HIGH AFFINITY HUMAN ANTIBODIES TO HUMAN ANGIOPOIETIN-2

FIELD OF THE INVENTION

[0001] The present invention relates to antibodies, and antigen-binding fragments thereof, which are specific for angiopoietin-2 (Ang-2).

BACKGROUND

[0002] Angiogenesis is the biological process whereby new blood vessels are formed. Aberrant angiogenesis is associated with several disease conditions including, *e.g.*, proliferative retinopathies, rheumatoid arthritis and psoriasis. In addition, it is well established that angiogenesis is critical for tumor growth and maintenance. Angiopoietin-2 (Ang-2) is a ligand for the Tie-2 receptor (Tie-2) and has been shown to play a role in angiogenesis. Ang-2 is also referred to in the art as Tie-2 ligand. (US 5,643,755; Yancopoulos et al., 2000, Nature 407:242-248).

[0003] Antibodies and other peptide inhibitors that bind to Ang-2 are mentioned in US 6,166,185; 7,521,053; 7,205,275; 2006/0018909 and 2006/0246071. There is a need in the art for novel Ang-2 modulating agents, including Ang-2 antibodies, that can be used to treat diseases and conditions caused by or exacerbated by angiogenesis.

BRIEF SUMMARY OF THE INVENTION

[0004] The present invention provides human antibodies that bind to human Ang-2. The present inventors, in view of various lines of evidence and investigation, have recognized a need for Ang-2 inhibitors which do not bind to or antagonize the related molecule Ang-1. For example, previous studies have demonstrated or suggested a beneficial role for Ang-1 in hemostasis (*see, e.g.*, Li *et al.*, 2001, *Thrombosis and Haemostasis 85*:191-374) and in protecting the adult vasculature against plasma leakage (*see, e.g.*, Thurston *et al.*, 2000, *Nature Medicine 6*:460-463; Thurston *et al.*, 1999, *Science 286*:2511-2514). Thus, the present inventors recognized that, in certain anti-angiogenic therapeutic situations, it may be beneficial to preserve Ang-1 activity. Accordingly, the present invention provides antibodies which bind specifically to Ang-2 but do not substantially bind to Ang-1. The present invention also includes antibodies that block the interaction between Ang-2 and its receptor Tie-2 but do not substantially block the interaction between Ang-1 and Tie-2. The antibodies of the invention are useful, *inter alia*, for inhibiting the angiogenesis-promoting activities of Ang-2 and for treating diseases and disorders caused by or related to the process of angiogenesis.

[0005] The antibodies of the invention can be full-length (for example, an IgG1 or IgG4 antibody) or may comprise only an antigen-binding portion (for example, a Fab, $F(ab')_2$ or scFv fragment), and may be modified to affect functionality, e.g., to eliminate residual effector functions.

[0006] In one embodiment, the invention comprises an antibody or antigen-binding fragment of an antibody comprising a heavy chain variable region (HCVR) having an amino acid sequence selected from the group consisting of SEQ ID NO: 2, 18, 22, 26, 42, 46, 50, 66, 70, 74, 90, 94, 98, 114, 118, 122, 138, 142, 146, 162, 166, 170, 186, 190, 194, 210, 214, 218, 234, 238, 242, 258, 262, 266, 282, 286, 290, 306, 310, 314, 330, 334, 338, 354, 358, 362, 378, 382, 386, 402, 406, 410, 426, 430, 434, 450, 454, 458, 474, 478, 482, 498, 502, 506, 514, and 516, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity. In one embodiment, the antibody or antigen-binding portion of an antibody comprises a HCVR having an amino acid sequence selected from the group consisting of SEQ ID NO: 18, 42, 66, 162, 210, 266, and 434.

[0007] In one embodiment, the invention comprises an antibody or antigen-binding fragment of an antibody comprising a light chain variable region (LCVR) having an amino acid sequence selected from the group consisting of SEQ ID NO: 10, 20, 24, 34, 44, 48, 58, 68, 72, 82, 92, 96, 106, 116, 120, 130, 140, 144, 154, 164, 168, 178, 188, 192, 202, 212, 216, 226, 236, 240, 250, 260, 264, 274, 284, 288, 298, 308, 312, 322, 332, 336, 346, 356, 360, 370, 380, 384, 394, 404, 408, 418, 428, 432, 442, 452, 456, 466, 476, 480, 490, 500, and 504, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity. In one embodiment, the antibody or antigen-binding portion of an antibody comprises a LCVR having an amino acid sequence selected from the group consisting of SEQ ID NO: 20, 44, 68, 164, 212, 274, and 442.

[0008] In specific embodiments, the antibody or antigen-binding fragment thereof comprises a HCVR and LCVR (HCVR/LCVR) amino acid sequence pair selected from the group consisting of SEQ ID NO: 2/10, 18/20, 22/24, 26/34, 42/44, 46/48, 50/58, 66/68, 70/72, 74/82, 90/92, 94/96, 98/106, 114/116, 118/120, 122/130, 138/140, 142/144, 146/154, 162/164, 166/168, 170/178, 186/188, 190/192, 194/202, 210/212, 214/216, 218/226, 234/236, 238/240, 242/250, 258/260, 262/264, 266/274, 282/284, 286/288, 290/298, 306/308, 310/312, 314/322, 330/332, 334/336, 338/346, 354/356, 358/360, 362/370, 378/380, 382/384, 386/394, 402/404, 406/408, 410/418, 426/428, 430/432, 434/442, 450/452, 454/456, 458/466, 474/476, 478/480, 482/490, 498/500, and 502/504. In one embodiment, the antibody or fragment thereof comprises a HCVR and LCVR selected from the amino acid sequence pairs of SEQ ID NO: 18/20, 42/44, 66/68, 162/164, 210/212, 266/274, and 434/442.

[0009] In a next aspect, the invention provides an antibody or antigen-binding fragment of an antibody comprising a heavy chain CDR3 (HCDR3) domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 8, 32, 56, 80, 104, 128, 152, 176, 200, 224, 248, 272, 296, 320, 344, 368, 392, 416, 440, 464, 488, and 512, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and a light chain CDR3 (LCDR3) domain selected from the group consisting of SEQ ID NO: 16, 40, 64, 88, 112, 136, 160, 184, 208, 232, 256, 280, 304, 328, 352, 376, 400, 424, 448,

472, and 496, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0010] In certain embodiments, the antibody or antigen-binding portion of an antibody comprises a HCDR3/LCDR3 amino acid sequence pair selected from the group consisting of SEQ ID NO: 8/16, 32/40, 56/64, 80/88, 104/112, 128/136, 152/160, 176/184, 200/208, 224/232, 248/256, 272/280, 296/304, 320/328, 344/352, 368/376, 392/400, 416/424, 440/448, 464/472, and 488/496. In one embodiment, the antibody or antigen-binding portion of an antibody comprises a HCDR3/LCDR3 amino acid sequence pair selected from the group consisting of SEQ ID NO: 8/16, 32/40, 56/64, 152/160, 200/208, 272/280, and 440/448. Non-limiting examples of anti-Ang-2 antibodies having these HCDR3/LCDR3 pairs are the antibodies designated H1H685, H1H690, H1H691, H1H696, H1H706, H1M724, and H2M744, respectively.

[0011] In a further embodiment, the invention comprises an antibody or fragment thereof further comprising a HCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 4, 28, 52, 76, 100, 124, 148, 172, 196, 220, 244, 268, 292, 316, 340, 364, 388, 412, 436, 460, 484, and 508, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a heavy chain CDR2 (HCDR2) domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 6, 30, 54, 78, 102, 126, 150, 174, 198, 222, 246, 270, 294, 318, 342, 366, 390, 414, 438, 462, 486, and 510, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; LCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 12, 36, 60, 84, 108, 132, 156, 180, 204, 228, 252, 276, 300, 324, 348, 372, 396, 420, 444, 468, and 492, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and a LCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 14, 38, 62, 86, 110, 134, 158, 182, 206, 230, 254, 278, 302, 326, 350, 374, 398, 422, 446, 470, and 494, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[0012] Certain non-limiting, exemplary antibodies and antigen-binding fragments of the invention comprise HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 domains, respectively, selected from the group consisting of: (i) SEQ ID NO: 4, 6, 8, 12, 14 and 16 (*e.g.*, H1H685); (ii) SEQ ID NO: 28, 30, 32, 36, 38 and 40 (*e.g.*, H1H690); (iii) SEQ ID NO: 52, 54, 56, 60, 62 and 64 (*e.g.*, H1H691); (iv) SEQ ID NO: 148, 150, 152, 156, 158 and 160 (*e.g.*, H1H696); (v) SEQ ID NO: 196, 198, 200, 204, 206 and 208 (*e.g.*, H1H706); (vi) SEQ ID NO: 268, 270, 272, 276, 278 and 280 (*e.g.*, H1M724); and (vii) SEQ ID NO: 436, 438, 440, 444, 446 and 448 (*e.g.*, H2M744).

[0013] In a related embodiment, the invention comprises an antibody or antigen-binding fragment of an antibody which specifically binds Ang-2, wherein the antibody or fragment comprises the heavy and light chain CDR domains (*i.e.*, CDR1, CDR2 and CDR3) contained

within heavy and light chain variable domain sequences selected from the group consisting of SEQ ID NO: 2/10, 18/20, 22/24, 26/34, 42/44, 46/48, 50/58, 66/68, 70/72, 74/82, 90/92, 94/96, 98/106, 114/116, 118/120, 122/130, 138/140, 142/144, 146/154, 162/164, 166/168, 170/178, 186/188, 190/192, 194/202, 210/212, 214/216, 218/226, 234/236, 238/240, 242/250, 258/260, 262/264, 266/274, 282/284, 286/288, 290/298, 306/308, 310/312, 314/322, 330/332, 334/336, 338/346, 354/356, 358/360, 362/370, 378/380, 382/384, 386/394, 402/404, 406/408, 410/418, 426/428, 430/432, 434/442, 450/452, 454/456, 458/466, 474/476, 478/480, 482/490, 498/500, and 502/504. In one embodiment, the antibody or fragment thereof comprises the CDR sequences contained within HCVR and LCVR selected from the amino acid sequence pairs of SEQ ID NO: 18/20, 42/44, 66/68, 162/164, 210/212, 266/274, and 434/442.

[0014] In another aspect, the invention provides nucleic acid molecules encoding anti-Ang-2 antibodies or fragments thereof. Recombinant expression vectors carrying the nucleic acids of the invention, and host cells into which such vectors have been introduced, are also encompassed by the invention, as are methods of producing the antibodies by culturing the host cells under conditions permitting production of the antibodies, and recovering the antibodies produced.

[0015] In one embodiment, the invention provides an antibody or fragment thereof comprising a HCVR encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1, 17, 21, 25, 41, 45, 49, 65, 69, 73, 89, 93, 97, 113, 117, 121, 137, 141, 145, 161, 165, 169, 185, 189, 193, 209, 213, 217, 233, 237, 241, 257, 261, 265, 281, 285, 289, 305, 309, 313, 329, 333, 337, 353, 357, 361, 377, 381, 385, 401, 405, 409, 425, 429, 433, 449, 453, 457, 473, 477, 481, 497, 501, 505, 513, and 515, or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% identity thereto. In one embodiment, the antibody or fragment thereof comprises a HCVR encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO: 17, 41, 65, 161, 209, 265, and 433.

[0016] In one embodiment, the invention provides an antibody or fragment thereof comprising a LCVR encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO: 9, 19, 23, 33, 43, 47, 57, 67, 71, 81, 91, 95, 105, 115, 119, 129, 139, 143, 153, 163, 167, 177, 187, 191, 201, 211, 215, 225, 235, 239, 249, 259, 263, 273, 283, 287, 297, 307, 311, 321, 331, 335, 345, 355, 359, 369, 379, 383, 393, 403, 407, 417, 427, 431, 441, 451, 455, 465, 475, 479, 489, 499, and 503, or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% identity thereto. In one embodiment, the antibody or fragment thereof comprises a LCVR encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO: 19, 43, 67, 163, 211, 273, and 441.

[0017] In one embodiment, the invention provides an antibody or antigen-binding fragment of an antibody comprising a HCDR3 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO: 7, 31, 55, 79, 103, 127, 151, 175, 199, 223, 247, 271, 295, 319, 343, 367, 391, 415, 439, 463, 487, and 511, or a substantially identical sequence having at least

90%, at least 95%, at least 98%, or at least 99% identity thereto; and a LCDR3 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO: 15, 39, 63, 87, 111, 135, 159, 183, 207, 231, 255, 279, 303, 327, 351, 375, 399, 423, 447, 471, and 495, or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% identity thereto. In one embodiment, the antibody or fragment thereof comprises HCDR3 and LCDR3 sequences encoded by the nucleic acid sequence pairs selected from the group consisting of SEQ ID NO: 7/15, 31/39, 55/63, 151/159, 199/207, 271/279, and 439/447.

[0018] In a further embodiment, the antibody or fragment thereof further comprises: a HCDR1 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO: 3, 27, 51, 75, 99, 123, 147, 171, 195, 219, 243, 267, 291, 315, 339, 363, 387, 411, 435, 459, 483, and 507, or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% identity thereto; a HCDR2 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO: 5, 29, 53, 77, 101, 125, 149, 173, 197, 221, 245, 269, 293, 317, 341, 365, 389, 413, 437, 461, 485, and 509, or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% identity thereto; a LCDR1 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO: 11, 35, 59, 83, 107, 131, 155, 179, 203, 227, 251, 275, 299, 323, 347, 371, 395, 419, 443, 467, and 491, or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% identity thereto; and a LCDR2 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO: 13, 37, 61, 85, 109, 133, 157, 181, 205, 229, 253, 277, 301, 325, 349, 373, 397, 421, 445, 469, and 493, or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% identity thereto.

[0019] In one embodiment, the antibody or fragment thereof comprises the heavy and light chain CDR sequences encoded by the nucleic acid sequences of SEQ ID NO: 17 and 19; SEQ ID NO: 41 and 43; SEQ ID NO: 65 and 67; SEQ ID NO: 161 and 163; SEQ ID NO: 209 and 211; SEQ ID NO: 265 and 273; or SEQ ID NO: 433 and 441.

[0020] The invention encompasses anti-Ang-2 antibodies having a modified glycosylation pattern. In some applications, modification to remove undesirable glycosylation sites may be useful. For example, the present invention encompasses modified versions of any antibody set forth herein wherein the modified version lacks a fucose moiety present on the oligosaccharide chain, for example, to increase antibody dependent cellular cytotoxicity (ADCC) function (see Shield et al. (2002) JBC 277:26733). In other applications, modification of galactosylation can be made in order to modify complement dependent cytotoxicity (CDC).

[0021] In another aspect, the invention provides a pharmaceutical composition comprising a recombinant human antibody or fragment thereof which specifically binds Ang-2 and a pharmaceutically acceptable carrier or diluent. In a related aspect, the invention features a composition which is a combination of an Ang-2 inhibitor and a second therapeutic agent. In one embodiment, the Ang-2 inhibitor is an antibody or fragment thereof. In one embodiment,

the second therapeutic agent is any agent that is advantageously combined with an Ang-2 inhibitor. Exemplary agents that may be advantageously combined with an Ang-2 inhibitor include, without limitation, any agent that inhibits or reduces angiogenesis, other cancer therapeutic agents, anti-inflammatory agents, cytokine inhibitors, growth factor inhibitors, anti-hematopoietic factors, non-steroidal anti-inflammatory drugs (NSAIDs), antiviral agents, and antibiotics.

