDTAVFYCAR	DMGSGWF DYFDY	WGQGTL VTVSSA
5.13.1	229	"	"	"	EVQLVESGGGLVQ PGGSLRLSCAAS	GFTFS SYWMS	WVRQAPG KGLEWVA	NIKQDGSEKY YVDSVKG	RFTISRDNAKNSLYLQM NSLRAEDTAVYYCAR	DPGIAVA GPFDY	WGQGTL VTVSSA
5.13	229	"	"	"	EVQLVESGGGLVQ PGGSLRLSCAAS	GFTFS SYWMS	WVRQAPG KGLEWVA	NIKQDGSEKY YVDSVKG	RFTISRDNAKNSLYLQM NSLRAEDTAVYYCAR	DPGIAVA GPFDY	WGQGTL VTVSSA
5.62	381	"	"	"	EVQLVESGGGLVQ PGGSLRLSCAAS	GFTFS SYWMS	WVRQAPG KGLEWVA	NIKQDGSEKY YVDSVKG	RFTISRDNAKNSLYLQM NSLRAEDTAVYYCAR	DPGIAVA GPFDY	WGQGTL VTVSSA

3.28.1	97	"	"	"	EVQLVESGGGLVQ PGGSLRLSCAAS	GFTFS SSWMS	WVRQAPG KGLEWVA	NIKQDGSDKY YVDSVKG	RFTISRDNAKNSLYLQM NSLRAEDTAVYYCVR	DKGSGWF DY	WGQGTL VTVSSA
5.41	321	"	"	"	EVQLVESGGGLVQ PGGSLRLSCAAS	GFTFS SYWMS	WVRQAPG KGLEWVA	NIKEDGSEKY YVDSVKG	RFTISRDNAKNSLYLQM NSLRAEDTAVFYCAR	DRSSGFF DY	WGQGTL VTVSSA
5.109	517	"	"	"	EVQLVESGGGLVQ PGGSLRLSCAAS	GFTFS SYWMS	WVRQAPG KGLEWVA	NIKQDGSEQY SVDSVKG	RFTISRDNAKNSLYLQM NTLRAEDTAVYYCVR	DPGIEVA GPFDY	WGQGTL VTVSSA
3.3	15	"	"	"	EVQLVESGGGLVQ PGGSLRLSCAVS	GFTFS SYWMS	WVRQAPG KGLEWVA	NIKQDGSEKY YVDSVKG	RFTISRDNAKNSLYLQM NSLRAEDTAVYYCAR	DQGIAVA GPFDY	WGQGTL VTVSSA
3.3.1	19	"	"	"	EVQLVESGGGLVQ PGGSLRLSCAVS	GFTFS SYWMS	WVRQAPG KGLEWVA	NIKQDGSEKY YVDSVKG	RFTISRDNAKNSLYLQM NSLRAEDTAVYYCAR	DQGIAVA GPFDY	WGQGTL VTVSSA
5.41.1	321	"	"	"	EVQLVESGGGLVQ PGGSLRLSCAAS	GFTFS SYWMS	WVRQAPG KGLEWVA	NIKEDGSEKY YVDSVKG	RFTISRDNAKNSLYLQM NSLRAEDTAVFYCAR	DRSSGFF DY	WGQGTL VTVSSA
5.62.1	381	"	"	"	EVQLVESGGGLVQ PGGSLRLSCAAS	GFTFS SYWMS	WVRQAPG KGLEWVA	NIKQDGSEKY YVDSVKG	RFTISRDNAKNSLYLQM NSLRAEDTAVYYCAR	DPGIAVA GPFDY	WGQGTL VTVSSA
5.83	447	"	"	"	EVQLVESGGGLVQ PGGSLRLSCEAS	GFTFS TYWMT	WVRQAPG KGLEWVA	NIKQDGSEKY YVDSVKG	RFTISRDNAKNSLYLQM NSLRAEDTAVYYCAR	DAGMEVA GPFDY	WGQGTL VTVSSA
	592	Germline			EVQLLESGGGLVQ PGGSLRLSCAAS	GFTFS SYAMS	WVRQAPG KGLEWVS	AISGSGGSTY YADSVKG	RFTISRDNSKNTLYLQM NSLRAEDTAVYYCAK	QWLVFDY	WGQGTL VTVSSA
5.40.2	317	VH3-23	D6-19	JH4B	EVQLLESGGGLVQ PGGSLRLSCAAS	GFTFS SYAMS	WVRQAPG KGLEWVS	AISGSGYSTY YADSVKG	RFTISRDNSKNTLYLQM NSLRAEDTAVYYCAK	DLQQWLV PTVFDY	WGQGTL VTVSSA
	593	Germline			QVQLQESGPGLVK PSETLSLTCTVS	GGSIS SYYWS	WIRQPPG KGLEWIG	YIYYSGSTNY NPSLKS	RVTISVDTSKNQFSLKL SSVTAADTAVYYCAR	QWLDY	WGQGTL VTVSSA
5.15	235	VH4-59	D6-19	JH4B	QVQLQESGPGLVK PSETLSLTCTVS	GGSIS GYYWS	WIRQPPG KGLEWIG	YIYYSGSTNY NPSLKS	RVTISVDTSKNQFSLKL SSVTAADTAVYYCAR	DRQWLDY	WGQGTL VTVSSA
	594	Germline			QVQLQESGPGLVK PSETLSLTCTVS	GGSIS SYYWS	WIRQPPG KGLEWIG	YIYYSGSTNY NPSLKS	RVTISVDTSKNQFSLKL SSVTAADTAVYYCAR	AFDI	WGQGTM VTVSSA
3.13	55	VH4-59	-NA-	JH3B	QVQLQESGPGLVK PSETLSLTCTVS	GGSIS NYYWS	WIRQPPG KGLEWIG	YIYYSGSTNY NPSLKS	RVTISVDTSKNQFSLKL SSVTAADTAVYYCAR	DRADAFD I	WGQGTM VTVSSA
3.7	31	"	"	"	QVQLQESGPGLVK PSETLSLTCTVS	GGSIS NYYWS	WIRQPPG KGLEWIG	YIYYSGSTNY NPSLKS	RVTISVDTSKNQFSLKL SSVTAADTAVYYCAR	DRADAFD I	WGQGTM VTVSSA
3.12	51	"	"	"	QVQLQESGPGLVK PSETLSLTCTVS	GGSIS NYYWS	WIRQPPG KGLEWIG	YIYYSGSTNY NPSLKS	RVTISVDTSKNQFSLKL SSVTAADTAVYYCAR	DRADAFD I	WGQGTM VTVSSA
3.33	111	"	"	"	QVQLQESGPGLVK PSETLSLTCTVS	GDSIS NYYWS	WIRQPPG KGLEWIG	YIYYSGSTNY NPSLKS	RVTISVDTSKNQFSLKL SSVTAADTAVYYCAR	ERGDAFD I	WGQGRV VTVSSA
4.16	183	"	"	"	QVQLQESGPGLVK PSETLSLTCTVS	GGSIS NYYWS	WIRQPPG KGLEWIG	YIYYSGSTNY NPSLKS	RVTISVDTSKNQFSLKL SSVTAADTAVYYCAR	DRADAFD I	WGQGTM VTVSSA
3.35	119	"	"	"	QVQLQESGPGLVK PSETLSLTCTVS	GDSIS NYYWS	WIRQPPG KGLEWIG	YIYYSGSTNY NPSLKS	RVTISVDTSKNQLSLKL SSVTAADTAVYYCAR	ERGDAFD I	WGQGRV VTVSSA

3.32	107	"	"	"	QVQLQESGPGLVK PSETLSLTCTVS	GGSIS NYYWS	WIRQPPG KGLEWIG	YIYYSGSTNY NPSLKS	RVTISVDTSKNQFSLKL SSVTAADTAVYYCAR	DRADAFD I	WGQGTM VTVSSA
	595	Germline			QVQLVESGGGLVK PGGSLRLSCAAS	GFTFS DYYMS	WIRQAPG KGLEWVS	YISSSGSTIY YADSVKG	RFTISRDNAKNSLYLQM NSLRAEDTAVYYCAR	GAFDI	WGQGTM VTVSSA
5.90	475	VH3-11	D3-16	JH3B	QVQLVESGGGLVK PGGSLRLSCAAS	GFTFS DYYMS	WIRQAPG KGLEWVS	YISSSGSSKN YADSVKG	RITISRDNAKNSLYLQM NSLRAEDTAVYYCAR	ERGDAFD I	WGQGTM VTVSSA
	596	Germline			QVQLQESGPGLVK PSETLSLTCTVS	GGSIS SYYWS	WIRQPAG KGLEWIG	RIYTSGSTNY NPSLKS	RVTMSVDTSKNQFSLKL SSVTAADTAVYYCAR	YNWNYWY FDL	WGRGTL VTVSSA
5.18	247	VH4-4	D1-20	JH2	QVQLQESGPGLVK PSETLSLTCTVS	GGSIS SYYWS	WIRQPAG KGLEWIG	RIYTSGFTNY NPSLKS	RVTMSVDTSKNQFSLKL SSVTAADTAVYYCAR	YNWNYWY FDL	WGRGIL VTVSSA
	597	Germline			QVQLVQSGAEVKK PGASVKVSCKAS	GYTFT GYYMH	WVRQAPG QGLEWMG	WINPNSGGTN YAQKFQG	RVTMTRDTSISTAYMEL SRLRSDDTAVYYCAR	IAVAGFD Y	WGQGTL VTVSSA
5.54.1	357	VH1-2	D6-19	JH4B	QVQLVQSGAEVKK PGASVKVSCKAS	GYTFT GYYMH	WVRQAPG QGLEWMG	WINPNSGGTN YAQKFQG	RVTMTRDTSISTAYMEL SRLRSDDTAVYYCAR	DGGSIAV AGHFEY	WGQGTL VTVSSA
5.14.1	233	"	"	"	QVQLVQSGAEVKK PGASVKVSCKAS	GYTFT GYYMY	WVRQAPG QGLEWMG	WINPNSGGTN YAQKFQG	RVTMTRDTSISTAYMEL SRLRSDDTAVYYCAR	DQGITVA GPFDY	WGQGSL VTVSSA
5.101.1	497	"	"	"	QVQLVQSGAEVKK PGASVKVSCKAS	GYTFT GYYMH	WVPQAPG QGLEWMG	WINPNSGGTN YAQKFQG	RVTMTRDTSISTAYMEL RLRSDDTAVYYCAR	DGGSIPV SGHFDY	WGQGTL VTVSSA
5.83.1	449	"	"	"	QVQLVQSGAEVKK PGASVKVSCKAS	GYTFT GYYMH	WVRQAPG QGLEWMG	WINPNSGGTN YAQKFQG	RVTMTRDTSISTAYMEL SRLRSDDTAVYYCAR	DGGSIAV AGHFDY	WGQGTL VTVSSA
	598	Germline			EVQLVESGGGLIQ PGGSLRLSCAAS	GFTVS SNYMS	WVRQAPG KGLEWVS	VIYSGGSTYY ADSVKG	RFTISRDNSKNTLYLQM NSLRAEDTAVYYCA	YSSGWYY GMDV	WGQGTT VTVSSA
6.9	555	VH3-53	D6-19	JH6B	EVQLVESGGGLIQ SGGSLRLSCAAS	GFTVS SKYMS	WVRQAPG KGLEWVS	VIYSGGFTYY ADSVKG	RFTVSRDNSKNTLYLQM NSLGAEDTAVYYCAT	YSSGWHY YGMDV	WGQGTT VTVSSA
	599	Germline			QVQLVESGGGVVQ PGRSLRLSCAAS	GFTFS SYGMH	WVRQAPG KGLEWVA	VIWYDGSNKY YADSVKG	RFTISRDNSKNTLYLQM NSLRAEDTAVYYCAR	GDLLLRY GRL	GPRDHG HRLLS
3.10	43	VH3-33	D4-17	JH6B	QVQLVESGGGVVQ PGRSLRLSCAAS	GFTFS SYGMH	WVRQAPG KGLEWVA	VIRYDGSNKY YADSVKG	RFTISRDNSKNTLNLQM NSLRAEDTAVYYCAR	DRDGDYP LLLLGMD V	WGQGTT VTVSSA
4.13	171	"	"	"	QVQLVESGGGVVQ PGRSLRLSCAAS	GFTFS SYGMH	WVRQAPG KGLEWVA	VIWYDGSNKY YADSVKG	RFTISRDNSKNTLYLQM NSLRAEDTAVYYCAA	DYGDSDY YYYYGMD V	WGQGTT VTVSSA
	600	Germline			QVQLVESGGGVVQ PGRSLRLSCAAS	GFTFS SYGMH	WVRQAPG KGLEWVA	VIWYDGSNKY YADSVKG	RFTISRDNSKNTLYLQM NSLRAEDTAVYYCAR	DIVATIN YYYGMDV	WGQGTT VTVSSA
5.2	195	VH3-33	D5-12	JH6B	QVQLVESGGGVVQ PGRSLRLSCAAS	GFTFS SYGMH	WVRQAPG KGLEWVA	VIWFDGFNKY YADSVKG	RFTISRDNSKNTLYLQM NSLRAEDTAVYHCAR	DRGYSGY DHYYGMD V	WGQGTT VTVSSA
5.12	225	"	"	"	QVQLVESGGGVVQ PGRSLRLSCAAS	GFTFS SYGMH	WVRQAPG KGLEWVA	VIWYDGGNKY YTDSVKG	RFTISRDNSKNTLYLQM NSLRAEDTAVYYCAR	DEDIVAT INYYYGM	WGQGTT VTVSSA

	601	Germline			EVQLVESGGGLVQ PGGSLRLSCAAS	GFTFS SYWMS	WVRQAPG KGLEWVA	NIKQDGSEKY YVDSVKG	RFTISRDNAKNSLYLQM NSLRAEDTAVYYCAR	DIWYFDL	WGRGTL VTVSSA
5.4	201	VH3-7	D3-22	JH2	EVQLVESGGDLVQ PGGSLRLSCAAS	GFTFS SYWMS	WVRQAPG KGLEWVA	NIKQDGSEKY FDSVKG	RFTISRDNAKNSLYLQM NSLRAEDTAVYYCAR	DIRWYFD L	WGRGTL VTVSSA
	602	Germline			EVQLVESGGGLVQ PGGSLRLSCAAS	GFTFS SYWMS	WVRQAPG KGLEWVA	NIKQDGSEKY YVDSVKG	RFTISRDNAKNSLYLQM NSLRAEDTAVYYCAR	WYFDL	WGRGTL VTVSSA
5.91	479	VH3-7	-NA-	JH2	EVQLVESGGGLVQ PGGSLRLSCAAS	GFTFS SYWMS	WVRQAPG KGLEWVA	NIKQDGSEKY YVDSVKG	RFTISRDNAKNSLYLQM NSLRAEDTAVYYCAR	DSWWYFD L	WGRGTL VTVSSA
	603	Germline			EVQLVESGGGLVQ PGGSLRLSCAAS	GFTFS SYWMS	WVRQAPG KGLEWVA	NIKQDGSEKY YVDSVKG	RFTISRDNAKNSLYLQM NSLRAEDTAVYYCAR	AAAFDY	WGQGTL VTVSSA
5.88	461	VH3-7	D6-13	JH4B	EVQMVESGGGLVQ PGGSLRLSCAAS	GFTLR SYWMS	WVRQAPG KGLEWVA	NIKEDGSEKY HVDSVKG	RFTISRDNAENSLFLQM SSLRAEDTAVYYCAR	DMEASAG LFDY	WGQGTL VTVSSA
5.88.1	465	"	"	"	EVQMVESGGGLVQ PGGSLRLSCAAS	GFTLR SYWMS	WVRQAPG KGLEWVA	NIKEDGSEKY HVDSVKG	RFTISRDNAENSLFLQM SSLRAEDTAVYYCAR	DMEASAG LFDY	WGQGTL VTVSSA
	604	Germline			QVQLVESGGGVVQ PGRSLRLSCAAS	GFTFS SYGMH	WVRQAPG KGLEWVA	VIWYDGSNKY YADSVKG	RFTISRDNSKNTLYLQM NSLRAEDTAVYYCA	YSNYYFD Y	WGQGTL VTVSSA
5.89	473	VH3-33	D4-11	JH4B	QVQLVESGGGVVQ PGRSLRLSCAAS	GFTFR SYGMH	WVRQAPG KGLEWVA	VIWYDGSYKN YGDSVKG	RFTISRDNSKNTLYLQM NSLRAEDTAVYYCAR	DYSNYEE YFDY	WGQGTL VTVSSA
	605	Germline			QITLKESGPTLVK PTQTLTLTCTFS	GFSLS TSGVG VG	WIRQPPG KALEWLA	LIYWNDDKRY SPSLKS	RLTITKDTSKNQVVLTM TNMDPVDTATYYCAH	RSSSWFD Y	WGQGTL VTVSSA
5.115	529	VH2-5	D6-13	JH4B	QITLKESGPTLVK PTQTLTLTCTLS	GFSLS ISGVG VG	WIRQPPG KALEWLA	FIYWNDDKRY SPSLKS	RLTITKDTSKNQVVLTM TNMDPVDTATYYCAH	RPDSSSW DFDY	WGQGTL VTVSSA
	606	Germline			EVQLVESGGGLVK PGGSLRLSCAAS	GFTFS SYSMN	WVRQAPG KGLEWVS	SISSSSSYIY YADSVKG	RFTISRDNAKNSLYLQM NSLRAEDTAVYYCAR	GIAFDY	WGQGTL VTVSSA
5.64	385	VH3-21	D2-2	JH4B	EVQLVESGGGLVK PGGSLRLSCAAS	GFTFN SYRMN	WVRQAPG KGLEWVS	SITSSSHYIY YADSVKG	RFTISRDNAKNSLYLQM NSLRAEDTAVYYCAR	DRGIAAP FDY	WGQGTL VTVSSA
	607	Germline			QVQLVQSGAEVKK PGASVKVSCKAS	GYTFT GYYMH	WVRQAPG QGLEWMG	WINPNSGGTN YAQKFQG	RVTMTRDTSISTAYMEL SRLRSDDTAVYYCAR	GIAAAGF DY	WGQGTL VTVSSA
5.39.1	313	VH1-2	D6-13	JH4B	QVQLVQSGAEVTK PGASVKVSCKAS	GYTFT AYHMY	WVRQAPG QGLEWMG	WINPNSGGTN YAQKFQG	RVTMTRDTSISTAYMEL SRLRSDDSPVYYCAR	DQGIAAA GPFDY	WGQGTL VTVSSA
5.16.1	239	"	"	"	QVQLVQSGAEVKK PGASVKVSCKAS	GYTFT GFYMY	WVRQAPG QGLEWMG	WINPNSSGTN HAQKFQG	RVTMTRDTSISTAYMEL SRLRSDDTAVYYCAR	DQDIATA GPFDY	WGQGTL VTVSSA
5.86.1	453	"	"	"	QVQLVQSGAEVKK PGASVKVSCKAS	GYTFT GYHMY	WVRQAPG QGLEWLG	WINPNSGGTN YAQKFQG	RVTMTRDTSISTAYMEL SRLRSDDTAVYYCVR	DQGIAAA GPFDY	WCQGTL VTVSSA
	608	Germline			QVQLQESGPGLVK	GGSIS	WIRQPAG	RIYTSGSTNY	RVTMSVDTSKNQFSLKL	GITFDP	WGQGTL

					PSETLSLTCTVS	SYYWS	KGLEWIG	NPSLKS	SSVTAADTAVYYCAR		VTVSSA
5.79	433	VH4-4	D1-20	JH5B	QVQLQESGPGLVK PSETLSLTCTVS	GGSIS SYYWS	WIRQPAG KGLEWIG	RIYTSGSTNY NPSLKS	RVTMSVDTSKNQFSLKL SSVTAADTAVYYCAR	GITGYGG FDP	WGQGTL VTVSSA
5.23	263	"	"	"	QVQLQESGPGLVK PSETLSLTCTVS	GDSIN SYYWS	WIRQPAG KGLEWIG	RIYTSGSTNY NPSLKS	RVTMSVDTSKNQFSLKL SSVTAADTAVYYCAR	GITGYGG FDP	WGQGTL VTVSSA
	609	Germline			QVQLVESGGGVVQ PGRSLRLSCAAS	GFTFS SYGMH	WVRQAPG KGLEWVA	VIWYDGSNKY YADSVKG	RFTISRDNSKNTLYLQM NSLRAEDTAVYYCG	GMDV	WGQGTT VTVSSA
5.87	457	VH3-33	D3-16	JH6B	QVQLVESGGGVVQ PGRSLRLSCAAS	GFTFS SYGMH	WVRQAPG KGLEWVA	VIWYDGSNKY YADSVKG	RFTISRDNSKNTLYLQM NSLRAEDTAVYYCAG	GATAMDV	CGQGST GTVSSA
	610	Germline			EVQLVQSGAEVKK PGESLKISCKGS	GYSFT SYWIG	WVRQMPG KGLEWMG	IIYPGDSDTR YSPSFQG	QVTISADKSISTAYLQW SSLKASDTAMYYCAR	NWNFDI	WGQGTM VTVSSA
3.6	27	VH5-51	D1-7	JH3B	EVQLVQSGAEVKK PGESLKISCKGS	GYSFS NYWIA	WVRQMPG KGLEWMG	IIYPGDSDTR YSPSFQG	QVTISADKSISTAYLQW SSLKASDTAMYYCAR	HENWN FFDTFDI	WGQGTM VTVSSA
	611	Germline			EVQLVESGGGLVK PGGSLRLSCAAS	GFTFS SYSMN	WVRQAPG KGLEWVS	SISSSSSYIY YADSVKG	RFTISRDNAKNSLYLQM NSLRAEDTAVYYCAR	IFGVVNW YFDL	WGRGTL VTVSSA
4.6	155	VH3-21	D3-3	JH2	EVQLVESGGGLVK PGGSLRLSCAAS	GFTFS SYSMN	WVRQAPG KGLEWVS	SISSSSSYIY YADSVKG	RFTISRDNAKNSLYLQM NSLRAEDTAVYYCAR	DGAIFGV VNWYFDL	WGRGTL VTVSSA
5.94	487	"	"	"	EVQLVESGGGLFK PGGSLRLSCAAS	GFTFS SYSMN	WVRQAPG KGLEWVS	SISSSSSYIY YADSVKG	RFTISRDNAKNSLYLQM NSLRAEDTAVYYCAR	DGAIFGV VNWYFDL	WGRGTL VTVSSA
5.58	367	"	"	"	EVQLVESGGGLVK PGGSLRLSCAAS	GFTFS SYSMN	WVRQAPG KGLEWVS	SISSSSSYIY YADSVKG	RFTISRDNAKNSLYLQM NSLRAEDTAVYYCAR	DGAIFGV VNWYFDL	WGRGTL VTVSSA
4.9	163	"	"	"	EVQLVESGGGLVK PGGSLRLSCAAS	GFTFS SYSMN	WVRQAPG KGLEWVS	SISSSSSYIY YADSVKG	RFTISRDNAKNSLYLQM NSLRAEDTAVYYCAR	DGAIFGV VNWYFDL	WGRGTL VTVSSA
5.70	399	"	"	"	EVQLVESGGGLVK PGGSLRLSCAAS	GFTFS SYSMN	WVRQAPG KGLEWVS	SISSSSSYIY YADSVKG	RFTISRDNAKNSLYLQM NSLRAEDTAVYYCAR	DGAIFGV VNWYFDL	WGRGTL VTVSSA
4.15	179	"	"	"	EVQLVESGGGLVK PGGSLRLSCAAS	GFTFS SYSMN	WVRQAPG KGLEWVS	SISSSSSYIY YADSVKG	RFTISRDNAKNSLYLQM NSLRAEDTAVYYCAR	DGAIFGV VNWYFDL	WGRGTL VTVSSA
4.8	159	"	"	"	EVQLVESGGGLVK PGGSLRLSCAAS	GFTFS SYSMN	WVRQAPG KGLEWVS	SISSSSSYIY YADSVKG	RFTISRDNAKNSLYLQM NSLRAEDTAVYYCAR	DGAIFGV VNWYFDL	WGRGTL VTVSSA
4.7	157	"	"	"	EVQLVESGGGLVK PGGSLRLSCAAS	GFTFS SYSMN	WVRQAPG KGLEWVS	SISSSSSYIY YADSVKG	RFTISRDNAKNSLYLQM NSLRAEDTAVYYCAR	DGAIFGV VNWYFDL	WGRGTL VTVSSA
	612	Germline			QVQLVESGGGVVQ PGRSLRLSCAAS	GFTFS SYGMH	WVRQAPG KGLEWVA	VIWYDGSNKY YADSVKG	RFTISRDNSKNTLYLQM NSLRAEDTAVYYCAR	QNYDFWS GYGMDV	WGQGTT VTVSSA
4.11	167	VH3-33	D3-3- D3-3	JH6B	QVQLVESGGGVVQ PGRSLRLSCAAS	GFTFS SYGMY	WVRQAPG KGLEWVA	VIWYDGSNKY YADSVKG	RFTISRDNSKNTLYLQM NSLRAEDTAVYYCAR	DFFQNYD FWSGSPV GYGMDV	WGQGTT VTVSSA
	613	Germline			QVQLVQSGAEVKK PGASVKVSCKAS	GYTFT SYGIS	WVRQAPG QGLEWMG	WISAYNGNTN YAQKLQG	RVTMTTDTSTSTAYMEL RSLRSDDTAVYYCAR	VGADY	WGQGTL VTVSSA

6.6	547	VH1-18	D1-26	JH4B	LVQSGAEVKKPGA SVKVSCKAS	GYTFT SYGIS	WVRQAPG QGLEWMG	WISAYNGNTN YAQKLQD	RVTMTTDTSTSTAYMEL RSLRSDDTAVYYCAR	GVGAKDY	WGQGTL VTVSSA
3.34	115	"	"	"	QVQLVQSGAEVKK PGASVKVSCKAS	GYTFT SYGIS	WVRQAPG QGLEWMG	WISTYNDNTN YAQKLQG	RVTMTTDTSTSTAYMEL RSLRSDDTAVYYCAR	GVGATDY	WGQGTL VTVSSA
5.30	285	"	"	"	QVQLVQSGAEVKK PGASVKVSCKAS	GYTFT SYGIS	WVRQAPG QGLEWMG	WISAHNGNTN YAQKLQG	RVTMTTDTSTSTAYMEL RSLRSDDTAVYYCAR	GVGSKDY	WGQGTL VTVSSA
	614	Germline			EVQLVESGGGLVK PGGSLRLSCAAS	GFTFS NAWMS	WVRQAPG KGLEWVG	RIKSKTDGGT TDYAAPVKG	RFTISRDDSKNTLYLQM NSLKTEDTAVYYCT	SSGWYYY YYGMDV	WGQGTT VTVSSA
5.55	361	VH3-15	D6-19	JH6B	EVQLVESGGGLVK PGGSLRLSCAAS	GFTFS NAWMS	WVRQAPG KGLEWVG	RIKSKTDGGT TDYAAPVKG	RFTISRDDSKNTLYLQM NSLKTEDTAVYYCTI	GSSGWYE AYYYYGM DV	WGQGTT VTVSSA
5.7	209	"	"	"	EVQLVESGGGLVK PGGSLRLSCAAS	GFTFS NAWMS	WVRQAPG KGLEWVG	RIKSKTDGGT TDYAAPVKG	RFTISRDDSKYTLYLQM NSLKTEDTAVYYCTI	GSSGWYE AYYYYGM DV	WGQGTT VTVSSA
5.5	203	"	"	"	EVQLVESGGGLVK PGGSLRLSCAAS	GFTFS NAWMS	WVRQAPG KGLEWVG	RIKSKTDGGT TDYAAPVKG	RFTISRDDSKYTLYLQM NSLKTEDTAVYYCTI	GSSGWYE AYYYYGM DV	WGQGTT VTVSSA
	615	Germline			QVQLVESGGGVVQ PGRSLRLSCAAS	GFTFS SYGMH	WVRQAPG KGLEWVA	VISYDGSNKY YADSVKG	RFTISRDNSKNTLYLQM NSLRAEDTAVYYCAR	DFWSNWF DP	WGQGTL VTVSSA
3.19	75	VH3-30	D3-3	JH5B	QVQLVESGGGVVQ PGRSLRLSCAAS	GFTFT NYGMH	WGRQAPG KGLEWVA	VISHDGNNKY YVDSVKG	RFTISRDNSKNTLYLQM NSLRAEDTAVYYCAR	EGIDFWS GLNWFDP	WGQGTL VTVSSA
3.19.1	75	"	"	"	QVQLVESGGGVVQ PGRSLRLSCAAS	GFTFT NYGMH	WGRQAPG KGLEWVA	VISHDGNNKY YVDSVKG	RFTISRDNSKNTLYLQM NSLRAEDTAVYYCAR	EGIDFWS GLNWFDP	WGQGTL VTVSSA
	616	Germline			EVQLVESGGGLVK PGGSLRLSCAAS	GFTFS NAWMS	WVRQAPG KGLEWVG	RIKSKTDGGT TDYAAPVKG	RFTISRDDSKNTLYLQM NSLKTEDTAVYYCT	YGDAFDI	WGQGTM VTVSSA
5.72	405	VH3-15	D4-17	JH3B	EVQLVESGGGLVK PGGSLRLSCAAS	GFTFS NAWMS	WVRQAPG KGLEWVG	RIKSKTDGGT TDYAAPVKG	RFTISRDDSKNTLYLQM NSLKTEDTAVYYCTN	YYGDFYA FDI	WGQGTM VTVSSA
3.14	59	"	"	"	EVQLVESGGGLVK PGGSLRLSCAAS	GFTFS NAWMS	WVRQAPG KGLEWVG	RIKSKTDGGT TDYAAPVKG	RFTISRDDSKNTLYLQM NSLKTEDTAVYYCII	FYGDFDA FDI-	WGQGTM VTVSSA
3.17	67	"	"	"	EVQLVESGGGLVK PGGSLRLSCAAS	GFTFS NAWMH	WVRQAPG KGLDWVG	RIKSKTDGGT ADYAAPVKG	RFTISRDDSENTLYLQM NSLKTEDTAVYYCTN	DYGDFYA FDI	WGQGTM VTVSSA
	617	Germline			QVQLVESGGGVVQ PGRSLRLSCAAS	GFTFS SYGMH	WVRQAPG KGLEWVA	VIWYDGSNKY YADSVKG	RFTISRDNSKNTLYLQM NSLRAEDTAVYYCAR	GYCSGGY GMDV	WGQGTT VTVSSA
5.82	443	VH3-33	D2-15	JH6B	QVQLVESGGGVVQ PGRSLRLSCAAS	GFTFS SYGMH	WVRQAPG KGLEWVA	IIWFDGSNKY YADSVKG	RFTISRDNSKNTLYLQM VSLRAEDTAVYYCAR	KGYCSGG RCVYGMD V	WGQGTT VTVSSA
5.59	371	"	"	"	QVQLVESGGGVVQ PGRSLRLSCAAS	GFTFS SYGMH	WVRQAPG KGLEWVA	IIWYDGSNKY YADSVKG	RFTISRDNSKNTLHLQM NSLRAEDTAVYYCAR	KGYCSGG SCVYGMD V	WGQGTT VTVSSA

5.92	481	"	"	"	QVQLVESGGGVVQ PGRSLRLSCAAS	GFTFS SYGMH	WVRQAPG KGLEWVA	VIWYDGSNKY YADSVKG	RFTISRDNSKNTLYLQM NSLRAEDTAVYYCAR	KGYCSGG RCVYGMD V	WGQGTT VTVSSA
	618	Germline			QVQLVESGGGVVQ PGRSLRLSCAAS	GFTFS SYGMH	WVRQAPG KGLEWVA	VIWYDGSNKY YADSVKG	RFTISRDNSKNTLYLQM NSLRAEDTAVYYCAR	RYFDWDY	WGQGTL VTVSSA
6.5	545	VH3-33	D3-9	JH4B	QVQLVESGGGVVQ PGRSLRLSCAAS	GFTFS SYGMH	WVRQAPG KGLEWVA	VIWYDGSNKY YADSVKG	RFTISRDNSKNTLYLQM NSLRAEDTAVYYCAR	GPLRYFD WPSDY	WGQGTL VTVSSA
6.2	535	"	"	"	QVQLVESGGGVVQ PGRSLRLSCAAS	GFTFS SYGMH	WVRQAPG KGLEWVS	LIWYAGSNKY YADSVKG	RFTISRDNSKNTLYLQM NSLRAEDTAVYYCAR	GPLRYFD WPSDY	WGQGTL VTVSSA
3.8	35	"	"	"	QVQLVESGGGVVQ PGRSLRLSCAAS	GFTFS SYGMH	WVRQAPG KGLEWVA	VIWYDGSNKY YADSVKG	RFTISRDNSKNTLYLQM NSLRAEDTAVYYCAR	GPLRYFD WPPDY	WGQGTL VTVSSA
	619	Germline			QVQLVQSGAEVKK PGASVKVSCKAS	GYTFT SYGIS	WVRQAPG QGLEWMG	WISAYNGNTN YAQKLQG	RVTMTTDTSTSTAYMEL RSLRSDDTAVYYCAR	YSSFDY	WGQGTL VTVSSA
5.74	413	VH1-18	D6-19	JH4B	QVQLVQSGAEVKK PGASVKVSCKAS	GYTFT SYCIS	WVRQAPG RGLEWMG	WICSYNGNTN CAQKLQG	RVTMTTDTSTTTAYMEL RGLRSDDTAVYYCAR	ESLYSSG WFDY	WGQGTL VTVSSA
	620	Germline			QVQLVESGGGVVQ PGRSLRLSCAAS	GFTFS SYGMH	WVRQAPG KGLEWVA	VIWYDGSNKY YADSVKG	RFTISRDNSKNTLYLQM NSLRAEDTAVYYCAR	DFWSNWF DP	WGQGTL VTVSSA
5.71	401	VH3-33	D3-3	JH5B	QVQLVESGGGVVQ PGRSLRLSCAAS	GFTFS SYGMH	WVRQAPG KGLEWVA	VIWYDGSNKY YADSVKG	RFTISRDNSKNTLYLQM NSLRAEDTAVYYCAR	EGLDFWS DFYNWFD P	WGQGTL VTVSSA
	621	Germline			QVQLVESGGGVVQ PGRSLRLSCAAS	GFTFS SYGMH	WVRQAPG KGLEWVA	VIWYDGSNKY YADSVKG	RFTISRDNSKNTLYLQM NSLRAEDTAVYYCAR		WGQGTL VTVSSA
4.14	175	VH3-33	-NA-	JH5B	QVQLVESGGGVVQ PGRSLRLSCAAS	GFTFS SYGMH	WVRQAPG KGLEWVA	VIWYDGSNKY YADSVKG	RFTISRDNSKNTLYLQM NSLRAEDTAVYYCAR	ELAS	WGQGTL VTVSSA
6.1	533	"	"	"	QVLLVESGGGVVQ PGRSLRLSCAAS	GFTFS SYGMH	WVRQAPG KGLEWVA	VIWFDGSKKY YADSVKG	RFTISRDNSKNSLYLQM NSLRAEDTAVYYCAR	ELEL	WGLGTL VTVSSA
	622	Germline			QVQLQESGPGLVK PSETLSLTCTVS	GGSIS SYYWS	WIRQPPG KGLEWIG	YIYYSGSTNY NPSLKS	RVTISVDTSKNQFSLKL SSVTAADTAVYYCA	TGDY	WGQGTL VTVSSA
5.106	509	VH4-59	D7-27	JH4B	QVQLQESGPGLVK PSETLSLTCTVS	GGSIS SYYWS	WIRQPPG KGLEWIG	YIYYSGSTNF NPSLKS	RVTTSVDTSKNQFSLNL RSVTAADTAVYYCAR	GTGASDY	WGQGTL VTVSSA
5.48	341	"	"	"	QVQLQESAPGLVK PSETLSLTCTVS	GGSIS SYYWS	WIRQPPG KGLEWIG	YISYSGSTNY NPSLKS	RVTTSVDTSKNQFSLKL SSVTAADTAVYYCTR	GTGASDY	WGQGTL VTVSSA
	623	Germline			QVQLVESGGGLVK PGGSLRLSCAAS	GFTFS DYYMS	WIRQAPG KGLEWVS	YISSSGSTIY YADSVKG	RFTISRDNAKNSLYLQM NSLRAEDTAVYYCAR	AFDI	WGQGTM VTVSSA
5.102	501	VH3-11	-NA-	JH3B	QVQLVESGGGLVK PGGSLRLSCAAS	GFTFS DYYMS	WIRQAPG KGLEWVS	YISSSGSTIY YADSVKG	RFTISRDNAKNSLYLQM NSLRAEDTAVYYCAR	ERGDAFD I	WGQGTM VTVSSA
5.24	267	"	"	"	QVRLVESGGGLVK PGGSLRLSCAAS	GFTFS DYYMS	WIRQAPG KGLEWAS	YISSSGYSIY YADSVKG	RFTISRDNAKNSLYLQM NSLRAEDTAVYYCAR	ERGDAFD I	WGQGTM VTVSSA