[0022] In yet another aspect, the invention provides methods for inhibiting Ang-2 activity using the anti-Ang-2 antibody or antigen-binding portion of the antibody of the invention, wherein the therapeutic methods comprise administering a therapeutically effective amount of a pharmaceutical composition comprising an antibody or antigen-binding fragment of an antibody of the invention. The disorder treated is any disease or condition which is improved, ameliorated, inhibited or prevented by removal, inhibition or reduction of Ang-2 activity. Preferably, the anti-Ang-2 antibody or antibody fragment of the invention is useful to treat any disease or condition caused by, associated with, or perpetuated by the process of angiogenesis. In certain embodiments of the invention, the anti-Ang-2 antibodies or antigen-binding portions thereof are useful for the treatment of cancer. In the context of cancer therapies, the anti-Ang-2 antibodies of the invention or antigen-binding portions thereof can be administered alone or in combination with other anti-cancer therapeutic antibodies, chemotherapeutic agents and/or radiation therapy. In other embodiments of the present invention, the anti-Ang-2 antibodies or antigen-binding fragments thereof are useful for the treatment of one or more eye disorders, *e.g.*, age-related macular degeneration, diabetic retinopathy, etc., and/or one or more inflammatory or infectious diseases.

[0023] Other embodiments will become apparent from a review of the ensuing detailed description.

BRIEF DESCRIPTION OF THE FIGURES

[0024] **Fig. 1** is an alignment of the last 88 C-terminal amino acids of human Ang-2 (residues 409 to 496 of SEQ ID NO:518) with the corresponding amino acid sequence of human Ang-1 (SEQ ID NO:531). Residues that differ between hAng-1 and hAng-2 are indicated by white text and black shading. Asterisks (*) indicate the amino acids of hAng-2 which were shown to interact with human Tie-2 by crystal structure analysis. *See* Barton *et al.*, *Nat. Struct. Mol. Biol. 13*:524-532 (2006). Triangles (▲) indicate the Tie-2-interacting amino acid positions that differ between hAng-2 and hAng-1.

[0025] **Fig. 2 (Panels A-C)** depict the results of Western blots which illustrate the extent to which Ang-2 binding molecules inhibit, or fail to inhibit, Ang-1-induced Tie-2 phosphorylation.

[0026] **Fig. 3** is a summary of the Ang-2FD-mFc point mutant binding experiment of Example 13, showing the amino acid changes which resulted in greater than a five-fold reduction in T½

of dissociation (depicted by solid circles •) relative to wild-type for the various antibodies and peptibodies tested.

DETAILED DESCRIPTION

[0027] Before the present invention is described, it is to be understood that this invention is not limited to particular methods and experimental conditions described, as such methods and conditions may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

[0028] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. As used herein, the term "about," when used in reference to a particular recited numerical value, means that the value may vary from the recited value by no more than 1%. For example, as used herein, the expression "about 100" includes 99 and 101 and all values in between (*e.g.*, 99.1, 99.2, 99.3, 99.4, etc.).

[0029] Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described.

Definitions

[0030] As used herein, the term "angiopoietin-2" or "Ang-2", unless specified as being from a non-human species (*e.g.*, "mouse Ang-2," "monkey Ang-2," etc.), refers to human Ang-2 or a biologically active fragment thereof (*e.g.*, a fragment of the Ang-2 protein which is capable of inducing angiogenesis *in vitro* or *in vivo*). Human Ang-2 is encoded by the nucleic acid sequence shown in SEQ ID NO:517 and has the amino acid sequence of SEQ ID NO:518. The amino acid sequences of mouse and monkey Ang-2 proteins are available from the NCBI protein sequence database under Accession Nos. NP_031452 and BAE89705.1, respectively.

[0031] The term "angiopoietin-1" or "Ang-1", unless specified as being from a non-human species (*e.g.*, "mouse Ang-1," "monkey Ang-1," etc.), refers to human Ang-1 or a biologically active fragment thereof. Human Ang-1 has the amino acid sequence as set forth in the NCBI protein sequence database under Accession No. AAB50557. The term "Tie-2" (also referred to in the art as "TEK") unless specified as being from a non-human species (*e.g.*, "mouse Tie-2," "monkey Tie-2," etc.), refers to human Tie-2 or a biologically active fragment thereof. Human Tie-2 has the amino acid sequence as set forth in the NCBI protein sequence database under Accession No. AAA61130.

[0032] The term "antibody", as used herein, is intended to refer to immunoglobulin molecules comprising four polypeptide chains, two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds, as well as multimers thereof (*e.g.*, IgM). Each heavy chain

comprises a heavy chain variable region (abbreviated herein as HCVR or V_H) and a heavy chain constant region. The heavy chain constant region comprises three domains, C_H1, C_H2 and C_H3. Each light chain comprises a light chain variable region (abbreviated herein as LCVR or V_L) and a light chain constant region. The light chain constant region comprises one domain (C_L1). The V_H and V_L regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FR). Each V_H and V_L is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. In different embodiments of the invention, the FRs of the anti-Ang-2 antibody (or antigen-binding portion thereof) may be identical to the human germline sequences, or may be naturally or artificially modified. An amino acid consensus sequence may be defined based on a side-by-side analysis of two or more CDRs.

[0033] The term "antibody," as used herein, also includes antigen-binding fragments of full antibody molecules. The terms "antigen-binding portion" of an antibody, "antigen-binding fragment" of an antibody, and the like, as used herein, include any naturally occurring, enzymatically obtainable, synthetic, or genetically engineered polypeptide or glycoprotein that specifically binds an antigen to form a complex. Antigen-binding fragments of an antibody may be derived, *e.g.*, from full antibody molecules using any suitable standard techniques such as proteolytic digestion or recombinant genetic engineering techniques involving the manipulation and expression of DNA encoding antibody variable and optionally constant domains. Such DNA is known and/or is readily available from, *e.g.*, commercial sources, DNA libraries (including, *e.g.*, phage-antibody libraries), or can be synthesized. The DNA may be sequenced and manipulated chemically or by using molecular biology techniques, for example, to arrange one or more variable and/or constant domains into a suitable configuration, or to introduce codons, create cysteine residues, modify, add or delete amino acids, etc.

[0034] Non-limiting examples of antigen-binding fragments include: (i) Fab fragments; (ii) $F(ab')_2$ fragments; (iii) Fd fragments; (iv) Fv fragments; (v) single-chain Fv (scFv) molecules; (vi) dAb fragments; and (vii) minimal recognition units consisting of the amino acid residues that mimic the hypervariable region of an antibody (*e.g.*, an isolated complementarity determining region (CDR)). Other engineered molecules, such as diabodies, triabodies, tetrabodies and minibodies, are also encompassed within the expression "antigen-binding fragment," as used herein.

[0035] An antigen-binding fragment of an antibody will typically comprise at least one variable domain. The variable domain may be of any size or amino acid composition and will generally comprise at least one CDR which is adjacent to or in frame with one or more framework sequences. In antigen-binding fragments having a V_H domain associated with a V_L domain, the V_H and V_L domains may be situated relative to one another in any suitable arrangement. For example, the variable region may be dimeric and contain V_H-V_H, V_H-V_L or V_L-V_L dimers.

Alternatively, the antigen-binding fragment of an antibody may contain a monomeric V_H or V_L domain.

[0036] In certain embodiments, an antigen-binding fragment of an antibody may contain at least one variable domain covalently linked to at least one constant domain. Non-limiting, exemplary configurations of variable and constant domains that may be found within an antigen-binding fragment of an antibody of the present invention include: (i) V_H-C_H1; (ii) V_H-C_H2; (iii) V_H-C_H3; (iv) V_H-C_H1-C_H2; (v) V_H-C_H1-C_H2-C_H3; (vi) V_H-C_H2-C_H3; (vii) V_H-C_L; (viii) V_L-C_H1; (ix) V_L-C_H2; (x) V_L-C_H3; (xi) V_L-C_H1-C_H2; (xii) V_L-C_H1-C_H2-C_H3; (xiii) V_L-C_H2-C_H3; and (xiv) V_L-C_L. In any configuration of variable and constant domains, including any of the exemplary configurations listed above, the variable and constant domains may be either directly linked to one another or may be linked by a full or partial hinge or linker region. A hinge region may consist of at least 2 (*e.g.*, 5, 10, 15, 20, 40, 60 or more) amino acids which result in a flexible or semi-flexible linkage between adjacent variable and/or constant domains in a single polypeptide molecule. Moreover, an antigen-binding fragment of an antibody of the present invention may comprise a homo-dimer or hetero-dimer (or other multimer) of any of the variable and constant domain configurations listed above in non-covalent association with one another and/or with one or more monomeric V_H or V_L domain (*e.g.*, by disulfide bond(s)).

[0037] As with full antibody molecules, antigen-binding fragments may be monospecific or multispecific (*e.g.*, bispecific). A multispecific antigen-binding fragment of an antibody will typically comprise at least two different variable domains, wherein each variable domain is capable of specifically binding to a separate antigen or to a different epitope on the same antigen. Any multispecific antibody format, including the exemplary bispecific antibody formats disclosed herein, may be adapted for use in the context of an antigen-binding fragment of an antibody of the present invention using routine techniques available in the art.

[0038] The constant region of an antibody is important in the ability of an antibody to fix complement and mediate cell-dependent cytotoxicity. Thus, the isotype of an antibody may be selected on the basis of whether it is desirable for the antibody to mediate cytotoxicity.

[0039] The term "human antibody", as used herein, is intended to include antibodies having variable and constant regions derived from human germline immunoglobulin sequences. The human antibodies of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (e.g., mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*), for example in the CDRs and in particular CDR3. However, the term "human antibody", as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences.

[0040] The term "recombinant human antibody", as used herein, is intended to include all human antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies expressed using a recombinant expression vector transfected into a host cell

(described further below), antibodies isolated from a recombinant, combinatorial human antibody library (described further below), antibodies isolated from an animal (e.g., a mouse) that is transgenic for human immunoglobulin genes (see e.g., Taylor et al. (1992) Nucl. Acids Res. 20:6287-6295) or antibodies prepared, expressed, created or isolated by any other means that involves splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant human antibodies have variable and constant regions derived from human germline immunoglobulin sequences. In certain embodiments, however, such recombinant human antibodies are subjected to *in vitro* mutagenesis (or, when an animal transgenic for human Ig sequences is used, *in vivo* somatic mutagenesis) and thus the amino acid sequences of the V_H and V_L regions of the recombinant antibodies are sequences that, while derived from and related to human germline V_H and V_L sequences, may not naturally exist within the human antibody germline repertoire *in vivo*.

[0041] Human antibodies can exist in two forms that are associated with hinge heterogeneity. In one form, an immunoglobulin molecule comprises a stable four chain construct of approximately 150-160 kDa in which the dimers are held together by an interchain heavy chain disulfide bond. In a second form, the dimers are not linked via inter-chain disulfide bonds and a molecule of about 75-80 kDa is formed composed of a covalently coupled light and heavy chain (half-antibody). These forms have been extremely difficult to separate, even after affinity purification.

[0042] The frequency of appearance of the second form in various intact IgG isotypes is due to, but not limited to, structural differences associated with the hinge region isotype of the antibody. A single amino acid substitution in the hinge region of the human IgG4 hinge can significantly reduce the appearance of the second form (Angal et al. (1993) Molecular Immunology 30:105) to levels typically observed using a human IgG1 hinge. The instant invention encompasses antibodies having one or more mutations in the hinge, C_H2 or C_H3 region which may be desirable, for example, in production, to improve the yield of the desired antibody form.

[0043] An "isolated antibody", as used herein, is intended to refer to an antibody that is substantially free of other antibodies having different antigenic specificities (e.g., an isolated antibody that specifically binds human Ang-2 or a human Ang-2 fragment is substantially free of antibodies that specifically bind antigens other than human Ang-2). The term "specifically binds," or the like, means that an antibody or antigen-binding fragment thereof forms a complex with an antigen that is relatively stable under physiologic conditions. Specific binding can be characterized by a K_D of about $1x10^{-8}$ M or less. Methods for determining whether two molecules specifically bind are well known in the art and include, for example, equilibrium dialysis, surface plasmon resonance, and the like. An isolated antibody that specifically binds human Ang-2 may, however, have cross-reactivity to other antigens, such as Ang-2 molecules from other species. Moreover, an isolated antibody may be substantially free of other cellular

material and/or chemicals.

[0044] A "neutralizing" or "blocking" antibody, as used herein, is intended to refer to an antibody whose binding to Ang-2 blocks the interaction between Ang-2 and its receptor (Tie-2) and/or results in inhibition of at least one biological function of Ang-2. The inhibition caused by an Ang-2 neutralizing or blocking antibody need not be complete so long as it is detectable using an appropriate assay. Exemplary assays for detecting Ang-2 inhibition are described elsewhere herein.

[0045] The fully-human anti-Ang-2 antibodies disclosed herein may comprise one or more amino acid substitutions, insertions and/or deletions in the framework and/or CDR regions of the heavy and light chain variable domains as compared to the corresponding germline sequences. Such mutations can be readily ascertained by comparing the amino acid sequences disclosed herein to germline sequences available from, for example, public antibody sequence databases. The present invention includes antibodies, and antigen-binding fragments thereof, which are derived from any of the amino acid sequences disclosed herein, wherein one or more amino acids within one or more framework and/or CDR regions are back-mutated to the corresponding germline residue(s) or to a conservative amino acid substitution (natural or non-natural) of the corresponding germline residue(s) (such sequence changes are referred to herein as "germline back-mutations"). A person of ordinary skill in the art, starting with the heavy and light chain variable region sequences disclosed herein, can easily produce numerous antibodies and antigen-binding fragments which comprise one or more individual germline back-mutations or combinations thereof. In certain embodiments, all of the framework and/or CDR residues within the V_H and/or V_L domains are mutated back to the germline sequence. In other embodiments, only certain residues are mutated back to the germline sequence, *e.g.*, only the mutated residues found within the first 8 amino acids of FR1 or within the last 8 amino acids of FR4, or only the mutated residues found within CDR1, CDR2 or CDR3. Furthermore, the antibodies of the present invention may contain any combination of two or more germline back-mutations within the framework and/or CDR regions, *i.e.*, wherein certain individual residues are mutated back to the germline sequence while certain other residues that differ from the germline sequence are maintained. Once obtained, antibodies and antigen-binding fragments that contain one or more germline back-mutations can be easily tested for one or more desired property such as, improved binding specificity, increased binding affinity, improved or enhanced antagonistic or agonistic biological properties (as the case may be), reduced immunogenicity, etc. Antibodies and antigen-binding fragments obtained in this general manner are encompassed within the present invention.