	624	Germline			EVQLVESGGGLVK PGGSLRLSCAAS	GFTFS NAWMS	WVRQAPG KGLEWVG	RIKSKTDGGT TDYAAPVKG	RFTISRDDSKNTLYLQM NSLKTEDTAVYYCT	YGDYYFD Y	WGQGTL VTVSSA
5.1	191	VH3-15	D4-17	JH4B	EVQLVESGGGLVK PGGSLRLSCAAS	GFTFS NAWMS	WVRQAPG KGLEWVG	RIKSKTDGGT TDYAAPVKG	RFTISRDDSKNTLYLQM NSLKIEDTAVYYCTI	TYGDYPY FDC	WGQGTL VTVSSA
5.33	293	"	"	"	EVQLVESGGGLVK PGGSLRLSCAAS	GFTFS NTWMS	WVRQAPG KGLEWVG	RIKSKTDGGT TDYAAPVKG	RFTISRDDSKNTLCLQL NSLKTEDTAVYYCSA	GYGDYPY FDF	WGQGTL VTVSSA
	625	Germline			QVQLQESGPGLVK PSETLSLTCTVS	GGSIS SYYWS	WIRQPPG KGLEWIG	YIYYSGSTNY NPSLKS	RVTISVDTSKNQFSLKL SSVTAADTAVYYCAR	FDY	WGQGTL VTVSSA
4.3	145	VH4-59	-NA-	JH4B	QVQLQESGPGLVK PSETLSLTCTVS	GGSIN NYYWS	WIRQPPG KGLEWIG	YIYYSGSTNY NPSLKS	RVTISVDTSKNQFSLKL SSVTAADTAVYYCAR	ERGDSFD Y	WGQGTL VTVSSA
	626	Germline			QVQLVESGGGVVQ PGRSLRLSCAAS	GFTFS SYGMH	WVRQAPG KGLEWVA	VISYDGSNKY YADSVKG	RFTISRDNSKNTLYLQM NSLRAEDTAVYYCAR	QLWDY	WGQGTL VTVSSA
5.60	373	VH3-30	D5-5	JH4B	QVQLVESGGGVVQ PGRSLRLSCAAS	GFTFS GYDIH	WVRQAPG KGLEWVA	VISYDGSNKY YADSVKG	RFTISRDNSKNTLYLQM NSLRAEDTAVYYCAR	ERQLWLI DY	WGQGTL VTVSSA
	627	Germline			QVQLVESGGGVVQ PGRSLRLSCAAS	GFTFS SYGMH	WVRQAPG KGLEWVA	VISYDGSNKY YADSVKG	RFTISRDNSKNTLYLQM NSLRAEDTAVYYCAR	GIAVAYG MDV	WGQGTT VTVSSA
5.75	417	VH3-30	D6-19	JH6B	QVQLVESGGGVVQ PGRSLRLSCAAS	GFTFS GYGMH	WVRQAPG KGLEWVA	VISYDGSNKY YADSVKG	RFTISRDNSKNTLYLQM NSLRAEDTAVYYCAR	DRGIAVA GYYGMDV	WGQGNT VTVSSA

Table 12. Light chain analysis

Chain Name	SEQ ID NO:	V	J	FR1	CDR1	FR2	CDR2	FR3	CDR3	J
	628	Germline		ETVLTQSPGTLS LSPGERATLSC	RASQSVSSSYL A	WYQQKPGQ APRLLIY	GASSRAT	GIPDRFSGSGSGTDFT LTISRLEPEDFAVYYC	QQYGSSPWT	FGQGTKVEIKR
5.77	423	A27	JK1	EVVLTQSPGTLS LSPGDRATLSC	RASQSVSSSYL A	WYNQKPGQ APRLLIF	GASSRAT	GIPDRFSGSGSGTDFT LTISRLEPEDFAVYYC	LQYGSSPWT	FGQGTKVEIKR
	629	Germline		DIQMTQSPSSLS ASVGDRVTITC	QASQDISNYLN	WYQQKPGK APKLLIY	DASNLET	GVPSRFSGSGSGTDFT FTISSLQPEDIATYYC	QQYDN	FGQGTKLEIKR
5.61	379	O18	JK2	DIQMTQSPSSLS ASVGDRVTITC	QASQDISNYLN	WYQQKPGK APKLLIY	DASNLET	GVPSRFSGSGSGTDFT FTISRLQPEDIATYYC	QQYDNLCS	FGQGTKLEIKR
	630	Germline		DIQMTQSPSSVS ASVGDRVTITC	RASQGISSWLA	WYQQKPGK APKLLIY	AASSLQS	GVPSRFSGSGSGTDFT LTISSLQPEDFATYYC	QQANSFPWT	FGQGTKVEIKR
5.3	199	L5	JK1	DIQMTQSPSSVS ASVGDRVTITC	RASQGIRSWLA	WYQQKPGK APKLLIY	AASSLQS	GVPSRFSGSGSGTDFT LTISSLQPEDFATYYC	QQANSFPWT	FGQGTKVEIKR
3.2	13	L5	JK1	DIQMTQSPSSVS ASVGDRVTITC	RASQGISSWLA	WYQQKPGK APKLLIY	AASSLQS	GVPSRFSGSGSGTDFT LTISSLQPEDFATYYC	QQAYSFRT	FGQGTKVEIKR
5.34	295	L5	JK1	DIQMTQSPSSVS ASVGDRVTITC	RASQGISSWLA	WYQQKPGK APKLLIY	AASSLQS	GVPSRFSGSGSGTDFT LTISSLQPEDFATYYC	QQANSFPWT	FGQGTKVEIKR
3.22	91	L5	JK1	DIQMTQSPFSVS ASVGDRVTITC	RASQGISNWLA	WYQQKPGK APKLLIY	TASSLQN	GVPSRFSGSGSGTDFT LTISSLQPEDFATYYC	QQINSFPWT	FGQGTKVEIKR
5.73	411	L5	JK1	DIQMTQSPSSVS ASVGDRVTITC	RASQGISRWLA	WYQQKPGK APKLLIY	VASSLQS	GVPSRFSGSGSGTDFT LTISSLQPEDFATYYC	QQANSFPRT	FGQGTKVEIKR
5.1	193	L5	JK1	DIQMTQSPSSVS ASVGDRVTITC	RASQGIRSWLA	WYQQKPGK APKLLIY	AASSLQS	GVPSRFSGSGSGTDFT LTISSLQPEDFATYYC	QQANSFPWT	FGQGTKVEIKR
3.9	41	L5	JK1	DIQMTQSPSSVS ASVGDRVTITC	RASQGISSWLA	WYQQKPGK APNLLIY	AASSLQS	GVPSRFSGSGSGTDFI LTISSLQPEDFATYYC	QQSNSFPRT	FGQGTKVEIKR
5.81	441	L5	JK1	DIQMTQSPSSVS ASVGDRVTITC	RASQGISSWLA	WYQQKPGK APKLLIY	AASSLQS	GVPSRFSGSGSGTDFT LTISSLQPEDFATYYC	QQANSFPRT	FGQGTKVEIKR
	631	Germline		ETTLTQSPAFMS ATPGDKVNISC	KASQDIDDDMN	WYQQKPGE AAIFIIQ	EATTLVP	GIPPRFSGSGYGTDFT LTINNIESEDAAYYFC	LQHDNFPLT	FGGGTKVEIKR
5.24	269	B2	JK4	ETTLTQSPAFMS ATPGDKVNISC	KASQDIDDDMN	WYQQKPGE VAIFIIQ	EATTLVP	GIPPRFSGSGYGTDFT LTINNIESEDAAYYFC	LQHDNFPLT	FGGGTKVEIKR
	632	Germline		DIQMTQSPSSLS ASVGDRVTITC	RASQGISNYLA	WYQQKPGK VPKLLIY	AASTLQS	GVPSRFSGSGSGTDFT LTISSLQPEDVATYYC	QKYNSAPFT	FGPGTKVDIKR

5.56.1	365	A20	JK3	DIQMTQSPSSLS ASVGDRVTITC	RASQGISYYLA	WYQQKPGK VPKLLIY	AASTLQS	GVPSRFSGSGSGTDFT LTISSLQPEDVATYYC	QKYNSAPFT	FGPGTKVDIKR
	633	Germline		EIVMTQSPATLS VSPGERATLSC	RASQSVSSNLA	WYQQKPGQ APRLLIY	GASTRAT	GIPARFSGSGSGTEFT LTISSLQSEDFAVYYC	QQYNNWPLT	FGGGTKVEIKR
5.6	207	L2	JK4	EIVMTQSPATLS VSPGERATLSC	RASQSVSSNLA	WYQQKPGQ APRLLIY	GASTRAT	GIPARFSGSGSGTELT LTISSLQSEDFAVYYC	QQYNNWPLT	FGGGTKVEIKR
5.44	331	L2	JK4	EIVMTQSPATLS VSPGERATLSC	RASQSISSNLA	WYQQKPGQ APRLLIY	GASTRAT	GIPARFSGSGSGTEFT LTISSLQPEDFAVYYC	QQYNNWPLT	FGRGTKVEIKR
5.38	311	L2	JK4	EIVMTQSPATLS VSPGERVTLSC	RASQSVSGNLA	WYQQKPGQ APRLLIY	GASTRAT	GIPARFSGSGSGTEFT LTISSLQSEDFAVYYC	QHYNNWPLT	FGGGTKVEIKR
5.35	299	L2	JK4	EIVMTQSPATLS VSPGERATLSC	RASQSVSSNLA	WYQQKPGQ APRLLIY	GASTRAT	GIPARFSGSGSGTEFT LTISSLQSEDFAVYYC	EQYNNWPLT	FGGGTKVEIKR
5.8	213	L2	JK4	EIVMTQSPATLS VSLGERATLSC	RASQSVRSNLA	WYQQKPGQ APRLLIY	GASTRAT	GIPARFSGSGSGTELT LTISSLQSEDFAVYYC	HQYNNWPLT	FGGGTKVEIKR
5.35.1	299	L2	JK4	EIVMTQSPATLS VSPGERATLSC	RASQSVSSNLA	WYQQKPGQ APRLLIY	GASTRAT	GIPARFSGSGSGTEFT LTISSLQSEDFAVYYC	EQYNNWPLT	FGGGTKVEIKR
	634	Germline		DIQMTQSPSSLS ASVGDRVTITC	RASQGIRNDLG	WYQQKPGK APKRLIY	AASSLQS	GVPSRFSGSGSGTEFT LTISSLQPEDFATYYC	LQHNSYPFT	FGPGTKVDIKR
4.13	173	A30	JK3	DIQMTQSPSSLS ASVGDRVTITC	RASQGIRDDLG	WFQQKPGK APKRLTY	AASSLQS	GVPSRFSGSGSGTEFT LTISSLQPEDFATYYC	LQYNSYPFT	FGPGTKVDIKR
	635	Germline		EIVMTQSPATLS VSPGERATLSC	RASQSVSSNLA	WYQQKPGQ APRLLIY	GASTRAT	GIPARFSGSGSGTEFT LTISSLQSEDFAVYYC	QQYNNWPFT	FGPGTKVDIKR
5.28.1	279	L2	JK3	EIVMTQSPATLS VSPGERATLSC	RASQSVTSNLA	WYQQKPGQ APRLLIY	GALIRAT	GIPARFSGSGSGTEFT LTISSLQSEDFAVYYC	QQYNNWPFT	FGPGTKVDIKR
5.28	279	L2	JK3	EIVMTQSPATLS VSPGERATLSC	RASQSVTSNLA	WYQQKPGQ APRLLIY	GALIRAT	GIPARFSGSGSGTEFT LTISSLQSEDFAVYYC	QQYNNWPFT	FGPGTKVDIKR
	636	Germline		DIQMTQSPSSLS ASVGDRVTITC	RASQGIRNDLG	WYQQKPGK APKRLIY	AASSLQS	GVPSRFSGSGSGTEFT LTISSLQPEDFATYYC	LQHNSYP	FGQGTKLEIKR
4.4	149	A30	JK2	DIQMTQSPSSLS ASVGDRVTITC	RASQGIRNDLG	WYQQKPGK APKRLIY	AASSLQS	GVPSRFSGSRSGTEFT LTISSLQPEDFATYYC	LQHNSYPPS	FGQGTKLEIKR
3.33	113	A30	JK2	DIQMTQSPSSLS ASVGDRVTITC	RASQGIRNDLG	WYQQKPGK APKRLIY	AASSLQS	GVPSRFSGSRSGTEFT LTISSLQPEDFATYYC	LQHNSYPPS	FGQGTKLEIKR
3.39	127	A30	JK2	DIQMTQSPSSLS ASVGDRVTITC	RASQGIRNDLG	WYQQKPGK APKRLIY	AASSLQS	GVPSRFSGSRSGTEFT LTISSLQPEDFATYYC	LQHNSYPPS	FGQGTKLEIKR
	637	Germline		DIQMTQSPSSLS ASVGDRVTITC	RASQGIRNDLG	WYQQKPGK APKRLIY	AASSLQS	GVPSRFSGSGSGTEFT LTISSLQPEDFATYYC	LQHNSYPRT	FGQGTKVEIKR
3.26	95	A30	JK1	DIQMTQSPSSLS ASVGDRVTITC	RASQGIRNDLG	WYQQKPGK APKRLIY	AASSLQS	GVPSRFSGSGSGTDFT LTISSLQPEDFATYYC	LQHNNYPRT	FGQGTKVEIKR

3.11	49	A30	JK1	DIQMTQSPSSLS TSVGDRVTITC	RASQGIRNDLG	WYQQKPGK APKRLIY	AASSLQS	GVPSRFSGSGSGTEFT LTISSLRPEDFATYYC	LQLNSYPRT	FGQGTKVEIKR
3.1	9	A30	JK1	DIQMTQSPSSLS ASVGDRVTITC	RASQGIRNDLG	WYQQKPGK APKRLIY	AASSLQS	GVPSRFSGSGSGTEFT LTISSLQPEDFATYYC	LQHNSYPRT	FGQGTKVEIKR
4.15	181	A30	JK1	DIQMTQSPSSLS ASVGDRVTITC	RASQGIRNDLG	WYQQKPGK APKRLIY	AASSLQS	GVPSRFSGSGSGTEFT LTISSLQPEDFATYYC	LQHNSYPPT	FGQGTKVEIKR
3.18	73	A30	JK1	DIQMTQSPSSLS ASVGDRVTITC	RASQGIRNDLG	WYQQKPGK APKRLIY	AASSLQS	GVPSRFSGSGSGTEFT LTISSLQPEDFATYYC	LQYNSYPRT	FGQGTKVEIKR
	638	Germline		EIVMTQSPATLS VSPGERATLSC	RASQSVSSNLA	WYQQKPGQ APRLLIY	GASTRAT	GIPARFSGSGSGTEFT LTISSLQSEDFAVYYC	QQYNNWPRT	FGQGTKVEIKR
5.16.1	241	L2	JK1	EIVMTQSPATLS VSPGERATLSC	RASQSVSSNLA	WYQQKPGQ APRLLIF	GASTRAT	GIPARFSGSGSGTEFT LTISSLQSEDFAVYYC	QQYNNWWT	FGRGTKVEIKR
5.40.2	319	L2	JK1	EIVMTQSPATLS VSPGERATLSC	RASQSVSSNLV	WYQQKPGQ APRLLIY	DSSTRAT	GIPVRFSGSGSGTEFT LTISSLQSEDFAVYYC	QQYNHWWT	FGQGTKVEIKR
5.41.1	323	L2	JK1	EIVMTQSPATLS VSPGERATLSC	RASQSVSSNLA	WYQQKPGQ APRFLIY	GASTRAT	GIPARFSGSGSGTEFT LTISSLQSEDFAVYYC	QQYNHWWT	FGQGTKVEIKR
6.4	543	L2	JK1	EIVMTQSPATLS VSPGERATLSC	RASQSLISNLA	WYQQKPGQ APRQLLF	GASTRAT	GIPARFSGSGSGTEFT LTISSLQSEDFAVYYC	HQYNNWWT	FGQGTKVEIKR
5.103.1	505	L2	JK1	ETVMTQSPATLS VSPGERVTLSC	RASQSVISSLA	WYQQKPGQ APRLLIY	GASTRAT	GIPARFSGSGSGTEFT LTISSLQSEDFAVYYC	QQYNNWWT	FGQGTKVEIKR
5.54.1	359	L2	JK1	EIVMTQSPATLS VSPGERATLSC	RASQSLISNLA	WYQQKPGQ APRLLIF	GASTRAT	GIPARFSGSGSGTEFT LTISSLQSEDFAIYYC	HQYNNWWT	FGQGTKVEIKR
5.13	231	L2	JK1	EIVMTQSPATLS VSPGERATLSC	RASQSVSSNLA	WYQQKPGQ APRLLIY	GASTRAT	GIPARFSGSGSGTEFT LTISSLQSEDFAVYYC	QQYNHWWT	FGQGTKVEIKR
3.31.1	101	L2	JK1	EVVMTQSPATLS VSPGERATLSC	RASQSVGSNLA	WYQQKPGQ APRLLIY	GASTRAT	GIPARFSGSGSGTEFT LTISSLQSEDFAVYCC	QQYNHWWT	FGQGTKVEIKR
5.88.1	467	L2	JK1	EIVMTQSPATLS VSPGERAILSC	RASQSISSNLA	WYQQKPGQ APRLLIY	GASTRAT	GIPARFSGSGSGTEFT LTISSLQSEDFAVYYC	QQYNYWWT	FGQGTKVEIKR
3.3	17	L2	JK1	EIVMTQSPATLS VSPGERATLSC	RASQTVSSDLA	WYQQKPGQ APRLLIY	GASIRAT	GIPARFSGSGSGTEFT LTISSLQSEDFAVYSC	QQYYNWWT	FGQGTKVEIKR
5.62	383	L2	JK1	EIVMTQSPATLS VSPGERATLSC	RASQSVSSNLA	WYQQKPGQ APRLLIY	GAFTRAT	GIPARFSGSGSGTEFT LTISSLQSEDFAVYYC	QQYNHWWT	FGQGTKVGIKR
5.101	499	L2	JK1	EIVMTQSPATLS VSPGERATLSC	RASQSLISNLA	WYQQKPGQ APRLLIF	GASTRAT	GIPARFSGSGSGTEFT LTISSLQSEDFAVYYC	HQYNNWWT	FGQGTKVEIKR
5.52	349	L2	JK1	EIVMTQSPATLA VSPGERATLSC	RARQSVSSNLA	WYQQKPGQ APRLLIY	GASTMAT	GFPARFSGRGSGTEFT LTISSLQSEDFAVYYC	QQYNNWWT	FGQGTKVEIKR
5.16	241	L2	JK1	EIVMTQSPATLS VSPGERATLSC	RASQSVSSNLA	WYQQKPGQ APRLLIF	GASTRAT	GIPARFSGSGSGTEFT LTISSLQSEDFAVYYC	QQYNNWWT	FGRGTKVEIKR

5.39	315	L2	JK1	EIVMTQSPATLS VSPGDRATLSC	RASQSVSSNFA	WYQQKPGQ APRLLIY	GSSTRAT	GIPARFSGSGSGTEFT LTISSLQSEDFAVYYC	QQYHYWWT	FGQGTKVEFKR
3.6	29	L2	JK1	EIVMTQSPATLS VSPGERATLSC	RASQSISSNLA	WFQQKPGQ APRLLIY	GASTRAT	GIPARFSGSGSGTEFT LTISSLQSEDFAVYYC	QQYNNWPRT	FGQGTTVEIKR
5.39.1	315	L2	JK1	EIVMTQSPATLS VSPGDRATLSC	RASQSVSSNFA	WYQQKPGQ APRLLIY	GSSTRAT	GIPARFSGSGSGTEFT LTISSLQSEDFAVYYC	QQYHYWWT	FGQGTKVEFKR
5.15	237	L2	JK1	EIVMTQSPATLS VSPGERATLSC	RASQTVISNLA	WYQQKPGQ APRLLIY	GASTRAT	GIPARFSGSGSGTEFT LTISSLQSEDFALYYC	QQYNNWWT	FGQGTKVEIKR
5.83.1	451	L2	JK1	EIVMTQSPATLS VSPGERATLSC	RASQSLISNLA	WYQQKPGQ APRLLIF	GASTRAT	GIPARFSGSGSGTEFT LTISSLQSEDFAVYYC	HQYNNWWT	FGQGTKVEIKR
5.41	323	L2	JK1	ETVMTQSPATLS VSPGERATLSC	RASQSVSSNLA	WYQQKPGQ APRFLIY	GASTRAT	GIPARFSGSGSGTEFT LTISSLQSEDFAVYYC	QQYNHWWT	FGQGTKVEIKR
5.62.1	383	L2	JK1	ETVMTQSPATLS VSPGERATLSC	RASQSVSSNLA	WYQQKPGQ APRLLIY	GAFTRAT	GIPARFSGSGSGTEFT LTISSLQSEDFAVYYC	QQYNHWWT	FGQGTKVGIKR
5.64	387	L2	JK1	EIVMTQSPATLS VSPGERATLSC	RASQSVSSNLA	WYQQKPGQ APRLLIY	GASTRAT	GFPARFSGSGSGTEFT LTISSLQSEDFAVYYC	QQYNNWWT	FGQGTKVEIKR
5.101.1	499	L2	JK1	ETVMTQSPATLS VSPGERATLSC	RASQSLISNLA	WYQQKPGQ APRLLIF	GASTRAT	GIPARFSGSGSGTEFT LTISSLQSEDFAVYYC	HQYNNWWT	FGQGTKVEIKR
5.103	505	L2	JK1	ETVMTQSPATLS VSPGERVTLSC	RASQSVISSLA	WYQQKPGQ APRLLIY	GASTRAT	GIPARFSGSGSGTEFT LTISSLQSEDFAVYYC	QQYNNWWT	FGQGTKVEIKR
5.54	359	L2	JK1	EIVMTQSPATLS VSPGERATLSC	RASQSLISNLA	WYQQKPGQ APRLLIF	GASTRAT	GIPARFSGSGSGTEFT LTISSLQSEDFAIYYC	HQYNNWWT	FGQGTKVEIKR
5.13.1	231	L2	JK1	EIVMTQSPATLS VSPGERATLSC	RASQSVSSNLA	WYQQKPGQ APRLLIY	GASTRAT	GIPARFSGSGSGTEFT LTISSLQSEDFAVYYC	QQYNHWWT	FGQGTKVEIKR
5.109	519	L2	JK1	EIVMTQSPATLS VSPGERATLSC	RASQSVSSYLA	WYQQKPGQ APRLLIY	GAFTRAT	GIPARFRGSGSGPEFT LTISSLQSEDFAVYYC	QQYSHWWT	FGQGTKVEIKR
5.108.1	515	L2	JK1	EIVMTQSPATLS VSPGERATLSC	RASQSVSSYLA	WYQQKPGQ APRLLIY	GAFTRAT	GIPARFRGSGSGPEFT LTISSLQSEDFAVYYC	QQYSHWWT	FGQGTKVEIKR
3.3.1	21	L2	JK1	EIVMTQSPATLS VSPGERATLSC	RASQTVSSDLA	WYQQKPGQ APRLLIY	GASIRAT	GIPARFSGSGSGTEFT LTISSLQSEDFAVYSC	QQYYNWWT	FGQGTKVEIKR
	639	Germline		DIVMTQSPLSLP VTPGEPASISC	RSSQSLLHSNG YNYLD	WYLQKPGQ SPQLLIY	LGSNRAS	GVPDRFSGSGSGTDFT LKISRVEAEDVGVYYC	MQALQTPIT	FGQGTRLEIKR
5.92	483	A3	JK5	DIVMTQSPLSLP VTPGEPASISC	RSSQSLLYSNG YNYLD	WYLQKPGQ SPQVLIY	LGSNRAS	GVPDRFSGSGSGTDFT LKISRVEAEDVGVYYC	MQALQTPIT	FGQGTRLEIKR
5.82	445	A3	JK5	DIVMTQSPLSLP VTPGEPASISC	RSSQSLLHSNG YNYLD	WYLQKPGQ SPQLLIY	LGSNRAS	GVPDRFGGSGSGTDFT LKISRVEAEDVGVYYC	MQALQTPIT	FGQGTRLEIKR
	640	Germline		DVVMTQSPLSLP VTLGQPASISC	RSSQSLVYSDG NTYLN	WFQQRPGQ SPRRLIY	KVSNWDS	GVPDRFSGSGSGTDFT LKISRVEAEDVGVYYC	MQGTHWPPL T	FGGGTKVEIKR

3.41	135	A1	JK4	DVVMTQSPLSLP VTLGQPASISC	RSSQSLVYSDG NTYLN	WFQQRPGQ SPRRLIY	KVSNWDS	GVPDRFSGSGSGTDFT LKISRVEAEDVGVYYC	MQGTHWPLT	FGGGTNVEIKR
3.40	131	A1	JK4	DVVMTQSPLSLP VTLGQPASISC	RSSQSLVYSDG NTYLN	WFQQRPGQ SPRRLIY	KDSNWDS	GVPDRFSGSGSGTDFT LKISRVEAEDVGVYYC	MQGTHWPLT	FGGGTNVEIKR
3.13	57	A1	JK4	DVVMTQSPLSLP VTLGQPASISC	RSSQSLVYSDG NTYLN	WFQQRPGQ SPRRLIY	KVSNWDS	GVPDRFSGSGSGTDFT LKISRVEAEDVGVYYC	MQGTHWPPL T	FGGGTKVEIKR
3.12	53	A1	JK4	DVVMTQSPLSLP VTLGQPASISC	RSSQSLVYSDG NTYLN	WFQQRPGQ SPRRLIY	KVSNWDS	GVPDRFSGSGSGTDFT LKISRVEAEDVGVYYC	MQGTHWPPL T	FGGGTKVEIKR
3.32	109	A1	JK4	DVVMTQSPLSLP VTLGQPASISC	RSSQSLVYSDG NTYLN	WFQQRPGQ SPRRLIY	KVSNWDS	GVPDRFSGSGSGTDFT LKISRVEAEDVGVYYC	MQGTHWPPL T	FGGGTKVEIKR
4.18	189	A1	JK4	DVVMTQSPLSLP VTLGQPASISC	RSSQSLVYSDG NTYLN	WFQQRPGQ SPRRLIY	KVSNWDS	GVPDRFSGSGSGTDFT LKISRVEAEDVGVYYC	MQGTHWPLT	FGGGTNVEIKR
4.16	185	A1	JK4	DVVMTQSPLSLP VTLGQPASISC	RSSQSLVYSDG NTYLN	WFQQRPGQ SPRRLIY	KVSNWDS	GVPDRFSGSGSGTDFT LKISRVEAEDVGVYYC	MQGTHWPPL T	FGGGTKVEIKR
3.7	33	A1	JK4	DVVMTQSPLSLP VTLGQPASISC	RSSQSLVYSDG NTYLN	WFQQRPGQ SPRRLIY	KVSNWDS	GVPDRFSGSGSGTDFT LKISRVEAEDVGVYYC	MQGTHWPPL T	FGGGTKVEIKR
4.14	177	A1	JK4	DVVMTQSPLSLP VTLGQPASISC	RSSQSLVYSDG NTYLN	WFQQRPGQ SPRRLIY	KVSNWDS	GVPDRFSGSGSGTDFT LKISRVEAEDVGVYYC	MQGTHWPPL T	FGGGTKVEIKR
3.15	63	A1	JK4	DVVMTQSPLSLP VTLGQPASISC	RSSQSLVYSDG NTYLN	WFQQRPGQ SPRRLIY	KVSNWDS	GVPDRFSGSGSGTDFT LKISRVEAEDVGVYYC	MQGTHWPPL T	FGGGTKVEIKR
	641	Germline		DIVMTQSPLSLP VTPGEPASISC	RSSQSLLHSNG YNYLD	WYLQKPGQ SPQLLIY	LGSNRAS	GVPDRFSGSGSGTDFT LKISRVEAEDVGVYYC	MQALQTPLT	FGGGTKVEIKR
3.10	45	A3	JK4	DIVMTQSPLSLP VTPGEPASISC	RSSQSLLHSNG YNYLD	WYLQKPGQ SPQLLIY	LGSNRAS	GVPDRFSGSGSGTDST LKISRVEAEDVGVYYC	MQALQTPLT	FGGGTKVEIKR
3.37	123	A3	JK4	DIVMTQSPLSLP VTPGEPASISC	RSSQSLLHSDG YNYLD	WYLQKPGQ SPQLLIY	LGSNRAS	GVPDRFSGSGSCTDFT LKISRVEAEDVGIYYC	MQALQTPHF	FGGGTKVEIKR
	642	Germline		DIVMTQSPDSLA VSLGERATINC	KSSQSVLYSSN NKNYLA	WYQQKPGQ PPKLLIY	WASTRES	GVPDRFSGSGSGTDFT LTISSLQAEDVAVYYC	QQYYSTPIT	FGQGTRLEIKR
5.78	427	B3	JK5	DIVMTQSPDSLA VSLGERATINC	KSSQSVLYSSN NQNFLA	WYQQKPGQ PPKLLIY	WASTRES	GVPDRFSGSGSGTDFT LTISSLQAEDVAVYYC	HQYYSTPIT	FGQGTRLEIKR
5.90	477	B3	JK5	DIVMTQSPDSLA VSLGEKATINC	KSSQSVIYSSN NQNFLA	WYQHKPGQ PPKLLIY	WASTRES	GVPDRFSDSGSGTDFT LTISSLQAEDVAVYYC	HQYYSTPIT	FGQGTRLEIKR
5.78.1	431	B3	JK5	DIVMTQSPDSLA VSLGERATINC	KSSQSVLYSSN NQNFLA	WYQQKPGQ PPKLLIY	WASTRES	GVPDRFSGSGSGTDFT LTISSLQAEDVAVYYC	HQYYSTPIT	FGQGTRLEIKR
	643	Germline		DVVMTQSPLSLP VTLGQPASISC	RSSQSLVYSDG NTYLN	WFQQRPGQ SPRRLIY	KVSNWDS	GVPDRFSGSGSGTDFT LKISRVEAEDVGVYYC	MQGTHW	FGQGTKLEIKR
4.3	147	A1	JK2	DVVMTQSPLSLP VTLGQPASISC	RSSRSLVYSDG NTYLN	WFQQRPGQ SPRRLIY	KVSNWDS	GVPDRFSGSGSGTDFT LKISRVEAADVGVYYC	MQGTHWPCS	FGQGTKLEIKR

	644	Germline		DIVMTQSPDSLA VSLGERATINC	KSSQSVLYSSN NKNYLA	WYQQKPGQ PPKLLIY	WASTRES	GVPDRFSGSGSGTDFT LTISSLQAEDVAVYYC	QQYYSTT	FGGGTKVEIKR
5.43	327	B3	JK4	DIVMTQSPDSLA VSLGERATINC	KSSQSVLYSSN NKNYLA	WYQQKPGQ PPKLLIY	WSSTRES	GVPDRFSGSGSGTDFT LTISSLQAEDVAVYYC	QQYYSTPLT	FGGGTKVEIKR
	645	Germline		DVVMTQSPLSLP VTLGQPASISC	RSSQSLVYSDG NTYLN	WFQQRPGQ SPRRLIY	KVSNWDS	GVPDRFSGSGSGTDFT LKISRVEAEDVGVYYC	MQGTHWPPT	FGQGTKVEIKR
3.20	83	A1	JK1	DVVMTQSPLSLP VTLGQPASISC	RSSQSLVYSDG NTYLN	WFQQRPGQ SPRRLIY	KVSKWDS	GVPDRFSGSGSGTDFT LKISRVEAEDVGVYYC	MQGTHWPPT	FGQGTKVEIKR
	646	Germline		DIVMTQSPLSLP VTPGEPASISC	RSSQSLLHSNG YNYLD	WYLQKPGQ SPQLLIY	LGSNRAS	GVPDRFSGSGSGTDFT LKISRVEAEDVGVYYC	MQALQWT	FGQGTKVEIKR
5.9	647	A3	JK1	DIVMTQSPLSLP VTPGEPASISC	RSSQSLLHSNG YNYLD	WYLQKPGQ SPQLLIY	LGSNRAS	GVPDRFSGSGSGTDFT LKISRVEAEDVGVYYC	MQALQTPWT	FGQGTKVEIKR
4.11	169	A3	JK1	DIVMTQSPLSLP VTPGEPASISC	RSSQSLLYSNG YNYLD	WYLQKPGQ SPQLLIY	LGSNRAS	GVPDREGGSGSGTDFT LKISRVEAEDVGVYYC	MQALQTPWT	FGQGTKVEIKR
	648	Germline		DIQMTQSPSSLS ASVGDRVTITC	RASQSISSYLN	WYQQKPGK APKLLIY	AASSLQS	GVPSRFSGSGSGTDFT LTISSLQPEDFATYYC	QQSYSTT	FGGGTKVEIKR
5.115	531	O12	JK4	DIEMTQSPSSLS ASVGDRVTITC	RASQNISSYLN	WYQQKPGK APKLLIY	AASSLQS	GVPSRFSGSGSGTDFT LTISSLQPEDFATYYC	QQSYSSPLT	FGGGTKVEIKR
5.66	391	O12	JK4	DIQMTQSPSSLS ASVGDRVTITC	RASQSISSYFN	WYQQKPGK APKLLIY	AASSLQS	GVPSRFSGSGSGTDFT LTISSLQPEDFATYYC	QQSYSTPLT	FGGGTKVEIKR
5.72	407	O12	JK4	DIQMTQSPSSLS ASVGDRVTITC	RASQTISSYLN	WYQQKPGK APKLLIY	AASSLQS	GVPSRFSGSGSGTDFT LTISSLQPEDFATYYC	QQTYSKSLT	FGGGTKVEIKR
5.114	527	O12	JK4	DIEMTQSPSSLS ASVGDRVTITC	RASQNISSYLN	WYQQKPGK APKLLIY	AASSLQS	GVPSRFSGSGSGTDFT LTISSLQPEDFATYYC	QQSYSSPLT	FGGGTKVEIKR
4.5	153	O12	JK4	DIQMTQSPSSLS ASVGDRVTITC	RASQSISSYLN	WYQQRPGK APKLLIY	AASSLQS	GVPSRFSGSGSGTDFT LTISSLQPEDFATYYC	QQSYSTPLT	FGGGTKVEIKR
	649	Germline		DIVMTQSPDSLA VSLGERATINC	KSSQSVLYSSN NKNYLA	WYQQKPGQ PPKLLIY	WASTRES	GVPDRFSGSGSGTDFT LTISSLQAEDVAVYYC	QQYYST	FGQGTKLEIKR
3.42	139	B3	JK2	DIVMTQSPDSLA VSLGERATINC	RSSQSILFSSN NKNYLA	WYQQKPGQ PPKLLLY	WASTRES	GVPARFSGSGSGTDFT LTISSLQAEDVAVYYC	QQYYSTPCS	FGQGTRLEIKR
5.60	375	B3	JK2	DIVMTQSPDSLA VSLGERATINC	KSSQSVLYSSN NKNYLV	WYQQKPGQ PPKLLIY	WASTRES	GVPDRFSGSGSGTDFT LTISSLQAEDVAVYYC	QQYYNTPCS	FGQGTKLEIKR
	650	Germline		EIVLTQSPGTLS LSPGERATLSC	RASQSVSSSYL A	WYQQKPGQ APRLLIY	GASSRAT	GIPDRFSGSGSGTDFT LTISRLEPEDFAVYYC	QQYGIT	FGQGTRLEIKR
5.58	369	A27	JK5	EIVLTQSPGTLS LSPGERATLSC	RASQSVSSSYL A	WYQQKAGQ APRLLIY	GASSRAT	GIPDRFSGSGSGTDFT LTISRLEPEDFAVYYC	QQYGWSSIT	FGQGTRLEIKR
3.19.1	77	A27	JK5	EIVLTQSPGTLS LSPGERATLSC	RASQSITGSYL A	WYQQKPGQ APRLLIC	GASSWAT	GIPDRFSGSGSGTDFT LTISRLEPEDFAVYYC	QQYSSSPIT	FGQGTRLEIKR