[0046] The present invention also includes anti-Ang-2 antibodies comprising variants of any of the HCVR, LCVR, and/or CDR amino acid sequences disclosed herein having one or more conservative substitutions. For example, the present invention includes anti-Ang-2 antibodies having HCVR, LCVR, and/or CDR amino acid sequences with, *e.g.*, 10 or fewer, 8 or fewer, 6 or

fewer, 4 or fewer, etc. conservative amino acid substitutions relative to any of the HCVR, LCVR, and/or CDR amino acid sequences disclosed herein. In one embodiment, the antibody comprises an HCVR having the amino acid sequence of SEQ ID NO:18 with 8 or fewer conservative amino acid substitutions. In another embodiment, the antibody comprises an HCVR having the amino acid sequence of SEQ ID NO:18 with 6 or fewer conservative amino acid substitutions. In another embodiment, the antibody comprises an HCVR having the amino acid sequence of SEQ ID NO:18 with 4 or fewer conservative amino acid substitutions. In another embodiment, the antibody comprises an HCVR having the amino acid sequence of SEQ ID NO:18 with 2 or fewer conservative amino acid substitutions. In one embodiment, the antibody comprises an LCVR having the amino acid sequence of SEQ ID NO:20 with 8 or fewer conservative amino acid substitutions. In another embodiment, the antibody comprises an LCVR having the amino acid sequence of SEQ ID NO:20 with 6 or fewer conservative amino acid substitutions. In another embodiment, the antibody comprises an LCVR having the amino acid sequence of SEQ ID NO:20 with 4 or fewer conservative amino acid substitutions. In another embodiment, the antibody comprises an LCVR having the amino acid sequence of SEQ ID NO:20 with 2 or fewer conservative amino acid substitutions.

[0047] The term "surface plasmon resonance", as used herein, refers to an optical phenomenon that allows for the analysis of real-time interactions by detection of alterations in protein concentrations within a biosensor matrix, for example using the BIAcore™ system (Biacore Life Sciences division of GE Healthcare, Piscataway, NJ).

[0048] The term "K_D ", as used herein, is intended to refer to the equilibrium dissociation constant of a particular antibody-antigen interaction.

[0049] The term "epitope" refers to an antigenic determinant that interacts with a specific antigen binding site in the variable region of an antibody molecule known as a paratope. A single antigen may have more than one epitope. Thus, different antibodies may bind to different areas on an antigen and may have different biological effects. Epitopes may be either conformational or linear. A conformational epitope is produced by spatially juxtaposed amino acids from different segments of the linear polypeptide chain. A linear epitope is one produced by adjacent amino acid residues in a polypeptide chain. In certain circumstance, an epitope may include moieties of saccharides, phosphoryl groups, or sulfonyl groups on the antigen.

[0050] The term "substantial identity" or "substantially identical," when referring to a nucleic acid or fragment thereof, indicates that, when optimally aligned with appropriate nucleotide insertions or deletions with another nucleic acid (or its complementary strand), there is nucleotide sequence identity in at least about 95%, and more preferably at least about 96%, 97%, 98% or 99% of the nucleotide bases, as measured by any well-known algorithm of sequence identity, such as FASTA, BLAST or Gap, as discussed below. A nucleic acid molecule having substantial identity to a reference nucleic acid molecule may, in certain instances, encode a polypeptide having the same or substantially similar amino acid sequence

as the polypeptide encoded by the reference nucleic acid molecule.

[0051] As applied to polypeptides, the term "substantial similarity" or "substantially similar" means that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 95% sequence identity, even more preferably at least 98% or 99% sequence identity. Preferably, residue positions which are not identical differ by conservative amino acid substitutions. A "conservative amino acid substitution" is one in which an amino acid residue is substituted by another amino acid residue having a side chain (R group) with similar chemical properties (e.g., charge or hydrophobicity). In general, a conservative amino acid substitution will not substantially change the functional properties of a protein. In cases where two or more amino acid sequences differ from each other by conservative substitutions, the percent sequence identity or degree of similarity may be adjusted upwards to correct for the conservative nature of the substitution. Means for making this adjustment are well-known to those of skill in the art. *See, e.g.*, Pearson (1994) Methods Mol. Biol. 24: 307-331. Examples of groups of amino acids that have side chains with similar chemical properties include (1) aliphatic side chains: glycine, alanine, valine, leucine and isoleucine; (2) aliphatic-hydroxyl side chains: serine and threonine; (3) amide-containing side chains: asparagine and glutamine; (4) aromatic side chains: phenylalanine, tyrosine, and tryptophan; (5) basic side chains: lysine, arginine, and histidine; (6) acidic side chains: aspartate and glutamate, and (7) sulfur-containing side chains are cysteine and methionine. Preferred conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, glutamate-aspartate, and asparagine-glutamine. Alternatively, a conservative replacement is any change having a positive value in the PAM250 log-likelihood matrix disclosed in Gonnet *et al.* (1992) Science 256: 1443-1445. A "moderately conservative" replacement is any change having a nonnegative value in the PAM250 log-likelihood matrix.

[0052] Sequence similarity for polypeptides, which is also referred to as sequence identity, is typically measured using sequence analysis software. Protein analysis software matches similar sequences using measures of similarity assigned to various substitutions, deletions and other modifications, including conservative amino acid substitutions. For instance, GCG software contains programs such as Gap and Bestfit which can be used with default parameters to determine sequence homology or sequence identity between closely related polypeptides, such as homologous polypeptides from different species of organisms or between a wild type protein and a mutein thereof. *See, e.g.*, GCG Version 6.1. Polypeptide sequences also can be compared using FASTA using default or recommended parameters, a program in GCG Version 6.1. FASTA (e.g., FASTA2 and FASTA3) provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences (Pearson (2000) supra). Another preferred algorithm when comparing a sequence of the invention to a database containing a large number of sequences from different organisms is the computer program

BLAST, especially BLASTP or TBLASTN, using default parameters. *See, e.g.*, Altschul *et al.* (1990) J. Mol. Biol. 215:403-410 and Altschul *et al.* (1997) Nucleic Acids Res. 25:3389-402.

Preparation of Human Antibodies

[0053] Methods for generating monoclonal antibodies, including fully human monoclonal antibodies are known in the art. Any such known methods can be used in the context of the present invention to make human antibodies that specifically bind to human Ang-2 and which possess one or more of the antigen-binding and/or functional characteristics of any of the exemplary anti-Ang-2 antibodies disclosed herein.

[0054] Using VELOCIMMUNE™ technology or any other known method for generating monoclonal antibodies, high affinity chimeric antibodies to Ang-2 are initially isolated having a human variable region and a mouse constant region. As in the experimental section below, the antibodies are characterized and selected for desirable characteristics, including affinity, selectivity, epitope, etc. The mouse constant regions are replaced with a desired human constant region to generate the fully human antibody of the invention, for example wild-type or modified IgG1 or IgG4. While the constant region selected may vary according to specific use, high affinity antigen-binding and target specificity characteristics reside in the variable region.

Bioequivalents

[0055] The anti-Ang-2 antibodies and antibody fragments of the present invention encompass proteins having amino acid sequences that vary from those of the described antibodies, but that retain the ability to bind human Ang-2. Such variant antibodies and antibody fragments comprise one or more additions, deletions, or substitutions of amino acids when compared to parent sequence, but exhibit biological activity that is essentially equivalent to that of the described antibodies. Likewise, the anti-Ang-2 antibody-encoding DNA sequences of the present invention encompass sequences that comprise one or more additions, deletions, or substitutions of nucleotides when compared to the disclosed sequence, but that encode an anti-Ang-2 antibody or antibody fragment that is essentially bioequivalent to an anti-Ang-2 antibody or antibody fragment of the invention. Examples of such variant amino acid and DNA sequences are discussed above.

[0056] Two antigen-binding proteins, or antibodies, are considered bioequivalent if, for example, they are pharmaceutical equivalents or pharmaceutical alternatives whose rate and extent of absorption do not show a significant difference when administered at the same molar dose under similar experimental conditions, either single does or multiple dose. Some antibodies will be considered equivalents or pharmaceutical alternatives if they are equivalent in the extent of their absorption but not in their rate of absorption and yet may be considered bioequivalent because such differences in the rate of absorption are intentional and are reflected in the labeling, are not essential to the attainment of effective body drug concentrations

on, e.g., chronic use, and are considered medically insignificant for the particular drug product studied.

[0057] In one embodiment, two antigen-binding proteins are bioequivalent if there are no clinically meaningful differences in their safety, purity, and potency.

[0058] In one embodiment, two antigen-binding proteins are bioequivalent if a patient can be switched one or more times between the reference product and the biological product without an expected increase in the risk of adverse effects, including a clinically significant change in immunogenicity, or diminished effectiveness, as compared to continued therapy without such switching.

[0059] In one embodiment, two antigen-binding proteins are bioequivalent if they both act by a common mechanism or mechanisms of action for the condition or conditions of use, to the extent that such mechanisms are known.

[0060] Bioequivalence may be demonstrated by in vivo and in vitro methods. Bioequivalence measures include, e.g., (a) an in vivo test in humans or other mammals, in which the concentration of the antibody or its metabolites is measured in blood, plasma, serum, or other biological fluid as a function of time; (b) an in vitro test that has been correlated with and is reasonably predictive of human in vivo bioavailability data; (c) an in vivo test in humans or other mammals in which the appropriate acute pharmacological effect of the antibody (or its target) is measured as a function of time; and (d) in a well-controlled clinical trial that establishes safety, efficacy, or bioavailability or bioequivalence of an antibody.

[0061] Bioequivalent variants of anti-Ang-2 antibodies of the invention may be constructed by, for example, making various substitutions of residues or sequences or deleting terminal or internal residues or sequences not needed for biological activity. For example, cysteine residues not essential for biological activity can be deleted or replaced with other amino acids to prevent formation of unnecessary or incorrect intramolecular disulfide bridges upon renaturation.

Biological and Therapeutic Characteristics of the Antibodies

[0062] In general, the antibodies of the instant invention bind to human Ang-2 with a K_D of less than 100 pM, typically with a K_D of less than 50 pM, and in certain embodiments, with a K_D of less than 40 pM, when measured by binding to antigen either immobilized on solid phase or in solution phase.

[0063] In addition, certain exemplary anti-Ang-2 antibodies of the invention may exhibit one or more of the following characteristics: (1) ability to bind to human Ang-2 but not to mouse Ang-2; (2) ability to bind to human Ang-2 and to mouse Ang-2; (3) ability to bind to human Ang-2 but not to human Ang-1, -3 or -4; (4) ability to bind to human Ang-2 but not to mouse Ang-1, -3 or -4; (5) ability to bind to human Ang-2 and to human Ang-1, -3 or -4; (6) ability to bind to human Ang-2 and to mouse Ang-1, -3 or -4; (7) ability to block binding of human Ang-2 to human Tie-2;

(8) ability to block binding of human Ang-2 to mouse Tie-2; (9) ability to block binding of mouse Ang-2 to human Tie-2; (10) ability to block binding of mouse Ang-2 to mouse Tie-2; (11) ability to block binding of human Ang-1 to human Tie-2; (12) ability to block binding of human Ang-1 to mouse Tie-2; (13) ability to block binding of mouse Ang-1 to human Tie-2; (14) ability to block binding of mouse Ang-1 to mouse Tie-2; (15) ability to inhibit human Ang-2-induced phosphorylation of human Tie-2; (16) ability to inhibit human Ang-2-induced phosphorylation of mouse Tie-2; (17) ability to inhibit mouse Ang-2-induced phosphorylation of human Tie-2; (18) ability to inhibit mouse Ang-2 induced phosphorylation of mouse Tie-2; (19) ability to inhibit human Ang-1-induced phosphorylation of human Tie-2; (20) ability to inhibit human Ang-1-induced phosphorylation of mouse Tie-2; (21) ability to inhibit mouse Ang-1-induced phosphorylation of human Tie-2; (22) ability to inhibit mouse-Ang-1-induced phosphorylation of mouse Tie-2; (23) ability to inhibit *in vivo* angiogenesis in an experimental model (*e.g.*, angiogenesis induced by a Matrigel plug containing MCF-7 cells implanted subcutaneously into nude mice); and/or (24) ability to inhibit or decrease tumor volume in a mouse xenograft model.

[0064] The present invention also includes antibodies that bind with high affinity to a construct comprising the Ang-2 fibronectin-like domain but lacking the Ang-2 N-terminal coiled-coil domain (such constructs are referred to herein as "Ang-2FD"). Exemplary Ang-2FD constructs include human Ang-2FD (SEQ ID NO:519), mouse Ang-2FD (SEQ ID NO:520), and monkey Ang-2FD (SEQ ID NO:521). The human, mouse and monkey Ang-2FD constructs may be monomeric or dimeric. Ang-2FD constructs may also include other non-Ang-2 amino acid sequences such as a human or mouse Fc domain linked to the Ang-2FD molecules. Another exemplary Ang-2FD construct is referred to herein as "hBA2" (or human "bow-Ang2") which is a tetramer of human Ang-2 fibrinogen-like domains associated with one another via a human or mouse Fc domain to form a bow-tie-like configuration. Typically, hBA2 consists of two Ang-2 dimers, wherein each Ang-2 dimer contains two Ang-2 fibronectin-like domains connected to one another via an Fc domain. Exemplary hBA2 components include the polypeptides designated hBA2-hIgG1 (SEQ ID NO:522) and hBA2-mIgG2a (SEQ ID NO:523). Unexpectedly, certain anti-Ang-2 antibodies of the present invention were found to bind to Ang-2FD constructs with much higher affinities than an known Ang-2 control antibody (*see* Examples set forth herein).

[0065] High affinity binding, in the context of anti-Ang-2 antibody binding to a human or mouse dimeric Ang-2FD construct, means that the anti-Ang-2 antibody binds the human or mouse dimeric Ang-2FD with a K_D of less than 300 pM. For example, anti-Ang-2 antibodies that bind with high affinity to human or mouse dimeric Ang-2FD include antibodies that bind to human or mouse dimeric Ang-2-FD with a K_D of less than 300 pM, less than 250 pM, less than 200 pM, less than 190 pM, less than 180 pM, less than 170 pM, less than 160 pM, less than 150 pM, less than 140 pM, less than 130 pM, less than 120 pM, less than 110 pM, less than 100 pM,

less than 90 pM, less than 80 pM, less than 70 pM, less than 60 pM or less than 50 pM, as measured at 25°C in a surface Plasmon resonance assay.

[0066] High affinity binding, in the context of anti-Ang-2 antibody binding to a monkey dimeric Ang-2FD construct, means that the anti-Ang-2 antibody binds the monkey dimeric Ang-2FD with a K_D of less than 500 pM. For example, anti-Ang-2 antibodies that bind with high affinity to monkey dimeric Ang-2FD include antibodies that bind to monkey Ang-2-FD with a K_D of less than 500 pM, less than 450 pM, less than 400 pM, less than 350 pM, less than 300 pM, less than 250 pM, less than 200 pM, less than 190 pM, less than 180 pM, less than 170 pM, less than 160 pM, less than 150 pM, less than 140 pM, less than 130 pM, less than 120 pM, less than 110 pM, less than 100 pM, less than 90 pM, or less than 80 pM, as measured at 25°C in a surface Plasmon resonance assay.