	651	Germline		DIQMTQSPSSLS ASVGDRVTITC	RASQGISNYLA	WFQQKPGK APKSLIY	AASSLQS	GVPSRFSGSGSGTDFT LTISSLQPEDFATYYC	QQYNSYPLT	FGGGTKVEIKR
3.16	65	L1	JK4	DIQMTQSPSSLS ASVGDRVTITC	RASQGISSYLA	WFQQKPGK APKSLIY	AASSLQS	GVPSKFSGSGSGTDFT LTISSLQPEDFATYYC	QQYNSYPLT	FGGGTKVEIKR
5.67	395	L1	JK4	DIQMTQSPSSLS ASVGDRVTITC	RASQGISNYLA	WFQQKPGK APESLIY	AASSLQT	GVPSKFSGNGSGTDFT LTISSLQPEDFATYYC	QQYNSYPLT	FGGGTKVEIKR
	652	Germline		DIQMTQSPSSLS ASVGDRVTITC	RASQSISSYLN	WYQQKPGK APKLLIY	AASSLQS	GVPSRFSGSGSGTDFT LTISSLQPEDFATYYC	QQSYSTPFT	FGPGTKVDIKR
5.86	455	O12	JK3	DIRMTQSPSSLS ASVGDRVTITC	RASQRISTYLN	WYQQKPGK APKFLIY	AASSLQS	GVPSRFSGSGSGTDFT LTISSLQPEDFATYYC	QQSYTTPFT	FGPGTKVDIKR
5.71	403	O12	JK3	DIQMTQSPSSLS ASVGDRVTITC	RASQSISNYLN	WYQQKPGK APKLLIE	TASSLQS	GVPSRFSGSGSGTDFT LTISSLQPEDFATYFC	QQSYSTPFT	FGPGTKVGIKR
5.25	271	O12	JK3	DIRMTQSPSSLS ASVGDRVTITC	RASQRISSYLN	WFQQKPGK APKFLIY	AASSLQS	GVPSRFSGSGSGTDFT LTISSLQPEDFATYYC	QQSYSTPFT	FGPGTKVDIKR
5.74	415	O12	JK3	DIRMTQSPSSLS ASVGDRVTITC	RASQRISTYLN	WYQQKPGK APKFLIY	AASSLQS	GVPSRFSGSGSGTDFT LTISSLQPEDFATYYC	QQSYTTPFT	FGPGTKVDIKR
3.14	61	O12	JK3	DIQMTQSPSSLS ASVGDRVTITC	RASQSISSYLN	WYQQKPGK APKLLIY	AASSLQS	GVPSRFSGSGSGTDFT LTISSLQAEDFATYYC	QQSLT	FGPGTKVDIKR
5.111	523	O12	JK3	DIQMTQSPSSLS ASVGDRVTFTC	RASQSIITFLN	WFQHKPGK APKLLFY	GASSLES	GVPSRFSGSGSGTNFT LTISSLQPEDFATYYC	QQSYSDPFT	FGPGTKVDIKR
5.87	459	O12	JK3	DIRMTQSPSSLS ASVGDRVTITC	RASQGIRTYLN	WYQQKPGK APKLLIY	AASSLQS	GVPSRFSGSGSGTDFT LTISSLQPEDFATYYC	QQNYTTPFT	FGPGTKVDIKR
4.2	143	O12	JK3	DIQMTQSPSSLS ASVGDRVTITC	RASQSISFYLN	WYQQKPGK APKLLIY	AASSLQS	GVPSRFSGSGSGTDFT LTISSLQPEDFATYYC	QQSYSSPFT	FGPGTKVDIKR
5.86.1	455	O12	JK3	DIRMTQSPSSLS ASVGDRVTITC	RASQRISTYLN	WYQQKPGK APKFLIY	AASSLQS	GVPSRFSGSGSGTDFT LTISSLQPEDFATYYC	QQSYTTPFT	FGPGTKVDIKR
3.17	69	O12	JK3	DIQMTQSPSSLS ASVGDRITITC	RARQSISSYLN	WYQQKPGK APKLLIY	AASSLQS	GVPSRFSGSGSGTDFT LTISSLQPEDFATYFC	QQSYSTPFT	FGPGAKVDIKR
5.11	223	O12	JK3	DIQMTQSPSSLS ASVGDRVTITC	RASQSISNYLN	WYQQKPGK APKLLIY	AASSLQS	GVPSRFSGSGSGTDFT LTISSLQPEDFATYYC	QQSYSIPFT	FGPGTKVDIKR
5.45	335	O12	JK3	DIQMTQSPSSLS ASVGDRVTITC	RASQSISSYLN	WYQQKPGK APELLIY	AASSLQS	GVPSRFSGSGSGTDFT LTISSLQPEDFATYYC	QQSYINPFT	FGPGTKVDIKR
5.12	227	O12	JK3	DIQMTQSPSSLS ASVGDRVTITC	RASQSISNYLN	WYQQKPGK APKLLIY	AASSLQS	GVPSRFSGSGSGTDFT LTISSLQPEDFATYYC	QQSYSIPFT	FGPGTKVDIKR
	653	Germline		EIVLTQSPGTLS LSPGERATLSC	RASQSVSSSYL A	WYQQKPGQ APRLLIY	GASSRAT	GIPDRFSGSGSGTDFT LTISRLEPEDFAVYYC	QQYGSST	FGGGTKVEIKR
6.2	537	A27	JK4	EIVLTQSPGTLS LSPGERATLSC	RASQSFSSSYL A	WFQQKPGQ APRLLIY	GASNRAT	GIPDRFSGSGSGTDFT LTISRLEPEDFAVYYC	HHFGTSPLT	FGGGTKVEIKR

	654	Germline		DIQMTQSPSSLS ASVGDRVTITC	QASQDISNYLN	WYQQKPGK APKLLIY	DASNLET	GVPSRFSGSGSGTDFT FTISSLQPEDIATYYC	QQYDNLPIT	FGQGTRLEIKR
5.23	265	O18	JK5	DIQMTQSPSSLS ASVGDRVTITC	QASQDISNYLN	WYQQKPGK APNLLIY	DASNLET	GVPSRFSGSGSGTDFT FTISSLQPEDIATYYC	QQYYNLPIT	FGQGTRLEIKR
5.17	245	O18	JK5	DIQMTQSPSSLS TSVGDRVTITC	QASQDIRNYLN	WYQQKPGK APKLLIH	DASNLET	GVPSRFSGNGSGTDFS FTITSLQPEDIATYYC	QQYANLPIT	FGQGTRLEIKR
5.29	283	O18	JK5	DIQMTQSPSSLS ASVGDRVTITC	QASQDIRNYLN	WYQQKPGK APKLLIY	DASNLET	GVPSRFSGSGSGTDFT FTISSLQPEDIATYYC	QQYDNLPIT	FGQGTRLEIKR
5.76	421	O18	JK5	DIQMTQSPSSLS ASVGDRVTITC	QASQDIRNYLN	WYQQKPGT APKLLIY	DASNLET	GVPSRFSGSGSGTDFT FTISSLQPEDIATYYC	QQYDNLPIT	FGQGTRLEIKR
5.10	219	O18	JK5	DIQMTQSPSSLS ASVGDRVTITC	QASQDIRNYLN	WYQQKPGK APKLLIY	DASNLET	GVPSRFSGSGSGTDFT FTISSLQPEDIATYYC	QQYDNLPIT	FGQGARLEIKR
5.22	261	O18	JK5	DIRMTQSPSSLS ASVGDSVTITC	QASQDIRNYLN	WYQQKPGK APKLLIY	DASNLET	GVPSRFSGSGSGTDFT FTISSLQPEDIATYYC	QQYDNLPIT	FGQGTRLEIKR
5.37	307	O18	JK5	DIQMTQSPSSLS ASVGDRVTITC	QASQDIRNYLN	WYQQKPGK APKLLIY	DASNLET	GVPSRFSGSGSGTDFT FTISSLQPEDIATYYC	QQYDNLPIT	FGQGTRLEIKR
5.31	291	O18	JK5	DIQMTQSPSSLS ASVGDRVTITC	QASQDIRNYLN	WYQQKPGK APKLLIY	DASNLET	GVPSRFSGSGSGTDFT FTISSLQPEDIATYYC	QQYDNLPIT	FGQGTRLEIKR
	655	Germline		DIQMTQSPSSLS ASVGDRVTITC	RASQGISNYLA	WFQQKPGK APKSLIY	AASSLQS	GVPSRFSGSGSGTDFT LTISSLQPEDFATYYC	QQYNSYT	FGPGTKVDIKR
3.34	117	L1	JK3	DIQMTQSPSSLS ASVGDRVSIIC	RASQGISNYLA	WFQQKPGK APKSLIY	AASSLQS	GVPSKFSGSGSGTDFT LTISSLQPEDFATYYC	QQYNSYPST	FGPGTKVDIKR
5.99	495	L1	JK3	DIQMTQSPSSLS ASVGDRVTITC	RASQDISNYLA	WFQQKPGK APKSLIY	AASSLQS	GVPSQFSGSGSGTDFT LTISSLQPEDFATYYC	QQYNNYPFT	FGPGTKVDVKR
	656	Germline		DIQMTQSPSSLS ASVGDRVTITC	RASQSISSYLN	WYQQKPGK APKLLIY	AASSLQS	GVPSRFSGSGSGTDFT LTISSLQPEDFATYYC	QQSYSTP	FGQGTKLEIKR
6.3.1	541	O12	JK2	DIQMTQSPSSLS ASVGDRVTITC	RASQSIRSYLN	WYQQKPGK APKVLIY	AASSLQS	GVPSRFSGSGSGTDFT LTISSLQPEDFATYYC	QQSYSTLCS	FGQGTKLEIKR
5.97	493	O12	JK2	DIQMTQSPSSLS ASVGTRVTITC	RASQSIRSYLN	WYQQKPGK APKLLIY	AASSLQR	GVPSRFSGSGSGTDFT LTISSLQAEDFATYYC	QQSYTTPLC S	FGQGTRLEIKR
5.27	275	O12	JK2	DIQMTQSPSSLS ASVGDRVTITC	RASQSISSYLN	WYQQKPGK APKILIY	AASSLQS	GVPSRFSGSGSGTDFT LTISSLQPEDFATYYC	QQSCSTPPE CS	FGQGTKLEIKR
5.21	257	O12	JK2	DIQMTQSPSSLS ASVGDRVTITC	RASQSISSYLN	WYQQKPGK APKLLIY	AASSLQS	GVPSRFSGSRSGTDFT LTISSLQPEDFATYYC	QQSYSVPCS	FGQGTKLEIKR
6.3	657	O12	JK2	DIQMTQSPSSLS ASVGDRVTITC	RASQSIRSYLN	WYQQKPGK APKVLIY	AASSLQS	GVPSRFSGSGSGTDFT LTISSLQPEDFATYYC	QQSYSTLCS	FGQGTKLEIKR
5.93	485	O12	JK2	DIQMTQSPSSLS ASVGDRVTITC	RASQSISSYLN	WYQQKPGK APKVLIY	GASSLQS	GVPSRFSGSGSGTDFT LTISSLQPEDFATYYC	QQSYSTLCS	FGQGTKLEIKR

	658	Germline		DIQMTQSPSSVS ASVGDRVTITC	RASQGISSWLA	WYQQKPGK APKLLIY	AASSLQS	GVPSRFSGSGSGTDFT LTISSLQPEDFATYYC	QQANSFPLT	FGGGTKVEIKR
5.95	489	L5	JK4	DIQMTQSPSSVS ASVGDRVTITC	RASQGISSWLA	WYQQKPGK APKLLIF	AASSLQS	GVPSRFSGSGSGTDFT LTISSLQPEDFATYYC	QQANSFPLT	FGGGTKVEIKR
5.52.1	353	L5	JK4	DIQMTQSPSSVS ASVGDRVTITC	RASQGISSWLA	WYQQKPGK APKLLIY	AVSSLES	GVPSRFSGSGSGTDFT LTISSLQPEDFATYYC	QQALT	FGGGTKVEIKR
	659	Germline		EIVLTQSPGTLS LSPGERATLSC	RASQSVSSSYL A	WYQQKPGQ APRLLIY	GASSRAT	GIPDRFSGSGSGTDFT LTISRLEPEDFAVYYC	QQYGSSPFT	FGPGTKVDIKR
3.8	37	A27	JK3	EIVLTQSPGTLS LSPGERATLSC	RASQSVSSSYL A	WYQQKPGQ APRLLIY	GASNRAT	GIPDRFSASGSGTDFT LTISRLEPEDFAVYYC	QQFGTSPFT	FGPGTKVDIKR
3.21	87	A27	JK3	EIVLTQSPGTLS LSPGERATLSC	RASQSVSSSYL A	WYQQKPGQ APRLLIY	GTSSRAT	GIPDRFSGSGSGTDFT LTISRLEPEDFAVYYC	QQYGSSLFT	FGPGTKVDIKR
	660	Germline		DIQMTQSPSSLS ASVGDRVTITC	QASQDISNYLN	WYQQKPGK APKLLIY	DASNLET	GVPSRFSGSGSGTDFT FTISSLQPEDIATYYC	QQYDNLT	FGGGTKVEIKR
5.18	249	O18	JK4	DIQMTQSPSSLS ASVGDRVTITC	QASQDITNYLN	WYQKKPGK APKVLIY	DASNLET	GVPSRFSGSGSGTGFT FTISSLQPEDIATYYC	QQYDHIPLT	FGGGTKVEIKR
5.48	343	O18	JK4	DIQMTQSPSSLS ASVGDRVTITC	QASQDITNYLN	WYQQKPGK APKLLIY	DASNLET	GVPSRFSGSGSGTDFT FTISSLQPEDIATYFC	QQYDNLPLT	FGGGTKVEIKR
	661	Germline		DIQMTQSPSSLS ASVGDRVTITC	RASQSISSYLN	WYQQKPGK APKLLIY	AASSLQS	GVPSRFSGSGSGTDFT LTISSLQPEDFATYYC	QQSYSTPT	FGQGTKVEIKR
5.36	303	O12	JK1	DIQMTQSPSSLS ASVGDRVTITC	RASQSISSYLN	WYQQKPGK APKLLIY	GASSLQS	GVPSRFSGSGSGTDFT LTISSLQPEDFATYYC	QQSYSIPRT	FGQGTKVEIKR
	662	Germline		DIQMTQSPSSLS ASVGDRVTITC	RASQGISNYLA	WFQQKPGK APKSLIY	AASSLQS	GVPSRFSGSGSGTDFT LTISSLQPEDFATYYC	QQYNSYPRT	FGQGTKVEIKR
5.80	437	L1	JK1	DIQMTQSPSSLS ASVGDRVTITC	RASQDISNYLA	WFQQKPGK APKSLIY	AASSLQS	GVPSKFSGSGSGTDFT LTISSLQPEDFATYYC	QQYNSYPRT	FGQGTKVEIKR
6.10	557	L1	JK1	DIQMTQSPSSLS ASVGDRVTITC	RASQDISNYLA	WFQQKPGK APKSLIY	AVSSLQS	GVPSKFSGSGSGTDFT LTISSLQPEDFATYYC	QQYNSYPRT	FGQGTKVEIKR
4.9	165	L1	JK1	DIQMTQSPSSLS ASVGDRVTITC	RASQGISNYLA	WFQQKPGK APKSLIY	AASSLQS	GVPSKFSGSGSGTDFT LTISSLQPEDFATYYC	QQYNSYPST	FGQGTKVEIKR
4.8	161	L1	JK1	DIQMTQSPSSLS ASVGDRVTITC	RASQGISNYLA	WFQQKPGK APKSLIY	AASSLQS	GVPSKFSGSGSGTDFT LTISSLQPEDFATYYC	QQYNSYPST	FGQGTKVEIKR
5.30	287	L1	JK1	DIQMTQSPSSLS ASVGDRVTITC	RASQDISNYLA	WFQQKPGK APKSLIY	AASSLQS	GVPSKFSGSGSGTDFT LTISSLQPEDFATYYC	QQYNSYPRT	FGQGTKVEIKR
6.6	549	L1	JK1	DIQMTQSPSSLS ASVGDRVTITC	RASQGISNYLA	WFQQKPGK APKSLIY	AASSLES	GVPSKFSGSGSGTDFN LTISSLQPEDFATYYC	QQYNSYPRT	FGQGTKVESKR

EXAMPLE 14

DETERMINATION OF CANONICAL CLASSES OF ANTIBODIES

[0262] Chothia, *et al.* have described antibody structure in terms of "canonical classes" for the hypervariable regions of each immunoglobulin chain (*J Mol Biol.* 1987 Aug 20; 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 beta-sheet framework. By examining sequences of immunoglobulins having unknown structure, Chothia, *et al.* show that many immunoglobuins 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.