[0067] High affinity binding, in the context of anti-Ang-2 antibody binding to a human monomeric Ang-2FD construct, means that the anti-Ang-2 antibody binds the human monomeric Ang-2FD with a K_D of less than 40 nM. For example, anti-Ang-2 antibodies that bind with high affinity to human monomeric Ang-2FD include antibodies that bind to human monomeric Ang-2-FD with a K_D of less than 40 nM, less than 30 nM, less than 25 nM, less than 20 nM, less than 15 nM, less than 10 nM, less than 9 nM, less than 8 nM, less than 7 nM, less than 6 nM, less than 5 nM, less than 4 nM, less than 3 nM, less than 2 nM, less than 1 nM, less than 0.9 nM, less than 0.8 nM, less than 0.7 nM, or less than 0.6 nM as measured at 25°C in a surface Plasmon resonance assay.

[0068] High affinity binding, in the context of anti-Ang-2 antibody binding to a hBA2 construct, means that the anti-Ang-2 antibody binds the hBA2 with a K_D of less than 80 pM. For example, anti-Ang-2 antibodies that bind with high affinity to hBA2 include antibodies that bind to hBA2 with a K_D of less than 80 pM, less than 75 pM, less than 70 pM, less than 65 pM, less than 60 pM, less than 55 pM, less than 50 pM, less than 45 pM, less than 40 pM, less than 35 pM, less than 30 pM, less than 25 pM, less than 20 pM, less than 18 pM, less than 16 pM, less than 14 pM, or less than 12 pM, as measured at 25°C in a surface Plasmon resonance assay.

[0069] The present invention includes antibodies that bind Ang-2 but do not substantially bind Ang-1. As used herein, an antibody "does not substantially bind Ang-1" if the antibody, when tested for binding to Ang-1 in a surface plasmon resonance assay in which the antibody is captured on a surface and full-length wild-type human Ang-1 at a concentration of about 25 nM is injected over the captured antibody surface at a flowrate of about 60 µl/min for about 3 minutes at 25°C, exhibits a K_D of greater than about 1 nM, *e.g.*, a K_D of greater than about 5 nM, greater than about 10 nM, greater than about 50 nM, greater than about 100 nM, greater than about 150 nM, greater than about 200 nM, greater than about 250 nM, greater than about 300 nM, greater than about 350 nM, greater than about 400 nM, greater than about 450 nM, greater than about 500 nM, or more. (*See*, *e.g.*, Example 4). In addition, an antibody "does not

substantially bind Ang-1" if the antibody fails to exhibit any binding to Ang-1 when tested in such an assay or equivalent thereof.

[0070] The present invention also includes antibodies that block the binding of Ang-2 to Tie-2 but do not substantially block the binding of Ang-1 to Tie-2. As used herein, an antibody "does not substantially block the binding of Ang-1 to Tie-2" if, when the antibody is premixed with Ang-1 antigen at a ratio of about 100:1 (antibody:antigen) and allowed to incubate at 25°C for about 60 minutes and then the equilibrated mixture is tested for binding to Tie-2 by surface plasmon resonance over a Tie-2-coated surface (5 µl/min for 5 min. at 25°C), the amount of Ang-1 bound to Tie-2 is at least 50% the amount of Ang-1 bound to Tie-2 in the presence of an irrelevant control molecule. (*See, e.g.*, Example 6). For example, if the amount of Ang-1 bound to Tie-2 following preincubation with an antibody is at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or about 100% the amount of Ang-1 that binds to Tie-2 following preincubation with an irrelevant control molecule under the above noted experimental conditions, then the antibody is deemed to "not substantially block the binding of Ang-1 to Tie-2."

[0071] Moreover, the present invention includes antibodies that block or substantially attenuate a biological activity of Ang-2 (*e.g.*, Ang-2-mediated phosphorylation of Tie-2; Ang-2-induced angiogenesis; etc.) but do not block or substantially attenuate the corresponding biological activity of Ang-1 (*e.g.*, Ang-1-mediated phosphorylation of Tie-2; Ang-1-induced angiogenesis; etc). Assays and tests useful for determining whether an antibody satisfies one or more of the characteristics listed above will be readily known and easily practiced by persons of ordinary skill in the art and/or can be fully ascertained from the present disclosure. For example, the experimental procedures detailed below can be used to determine whether a given antibody binds or does not bind to Ang-2 and/or Ang-1; blocks or does not block binding of Ang-2 and/or Ang-1 to Tie-2; inhibits or does not inhibit Ang-2- and/or Ang-1-mediated phosphorylation of Tie-2; etc.

Epitope Mapping and Related Technologies

[0072] To screen for antibodies that bind to a particular epitope (e.g., those which block binding of IgE to its high affinity receptor), a routine cross-blocking assay such as that described "Antibodies," Harlow and Lane (Cold Spring Harbor Press, Cold Spring Harb., NY) can be performed. Other methods include alanine scanning mutants, peptide blots (Reineke (2004) Methods Mol Biol 248:443-63), or peptide cleavage analysis. In addition, methods such as epitope excision, epitope extraction and chemical modification of antigens can be employed (Tomer (2000) Protein Science 9: 487-496).

[0073] The term "epitope" refers to a site on an antigen to which B and/or T cells respond. B-cell epitopes can be formed both from contiguous amino acids or noncontiguous amino acids

juxtaposed by tertiary folding of a protein. Epitopes formed from contiguous amino acids are typically retained on exposure to denaturing solvents, whereas epitopes formed by tertiary folding are typically lost on treatment with denaturing solvents. An epitope typically includes at least 3, and more usually, at least 5 or 8-10 amino acids in a unique spatial conformation.

[0074] Modification-Assisted Profiling (MAP), also known as Antigen Structure-based Antibody Profiling (ASAP) is a method that categorizes large numbers of monoclonal antibodies (mAbs) directed against the same antigen according to the similarities of the binding profile of each antibody to chemically or enzymatically modified antigen surfaces (US 2004/0101920). Each category may reflect a unique epitope either distinctly different from or partially overlapping with epitope represented by another category. This technology allows rapid filtering of genetically identical antibodies, such that characterization can be focused on genetically distinct antibodies. When applied to hybridoma screening, MAP may facilitate identification of rare hybridoma clones that produce mAbs having the desired characteristics. MAP may be used to sort the anti-Ang-2 antibodies of the invention into groups of antibodies binding different epitopes.

[0075] Anti-Ang-2 antibodies can bind to an epitope within the amino-terminal coiled-coil domain or within the carboxy-terminal fibrinogen-like domain ("FD"). In preferred embodiments of the present invention, the anti-Ang-2 antibodies and antigen binding fragments thereof bind to an epitope within the FD.

[0076] The amino acids within the FD of Ang-2 that interact with Tie-2 have been ascertained from crystal structure analysis. *See* Barton *et al.*, *Nat. Struct. Mol. Biol. 13*:524-532 (May 2006). With regard to antibodies that block the binding of Ang-2 to Tie-2 but do not substantially block binding of Ang-1 to Tie-2 (*e.g.*, H1H685P, *see* Examples 5 and 6 below), the epitope to which such antibodies bind may include one or more amino acids of Ang-2 that (a) interact with Tie-2 and (b) are non-identical to the corresponding amino acid in Ang-1. (See Figure 1). Thus, the epitope to which such Ang-2 preferential antibodies bind may include one or more of the following amino acids of hAng-2 (SEQ ID NO:518): S-417; K-432; I-434; N-467; F-469; Y-475; or S-480. For example, the present inventors have discovered that antibodies which interact with amino acids F-469, Y-475, and S-480 of Ang-2 (SEQ ID NO:518) preferentially interact with Ang-2 over Ang-1, and this preferential binding may have therapeutic benefits. Thus, the present invention includes anti-Ang-2 antibodies which specifically bind human angiopoietin-2 (hAng-2) but do not substantially bind hAng-1, wherein the antibodies bind an epitope on hAng-2 (SEQ ID NO:518) comprising amino acids F-469, Y-475, and S-480. Similarly, the present invention includes anti-Ang-2 antibodies which block the binding of hAng-2 to hTie-2 but do not substantially block the binding of hAng-1 to hTie-2, wherein the antibodies bind an epitope on hAng-2 (SEQ ID NO:518) comprising amino acids F-469, Y-475, and S-480.

[0077] The present invention includes anti-Ang-2 antibodies that bind to the same epitope as any of the specific exemplary antibodies described herein (*e.g.*, H1H685, H1H690, H1H691, H1H696, H1H706, H1M724 and/or H2M744). Likewise, the present invention also includes anti-

Ang-2 antibodies that compete for binding to Ang-2 with any of the specific exemplary antibodies described herein (*e.g.*, H1H685, H1H690, H1H691, H1H696, H1H706, H1M724 and/or H2M744).

[0078] One can easily determine whether an antibody binds to the same epitope as, or competes for binding with, a reference anti-Ang-2 antibody by using routine methods known in the art. For example, to determine if a test antibody binds to the same epitope as a reference anti-Ang-2 antibody of the invention, the reference antibody is allowed to bind to an Ang-2 protein or peptide under saturating conditions. Next, the ability of a test antibody to bind to the Ang-2 molecule is assessed. If the test antibody is able to bind to Ang-2 following saturation binding with the reference anti-Ang-2 antibody, it can be concluded that the test antibody binds to a different epitope than the reference anti-Ang-2 antibody. On the other hand, if the test antibody is not able to bind to the Ang-2 molecule following saturation binding with the reference anti-Ang-2 antibody, then the test antibody may bind to the same epitope as the epitope bound by the reference anti-Ang-2 antibody of the invention. Additional routine experimentation (*e.g.*, peptide mutation and binding analyses) can then be carried out to confirm whether the observed lack of binding of the test antibody is in fact due to binding to the same epitope as the reference antibody or if steric blocking (or another phenomenon) is responsible for the lack of observed binding. Experiments of this sort can be performed using ELISA, RIA, Biacore, flow cytometry or any other quantitative or qualitative antibody-binding assay available in the art. In accordance with certain embodiments of the present invention, two antibodies bind to the same (or overlapping) epitope if, *e.g.*, a 1-, 5-, 10-, 20- or 100-fold excess of one antibody inhibits binding of the other by at least 50% but preferably 75%, 90% or even 99% as measured in a competitive binding assay (see, e.g., Junghans et al., Cancer Res. 1990:50:1495-1502). Alternatively, two antibodies are deemed to bind to the same epitope if essentially all amino acid mutations in the antigen that reduce or eliminate binding of one antibody reduce or eliminate binding of the other. Two antibodies are deemed to have "overlapping epitopes" if only a subset of the amino acid mutations that reduce or eliminate binding of one antibody reduce or eliminate binding of the other.

[0079] To determine if an antibody competes for binding with a reference anti-Ang-2 antibody, the above-described binding methodology is performed in two orientations: In a first orientation, the reference antibody is allowed to bind to an Ang-2 molecule under saturating conditions followed by assessment of binding of the test antibody to the Ang-2 molecule. In a second orientation, the test antibody is allowed to bind to an Ang-2 molecule under saturating conditions followed by assessment of binding of the reference antibody to the Ang-2 molecule. If, in both orientations, only the first (saturating) antibody is capable of binding to the Ang-2 molecule, then it is concluded that the test antibody and the reference antibody compete for binding to Ang-2. As will be appreciated by a person of ordinary skill in the art, an antibody that competes for binding with a reference antibody may not necessarily bind to the same epitope as the reference

antibody, but may sterically block binding of the reference antibody by binding an overlapping or adjacent epitope.

Species Selectivity and Species Cross-Reactivity

[0080] According to certain embodiments of the invention, the anti-Ang-2 antibodies bind to human Ang-2 but not to Ang-2 from other species. Alternatively, the anti-Ang-2 antibodies of the invention, in certain embodiments, bind to human Ang-2 and to Ang-2 from one or more non-human species. For example, the Ang-2 antibodies of the invention may bind to human Ang-2 and may bind or not bind, as the case may be, to one or more of mouse, rat, guinea pig, hamster, gerbil, pig, cat, dog, rabbit, goat, sheep, cow, horse, camel, cynomologous, marmoset, rhesus or chimpanzee Ang-2.

Immunoconjugates

[0081] The invention encompasses anti-Ang-2 monoclonal antibodies conjugated to a therapeutic moiety ("immunoconjugate"), such as a cytotoxin, a chemotherapeutic drug, an immunosuppressant or a radioisotope. Cytotoxic agents include any agent that is detrimental to cells. Examples of suitable cytotoxic agents and chemotherapeutic agents for forming immunoconjugates are known in the art, see for example, WO 05/103081).

Multispecific Antibodies

[0082] The antibodies of the present invention may be monospecific, bispecific, or multispecific. Multispecific mAbs may be specific for different epitopes of one target polypeptide or may contain antigen-binding domains specific for more than one target polypeptide. See, e.g., Tutt et al. (1991) J. Immunol. 147:60-69. The anti-Ang-2 antibodies of the present invention, or portions thereof, can be linked to or co-expressed with another functional molecule, e.g., another peptide or protein, to form a multispecific molecule. For example, an antibody or fragment thereof can be functionally linked (e.g., by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other molecular entities, such as another antibody or antibody fragment, to produce a bispecific or a multispecific antibody with a second binding specificity.

[0083] An exemplary bi-specific antibody format that can be used in the context of the present invention involves the use of a first immunoglobulin (Ig) C_H3 domain and a second Ig C_H3 domain, wherein the first and second Ig C_H3 domains differ from one another by at least one amino acid, and wherein at least one amino acid difference reduces binding of the bispecific antibody to Protein A as compared to a bi-specific antibody lacking the amino acid difference. In one embodiment, the first Ig C_H3 domain binds Protein A and the second Ig C_H3 domain contains a mutation that reduces or abolishes Protein A binding such as an H95R modification (by IMGT exon numbering; H435R by EU numbering). The second C_H3 may further comprise a

Y96F modification (by IMGT; Y436F by EU). Further modifications that may be found within the second C_H3 include: D16E, L18M, N44S, K52N, V57M, and V82I (by IMGT; D356E, L358M, N384S, K392N, V397M, and V422I by EU) in the case of IgG1 antibodies; N44S, K52N, and V82I (IMGT; N384S, K392N, and V422I by EU) in the case of IgG2 antibodies; and Q15R, N44S, K52N, V57M, R69K, E79Q, and V82I (by IMGT; Q355R, N384S, K392N, V397M, R409K, E419Q, and V422I by EU) in the case of IgG4 antibodies. Variations on the bi-specific antibody format described above are contemplated within the scope of the present invention.

Therapeutic Formulation and Administration

[0084] The invention provides therapeutic compositions comprising the anti-Ang-2 antibodies or antigen-binding fragments thereof of the present invention. The therapeutic compositions in of the present invention may further comprise one or more pharmaceutically acceptable carriers, excipients, and other agents that are incorporated into formulations to provide improved transfer, delivery, tolerance, and the like (herein collectively referred to as "pharmaceutically acceptable carriers or diluents"). A multitude of appropriate formulations can be found in the formulary known to all pharmaceutical chemists: Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, PA. 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. See also Powell et al. "Compendium of excipients for parenteral formulations" PDA, 1998, J Pharm Sci Technol 52:238-311.

[0085] The dose of antibody may vary depending upon the age and the size of a subject to be administered, target disease, conditions, route of administration, and the like. The preferred dose is typically calculated according to body weight or body surface area. When an antibody of the present invention is used for treating a condition or disease associated with Ang-2 activity in an adult patient, it may be advantageous to intravenously administer the antibody of the present invention normally at a single dose of about 0.01 to about 20 mg/kg body weight, more preferably about 0.02 to about 7, about 0.03 to about 5, or about 0.05 to about 3 mg/kg body weight. Depending on the severity of the condition, the frequency and the duration of the treatment can be adjusted. Effective dosages and schedules for administering Ang-2 antibodies may be determined empirically; for example, patient progress can be monitored by periodic assessment, and the dose adjusted accordingly. Moreover, interspecies scaling of dosages can be performed using well-known methods in the art (*e.g.*, Mordenti et al., 1991, Pharmaceut. Res. 8:1351).

[0086] Various delivery systems are known and can be used to administer the pharmaceutical composition of the invention, e.g., encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the mutant viruses, receptor mediated endocytosis

(see, e.g., Wu et al. 1987, J. Biol. Chem. 262:4429-4432). Methods of introduction include, but are not limited to, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The composition may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local.

[0087] A pharmaceutical composition of the present invention can be delivered, *e.g.*, subcutaneously or intravenously with a standard needle and syringe. In addition, with respect to subcutaneous delivery, a pen delivery device readily has applications in delivering a pharmaceutical composition of the present invention. Such a pen delivery device can be reusable or disposable. A reusable pen delivery device generally utilizes a replaceable cartridge that contains a pharmaceutical composition. Once all of the pharmaceutical composition within the cartridge has been administered and the cartridge is empty, the empty cartridge can readily be discarded and replaced with a new cartridge that contains the pharmaceutical composition. The pen delivery device can then be reused. In a disposable pen delivery device, there is no replaceable cartridge. Rather, the disposable pen delivery device comes prefilled with the pharmaceutical composition held in a reservoir within the device. Once the reservoir is emptied of the pharmaceutical composition, the entire device is discarded.

[0088] Numerous reusable pen and autoinjector delivery devices have applications in the subcutaneous delivery of a pharmaceutical composition of the present invention. Examples include, but certainly are not limited to AUTOPEN™ (Owen Mumford, Inc., Woodstock, UK), DISETRONIC™ pen (Disetronic Medical Systems, Burghdorf, Switzerland), HUMALOG MIX 75/25™ pen, HUMALOG™ pen, HUMALIN 70/30™ pen (Eli Lilly and Co., Indianapolis, IN), NOVOPEN™ I, II and III (Novo Nordisk, Copenhagen, Denmark), NOVOPEN JUNIOR™ (Novo Nordisk, Copenhagen, Denmark), BD™ pen (Becton Dickinson, Franklin Lakes, NJ), OPTIPEN™, OPTIPEN PRO™, OPTIPEN STARLET™, and OPTICLIK™ (sanofi-aventis, Frankfurt, Germany), to name only a few. Examples of disposable pen delivery devices having applications in subcutaneous delivery of a pharmaceutical composition of the present invention include, but certainly are not limited to the SOLOSTAR™ pen (sanofi-aventis), the FLEXPEN™ (Novo Nordisk), and the KWIKPEN™ (Eli Lilly).

[0089] For the treatment of eye disorders, the antibodies and antigen-binding fragments of the invention may be administered, *e.g.*, by eye drops, subconjunctival injection, subconjunctival implant, intravitreal injection, intravitreal implant, sub-Tenon's injection or sub-Tenon's implant.

[0090] The pharmaceutical composition can be also delivered in a vesicle, in particular a liposome (see Langer 1990 Science 249:1527-1533; Treat et al. (1989) in Liposomes in the Therapy of Infectious Disease and Cancer, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353-365; Lopez-Berestein, ibid., pp. 317-327; see generally ibid.).

[0091] In certain situations, the pharmaceutical composition can be delivered in a controlled release system. In one embodiment, a pump may be used (see Langer, supra; Sefton 1987 CRC Crit. Ref. Biomed. Eng. 14:201). In another embodiment, polymeric materials can be used. In yet another embodiment, a controlled release system can be placed in proximity of the composition's target, thus requiring only a fraction of the systemic dose (see, *e.g.*, Goodson, 1984, in Medical Applications of Controlled Release, supra, vol. 2, pp. 115-138).

[0092] The injectable preparations may include dosage forms for intravenous, subcutaneous, intracutaneous and intramuscular injections, drip infusions, etc. These injectable preparations may be prepared by methods publicly known. For example, the injectable preparations may be prepared, *e.g.*, by dissolving, suspending or emulsifying the antibody or its salt described above in a sterile aqueous medium or an oily medium conventionally used for injections. As the aqueous medium for injections, there are, for example, physiological saline, an isotonic solution containing glucose and other auxiliary agents, etc., which may be used in combination with an appropriate solubilizing agent such as an alcohol (e.g., ethanol), a polyalcohol (e.g., propylene glycol, polyethylene glycol), a nonionic surfactant [e.g., polysorbate 80, HCO-50 (polyoxyethylene (50 mol) adduct of hydrogenated castor oil)], etc. As the oily medium, there are employed, e.g., sesame oil, soybean oil, etc., which may be used in combination with a solubilizing agent such as benzyl benzoate, benzyl alcohol, etc. The injection thus prepared is preferably filled in an appropriate ampoule.

[0093] Advantageously, the pharmaceutical compositions for oral or parenteral use described above are prepared into dosage forms in a unit dose suited to fit a dose of the active ingredients. Such dosage forms in a unit dose include, for example, tablets, pills, capsules, injections (ampoules), suppositories, etc. The amount of the aforesaid antibody contained is generally about 5 to about 500 mg per dosage form in a unit dose; especially in the form of injection, it is preferred that the aforesaid antibody is contained in about 5 to about 100 mg and in about 10 to about 250 mg for the other dosage forms.

Therapeutic Uses of the Antibodies

[0094] The antibodies of the invention are useful, *inter alia*, for the treatment, prevention and/or amelioration of any disease or disorder associated with Ang-2 activity, including diseases or disorders associated with angiogenesis. The antibodies and antigen-binding fragments of the present invention may be used to treat, *e.g.*, primary and/or metastatic tumors arising in the brain and meninges, oropharynx, lung and bronchial tree, gastrointestinal tract, male and female reproductive tract, muscle, bone, skin and appendages, connective tissue, spleen, immune system, blood forming cells and bone marrow, liver and urinary tract, and special sensory organs such as the eye. In certain embodiments, the antibodies and antigen-binding fragments of the invention are used to treat one or more of the following cancers: renal cell carcinoma, pancreatic carcinoma, breast cancer, prostate cancer, malignant gliomas, osteosarcoma,

colorectal cancer, malignant mesothelioma, multiple myeloma, ovarian cancer, small cell lung cancer, non-small cell lung cancer, synovial sarcoma, thyroid cancer, or melanoma.

[0095] The antibodies and antigen-binding fragments of the present invention may also be useful for the treatment of one or more eye disorders. Exemplary eye disorders that can be treated with the antibodies and antigen-binding fragments of the invention include, *e.g.*, age-related macular degeneration, diabetic retinopathy, and other eye disorders associated with choroidal neovascularization, vascular leak, retinal edema and inflammation. Additionally, the anti-Ang-2 antibodies of the invention may be administered as an adjuvant to glaucoma surgery to prevent early hem- and lymphangiogenesis and macrophage recruitment to the filtering bleb after glaucoma surgery, and improve clinical outcome.

[0096] In other embodiments of the present invention, the antibodies or antigen-binding fragments are used to treat hypertension, diabetes (including non insulin dependent diabetes mellitus), psoriasis, arthritis (including rheumatoid arthritis), asthma, sepsis, kidney disease and edema associated with injury, stroke or tumor.

[0097] Ang-2 expression has been shown to correlate with the severity of various inflammatory and/or infectious diseases (*see*, *e.g.*, Siner et al., 2009, Shock 31:348-353; Yeo et al., 2008, Proc. Natl. Acad. Sci. (USA):105:17097-17102). Accordingly, the anti-Ang-2 antibodies of the present invention can be used to treat, prevent or ameliorate one or more inflammatory or infectious diseases. Exemplary infectious diseases that can be treated, prevented or ameliorated by administration of the anti-Ang-2 antibodies of the invention include, but are not limited to: malaria (*Plasmodium falciparum* infection), viral hemorrhagic fevers (*e.g.*, dengue fever), rickettsial infection, toxic shock syndrome, sepsis, hepatitis C, *Bartonella bacilliformis* infection, leishmaniasis, mycobacterial infection, and Epstein-Barr virus infection.

Combination Therapies

[0098] Combination therapies may include an anti-Ang-2 antibody of the invention and, for example, another Ang-2 antagonist (*e.g.*, an anti-Ang-2 antibody, peptibody, or CovX-body such as CVX-060 (*see* US 7,521,425)). The anti-Ang-2 antibodies of the invention may also be administered together with another anti-angiogenic agent such as, *e.g.*, a VEGF antagonist (*e.g.*, a VEGF-Trap, *see*, *e.g.*, US 7,087,411 (also referred to herein as a "VEGF-inhibiting fusion protein"), anti-VEGF antibody (*e.g.*, bevacizumab), a small molecule kinase inhibitor of VEGF receptor (*e.g.*, sunitinib, sorafenib or pazopanib), an anti-DLL4 antibody (*e.g.*, an anti-DLL4 antibody disclosed in US 2009/0142354 such as REGN421), etc.), or with an antagonist of epidermal growth factor receptor (EGFR) (*e.g.*, anti-EGFR antibody or small molecule inhibitor of EGFR activity). Other agents that may be beneficially administered in combination with the anti-Ang-2 antibodies of the invention include cytokine inhibitors, including small-molecule cytokine inhibitors and antibodies that bind to cytokines such as IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, IL-9, IL-11, IL-12, IL-13, IL-17, IL-18, or to their respective receptors. The

present invention also includes therapeutic combinations comprising any of the anti-Ang-2 antibodies mentioned herein and an inhibitor of one or more of VEGF, DLL4, EGFR, or any of the aforementioned cytokines, wherein the inhibitor is an aptamer, an antisense molecule, a ribozyme, an siRNA, a peptibody, a nanobody or an antibody fragment (*e.g.*, Fab fragment; $F(ab')_2$ fragment; Fd fragment; Fv fragment; scFv; dAb fragment; or other engineered molecules, such as diabodies, triabodies, tetrabodies, minibodies and minimal recognition units). The anti-Ang-2 antibodies of the invention may also be administered in combination with antivirals, antibiotics, analgesics, corticosteroids and/or NSAIDs. The anti-Ang-2 antibodies of the invention may also be administered as part of a treatment regimen that also includes radiation treatment and/or conventional chemotherapy. When combined with one or more additional agents, the anti-Ang-2 antibodies of the invention may be administered prior to, simultaneous with (*e.g.*, in the same formulation or in separate formulations), or subsequent to the administration of the other agent(s).

Diagnostic Uses of the Antibodies

[0099] The anti-Ang-2 antibodies of the present invention may also be used to detect and/or measure Ang-2 in a sample, *e.g.*, for diagnostic purposes. For example, an anti-Ang-2 antibody, or fragment thereof, may be used to diagnose a condition or disease characterized by aberrant expression (*e.g.*, over-expression, under-expression, lack of expression, etc.) of Ang-2. Exemplary diagnostic assays for Ang-2 may comprise, *e.g.*, contacting a sample, obtained from a patient, with an anti-Ang-2 antibody of the invention, wherein the anti-Ang-2 antibody is labeled with a detectable label or reporter molecule. Alternatively, an unlabeled anti-Ang-2 antibody can be used in diagnostic applications in combination with a secondary antibody which is itself detectably labeled. The detectable label or reporter molecule can be a radioisotope, such as ^{3}H, ^{14}C, ^{32}P, ^{35}S, or ^{125}I; a fluorescent or chemiluminescent moiety such as fluorescein isothiocyanate, or rhodamine; or an enzyme such as alkaline phosphatase, ß-galactosidase, horseradish peroxidase, or luciferase. Specific exemplary assays that can be used to detect or measure Ang-2 in a sample include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluorescence-activated cell sorting (FACS).

EXAMPLES

[0100] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the methods and compositions of the invention, and are not intended to limit the scope of what the inventors regard as their invention. Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is

average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric.

Example 1. Generation of Human Antibodies to Human Ang-2

[0101] Human Ang-2 antigen was administered directly, with an adjuvant to stimulate the immune response, to a VELOCIMMUNE® mouse comprising DNA encoding human Immunoglobulin heavy and kappa light chain variable regions. The antibody immune response was monitored by an Ang-2-specific immunoassay. When a desired immune response was achieved splenocytes were harvested and fused with mouse myeloma cells to preserve their viability and form hybridoma cell lines. The hybridoma cell lines were screened and selected to identify cell lines that produce Ang-2-specific antibodies. Using this technique several anti-Ang-2 chimeric antibodies (*i.e.*, antibodies possessing human variable domains and mouse constant domains) were obtained; exemplary antibodies generated in this manner were designated as follows: H1M724, H1M727, H1M728, H2M730, H1M732, H1M737, H2M742, H2M743, H2M744, H1M749, H2M750 and H1M810.

[0102] Anti-Ang-2 antibodies were also isolated directly from antigen-positive B cells without fusion to myeloma cells, as described in U.S. 2007/0280945A1. Using this method, several fully human anti-Ang-2 antibodies (*i.e.*, antibodies possessing human variable domains and human constant domains) were obtained; exemplary antibodies generated in this manner were designated as follows: H1H685, H1H690, H1H691, H1H693, H1H694, H1H695, H1H696, H1H704, H1H706 and H1H707.

[0103] The biological properties of the exemplary anti-Ang-2 antibodies generated in accordance with the methods of this Example are described in detail in the Examples set forth below.

Example 2. Variable Gene Utilization Analysis

[0104] To analyze the structure of antibodies produced, the nucleic acids encoding antibody variable regions were cloned and sequenced. From the nucleic acid sequence and predicted amino acid sequence of the antibodies, gene usage was identified for each heavy chain variable region (HCVR) and light chain variable region (LCVR) (Table 1).

Table 1

Antibody	HCVR			LCVR		Antibody Identifier
	V_H	D_H	J_H	V_K	J_K	HCVR/LCVR SEQ ID NOs
H1H685	3-13	3-16	4	3-20	1	2/10
H1H690	3-23	4-4	3	3-11	4	26/34
H1H691	3-9	4-17	6	3-20	4	50/58
H1H693	3-23	4-4	3	1-12	1	74/82
H1H694	3-15	6-6	4	1-5	1	98/106

H1H695	3-33	5-12	6	3-15	5	122/130
H1H696	3-11	4-17	4	1-16	4	146/154
H1H704	3-33	6-6	4	1-16	5	170/178
H1H706	3-33	3-3	3	1-16	1	194/202
H1H707	3-33	3-3	3	3-20	4	218/226
H1M724	3-33	3-3	5	1-17	4	266/274
H1M727	1-18	3-3	6	2-28	2	338/346
H1M728	3-7	6-19	4	1-5	1	290/298
H2M730	3-7	6-13	4	1-5	1	362/370
H1M732	3-15	1-7	4	1-17	3	242/250
H2M742	3-23	5-5	5	2-28	4	386/394
H2M743	3-23	2-8	4	1-12	4	410/418
H2M744	1-18	4-4	5	1-12	4	434/442
H1M749	3-33	5-5	4	3-15	1	314/322
H2M750	3-33	6-6	4	1-16	4	458/466
H1M810	3-23	3-3	3	1-12	1	482/490

[0105] Table 2 sets forth the heavy and light chain variable region amino acid sequence pairs of selected anti-Ang-2 antibodies and their corresponding antibody identifiers. The N, P and G designations refer to antibodies having heavy and light chains with identical CDR sequences but with sequence variations in regions that fall outside of the CDR sequences (*i.e.*, in the framework regions). Thus, N, P and G variants of a particular antibody have identical CDR sequences within their heavy and light chain variable regions but contain modifications within the framework regions.