[0263] Their discovery implied 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.* (*Nature* 1989 Dec 21-28; 342(6252):877-83) and others (Martin, *et al. J Mol Biol.* 1996 Nov 15; 263(5):800-15) confirmed that there is a small repertoire of main-chain conformations for at least five of the six hypervariable regions of antibodies.

[0264] The CDRs of each antibody described above 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 table below (Table 13) summarizes the results of the analysis. The Canonical Class data is in the form of *HCDR1-HCDR2-LCDR1-LCDR2-LCDR3, wherein "HCDR" refers to the heavy chain CDR and "LCDR" refers to the light chain CDR. Thus, for example, a canonical class of 1-3-2-1-5 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 5.

[0265] Assignments were made to a particular canonical class where there was 70% or greater identity of the amino acids in the antibody with the amino acids defined for each canonical class. Where there was less than 70% identity, the canonical class assignment is marked with an asterisk ("*") to indicate that the best estimate of the proper canonical class was made, based on the length of each CDR and the totality of the data. Where there was no matching canonical class with the same CDR length, the canonical class assignment is marked with a "Y." The amino acids defined for each antibody can be found, for example, in the articles by Chothia, *et al.* referred to above. Table 13 reports the canonical class data for each of the Ang-2 antibodies.

Table 13. Canonical classes of antibodies against Ang-2

Antibody	Canonical Class
5.18	1-1-2-1-1
5.81	1-1-2-1-1
5.66	1-1-2-1-1
5.48	1-1-2-1-1
5.23	1-1-2-1-1
3.33	1-1-2-1-1
5.15	1-1-2-1-3*
4.3	1-1-4-1-1
3.12	1-1-4-1-5*
4.16	1-1-4-1-5*
3.13	1-1-4-1-5*
3.7	1-1-4-1-5*
3.32	1-1-4-1-5*
3.37	1-1-4-1-Y
5.30	1-2-2-1-1
3.6	1-2-2-1-1
6.6	1-2-2-1-1
3.34	1-2*-2-1-1
5.74	1-2*-2-1-1
5.38	1-3-2-1-1
5.28.1	1-3-2-1-1
5.6	1-3-2-1-1
5.44	1-3-2-1-1
5.28	1-3-2-1-1
5.86.1	1-3-2-1-1
5.35.1	1-3-2-1-1
5.35	1-3-2-1-1
5.8	1-3-2-1-1
5.22	1-3-2-1-1
4.15	1-3-2-1-1
5.67	1-3-2-1-1

5.87	1-3-2-1-1
5.10	1-3-2-1-1
5.71	1-3-2-1-1
5.21	1-3-2-1-1
5.80	1-3-2-1-1
3.18	1-3-2-1-1
3.11	1-3-2-1-1
3.26	1-3-2-1-1
5.31	1-3-2-1-1
5.76	1-3-2-1-1
4.9	1-3-2-1-1
5.17	1-3-2-1-1
3.39	1-3-2-1-1
5.37	1-3-2-1-1
3.22	1-3-2-1-1
5.29	1-3-2-1-1
5.73	1-3-2-1-1
5.12	1-3-2-1-1
3.1	1-3-2-1-1
5.11	1-3-2-1-1
4.8	1-3-2-1-1
5.24	1-3-2-1-1
6.3.1	1-3-2-1-1*
5.56.1	1-3-2-1-1*
5.111	1-3-2-1*-1
4.13	1-3-2-1*-1
5.52	1-3*-2-1-3*
5.16.1	1-3-2-1-3*
5.39.1	1-3-2-1-3*
5.103.1	1-3-2-1-3*
5.101.1	1-3-2-1-3*
5.54.1	1-3-2-1-3*
5.83.1	1-3-2-1-3*
5.62	1-3-2-1-3*
5.88.1	1-3-2-1-3*
5.40.2	1-3-2-1-3*
5.109	1-3-2-1-3*
5.64	1-3-2-1-3*
5.13	1-3-2-1-3*
3.3	1-3-2-1-3*
5.41	1-3-2-1-3*
3.3.1	1-3-2-1-3*
3.31.1	1-3-2-1-3*
5.41.1	1-3-2-1-3*
5.62.1	1-3-2-1-3*
5.108.1	1-3-2-1-3*
5.13.1	1-3-2-1-3*
5.97	1-3-2-1-5*

5.52.1	1-3-2-1-Y
5.78.1	1-3-3-1-1
5.60	1-3-3-1-1
5.43	1-3-3-1-1
5.90	1-3-3-1*-1
3.42	1-3-3-1*-1
4.11	1-3-4-1-1
3.40	1-3-4-1-1
5.82	1-3-4-1-1
4.18	1-3-4-1-1
3.41	1-3-4-1-1
5.92	1-3-4-1-1
3.10	1-3-4*-1-1
4.14	1-3-4-1-5*
3.19.1	1-3-8*-1-1
6.2	1-3-8*-1-1
3.8	1-3-8*-1-1
5.58	1-3-8-1-1*
4.5	1-4*-2-1-1
5.1	1-4*-2-1-1
4.2	1-4*-2-1-1
3.9	1-4*-2-1-1
5.45	1-4*-2-1-1
3.17	1-4*-2-1-1
5.72	1-4*-2-1-1*
3.14	1-4*-2-1-Y
5.115	3-1-2-1-1
5.36	3-1*-2-1-1
3.2	3-1-2-1-3*
3.21	3-1-8-1-1*
5.61	3-Y-2-1-3*

[0266] Table 14 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.

Table 14. Number of anti-Ang-2 antibodies in each canonical class

H1-H2-L1-L2-L3	Number of mAbs
1-1-2-1-1	6
1-1-2-1-3*	1
1-1-4-1-1	1
1-1-4-1-5*	5
1-1-4-1-Y	1
1-2-2-1-1	5
1-3-2-1-1	38
1-3-2-1-3*	21

1-3-2-1-5*	1
1-3-2-1-Y	1
1-3-3-1-1	5
1-3-4-1-1	7
1-3-4-1-5*	1
1-3-8*-1-1	4
1-4*-2-1-1	7
1-4*-2-1-Y	1
3-1-2-1-1	2
3-1-2-1-3*	1
3-1-8-1-1*	1
3-Y-2-1-3*	1

Notes:
1. Those with * means assignment has been given to the best matching class, although there are some violations at the defining positions.
2. Y means there is no matching canonical class with the same CDR length.

EXAMPLE 15

EPITOPE MAPPING OF ANG-2 ANTIBODIES

[0267] The binding domain of 27 antibodies neutralizing the activity of Ang-2 was analyzed.

[0268] Recombinant Human Ang-2 was purchased from R&D systems (623-AN). Goat anti-human Ang-2 polyclonal antibodies (R&D systems AF623) were selected for their ability to recognize rhAng-2 in direct ELISA and Western blots. The polyclonal antibodies were biotinylated for detection with HRP conjugated - Streptavidin

[0269] All restriction enzymes were supplied by New England Biolabs and were used according to the manufacture's instructions. All plasmids DNA were purified using spin mini columns (Invitrogen, Carlsbad, CA). Oligonucleotide primers used for cloning and site directed mutagenesis were synthesized by Qiagen Operon.

[0270] Antibodies: 27 hybridoma derived human anti Ang-2 antibodies were selected based on their ability to inhibit binding of rhAng-2 to its receptor. The antibodies are listed below in Table 15.

Table 15.

	Hybridoma code	OD650 in inibition assay
1	**x5.56**	**0.0863**
2	**x3.38**	**0.0792**
3	**x3.19**	**0.0633**
4	**x3.28***	**0.0588**
5	**x3.3**	**0.0558**
6	**x3.31***	**0.0516**
7	**x5.88***	**0.0874**
8	**x5.49***	**0.0856**
9	**x5.101**	**0.0824**
10	**x5.41***	**0.0776**
11	**x5.108***	**0.0688**
12	**x5.62**	**0.0650**
13	**x5.39**	**0.0519**
14	**x5.16***	**0.0500**
15	**x5.83**	**0.0484**
16	**x5.54**	**0.0440**
17	**x5.14**	**0.0430**
18	**x5.86**	**0.0419**
19	x5.78	0.0984
20	x5.103*	0.1013
21	**x5.28**	**0.0821**
22	**x5.40**	**0.0691**
23	**x5.35***	**0.0663**
24	**x6.3**	**0.0617**
25	x5.13	0.0744
26	x5.2	0.0690
27	x5.52	0.0627

Epitope Characterization of the 27 neutralizing anti-Ang-2 antibodies

Dot Blots

[0271] RhAng-2 (R&D systems) was spotted on nitrocellulose membrane in its native or reduced form, using Bio-Dot Microfiltration unit. All human monoclonal antibodies (MAbs) raised against human Ang-2 had bound to non-reduced Ang-2, but not to reduced form, indicating that all mAbs recognize conformational epitopes, which are apparently destroyed upon reduction of the protein.

Cloning and Expression of Ang1 and Ang-2 Proteins

[0272] To better understand the structural basis for interaction of mAbs with Ang-2, a set of chimeric Ang1/Ang-2 molecules were used. This approach takes advantage of the fact that members of the angiogenic family are structurally related.

Although Ang-2 and Angl show only 60% homology in their protein sequence, both share the same modular structure composed of an amino terminal coiled-coil domain and a carboxyl terminal fibrinogen like-domain.

Cloning of human Ang-1 and Ang-2

[0273] Two alternatively spliced forms of human Ang-2 cDNAs were amplified from human umbilical vein endothelial cell line (HUVEC). PCR amplification of HUVEC cDNA using Ang-2 specific primers reveled both the full length Ang-2 (1491 bp) and a 1330 base pain variant Ang-2_{443} (Injune et al., (2000) *JBC* 275: 18550). Ang-2_{443} is a variant generated by alternative splicing of exon B and missing part of the coiled-coil domain (amino-acids 96-148). Both Ang-2 cDNAs were cloned into pCR3.1 expression vector and expressed in 293F cells as shown in Figure 6. Human Ang-1 cDNA was obtained by RT-PCR using total RNA extracted from human breast cell line MDA-MB-231. A 1.5 Kb cDNA was cloned into pCR3.1 expression vector and expression was detected in the supernatant of transiently transfected 293F cells.

ELISA

[0274] Binding of the 27 mAbs to supernatants generated from transient transfection of Ang-2 and Angl cDNAs was tested using antibody capture ELISA. Ang-2, Ang-2_{443} and Ang-1 were bound to an ELISA plate coated with goat polyclonal antibodies against human Ang-2 or Ang-1 (respectively). The binding of the top 27 human monoclonal antibodies was detected with a HRP-conjugated goat anti-human antibody, followed by colorimetric horseradish peroxidase substrate (Enhanced K-Blue TMB substrate Neogen Corporation). The absorbance of each well of the ELISA plates was measured at 450 nm on a microplate autoreader.

Transfection of 293F Cells

[0275] 293F human embryonic kidney cells were maintained in 10% fetal bovine serum in Dulbecco's modified eagles medium supplemented with penicillin and streptomycin. 293F cells were transiently transfected using Calcium phosphate. At 72 hours, the medium was harvested and filtered for ELISA and Western Blot analysis.

[0276] All 27 antibodies were shown to bind specifically Ang-2/Ang-2_{443} antigens. No cross reactivity was detected with human Ang-1. Amino acids 96-148 in the

coiled-coil domain of Ang-2, which are missing in the Ang-2_{443} protein sequence, were excluded as the binding domain for each of the 27 antibodies.

Construction of Ang-1/Ang-2 Chimeric Molecules

[0277] Restriction cleavage sites common in human Ang-1 and Ang-2 genes were used for construction of In-frame fusion Angiopoietin chimeric proteins.

[0278] Four constructs were made: Human Ang-1/2 *BsmI*, Ang-2/1*BsmI*, Ang-1/2*SspI* and Ang-2/1 *SspI*. All proteins were expressed and secreted in detectable levels measured by ELISA assay using polyclonal antibodies against human Ang-1 and Ang-2.

[0279] The Amino acid joining points are at the following positions:

BsmI- 117(Ang-2)/119(Ang-1)

SspI- 353(Ang-2)/354(Ang-1)

[0280] The difference of one amino acid is due to the presence of 497 residues in the human Ang-1 compared to 496 residues in the human Ang-2. All constructs were expressed in 293F cells, and detected by goat anti-human polyclonal antibodies against Ang-1 and Ang-2. The top 27 antibodies were tested for their ability to bind chimeric Ang-1/2 molecules. All 27 antibodies showed a similar pattern of binding to the Ang-1/2*BsmI* construct only. The results of these experiments indicate that the binding domain for all antibodies is between residues 117-496, most likely in the fibrinogen binding domain, where the epitope is disrupted in the *SspI* fusion Ang proteins around amino acid 353.

Construction of Mouse/Human Ang-2 Chimeric Molecules

[0281] Since Ang-2 shares ~55% amino acid identity with Ang-1, it was difficult to find a common restriction site to be used for cloning of chimeric molecules. Mouse and human Ang-2 are more similar, having about 85% sequence homology. Mouse Ang-2 cDNA cloned in the pCMCsport expression vector was purchased from Invitrogen. The 27 selected antibodies were tested for their immunoreactivity with recombinant mouse Ang-2. Six out of the 27 cross reacted with mouse Ang-2 with 100% of their immunoreactivity on human Ang-2, indicating that the murine antigen retains most of the immunoreactivity of human Ang-2 (Data are summarized in Table 16).

[0282] The human-Mouse chimeric system was chosen for epitope mapping based on the findings that most antibodies bind specifically to the human Ang-2 antigen

and do not cross react with mouse Ang-2. Various cDNA constructs of Ang-2 were generated and cloned into a mammalian expression vector.

[0283] Constructs of Mouse/human Ang-2 were made using the common *StuI* restriction site, located in the fibrinogen-binding domain, with the amino acid joining point at residue 311. All mAbs specific to the human Ang-2 were able to bind to the Mouse/Human Ang-2 *StuI*, indicating that the binding domain is in the fibrinogen-binding domain between residues 311-496. In order to narrow down the binding domain, a new construct was prepared in which the human fragment *StuI-TfiI* replaced the mouse sequence in the mouse Ang-2 cDNA. (Figure 9).

[0284] All antibodies specific to human Ang-2 showed a positive ELISA signal, with 15-100% of their immunoreactivity on human Ang-2. The binding domain of two antibodies with a unique VH gene usage 5.35.1 (VH3-20) and 5.28.1 (VH3-43) as shown in Table 17, was mapped to a region between amino-acids 310-400.

[0285] Antibodies that cross reacted with mouse Ang-2 were expected to show 100% reactivity and could not be mapped using Mouse/human chimeric constructs.

Site Directed Mutagenesis

[0286] In order to define the important residues involved in the binding site of different antibodies, a few residues of Human Ang-2 were mutated, and screened against the entire panel of antibodies for binding by ELISA assay.

[0287] Because direct binding detected by ELISA is insensitive to small and moderate differences in affinity, large changes in binding observed after substitution of single amino acid probably identify key sites that interact with the antibody. In addition, polyclonal antibodies against human Ang-2 maintain 100% reactivity with each construct, indicating that the mutagenesis procedure did not introduce any broad structural changes across the Ang-2 molecule. Two independent changes of Val to Met in position 345 (V345M) and His to Gln at position 375 (H375Q) were ignored by all 27 antibodies, indicating that these residues are not reactive, or that the changes in the conformational epitopes require more than single amino acid substitution. Changing of two residues at positions 365 and 367 dramatically changed the binding of single antibody, Mab 5.35.1. Sequence analysis of 5.35.1 showed the unique usage of VH3-20 and unique CDR3 on both the heavy and light chains. All fusion points of Ang-2 chimeric molecules and point mutations are highlighted in Figure 8. Figure 9 is an amino acid sequence comparison of mouse Ang-1 (SEQ ID NO: 5), human Ang-1 (SEQ ID NO: 2), mouse Ang-2 (SEQ ID

NO: 4), and human Ang-2 (SEQ ID NO: 3). The arrowhead shows the cleavage site for hydrophobic leader sequences. The arrows define the limits of the coiled-coil and fibrinogen like domains. The solid circles show the conserved cysteine residues (image taken from Maisonpierre et al., 1997, *Science* 277:55).

[0288] Binding data to all Ang-2 molecules is summarized below in Table 16:

Table 16.