Table 2

Name	HCVR/LCVR SEQ ID NOs	Name	HCVR/LCVR SEQ ID NOs	Name	HCVR/LCVR SEQ ID NOs
H1H685N	2/10	**H1H685P**	18/20	**H1H685G**	22/24
H1H690N	26/34	**H1H690P**	42/44	**H1H690G**	46/48
H1H691N	50/58	**H1H691P**	66/68	**H1H691G**	70/72
H1H693N	74/82	**H1H693P**	90/92	**H1H693G**	94/96
H1H694N	98/106	**H1H694P**	114/116	**H1H694G**	118/120
H1H695N	122/130	**H1H695P**	138/140	**H1H695G**	142/144
H1H696N	146/154	**H1H696P**	162/164	**H1H696G**	166/168
H1H704N	170/178	**H1H704P**	186/188	**H1H704G**	190/192
H1H706N	194/202	**H1H706P**	210/212	**H1H706G**	214/216
H1H707N	218/226	**H1H707P**	234/236	**H1H707G**	238/240
H1M724N	266/274	**H1M724P**	282/284	**H1M724G**	286/288
H1M727N	338/346	**H1M727P**	354/356	**H1M727G**	358/360
H1M728N	290/298	**H1M728P**	306/308	**H1M728G**	310/312
H2M730N	362/370	**H2M730P**	378/380	**H2M730G**	382/384

H1M732N	242/250	**H1M732P**	258/260	**H1M732G**	262/264
H1M737N	506/X*	**H1M737P**	514/X*	**H1M737G**	516/X*
H2M742N	386/394	**H2M742P**	402/404	**H2M742G**	406/408
H2M743N	410/418	**H2M743P**	426/428	**H2M743G**	430/432
H2M744N	434/442	**H2M744P**	450/452	**H2M744G**	454/456
H1M749N	314/322	**H1M749P**	330/332	**H1M749G**	334/336
H2M750N	458/466	**H2M750P**	474/476	**H2M750G**	478/480
H1M810N	482/490	**H1M810P**	498/500	**H1M810G**	502/504

* The amino acid sequence of the LCVR of H1M737 is not shown.

Control Constructs Used in the Following Examples

[0106] Various control constructs (anti-Ang-2 antibodies and anti-Ang-2 peptibodies) were included in the following experiments for comparative purposes. The control constructs are designated as follows: Control I: a human anti-Ang-2 antibody with heavy and light chain variable domains having the amino acid sequences of the corresponding domains of "Ab536(THW)," as set forth in US 2006/0018909 (*see also* Oliner et al., 2004, Cancer Cell 6:507-516); Control II: a peptibody that binds human Ang-2 having the amino acid sequence of "2XCon4(C)," as set forth in US 7,205,275, (*see also* Oliner et al., 2004, Cancer Cell 6:507-516); Control III: a peptibody that binds human Ang-2 having the amino acid sequence of "L1-7," as set forth in US 7,138,370; Control IV: a human anti-Ang-2 antibody with heavy and light chain variable regions having the amino acid sequences of the corresponding domains of "3.19.3" as set forth in US 2006/0246071; and Control V: a human anti-Ang-2 antibody with heavy and light chain variable regions having the amino acid sequences of the corresponding domains of "MEDI1/5" as set forth in WO 2009/097325. (Not all control constructs were used in every Example). In the tables that follow, the notations "Ab" and "Pb" are included to identify antibody and peptibody controls, respectively (*i.e.*, Control 1 = Ab; Control II = Pb; Control III = Pb; Control IV = Ab; and Control V = Ab).

Example 3. Antigen Binding Affinity Determination.

[0107] Equilibrium dissociation constants (K_D values) for the binding of selected purified Ang-2 antibodies to dimeric fibrinogen-like domain of human (SEQ ID NO: 519), mouse (*Mus musculus*; SEQ ID NO: 520) and monkey (*Macca fascicularis;* SEQ ID NO: 521) Ang-2 (Ang-2FD) conjugated to human IgG1 (SEQ ID NO:528) were determined by surface kinetics using a real-time biosensor surface plasmon resonance assay. Antibody was captured on a goat anti-mouse IgG polyclonal antibody surface, a goat anti-human κ polyclonal antibody (Southern Biotech, Birmingham, AL) surface or a goat anti-human IgG polyclonal antibody (Jackson Immuno Research Lab, West Grove, PA) surface created through direct amine coupling to a BIACORE™ CM5 sensor chip to form a captured antibody surface. Varying concentrations (ranging from 50nM to 6.25nM) of protein were injected at 100µl/min over captured antibody

surface for 90 seconds. Antigen-antibody binding and dissociation were monitored in real time at room temperature. Kinetic analysis was performed to calculate K_D and half-life of antigen/antibody complex dissociation. The results are summarized in Table 3 below.

Table 3

Antibody	Dimeric Human Ang-2FD		Dimeric Mouse Ang-2FD		Dimeric Monkey Ang-2FD	
	K_D (pM)	$T_{1/2}$ (min)	K_D (pM)	$T_{1/2}$ (min)	K_D (pM)	$T_{1/2}$ (min)
H1M724N	179	42.7	694	16	730	25.7
H1M728N	137	58.4	5650	9.9	1580	69.5
H2M730N	210	47	-	-	842	36.6
H1M732N	484	35.5	1700	21.4	7330	24.1
H1M737N	251	34.5	1740	6.3	3810	16
H2M742N	295	38	610	30.8	6170	28.5
H2M743N	154	167	882	195.2	234	169.2
H2M744N	98.9	109.1	143	223.1	500	281.7
H2M749N	165	42.9	529	25.5	1500	40.9
H2M750N	362	32.2	-	-	1470	23

[0108] The above experiment was repeated using selected purified anti-Ang-2 antibodies cloned onto human IgG1. The results are summarized in Table 4 below.

Table 4

Antibody	Dimeric Human Ang-2FD		Dimeric Mouse Ang-2FD		Dimeric Monkey Ang-2FD	
	K_D (pM)	$T_{1/2}$ (min)	K_D (pM)	$T_{1/2}$ (min)	K_D (pM)	$T_{1/2}$ (min)
H1H685P	71.4	229.4	148	128.7	99.4	177.1
H1H690P	79	126.1	91.3	105.2	55.6	195.2
H1H691P	220	38.5	220	43.8	290	41
H1H693P	500	37.1	446	63.7	1170	17.6
H1H694P	126	265.6	237	166.5	356	85.6
H1H695P	245	147	347	124.2	440	84.1
H1H696P	289	38.8	402	37.6	354	36.6
H1H704P	331	86.1	484	61.9	818	33.5
H1H706P	201	50.4	357	47	164	53.3
H1H707P	262	26.6	328	34.4	283	22.3
H1H724N	115	107	185	84	239	173
H1H728N	162	81	5760	20	2000	77
H1H730N	234	62	97.1	90	3400	87
H1H732N	386	57	529	51	439	118
H1H742N	186	65	276	58	683	93
H1H743N	88.2	254	124	233	96.5	780
H1H744N	114	127	158	115	346	164

H1H749N	118	109	177	96	407	143
H1H750N	164	127	218	121	199	244
Control I (Ab)	339	34.8	339	47.1	537	27.1

[0109] Additional binding experiments were conducted using selected anti-Ang-2 antibodies at two different temperatures to further assess cross-species affinity. Each selected antibody or control construct was captured at a flow rate of 40 µL/min for 1 minute on a goat anti-human kappa polyclonal antibody surface created through direct chemical coupling to a BIACORE™ chip to form a captured antibody surface. Human, monkey and mouse Ang-2FD-Fc at a concentration of 25 nM or 0.78 nM was injected over the captured antibody surface at a flowrate of 60 µL/min for 3 minutes, and antigen-antibody dissociation was monitored in real time for 20 minutes at either 25°C or 37°C.

[0110] Results are summarized in Tables 5 (25°C binding) and 6 (37°C binding) below.

Table 5

Binding at 25°C

Antibody	**Dimeric Human Ang-2FD-mFc**		**Dimeric Mouse Ang-2FD-hFc**		**Dimeric Monkey Ang-2FD-hFc**	
	K_D (Molar)	**$T_{1/2}$ (min)**	**K_D (Molar)**	**$T_{1/2}$ (min)**	**K_D (Molar)**	**$T_{1/2}$ (min)**
H1H685P	1.17E-11	227	6.51E-11	208	2.20E-11	275
H1H744N	1.16E-10	23	3.85E-10	33	2.44E-10	24
Control I (Ab)	1.07E-09	15	1.07E-09	15	1.03E-09	4
Control IV (Ab)	1.27E-11	269	4.02E-11	289	1.55E-11	342

Table 6

Binding at 37°C

Antibody	**Dimeric Human Ang-2FD-mFc**		**Dimeric Mouse Ang-2FD-hFc**		**Dimeric Monkey Ang-2FD-hFc**	
	K_D (Molar)	**$T_{1/2}$ (min)**	**K_D (Molar)**	**$T_{1/2}$ (min)**	**K_D (Molar)**	**$T_{1/2}$ (min)**
H1H685P	2.70E-11	60	9.39E-11	64	7.21E-11	65
H1H744N	1.05E-10	18	2.15E-10	26	3.20E-10	11
Control I (Ab)	---	---	3.90E-10	12	---	---
Control IV (Ab)	9.91E-12	184	5.40E-11	119	4.74E-11	107

[0111] In another experiment, K_D values for selected purified antibodies that bind to a human "bow-Ang-2" tetrameric construct ("hBA2") were determined (using the methods described above). hBA2 consists of two dimers, each dimer containing two Ang-2 fibronectin-like domains connected to one another by a human Fc domain. The amino acid sequence of the dimer constituents of hBA2 is represented by SEQ ID NO:522. The results are summarized in Table 7 below.

Table 7

Antibody	hBA2	
	K_D (pM)	$T_{1/2}$ (min)
H1H685P	11.9	587.2
H1H690P	17.9	299.3
H1H691P	106	50.6
H1H693P	299	28.7
H1H694P	68.4	111.3
H1H695P	40.1	254.3
H1H696P	111	51.5
H1H704P	93.9	117.7
H1H706P	79.1	63.9
H1H707P	75.2	51.4
H1H724N	23.3	323
H1H728N	41.8	185
H1H730N	55.9	152
H1H732N	132	73
H1H742N	72.1	87
H1H743N	9.71	1118
H1H744N	17.2	442
H1H749N	32.5	235
H1H750N	36.9	284
Control I (Ab)	83	57.5

[0112] In yet another experiment, K_D values for selected purified antibodies that bind to wild-type human Ang-2 (hAng-2-WT; SEQ ID NO: 518) and the fibrinogen-like domain of human Ang2 (hAng-2FD) were determined (as described above). The results are summarized in Table 8 below.

Table 8

Antibody	Monomeric hAng-2FD		hAng-2-WT	
	K_D (nM)	$T_{1/2}$ (min)	K_D (pM)	$T_{1/2}$ (min)
H1M724N	1.75	17.4	33.1	568
H1M728N	1.17	33.9	33.8	725
H2M730N	2.06	24.4	49.2	519
H1M732N	6.13	18.7	131	333
H1M737N	2.82	13.1	59.3	282
H2M742N	4.81	18.0	67.9	437
H2M743N	0.399	156.7	14.3	2366
H2M744N	0.475	89.3	28.9	846

H2M749N	1.38	27.9	49	479
H2M750N	4.42	21.5	40.8	991
H1H685P	0.578	55	47.6	1000
H1H691P	11	0.57	19.1	684.6
H1H690P	0.594	25.16	12.4	1568
H1H693P	44.8	0.61	425	100
H1H694P	7.89	9.85	158	209.7
H1H695P	1.12	50.59	31.1	1770.7
H1H696P	38.4	0.20	40.3	642.7
H1H704P	0.39	3.31	36.2	747.6
H1H706P	11	1.02	27.4	661.9
H1H707P	145	-	77.1	217.4
H1H724N	2.4	13.34	22.6	895
H1H728N	1.18	5.86	43	566
H1H730N	2.84	3.44	47.5	534
H1H732N	264	0.22	202	264
H1H742N	486	2.29	44.9	666
H1H743N	2.35	33.03	9.48	3927
H1H744N	1.02	42.14	30.8	837
H1H749N	1.13	33.48	12.5	1833
H1H750N	0.787	30.20	9.5	4442
Control I (Ab)	44.5	0.03	47.6	512
Control II (Pb)	90	-	44.7	334.8

[0113] Additional experiments were conducted to measure the binding properties of selected anti-Ang-2 antibodies to monomeric hAng-2FD at 25°C and 37°C. Each selected antibody or control construct was captured at a flow rate of 40 µL/min for 1 minute on a goat anti-human IgG polyclonal antibody surface created through direct chemical coupling to a BIACORE™ chip to form a captured antibody surface. Human Ang-2FD at a concentration of 500 nM or 7.8 nM was injected over the captured antibody surface at a flowrate of 60 µL/min for 3 minutes, and antigen-antibody dissociation was monitored in real time for 20 minutes at either 25°C or 37°C.

[0114] Results are summarized in Tables 9 (25°C) and 10 (37°C) below. N/D = not determined.

Table 9

Binding to monomeric hAng-2FD at 25°C				
	ka (Ms^{-1})	kd (s^{-1})	K_D (Molar)	T½
H1H685P	2.44E+05	7.96E-05	3.36E-10	145 minutes
H1H744N	2.92E+05	1.24E-04	4.24E-10	93 minutes
Control I (Ab)	4.00E+05	5.10E-02	1.28E-07	14 seconds
Control II (Pb)	*steady-state*	*steady-state*	9.00E-08	*steady-state*
Control III (Pb)	5.40E+05	6.30E-02	1.17E-07	11 seconds

Control IV (Ab)	2.84E+05	3.56E-02	1.25E-07	19 seconds

Table 10

Binding to monomeric hAng-2FD at 37°C				
	ka (Ms^{-1})	kd (s^{-1})	K_D (Molar)	T½
H1H685P	4.06E+05	1.39E-04	3.42E-10	83 minutes
H1H744N	3.86E+05	5.48E-04	1.42E-09	21 minutes
Control I (Ab)	*steady-state*	*steady-state*	1.51E-07	*steady-state*
Control II (Pb)	N/D	N/D	N/D	N/D
Control III (Pb)	*steady-state*	*steady-state*	2.94E-07	*steady-state*
Control IV (Ab)	*steady-state*	*steady-state*	9.40E-08	*steady-state*

[0115] As shown in this Example, several of the anti-Ang-2 antibodies generated in accordance with the methods of Example 1 bound to Ang-2 constructs with equivalent or higher affinities than the controls. For example, antibodies H1H685, H1H690, H1H724 and H1H744 bound to dimeric human Ang-2-FD with K_D's of 71.4, 79, 115, and 114 pM, respectively, whereas Control I antibody bound to dimeric human Ang-2-FD with a K_D of 339 pM (*see* Table 4). Similarly, antibodies H1H685, H1H690, H1H724 and H1H744 bound to human BA2 (a tetrameric Ang-2 fibrinogen-like domain construct) with K_D's of 11.9, 17.9, 23.3 and 17.2 pM, respectively, whereas Control I antibody bound to hBA2 with a K_D of 83 pM (*see* Table 7). Thus, as compared to the control constructs, many of the antibodies of the invention exhibit enhanced binding to Ang-2. Antibody H1H685P showed especially robust binding properties to Ang-2 as compared to the control constructs.