Clone	Bin	binding domain	Human Ang-2-443	Mouse CX	*M/H BsmI*	*M/H StuI 310-496*	*Stu-TfiI 310-400*	V345M	*N365Q367*	*H375Q*
5.39.1	1	Fib.Like Domain	100%	No	100%	100%	25%	100%	100%	100%
5.16.1*	1	Fib.Like Domain	100%	No	100%	100%	30%	100%	100%	100%
5.86.1	1	Fib.Like Domain	100%	No	100%	100%	23%	100%	100%	100%
5.54.1	1	Fib.Like Domain	100%	No	100%	100%	77%	100%	100%	100%
5.14.1	1	Fib.Like Domain	100%	No	100%	100%	31%	100%	100%	100%
5.83.1	1	Fib.Like Domain	100%	No	100%	100%	86%	100%	100%	100%
5.101.1	1	Fib.Like Domain	100%	No	100%	100%	28%	100%	100%	100%
6.3.1	7	Fib.Like Domain	100%	No	100%	100%	27%	100%	100%	100%
5.103.1*	2	Fib.Like Domain	100%	No	100%	100%	40%	100%	100%	100%
5.78.1	5	Fib.Like Domain	100%	Yes	100%	100%	100%	100%	100%	100%
5.35.1*	8	Fib.Like Domain	100%	No	100%	100%	100%	100%	15%	100%
5.40.2*	8	Fib.Like Domain	100%	No	100%	100%	65%	100%	100%	100%
3.19.1	6	Fib.Like Domain	100%	Yes	100%	100%	100%	100%	100%	100%
5.108.1*	1	Fib.Like Domain	100%	No	100%	100%	36%	100%	100%	100%
5.52.1		Fib.Like Domain	100%	Yes	100%	100%	100%	100%	100%	100%
5.56.1	3	Fib.Like Domain	100%	Yes	100%	100%	100%	100%	100%	100%
5.2		Fib.Like Domain	100%	Yes	100%	100%	100%	100%	100%	100%
5.28.1	4	Fib.Like Domain	100%	No	100%	100%	100%	100%	100%	100%
5.41.1*	1	Fib.Like Domain	100%	No	100%	100%	30%	100%	100%	100%
5.13.1		Fib.Like Domain	100%	No	100%	100%	27%	100%	100%	100%
3.3.1	1	Fib.Like Domain	100%	No	100%	100%	15%	100%	100%	100%
3.31.1*	1	Fib.Like Domain	100%	No	100%	100%	15%	100%	100%	100%
5.62.1	1	Fib.Like Domain	100%	No	100%	100%	30%	100%	100%	100%
3.28.1*	1	Fib.Like Domain	100%	No	100%	100%	31%	100%	100%	100%
5.88.1*	1	Fib.Like Domain	100%	No	100%	100%	20%	100%	100%	100%
3.38		Fib.Like Domain	100%	Yes	100%	100%	ND	100%	100%	100%
5.49*		Fib.Like Domain	100%	No	100%	100%	35%	100%	100%	100%

Data are presented as percent binding compared to human Ang-2.

Table 17: Sequence analysis and cross reactivity with mouse Ang-2.

Hybridoma code	OD650 in inibition assay	Mouse Ang-2 XR	Bin	VH	DH	JH	VK	JK
x5.56	**0.0863**	Yes	3	VH3-33	D1-7	JH4b	A20	JK3
x3.38	**0.0792**	Yes	2					
x3.19	**0.0633**	Yes	6	VH3-30	D3-3	JH5b	A27	JK5
x3.28*	**0.0588**	No	1	VH3-7	D6-19	JH4b	L2	JK1
x3.3	**0.0558**	No	1	VH3-7	D6-19	JH4b	L2	JK1
x3.31*	**0.0516**	No	1	VH3-7	D6-19	JH4b	L2	JK1
x5.88*	**0.0874**	No	1	VH3-7	D6-19	JH4b	L2	JK1
x5.49*	**0.0856**	No	1					
x5.101	**0.0824**	No	1	VH1-2	D6-19	JH4b	L2	JK1
x5.41*	**0.0776**	No	1	VH3-7	D6-19	JH4b	L2	JK1
x5.108*	**0.0688**	No	1	VH3-33	D1-7	JH5b	L2	JK1
x5.62	**0.0650**	No	1	VH3-7	D6-19	JH4b	L2	JK1
x5.39	**0.0519**	No	1	VH1-2	D6-13	JH4b	L2	JK1
x5.16*	**0.0500**	No	1	VH1-2	D6-13	JH4b	L2	JK1
x5.83	**0.0484**	No	1	VH1-2	D6-19	JH4b	L2	JK1
x5.54	**0.0440**	No	1	VH1-2	D6-19	JH4b	L2	JK1
x5.14	**0.0430**	No	1	VH1-2	D6-19	JH4b		
x5.86	**0.0419**	No	1	VH1-2	D6-13	JH4b	O12	JK3
x5.78	0.0984	Yes	5	VH1-2	D2-2	JH6b	B3	JK5
x5.103*	0.1013	No	2	VH1-2	D6-19	JH4a	L2	JK1
x5.28	**0.0821**	No	4	VH3-43	D6-19	JH4b	L2	JK3
x5.40	**0.0691**	No	8	VH3-23	D6-19	JH4B	L2	JK1
x5.35*	**0.0663**	No	8	VH3-20	D6-19	JH2	L2	JK4
x6.3	**0.0617**	No	7	VH1-2	D1-7	JH4b	O12	JK2
x5.13	0.0744	No		VH3-7	D6-19	JH4b	L2	JK1
x5.2	0.0690	Yes		VH3-33	D5-12	JH6b		
x5.52	0.0627	Yes		VH3-33	D1-1	JH4b	L5	JK4

[0289] The sequence analysis of IgH and IgL sequences was accomplished using sequence analysis software tools, and by alignment of VH genes to a germ line database. The software also evaluates D elements, reading frame, N region insertion, P nucleotide addition, nucleotide loss and CDR3 length. Analysis of 27 individual antibodies specific to CR64 yielded only 7 germline VH genes, 10 of them from the same VH1 family. Selection of neutralizing antibodies showed that antibodies expressing same Ig V_H and in some cases same V_HDJ_H rearrangements and that pairs of H and L chain were conserved. This finding suggests that, for any given epitope, only a few members of the germ line repertoire are used to form the corresponding paratope, and for each antigenic epitope a limited number of L- and H –chain genes can pair to form a specific paratope.

[0290] Recurrent usage of similar V_H, V_K and complementary determining region (CDR) structures by different monoclonal antibodies is linked to the fact that all Ang-2 neutralizing activity is restricted to the fibrinogen like domain, and is in agreement with work published by Procopio et al (1999, *JBC* 274: 30196), showing that the effect of Ang-2 on Tie2 is linked to the Fibrinogen-like domain. The epitope mapping data indicates that the monoclonal antibodies bind Ang-2 through a broad interface that includes most of the fibrinogen like domain.

EXAMPLE 16

DETERMINATION OF CROSS-REACTIVITY WITH MOUSE ANG-2

[0291] The cross-reactivity of the anti-human Ang-2 mAbs to mouse Ang-2 was tested by ELISA. For this purpose, a mouse Ang-2 expression vector was constructed, and eukaryotic cells were transfected transiently to produce mouse Ang-2.

[0292] The mouse Angiopoietin-2 (mAng-2) expression construct was obtained from Research Genetics, a distributor of the I.M.A.G.E consortium (see world wide web at image.llnl.gov). Mouse Ang-2 cDNA (GenBank Accession No. BC027216, IMAGE:3494566) was derived from the library NCI_CGAP_Lu29, which is a lung tumor library. The cDNA was cloned into the pCMV-SPORT6 expression vector (Invitrogen Carlsbad, CA) through SalI(5') and NotI(3') sites and contained the full length mouse Ang-2 (mAng-2) open reading frame of 496 amino acids, as well as the 5' and 3' untranslated flanking regions for a total of 2471 base pairs.

[0293] Ten μg of the above mAng-2 plasmid was transfected into HEK293F cells using the calcium phosphate method. Approximately, 1 X 10^6 HEK293F cells were seeded on a 10 cm tissue culture plate on the previous day. The medium was changed after 5 hours or overnight transfection and the cells were grown for another 2-3 days before the supernatants containing the secreted mAng-2 protein were collected. The expression of mAng-2 was confirmed by ELISA using a polyclonal antibody obtained from R&D Systems (catalog No. AF623).

[0294] 96-well Nunc Immplates were coated with conditioned-medium collected from HEK293F/mouse Ang-2 transfectants, 100 μl in each well. The plates were incubated at 4°C overnight, followed by washing four times using Phosphate Buffer Saline with a Skan Washer 300 station (SKATRON). The wells were blocked by 100 μl of ABX-block buffer (0.5% BSA, 0.1%Tween, 0.01% Thimerosal in PBS) for 1 hour.

Anti-Ang-2 mAbs with appropriate concentrations and diluted in the blocking buffer were added into the wells with a volume of 100 μl/well, and incubated at room temperature for at least 1 hour. The mAbs and each of their dilutes were tested in duplicate. After washing twice, the bound mAbs were detected by goat anti-human Fc-HPPO-conjugated antibody (Caltag, Code H10507) at 1/1,000 dilution at room temperature for an hour. To set up the detecting chromagenic reaction, 100 μl of TMB substrate (TMB-microwell, BioFX, Cat. No. TMSK-1000-01) was added after washing the wells three times using PBS. The plates were incubated for 30 minutes before 650 stop solution (100 μl/well, BioFX, Cat. No. BSTP-0100-01) was added to terminate the reaction. The light absorbance at 650 nm was determined by a Spectramax Plus reader.

[0295] The top 27 neutralizing mAbs were tested in this assay. The light absorbance demonstrated that monoclonal antibodies 3.19.3, 3.38, 5.2.1, 5.52.1, 5.56.1, and 5.78.1 were capable of binding to mouse Ang-2 under the experimental conditions. For confirmation, each binding antibody was titered by ELISA. The mean OD 650 nm (± S.D.) values were plotted against Log concentration of mAbs (μg/ml) is shown in Figure 10. The clones 5.2.1, 5.28.1, 3.19.3 and 3.31.2 are shown in the figure. Monoclonal antibodies 5.2.1 and 3.19.3 bound mouse Ang-2 in a dose-dependent manner, reaching saturation at about 10 μg/ml (Figure 10). The binding curves of these two mAbs were typical Sigmoidal dose-response relationship curves. Dose-dependency and saturation was not observed in the antibody concentration range tested, except for clones 5.2.1 and 3.19.3. Based on this result, it appears that only mAbs 5.2.1 and 3.19.3 have cross-reactivity to mouse Ang-2.

EXAMPLE 17

INHIBITION OF MURINE ANG-2 BINDING TO HUMAN TIE2

[0296] The monoclonal antibody 3.19.3 was selected for further testing of its ability to inhibit the binding of mouse Ang-2 to human Tie2. For this purpose, an ELISA plate was coated with 4 μg/ml of hTie2/Fc (R&D Systems, Inc.) at 100 μl/well, and the wells were blocked as routine at 4°C overnight. The recombinant mouse Ang-2 (mAng-2) in the culture supernatant of the 293T/mAng-2 transfectants described above was employed. 100 μl of mAng-2 containing supernatant with mAb 3.19.3 at various concentrations was added into the pre-coated wells, and incubated at room temperature for 1 hr.

[0297] As a control, recombinant human Ang-2 (R&D Systems, Inc.) mixed with the antibody was also included. Each concentration of the mAb was tested in triplicate. The bound mouse and human Ang-2 were detected using a goat anti-human Ang-2 polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA) that cross-react with mouse Ang-2, coupled with a secondary rabbit anti-goat IgG-HRP. OD650 was determined 30 min after the HRP substrate was added. It was discovered that mAb 3.19.3 inhibited binding of both human and mouse Ang-2 to human Tie2 in a dose-dependent manner (Figure 11).

EXAMPLE 18

DETERMINATION OF CROSS-REACTIVITY WITH MONKEY VASCULATURE

[0298] Because Ang-2 is specifically expressed in angiogenic endothelial cells, these cells from monkeys were immunohistochemically stained with anti-Ang-2 antibodies as a way to indirectly determine whether each antibody cross-reacted with monkey Ang-2.

[0299] The top 10 neutralizing mAbs selected as described in Example 4 (Table 4) were examined in this experiment using endothelial cell-rich ovarian tissue from monkeys. Completely dried 6 μm frozen monkey (Cynomolgus macaque) ovary tissue sections were fixed with 4°C acetone for 5 minutes. After washing the slides three times with PBS, the endogenous peroxidase of the tissues was blocked with 0.3 % of H_2O_2 for 10 minutes. Subsequently, the tissues were washed with PBS and blocked with 10 μg/ml goat anti-human IgG Fab for 15 minutes. The tissue sections were washed again with PBS followed by treatment with 10% normal goat serum for 10 minutes. After draining the serum, each of the 10 anti-Ang-2 mAbs (10 μg/ml) were applied to the sections and incubated for 2 hours. The bound Ang-2 mAbs were detected with 10 μg/ml mouse anti-human IgG for 15 minutes followed by incubation with peroxidase conjugated goat anti-mouse IgG for 30 minutes. Staining was performed using AEC-substrate system (DAKO, Cat. No. 3464) under microscopic observation for optimal result.

[0300] All 10 mAbs were found to stain the angiogenic vascular endothelial cells among the ovary tissue; whereas, the isotype control mAbs did not stain. This demonstrated that the 10 mAbs from Table 4 would cross react with monkey Ang-2.

EXAMPLE 19

MAB 3.19.3 INHIBITS *IN VIVO* ANGIOGENESIS IN MATRIGEL PLUG ASSAY

[0301] To evaluate the *in vivo* anti-angiogenic potential of anti-Ang-2 monoclonal antibodies, a Matrigel plug angiogenesis assay was conducted. MCF-7 cells were found to produce Ang-2 when cultured *in vitro*, or implanted in an immunodeficient mouse as a xenograft. When MCF-7 was incorporated into Matrigel and implanted subcutaneously into nude mice, robust vascular in growth into the gel was found. To establish the Matrigel plug model, 6-8 week old female BALB/c/nu/nu mice, with body weights ranging from 18 to 20 g (Charles River Laboratories, Wilmington, MA) were employed. A total of 0.5 mL of Matrigel containing $2X10^6$ MCF-7 cells, with or without Ang-2 antibodies, or control agents (including Matrigel alone, Tie2/Fc, IgG2 and IgG4 isotype controls, and anti-VEGF mAb), were subcutaneously injected into the right flank of the nude mice. Five mice were used for each test group. All the mAbs tested were adjusted to a concentration of 100 μg/ml.

[0302] After seven days, Matrigel plugs were harvested and scored for blood vessel density. For this purpose, cervical dislocation of mice under deep anesthesia was performed. The Matrigel plugs were exposed through removal of the covering skin flap. The Matrigel plugs were then removed and digital images were then recorded. The Matrigel plugs were resected carefully and cut into two parts. One part was snap frozen in TissueTek and the other fixed in buffered formalin. Both parts were then embedded in paraffin for sectioning. Three 5 to 7 μm thick sections from each mouse were cut and stained with hematoxylin and eosin. The sections were then examined under a phase contrast microscope. Representative photomicrographs were recorded [two frames (100 X and 400 X)] and endothelial cell and blood vessel infiltration was recorded.

[0303] The frozen Matrigel plugs were sectioned (10 μm sections) in a Cryocut microtome. Two independent sections per mouse were made and used for staining. Sections were blocked with BSA (0.1 %) and then treated with monoclonal antibody reactive to mouse. CD31 conjugated to Phycoerythin (dilutions as recommended by the manufacturer). After thorough washings, sections were mounted under anti-fading reagent (Vecta Shield) and observed under a UV microscope using a red filter. Representative Digital images were captured (two images at 100 X and 200 X magnification). Nuclei were counterstained with DAPI. Immunofluorescence images of CD31 staining were analyzed by a Skeletinization program. Data were processed to

provide mean vessel density, node and length for each group. The results can be found in Figures 12A and 12B, which show the effect of anti-Ang-2 antibodies on the number of blood vessels ends (Figure 12A) and blood vessel length (Figure 12B).

[0304] This experiment demonstrated that, in comparison with Matrigel alone, MCF-7 cells that were incorporated in the Matrigel were able to induce a significant level of angiogenesis. The induced angiogenesis could be inhibited by a positive control anti-VEGF antibody. The angiogenesis was also significantly inhibited by soluble recombinant Tie2/Fc protein, suggesting that Ang-2 produced by MCF-7 cells plays a role in the angiogenesis in this model. By binding to any Ang-2, the Tie2/Fc would effectively reduce the level of Ang-2 that is exposed to the MCF-7 cells.

[0305] It is not clear how the IgG2 isotype negative control antibody, PK16.1.3, impacted angiogenesis, although this antibody was also found to occasionally interfere with tumor growth in some xenograft models (data not shown). The IgG4 isotype control antibody did not have any effect on the angiogenesis in this model. As seen in Figures 12A and 12B, clones 5.88.3, 3.3.2, 3.19.3 and 5.28.1 significantly inhibited angiogenesis ($P<0.05$, t-test performed by VasculoGen), while others had lesser effects.

[0306] It is well established that Ang-2 is expressed by endothelial cells in the tumor, and thus has been considered as a autocrine angiogenic factor. However, Ang-2 has also been found to be expressed by many types of tumor cells *in vitro* and *in vivo*. Except 3.19.3, the mAbs tested here do not cross-react with mouse Ang-2. In this *in vivo* model, the mAbs only neutralized human Ang-2 produced by the MCF-7 cell, but not the mouse Ang-2. The inhibitory effect of these mAbs suggests that tumor expressed Ang-2 can be a paracrine angiogenesis factor. The overall anti-angiogenic activity of the mAb was partially attributable to the neutralization of the tumor Ang-2, in addition to neutralization of vascular endothelium expressed Ang-2.

EXAMPLE 20

DETERMINATION OF THE THERAPEUTIC EFFICACY OF MAB 3.19.3 IN A431 PREVENTIONAL XENOGRAFT MODEL

[0307] Anti-Ang-2 mAb clone 3.19.3 not only bound to mouse Ang-2, but also inhibited binding of mouse Ang-2 to human Tie2. The anti-tumor activity of this monoclonal antibody was tested in a mouse xenograft model of human skin epidermoid carcinoma by using the A431 cell line.

[0308] A431 cells were cultured in flasks as routine until the cells reached sub-confluence. Immunodeficient 6-8 week old female mice (Balb/c/nu/nu) were employed for model development. The A431 cells were harvested and suspended in Matrigel. A cell suspension containing 5 x 10^6 cells was injected intradermally into the flank of the mice. The mice were randomized into different groups, each containing 11 mice. On the same day, the mice were injected intraperitoneally with 0.5 mg of mAb 3.19.3, or isotype control antibody, and twice per week thereafter. The dimensions of each tumor were measured twice per week. The volume of the tumor was calculated as: Volume = Length x $(Width)^2$ x 0.5 (cm^3).

[0309] As illustrated in Figure 13, mAb 3.19.3 significantly delayed A431 xenograft tumor growth. The average tumor volume of the isotype control group reached about 1.5 cm^3 at the end of the experiment, whereas the growth rate of the treated group significantly slowed after Day 10, and was about 0.5 cm^3 at the end. At Day 23, the volume ration of T/C (Treatment/Control) is 1/3, indicating a 66% inhibition of the growth.

[0310] The results suggest that at the dosage used in this experiment, by binding to mouse Ang-2 and blocking the binding of this ligand to its receptor Tie2, mAb 3.16.3 was able to significantly delay the growth of A431 xenograft in nude mice. It is likely that the anti-tumor effect of the monoclonal antibody is due to inhibiting angiogenesis in the host, as demonstrated by the Matrigel plug assays. Using the Microvessel Density (MVD) in the tumor as a pharmacodynamic marker, the mechanism of action with respect to anti-angiogenesis is further demonstrated in Example 22.

[0311] The mechanism of action of mAb 3.19.3 may not be limited to its blockage of Ang-2/Tie2 association and consequent signaling. As indicated in Example 7, this mAb is also found to bind to Ang-1 and block binding of Ang-1 to Tie2. Interestingly, the mAb also blocks Ang-1-induced Tie2 phosphorylation. It is known that Ang-1 is involved in vessel maturation. When comparing the potency of mAb 3.19.3 for its inhibition in the binding of Ang-1 versus Ang-2 to Tie2 (Example 12), it is apparent that mAb 3.19.3 is predominantly an Ang-2 antagonist. Without being bound to any particular theory, it is possible that dual blockage of signaling from Ang-2 and Ang-1 impairs angiogenesis and consequently tumor growth.

EXAMPLE 21

MAB 3.19.3 INHIBITS TUMOR GROWTH IN ESTABLISHED XENOGRAFT MODELS

[0312] Ang-2 is upregulated by angiogenic endothelial cells, and is correlated to progression of many types of tumor. It is reasonable to postulate that a monoclonal antibody that blocks binding of Ang-2/Tie2 association will be able to inhibit angiogenesis, and therefore, inhibit the tumor growth. In this experiment, the therapeutic efficacy of an anti-Ang-2 mAb was demonstrated. Since mAb 3.19.3 cross-reacts and neutralizes mouse Ang-2/Tie2 signaling, this mAb was chosen to demonstrate the *in vivo* efficacy of inhibiting tumor growth.

[0313] To test whether anti-Ang-2 mAb 3.19.3 also inhibits established tumor, and tumors other than A-431, the human colon adenocarcinoma LoVo xenograft model was employed. Doses of Mab 3.19.3 at 0.5, 2, and 10 mg/kg were administered twice per week intraperitoneally. The treatments did not start until the tumors were established with an average volume of 0.2 cm^3. On these established tumors, mAb 3.19.3 also demonstrated an inhibitory effect in comparison with the isotype control. Figure 14A shows that 79% inhibition at 0.5 and 2 mg/kg (p values are 0.022 and 0.027, respectively) was achieved, and 75% inhibition on tumor growth were found (p=0.006) at 10 mg/kg.

[0314] The tumor growth inhibitory effect was reproduced in an additional xenograft model, human colon adenocarcinoma SW480, which was allowed to grow to an average volume of 0.2 cm^3. Although at 0.5 mg/kg the mAb 3.19.3 was not found to have a significant effect, at both 2 and 10 mg/kg, the mAb inhibited tumor growth by 60% (p =0.003 and 0.006 respectively) at Day 53 after tumor implantation (Figure 14B).

[0315] In summary of the above results, anti-Ang-2 mAb 3.19.3 significantly inhibited tumor growth in the three models tested. Interestingly, both LoVo and SW480 express human Ang-2. However, two other mAbs that have no mouse Ang-2 cross-reactivity did not show significant inhibitory activity on tumor growth (data not shown), despite the fact that human Ang-2 was expressed by the tumor cells. These results imply that an antagonist of the host Ang-2 is required to block angiogenesis and tumor growth.

[0316] As discussed above, Mab 3.19.3 cross-reacts with Ang-1. However, the potency of mAb 3.19.3 on Ang-1/Tie2 association was far lower than that on Ang-2/Tie2 association (Example 12). For this reason, it is reasonable to conclude that the therapeutic efficacy seen in these models is predominantly due to Ang-2 antagonism. However, blockage of Ang-1 *in vivo* in the models could not be completely excluded.

During the entire course of the experiments, no obvious toxicity effect, such as weight loss or bleeding, was observed.

EXAMPLE 22

IN VIVO EFFICACY OF MAB 3.19.3 IN ADDITIONAL TUMOR XENOGRAFT MODELS

[0314] The anti-tumor activity of the 3.19.3 monoclonal antibody was tested in mouse xenograft models of human cancer by using 9 different tumor cell lines.

[0317] Colon adenocarcinoma (Lovo, SW480, Colo205, HT29, HCT116), epidermoid carcinoma (A431), lung carcinoma (Calu-6) and breast adenocarcinoma (MCF7, MDA-MB-231) cells were cultured in flasks as routine until the cells reached sub-confluence. Immunodeficient 7-10 week old female mice were employed for model development. The cells were harvested, suspended in Matrigel, and then injected subcutaneously into each mouse. The mice were then randomized into cohorts containing 10-12 mice. The mice were injected intraperitoneally with 0.5 mg of mAb 3.19.3, or isotype control antibody, and twice per week thereafter. For all experiments, isotype control antibody treatment was included. The dimensions of each tumor were measured twice per week. The volume of the tumor was calculated as: Volume = Length x $(\text{Width})^2$ x 0.5 (cm^3). Graphical comparisons of tumor growth inhibition is shown for HT29 (Figure 15A) and Calu6 (Figure 15B) xenografts.

[0318] As seen in Table 16, mAb 3.19.3 showed significant activity in all 7 xenograft subcutaneous models tested and both orthotopic models with non-optimized dose and schedule.

Table 18. Summary of in vivo efficacy of mAb 3.19.3

Tumor models	**% Inhibition**	**% Inhibition**
SubQ Xenografts	**2 mg/kg twice weekly**	**10mg/kg twice weekly**
Colo205	35	46
A431	43	66
HT29	N.D.	54
Calu6	N.D	38
HCT116	N.D	33
Orthotopic models		
MCF7	35*	74
MDA-MB-231	50	58

$P<0.05$ in all cases

*Growth inhibition not statistically significant.

N.D. – not determined

[0319] The MDA-MB-231 tumor tissue was analyzed via CD31+ vessel staining density. CD31 staining density was measured by threshold and by manual grid counting methods. Eleven tumors per group and at least 20 images per tumor were analyzed. As seen in Figure 15C, treatment of mice with 3.19.3 antibody decreased the density of CD31 staining by 40% compared to a control IgG antibody. This was statistically significant with both counting methods, threshold ($p<0.015$) and manual grid counting ($p<0.00004$) by 1-tailed T-test. Similar CD31+ vessel counts were made on ex-vivo tissue for the Colo205 and HCT116 xenografts. These samples also exhibited a similar significant reduction in CD31+ vessels.

EXAMPLE 23

USES OF ANTI-ANG-2 ANTIBODIES FOR THE TREATMENT OF ANGIOGENESIS RELATED DISEASES

[0320] To determine the *in vivo* effects of anti-Ang-1 and anti-Ang-2 antibody treatment in human patients with various solid tumors, human patients are dosed periodically with an effective amount of anti-Ang-1 and anti-Ang-2 antibody. At periodic times during the treatment, the human patients are monitored to determine whether tumor growth is inhibited. Following treatment, it is found that patients undergoing treatment with the anti-Ang-1 and anti-Ang-2 antibody in comparison to patients that are not treated have relative improvements in one or more of the following including but not limited to smaller tumors, delayed time to progression or longer time of survival.

EXAMPLE 24

USE OF ANTI-ANG-2 ANTIBODIES AS A DIAGNOSTIC AGENT

Detection of Ang-2 Antigen in a Sample

[0321] An Enzyme-Linked Immunosorbent Assay (ELISA) for the detection of Ang-1 or Ang-2 antigen in a sample may be developed. In the assay, wells of a microtiter plate, such as a 96-well microtiter plate or a 384-well microtiter plate, are adsorbed for several hours with a first fully human monoclonal antibody directed against Ang-1 and Ang-2. The immobilized antibody serves as a capture antibody for any of the antigen that may be present in a test sample. The wells are rinsed and treated with a

blocking agent such as milk protein or albumin to prevent nonspecific adsorption of the analyte.

[0322] Subsequently the wells are treated with a test sample suspected of containing the antigen, or with a solution containing a standard amount of the antigen. Such a sample may be, for example, a serum sample from a subject suspected of having levels of circulating antigen considered to be diagnostic of a pathology.

[0323] After rinsing away the test sample or standard, the wells are treated with a second fully human monoclonal anti-Ang-1 and anti-Ang-2 antibody that is labeled by conjugation with biotin. A monoclonal or mouse or other species origin might also be used. The labeled anti-Ang-1 and anti-Ang-2 antibody serves as a detecting antibody. After rinsing away excess second antibody, the wells are treated with avidin-conjugated horseradish peroxidase (HRP) and a suitable chromogenic substrate. The concentration of the antigen in the test samples is determined by comparison with a standard curve developed from the standard samples.

[0324] This ELISA assay provides a highly specific and very sensitive assay for the detection of the Ang-1 and Ang-2 antigen in a test sample.

Determination of Ang-2 Antigen Concentration in Patients

[0325] A sandwich ELISA is developed to quantify Ang-1 and Ang-2 levels in human serum. The two fully human monoclonal anti-Ang-2 antibodies from the sandwich ELISA, recognizes different epitopes on the Ang-2 molecule. Alternatively, monoclonal antibodies of mouse or other species origin may be used. The ELISA could be but is not necessarily performed as follows: 50μL of capture anti-Ang-2 antibody in coating buffer (0.1 M $NaHCO_3$, pH 9.6) at a concentration of 2μg/mL is coated on ELISA plates (Fisher). After incubation at 4°C overnight, the plates are treated with 200μL of blocking buffer (0.5% BSA, 0.1% Tween 20, 0.01% Thimerosal in PBS) for 1 hour at 25°C. The plates are washed (3x) using 0.05% Tween 20 in PBS (washing buffer, WB). Normal or patient sera (Clinomics, Bioreclaimation) are diluted in blocking buffer containing 50% human serum. The plates are incubated with serum samples overnight at 4°C, washed with WB, and then incubated with 100μL/well of biotinylated detection anti-Ang-2 antibody for 1 hour at 25°C. After washing, the plates are incubated with HRP-Streptavidin for 15 minutes, washed as before, and then treated with 100μL/well of o-phenylenediamine in H_2O_2 (Sigma developing solution) for color generation. The

reaction is stopped with 50μL/well of H_2SO_4 (2M) and analyzed using an ELISA plate reader at 492 nm. Concentration of Ang-2 antigen in serum samples is calculated by comparison to dilutions of purified Ang-2 antigen using a four parameter curve fitting program.

INCORPORATION BY REFERENCE

[0326] All references cited herein, including patents, patent applications, papers, text books, and the like, and the references cited therein, to the extent that they are not already, are hereby incorporated herein by reference in their entirety.

EQUIVALENTS

[0327] The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the invention. The foregoing description and Examples detail certain preferred embodiments of the invention and describes the best mode contemplated by the inventors. It will be appreciated, however, that no matter how detailed the foregoing may appear in text, the invention may be practiced in many ways and the invention should be construed in accordance with the appended claims and any equivalents thereof.

WHAT IS CLAIMED IS:

1. A targeted binding agent that binds to Angiopoietin-2 with a Kd of less than 100 picomolar (pM).

2. The targeted binding agent of Claim 1, wherein said targeted binding agent also binds to Angiopoietin-1.

3. The targeted binding agent of Claim 1, wherein said targeted binding agent prevents Tie2 phosphorylation.

4. The targeted binding agent of Claim 1, wherein said targeted binding agent binds Angiopoietin-2 with a Kd of less than 50 picomolar (pM).

5. The targeted binding agent of Claim 1, wherein said targeted binding agent binds Angiopoietin-2 with a Kd of less than 20 picomolar (pM).

6. The targeted binding agent of Claim 1, wherein said targeted binding agent binds Angiopoietin-2 with a Kd of less than 6 picomolar (pM).

7. The targeted binding agent, of Claim 2, wherein said targeted binding agent binds Angiopoietin-1 with a Kd of less than 300 picomolar (pM).

8. The targeted binding agent of Claim 2, wherein said targeted binding agent binds Angiopoietin-1 with a Kd of less than 160 picomolar (pM).

9. The targeted binding agent of Claim 2, wherein said targeted binding agent binds Angiopoietin-1 with a Kd of less than 80 picomolar (pM).

10. The targeted binding agent of Claim 2, wherein said targeted binding agent binds Angiopoietin-1 with a Kd of less than 40 picomolar (pM).

11. The targeted binding agent of Claim 1 or Claim 2, wherein said targeted binding agent is a monoclonal antibody.

12. The targeted binding agent of Claim 11, wherein said targeted binding agent is a fully human monoclonal antibody.

13. The targeted binding agent of Claim 12, wherein said targeted binding agent is mAb 3.19.3 (ATCC Accession Number PTA-7260) or mAb 3.3.2 (ATCC Accession Number PTA-7258) or mAb 5.88.3 (ATCC Accession Number PTA-7259).

14. The targeted binding agent of Claim 1 or Claim 2, in association with a pharmaceutically acceptable carrier.

15. A nucleic acid molecule encoding the targeted binding agent of Claim 1 or Claim 2.

16. A vector comprising the nucleic acid molecule of Claim 15.

17. A host cell comprising the vector of Claim 16.

18. A method of treating a malignant tumor in an animal, comprising administering to said animal in need thereof a therapeutically effective dose of the targeted binding agent of Claim 1 or Claim 2.

19. The method of Claim 18, wherein said animal is human.

20. The method of Claim 18, wherein said targeted binding agent is the monoclonal antibody 3.19.3 (ATCC Accession Number PTA-7260) or 3.3.2 (ATCC Accession Number PTA-7258) or 5.88.3 (ATCC Accession Number PTA-7259).

21. The method of Claim 18, wherein said malignant tumor is selected from the group consisting of: melanoma, small cell lung cancer, non-small cell lung cancer, glioma, hepatocellular (liver) carcinoma, thyroid tumor, gastric (stomach) cancer, prostate cancer, breast cancer, ovarian cancer, bladder cancer, lung cancer, glioblastoma, endometrial cancer, kidney cancer, colon cancer, pancreatic cancer, and epidermoid carcinoma.

22. A method of treating Angiopoietin-2 induced angiogenesis, comprising administering to an animal in need thereof a therapeutically effective dose of the targeted binding agent of Claim 1 or Claim 2.

23. The method of Claim 22, wherein said animal is human.

24. The method of Claim 22, wherein said targeted binding agent is a fully human monoclonal antibody.

25. The method of Claim 22, wherein the targeted binding agent binds to Angiopoietin-1.

26. An antagonist of the biological activity of Angiopoietin-1 and Angiopoietin-2, wherein the antagonist does not bind to the ATP-binding site of Tie-2.

27. An antagonist according to Claim 26 wherein the Angiopoietin-1 antagonist activity and the Angiopoietin–2 antagonist activity is comprised within one molecule.

28. An antagonist according to Claim 26 wherein the Angiopoietin-1 antagonist activity and the Angiopoietin–2 antagonist activity is comprised within more than one molecule.

29. An antagonist according to Claim 26 wherein the antagonist binds to the Tie-2 receptor.

30. An antagonist according to Claim 26 wherein the antagonist binds to Angiopoietin-1 and Angiopoietin -2.

31. An antagonist according to any of Claims 26 to 30 wherein the antagonist is an antibody.

32. An antagonist according to Claim 31 wherein the antagonist is a monoclonal antibody.

33. An antagonist according to Claim 32 wherein the antagonist is a fully human monoclonal antibody.

34. An antagonist according to Claim 33 wherein the antagonist is the fully human monoclonal antibody 3.19.3 (ATCC Accession Number PTA-7260) or 3.3.2 (ATCC Accession Number PTA-7258) or 5.88.3 (ATCC Accession Number PTA-7259).

35. An antagonist according to Claim 32 wherein the antagonist is an antibody that binds to the same epitope as the fully human monoclonal antibody 3.19.3 (ATCC Accession Number PTA-7260) or 3.3.2 (ATCC Accession Number PTA-7258) or 5.88.3 (ATCC Accession Number PTA-7259).

36. Use of the targeted binding agent of Claim 1 or Claim 2 in the preparation of a medicament for the treatment of a malignant tumor.

37. The use of Claim 36, wherein said malignant tumor is selected from the group consisting of: melanoma, small cell lung cancer, non-small cell lung cancer, glioma, hepatocellular (liver) carcinoma, thyroid tumor, gastric (stomach) cancer, prostate cancer, breast cancer, ovarian cancer, bladder cancer, lung cancer, glioblastoma, endometrial cancer, kidney cancer, colon cancer, pancreatic cancer, and epidermoid carcinoma.

38. The use of Claim 36, wherein said targeted binding agent is a monoclonal antibody.

39. The use of Claim 38, wherein said antibody is a fully human monoclonal antibody.

40. The use of Claim 39, wherein said fully human monoclonal antibody is a fully human 3.19.3 antibody (ATCC Accession Number PTA-7260) or 3.3.2 (ATCC Accession Number PTA-7258) or 5.88.3 (ATCC Accession Number PTA-7259).