Example 4. Preferential Binding to Ang-2 Over Ang-1

[0116] Binding experiments (plasmon resonance assays) were conducted to ascertain whether selected antibodies bound to both Ang-2 and Ang-1 or if they preferentially bound to Ang-2 only. Each selected antibody or control construct was captured at a flow rate of 40 µL/min for 1 minute on a goat anti-human IgG polyclonal antibody surface created through direct chemical coupling to a BIACORE™ chip to form a captured antibody surface. Full-length wild-type human Ang-1 or Ang-2 at a concentration of 25 nM or 0.78 nM were injected over the captured antibody surface at a flowrate of 60 µL/min for 3 minutes, and antigen-antibody dissociation was monitored in real time for 20 minutes at either 25°C or 37°C.

[0117] The results of these experiments are summarized in Tables 11 - 14 below. N/D = not determined. "No binding" means that no detectable binding was observed under the particular experimental conditions used in these experiments.

Table 11a

Binding to hAng-2-WT at 25°C				
	ka (Ms^{-1})	kd (s^{-1})	K_D (Molar)	T½ (minutes)
H1H685P	6.59E+05	1.60E-05	2.42E-11	722
H1H744N	7.65E+05	2.57E-05	3.35E-11	450
Control I (Ab)	4.74E+05	2.26E-05	4.76E-11	512
Control II (Pb)	7.73E+05	3.45E-05	4.47E-11	335
Control III (Pb)	3.29E+05	1.98E-05	6.01E-11	584
Control IV (Ab)	3.80E+06	2.74E-04	7.22E-11	42

Table 11b

Binding to hAng-2-WT at 25°C				
	ka (Ms^{-1})	kd (s^{-1})	K_D (Molar)	T½ (minutes)
H1H685P	1.15E+05	8.50E-06	7.39E-11	1359
Control II (Pb)	8.30E+04	5.41E-05	6.52E-10	213
Control V (Ab)	1.12E+05	2.66E-05	2.73E-10	434

Table 12a

Binding to hAng-1-WT at 25°C				
	ka (Ms^{-1})	kd (s^{-1})	K_D (Molar)	T½ (minutes)
H1H685P	No binding	No binding	No binding	No binding
H1H744N	4.10E+05	3.81E-05	9.30E-11	303
Control I (Ab)	4.55E+05	2.49E-05	5.47E-11	464
Control II (Pb)	4.53E+05	3.54E-05	7.82E-11	326
Control III (Pb)	No binding	No binding	No binding	No binding
Control IV (Ab)	6.60E+05	1.11E-04	1.68E-10	105

Table 12b

Binding to hAng-1-WT at 25°C				
	ka (Ms^{-1})	kd (s^{-1})	K_D (Molar)	T½ (minutes)
H1H685P	No binding	No binding	No binding	No binding
Control II (Pb)	3.04E+05	2.51E-05	8.26E-11	460
Control V (Ab)	2.75E+05	6.68E-05	2.43E-10	173

Table 13a

Binding to hAng-2-WT at 37°C				
	ka (Ms^{-1})	kd (s^{-1})	K_D (Molar)	T½ (minutes)
H1H685P	8.54E+05	3.76E-05	4.40E-11	707
H1H744N	7.01E+05	2.43E-04	3.47E-10	48

Table 13b

Binding to hAng-2-WT at 37°C				
	ka (Ms^{-1})	kd (s^{-1})	K_D (Molar)	T½ (minutes)
H1H685P	1.36E+05	2.16E-05	1.59E-10	535
Control II (Pb)	3.79E+04	1.17E-04	3.09E-09	99
Control V (Ab)	9.42E+04	7.92E-05	8.41E-10	146

Table 14a

Binding to hAng-1-WT at 37°C				
	ka (Ms^{-1})	kd (s^{-1})	K_D (Molar)	T½ (minutes)
H1H685P	No binding	No binding	No binding	No binding
H1H744N	1.47E+06	5.20E-05	3.12E-11	222
Control III (Pb)	No binding	No binding	No binding	No binding

Table 14b

Binding to hAng-1-WT at 37°C				
	ka (Ms^{-1})	kd (s^{-1})	K_D (Molar)	T½ (minutes)
H1H685P	No binding	No binding	No binding	No binding
Control II (Pb)	2.81E+05	4.35E-05	1.55E-10	266
Control V (Ab)	4.42E+05	5.47E-05	1.24E-10	211

[0118] These results show that H1H685P is unique among the antibodies tested in this experiment in that it binds with high affinity to Ang-2 but does not bind to Ang-1. The only other construct that exhibits binding to Ang-2 but not to Ang-1 is Control III. It should be emphasized, however, that Control III is a peptibody and that all of the other antibodies tested in this experiment bound to both Ang-2 and Ang-1. The selectivity for Ang-2 binding may confer therapeutic benefits on H1H685P that are not possessed by antibodies that bind to both Ang-2 and Ang-1.

Example 5. Inhibition of Ang-2 Binding to Human Tie-2

[0119] Tie-2 is a natural receptor for Ang-2. Anti-Ang-2 antibodies were tested for their ability to block Ang-2 binding to human Tie-2 (hTie-2). hTie-2-mFc (a chimeric construct consisting of human Tie-2 conjugated to mouse IgG; SEQ ID NO:525) was coated onto 96-well plates at a concentration of 2μg/ml and incubated overnight followed by washing four times in wash buffer (PBS with 0.05% Tween-20). The plate was then blocked with PBS (Irvine Scientific, Santa Ana, CA) containing 0.5% BSA (Sigma-Aldrich Corp., St. Louis, MO) for one hour at room temperature. In a separate plate, purified anti-Ang-2 antibodies, at a starting concentration of 50nM, were serially diluted by a factor of three across the plate. Human, mouse or monkey Ang-2FD protein conjugated to human IgG (Ang-2FD-hFc) were added to final concentrations of 2nM, 8nM, or 2nM respectively and incubated for one hour at room temperature. The

antibody/Ang-2FD-Fc mixture was then added to the plate containing hTie-2-mFc and incubated for one hour at room temperature. Detection of Ang-2FD-hFc bound to hTie-2-mFc protein was determined with Horse-Radish Peroxidase (HRP) conjugated to a-human IgG antibody (Jackson Immuno Research Lab, West Grove, PA) and developed by standard colorimetric response using tetramethylbenzidine (TMB) substrate (BD Biosciences, San Jose, CA). Absorbance was read at OD_{450} for 0.1sec. Percent blocking of Ang-2FD-hFc binding to hTie-2-mFc by 16.67nM of selected anti-Ang-2 antibodies is shown in Table 15.

Table 15

Antibody	Percent Blocking of Ang-2FD Binding to Tie-2		
	Human Ang-2FD-hFc	Mouse Ang-2FD-hFc	Monkey Ang-2FD-hFc
H1M724N	99.5	96.6	95.2
H1M728N	98.5	83.9	97.1
H2M730N	98.9	55.0	97.3
H1M732N	97.7	90.9	95.8
H1M737N	99.1	95.4	90.5
H2M742N	99.6	98.6	94.1
H2M743N	99.6	98.4	95.1
H2M744N	99.5	98.4	95.5
H2M749N	99.5	97.3	97.4
H2M750N	99.4	53.7	97.4
Control I (Ab)	94.5	90.2	96.9

[0120] In a similar experiment, selected purified anti-Ang-2 antibodies cloned onto human IgG1 were tested for their ability to block Ang-2FD binding to hTie-2 (as described above). Percent blocking of Ang-2FD-hFc binding to hTie-2-mFc by 16.67nM of selected anti-Ang-2 antibodies is shown in Table 16. NT: not tested.

Table 16

Antibody	Percent Blocking of Ang-2FD Binding to Tie-2		
	Human Ang-2FD-hFc	Mouse Ang-2FD-hFc	Monkey Ang-2FD-hFc
H1H685P	93.8	97.1	62.2
H1H690P	97.2	98.0	99.6
H1H691P	97.4	96.7	99.8
H1H693P	73.9	63.6	NT
H1H694P	79.8	36.0	NT
H1H695P	98.4	97.6	NT
H1H696P	98.2	94.9	99.2
H1H704P	97.0	41.8	NT

H1H706P	97.1	95.9	99.8
H1H707P	95.1	93.8	NT
H1H724N	96.6	97.1	96.6
H1H744N	97.9	97.6	96.2
Control I (Ab)	97.3	82.2	98.5

[0121] In another experiment, selected purified anti-Ang-2 antibodies were tested for their ability to block binding of 20pM biotinylated hBA2 to hTie-2 (as described above). For this experiment, human Tie-2 conjugated to a histidine tag (hTie-2-His; SEQ ID NO:526) was used in a similar fashion to the hTie-2-mFc described above. Antibody concentrations from 5nM were serially diluted three-fold. An IC_{50} (Inhibitory Concentration) value was generated by calculating the amount of antibody required to block 50% of the signal from the binding of biotin-hBA2 to Tie-2. An average IC_{50} value for each antibody was calculated based on two separate experiments. The results are summarized in Table 17. NB: no blocking observed at 5nM concentration.

Table 17

Antibody	**Biotin - hBA2**
	Average IC_{50} (pM)
H1M724N	9.72
H1M728N	14.05
H2M730N	14.60
H1M732N	82.17
H1M737N	13.01
H2M742N	9.65
H2M743N	11.01
H2M744N	11.43
H2M749N	6.43
H2M750N	8.83
Control I (Ab)	30.23
Control II (Pb)	7.75
Control III (Pb)	16.49

[0122] In a similar experiment, selected purified anti-Ang-2 antibodies cloned onto human IgG1 were tested for their ability to block binding of biotinylated hBA2 to hTie-2 (as described above). The results are shown in Table 18. NB: no blocking observed at 5nM concentration.

Table 18

Antibody	Biotin - hBA2
	IC_{50} (pM)
H1H685P	20
H1H690P	17
H1H691P	13
H1H693P	NB
H1H694P	NB
H1H695P	59
H1H696P	22
H1H704P	56
H1H706P	8
H1H707P	22
H1H724N	4
H1H744N	25

[0123] This Example illustrates that several of the anti-Ang-2 antibodies generated in accordance with the methods of Example 1 blocked the interaction between the Ang-2 fibrinogen-like domain and its receptor (TIE-2) to an equivalent or greater extent than the control antibody. For example, antibodies H1H690, H1H691, H1H695, H1H696, H1H704, H1H706, H1H707, H1H724 and H1H744 each caused greater than 95% blocking of human, mouse and monkey Ang-2FD constructs to the TIE-2 receptor, similar to the results observed with the control constructs (*see* Table 16).

Example 6: Inhibition of Full-Length Ang-2 and Ang-1 Binding to Human Tie-2

[0124] Tie-2 is a receptor for Ang-1 as well as Ang-2. Therefore, in the present Example, the ability of certain anti-Ang-2 antibodies to block binding of Ang-2 or Ang-1 to human Tie-2 was measured and compared.

[0125] The ELISA experiments shown in this Example were conducted in a similar manner to the experiments of Example 5. Briefly, hTie-2-mFc (a chimeric construct consisting of human Tie-2 conjugated to mouse IgG; SEQ ID NO:525) was coated onto 96-well plates at a concentration of 2μg/ml and incubated overnight followed by washing four times in wash buffer (PBS with 0.05% Tween-20). The plate was then blocked with PBS (Irvine Scientific, Santa Ana, CA) containing 0.5% BSA (Sigma-Aldrich Corp., St. Louis, MO) for one hour at room temperature. In a separate plate, purified anti-Ang-2 antibodies and control constructs, at a starting concentration of 300nM, were serially diluted by a factor of three across the plate. Full-length human Ang-2 or Ang-1 protein conjugated to 6X histidine tag (R&D Systems, Minneapolis, MN) were added to a final concentration of 0.6nM and incubated for one hour at room temperature. The antibody/antigen mixture was then added to the plate containing hTie-2-mFc and incubated for one hour at room temperature. Detection of Ang-2-His or Ang-1-His

bound to hTie-2-mFc protein was determined with Horse-Radish Peroxidase (HRP) conjugated to a-Penta-His antibody (Qiagen, Valencia, CA) and developed by standard colorimetric response using tetramethylbenzidine (TMB) substrate (BD Biosciences, San Jose, CA). Absorbance was read at OD_{450} for 0.1sec. An IC_{50} (Inhibitory Concentration) value was generated by calculating the amount of antibody required to block 50% of the signal from the binding of human Ang-2 or Ang-1 to Tie-2. The results, expressed in terms of IC_{50} are shown in Table 19, columns (1) and (2). The extent to which the antibodies or control constructs block the hAng-2/Tie-2 interaction relative to the hAng-1/Tie-2 interaction is reflected in the fold difference in IC_{50} shown in column (3); that is, a higher number in column (3) indicates a greater capacity to block the hAng-2/Tie-2 interaction than the hAng-1/Tie-2 interaction.

Table 19

Antibody	(1) Blocking hAng-2 WT to Tie-2 IC_{50} (M)	(2) Blocking hAng-1 WT to Tie-2 IC_{50} (M)	(3) Fold Difference in hAng-1 Blocking IC_{50} Compared to h-Ang-2 Blocking IC_{50}*
H1H685P	1.294E-10	> 3.000E-07	> 2318
H1H744N	7.871E-11	1.872E-07	2378
Control I (Ab)	9.372E-11	6.171E-08	658
Control II (Pb)	3.096E-11	5.509E-11	1.8
Control III (Pb)	1.626E-10	> 1.000E-06	> 6150
Control IV (Ab)	1.476E-10	4.252E-09	28.8

* Calculated by dividing the hAng-1 blocking IC_{50} (column 2) by the hAng-2 blocking IC_{50} (column 1).

[0126] In an effort to further assess the ability of selected anti-hAng-2 antibodies to block the binding of Ang-1 to Tie-2, a biosensor surface plasmon resonance experiment was conducted. In this experiment, a human Tie-2 full-length extracellular domain construct (hTie-2-mFc-ecto) was amine-coupled on a BIACORE™ chip to create a receptor coated surface. Selected anti-hAng-2 antibodies and control constructs, at 1 μM (100-fold excess over antigen), were premixed with 10 nM of hAng-1-WT, followed by 60 minutes incubation at 25°C to allow antibody-antigen binding to reach equilibrium to form equilibrated solutions. The equilibrated solutions were injected over the receptor surfaces at 5 μL/min for 5 minutes at 25°C. Changes in resonance units (RU) due to the binding of the hAng-1-WT to hTie-2-mFc were determined. An irrelevant peptibody construct with no binding to hAng-1 was included in this experiment to establish the 0% blocking baseline, and a human Tie-2-mFc construct was used as a positive control for blocking. The amount of Ang-1 bound to Tie-2 following antibody preincubation, expressed as a percentage of the amount of Ang-1 bound to Tie-2 following negative control

preincubation, is shown in Table 20. (A greater amount of Ang-1 binding to Tie-2 signifies a lower degree of antibody blocking).

Table 20

Antibody	RU (average)	Percent of Negative Control Binding
Negative Control (irrelevant peptibody)	169	100
hTie-2-mFc	71	42
H1H685P	137	81
H1H744N	57	34
H1H691P	117	69
H1H706P	140	83
H1H724N	57	34
Control I (Ab)	48	28
Control II (Pb)	48	28
Control III (Pb)	160	95

[0127] The foregoing experiment was repeated using different amounts of Ang-2 blockers and controls. In particular, a human Tie-2 full-length extracellular domain construct (hTie-2-mFc-ecto) was amine-coupled on a BIACORE™ chip to create a receptor-coated surface. Selected anti-hAng-2 antibodies and control constructs (50 or 150 nM) were mixed with hAng-2-WT (25 nM) followed by 60 minutes incubation at 25°C to allow antibody-antigen binding to reach equilibrium. The equilibrated solutions were injected over the receptor surfaces at 10 µL/min for 5 minutes at 25°C. To evaluate the ability of the selected anti-hAng-2 antibodies to block Ang-1-WT binding to hTie-2, a similar procedure was followed except the antibodies were tested at three concentrations (50, 100 or 1000 nM) and incubated with 10 nM of hAng-1-WT. Changes in resonance units (RU) due to the binding of the Ang-2-WT or hAng-1-WT to hTie-2-mFc were determined. An irrelevant antibody with no binding to either angiopoietin was included in these experiments to establish the 0% blocking baseline, and a human Tie-2-mFc construct was used as a positive control for blocking. Results are summarized in Tables 21 (hAng-1 applied to a hTie-2 surface) and 22 (hAng-2 applied to a hTie-2 surface).

Table 21 (hAng-1 WT)

	Amount of Antibody or Control					
	50 nM		100 nM		1000 nM	
Antibody	Specific Bound RU	Percent of Neg. Ctrl Binding	Specific Bound RU	Percent of Neg. Ctrl Binding	Specific Bound RU	Percent of Neg. Ctrl Binding
Negative Control (irrelevant antibody)	316	100	307	100	276	100
hTie-2-mFc	70	22	39	13	-47	0
H1H685P	299	95	291	95	289	105
Control II (Pb)	8	2.5	4	1.3	-1	0
Control V (Ab)	150	48	114	37	29	11

Table 22 (hAng-2 WT)

	Amount of Antibody or Control			
	50 nM		150 nM	
Antibody	Specific Bound RU	Percent of Neg. Ctrl Binding	Specific Bound RU	Percent of Neg. Ctrl Binding
Negative Control (irrelevant antibody)	281	100	278	100
hTie-2-mFc	97	35	82	30
H1H685P	12	4.3	12	4.3
Control II (Pb)	10	3.6	10	3.6
Control V (Ab)	12	4.3	12	4.3

[0128] The results obtained from these experiments are in agreement with previous results which showed that H1H685P preferentially binds to Ang-2 over Ang-1 (see Example 4). In particular, the results from this Example show that several anti-Ang-2 antibodies (*e.g.*, H1H685P and H1H706P) do not significantly block the binding of human Ang-1 to human Tie-2, even though, in other experiments, it was demonstrated that these antibodies potently blocked the interaction between Ang-2 and Tie-2 (see Example 5, Table 16). Moreover, in these experiments none of the control constructs, except for the Control III peptibody, exhibited the same degree of preferential binding/blocking of Ang-2 over Ang-1 as the exemplary anti-Ang-2 antibodies of the present invention, such as H1H685P.

Example 7. Inhibition of Ang-2-Mediated Tie-2 Phosphorylation by anti-Ang-2 Antibodies

[0129] The inventors of the present invention have demonstrated that Ang-2 expression can be induced in human umbilical vein endothelial cells (HUVECs) by the transcription factor FOXO1 (Daly et al. 2006 PNAS 103:15491). Further, the inventors have shown that infection of HUVECs with an adenovirus encoding FOXO1 results in expression and secretion of Ang-2, followed by activation of Tie-2 phosphorylation (Daly et al. 2006 PNAS 103:15491).

[0130] Anti-Ang-2 antibodies were tested for their ability to inhibit Tie-2 phosphorylation. Briefly, $7x10^5$ HUVECs (Vec Technologies, Rensselaer, NY) were plated in 6cm cell culture

dishes in 3.5ml of MCDB131 Complete medium (Vec Technologies, Rensselaer, NY). The following day, the cells were washed with Opti-MEM (Invitrogen Corp., Carlsbad, CA) and fed with 2ml of Opti-MEM. Recombinant adenoviruses encoding either green fluorescent protein (GFP; control) or human FOXO1 (Daly et al. 2004 Genes Dev. 18:1060) were added to the cells at a concentration of 10 pfu/cell and incubated for four hours. Cells were then washed with MCDB131 and fed with 2ml of MCDB131 containing anti-Ang-2 antibodies at a concentration of 0.5µg/ml. At twenty hours post infection, cells were lysed and subjected to Tie-2 immunoprecipitation as described by Daly et al., *Proc. Natl. Acad. Sci. USA 103*:15491-15496 (2006). Immunoglobulin was collected on protein A/G beads (Santa Cruz Biotechnology, Santa Cruz, CA) for one hour. Beads were washed with cold lysis buffer and resuspended in SDS sample buffer for analysis by western blot with antibodies specific for phosphotyrosine (Millipore, Billerica, MA) or Tie-2. Signals were detected using HRP-conjugated secondary antibodies and ECL reagents (GE Healthcare, Piscataway, NJ). X-Ray films were scanned and the phospho-Tie-2 and Tie-2 signals were quantified using ImageJ software. The phospho-Tie-2/Tie-2 ratios were used to determine the % inhibition for each anti-Ang-2 antibody (i.e. Percent inhibition = Reduction in phospho-Tie-2/Tie-2 as compared to control). For example, a reduction in Tie-2 phosphorylation to the level observed in the control sample is considered to be 100% inhibition. Relative inhibition (+, ++, +++) for each anti-Ang-2 antibody tested according to the percent inhibition observed (25-50%, 50-75%, 75-100%, respectively) is shown in Table 23.

Table 23

Antibody	Inhibition of Tie-2 phosphorylation
H1H685P	+++
H1H690P	+++
H1H691P	+++
H1H693P	+++
H1H694P	++
H1H695P	+++
H1H696P	+++
H1H704P	+++
H1H706P	+++
H1H707P	+++
H1M724N	+++
H1M728N	++
H1M732N	++
H1M742N	++
H1M743N	+++
H1M744N	+++

H1M749N	++
H1M750N	+++
Control I (Ab)	+
Control II (Pb)	+++

[0131] As demonstrated in this Example, the anti-Ang-2 antibodies generated in accordance with the methods of Example 1 inhibited Tie-2 phosphorylation to a greater extent than the Control I antibody. Especially robust inhibition was observed with antibodies H1H685, H1H690, H1H691, H1H693, H1H695, H1H696, H1H704, H1H706, H1H707, H1M724, H1M744 and H1M750.

Example 8. Inhibition of Ang-1-Mediated Tie-2 Phosphorylation

[0132] As shown in the previous Example, Ang-2 can mediate the phosphorylation of Tie-2. Ang-1 is also capable of promoting Tie-2 phosphorylation. In the present Example, the ability of selected anti-Ang-2 antibodies to block Ang-1-mediated phosphorylation of Tie-2 was assessed.

[0133] EA.hy926 cells (Edgell *et al.*, *Proc. Natl. Acad. Sci. USA 80*:3734-3737 (1983)) were plated at $5x10^6$ cells per 10 cm dish in 10 ml DMEM with 10% FBS, HAT, L-glutamine and penicillin/streptomycin. After 24 hours, cells were serum-starved for 1 hour in 10 ml DMEM + 1 mg/ml BSA. Cells were then stimulated for 10 minutes with 500 ng/ml of recombinant human Ang-1 (R&D Systems) in the presence of either an irrelevant isotype control antibody ("9E10") at 400 nM or the anti-Ang-2 antibody H1H685P, or control agents (Control I, Control II, Control IV, or Control V) at concentrations ranging from 10 to 400 nM.

[0134] Following incubation, cells were lysed and Tie-2 was immunoprecipitated as described by Daly et al., *Proc. Natl. Acad. Sci. USA 103*:15491-15496 (2006). Immune complexes were collected by incubation with protein A/G beads (Santa Cruz Biotechnology, Santa Cruz, CA) for 60 min. Beads were washed with cold lysis buffer and bound proteins were eluted by heating in SDS sample buffer. Samples were then subjected to Western blot analysis with monoclonal antibodies against Tie-2 or phosphotyrosine (clone 4G10, Millipore, Billerica, MA). Results are shown in Figure 2.

[0135] Signals were detected using HRP-conjugated secondary antibodies and ECL reagents (GE Healthcare, Piscataway, NJ). X-ray films were scanned and the phospho-Tie-2 and Tie-2 signals were quantified using ImageJ software. The phospho-Tie-2/Tie-2 ratios were used to determine the % inhibition for each antibody or peptibody. Percent inhibition = reduction in phospho-Tie-2/Tie-2 as compared to the control sample (400 nM isotype control antibody).

[0136] In the presence of the control antibody 9E10, Ang-1 strongly activated Tie-2 phosphorylation (Figure 2, panel A – compare lanes 2 and 3 vs lane 1). All of the control agents that were tested significantly inhibited Tie-2 phosphorylation, with complete inhibition occurring at 50 nM for Control II (Figure 2, panel B – lane 17), 100 nM for Control IV (Figure 2, panel A – lane 11) and 200 nM for Control I (Figure 2, panel B – lane 24) and Control V (Figure 2, panel C

– lane 9). By contrast, H1H685P had no significant inhibitory effect even at 400 nM (Figure 2, panel A - lanes 4-8), These results provide additional confirmation of the specificity of H1H685P for Ang-2 over Ang-1.

Example 9. Inhibition of Tumor Growth by Anti-Ang-2 Antibodies

[0137] The effect of selected purified anti-Ang-2 antibodies on tumor growth was determined using two tumor cell lines.

[0138] PC3 (Human prostate cancer cell line) Briefly, $5x10^6$ PC3 cells in 100µl of growth factor-reduced Matrigel (BD Biosciences) were injected subcutaneously into the flanks of 6-8 week old male NCr nude mice (Taconic, Hudson, NY). After tumor volumes reached an average of about $200mm^3$, mice were randomized into groups for treatment. Mice in each treatment group were administered an anti-Ang-2 antibody, Fc protein, or control construct, at a concentration of 10mg/kg via intraperitoneal injection twice per week for approximately three weeks (Table 24) or at concentrations of 2.5, 12.5, or 25 mg/kg via subcuataneous injection twice per week for approximately three weeks (Table 25). Tumor volumes were measured twice per week over the course of the experiment and tumor weights were measured upon excision of tumors at the conclusion of the experiment. Averages (mean +/- standard deviation) of tumor weight and growth were calculated for each treatment group. Percent decrease of tumor weight and growth were calculated from comparison to Fc protein measurements. Results are summarized in Tables 24 and 25.

Table 24

Antibody	Avg Tumor Weight (g)	% Decrease in Tumor Weight	Avg Tumor Growth (mm^3)	% Decrease in Tumor Growth
Fc protein	0.66 ±0.26	-	509 ± 213	-
Control I (Ab)	0.47 ± 0.23	29	300 ± 242	41
H1H724N	0.55 ± 0.07	17	392 ± 169	23
H1H744N	0.43 ± 0.20	35	259 ± 212	49
H1H685P	0.44 ± 0.12	33	305 ± 143	40
H1H691P	0.59 ± 0.07	11	485 ± 141	5
H1H706P	0.52 ± 0.14	21	329 ± 125	35

Table 25

Antibody	Avg Tumor Growth (mm^3)	% Decrease in Tumor Growth
Fc protein	1031 ± 485	-

Control II (Pb) (2.5 mg/kg)	356 ± 196	65
Control II (Pb) (12.5 mg/kg)	360 ± 162	65
Control II (Pb) (25 mg/kg)	527 ± 218	49
H1H685P (2.5 mg/kg)	308 ± 274	70
H1H685P (12.5 mg/kg)	550 ± 150	47
H1H685P (25 mg/kg)	413 ± 208	60

[0139] As shown above, antibodies H1H744N and H1H685P demonstrated especially marked anti-tumor activity in the PC3 mouse tumor model as compared to the control constructs.

[0140] The results of similar experiments using the PC3 mouse tumor model and different experimental antibodies (dosed at 2 mg/kg, twice per week) are shown in Tables 26 and 27.

Table 26

Antibody	Avg Tumor Weight (g)	% Decrease in Tumor Weight	Avg Tumor Growth (mm^3)	% Decrease in Tumor Growth
Fc protein	0.626 ± 0.156	-	356 ± 93	-
Control I (Ab)	0.347 ± 0.093	45	250 ± 145	30
H2M742N	0.407 ± 0.076	35	220 ± 102	38
H2M743N	0.372 ± 0.122	41	179 ± 169	50

Table 27

Antibody	Avg Tumor Weight (g)	% Decrease in Tumor Weight	Avg Tumor Growth (mm^3)	% Decrease in Tumor Growth
Fc protein	0.552 ± 0.211	-	473 ± 202	-
H1M749N	0.383 ± 0.275	31	220 ± 261	54
H1M750N	0.348 ± 0.128	37	227 ± 195	52

[0141] COLO 205 (Human colorectal adenocarcinoma cell line) Briefly, $2x10^6$ COLO 205 cells in 100µl of serum-free medium were injected subcutaneously into the flank of 6-8 week old male NCr nude mice (Taconic, Hudson, NY). After tumor volumes reached an average of about $150mm^3$, mice were randomized into groups for treatment with antibody or Fc protein. Mice in each treatment group were administered an anti-Ang-2 antibody or Fc protein at a concentration of 4mg/kg via intraperitoneal injection twice per week for approximately two weeks. Tumor volumes were measured twice per week over the course of the experiment and tumor weights were measured upon excision of tumors at the conclusion of the experiment. Averages (mean

+/- standard deviation) of tumor weight and growth were calculated for each treatment group. "Avg. Tumor Growth" represents the average growth from the time of treatment initiation (when tumors were approximately 150 mm^3). Percent decrease of tumor weight and growth are calculated from comparison to Fc protein measurements. Results are summarized in Table 28.

Table 28

Antibody	Avg Tumor Weight (g)	% Decrease in Tumor Weight	Avg Tumor Growth (mm^3)	% Decrease in Tumor Growth
Fc protein	0.847 ± 0.180	-	731 ± 249	-
Control I (Ab)	0.503 ± 0.090	41	367 ± 121	50
Control II (Pb)	0.608 ± 0.085	28	492 ± 82	33
H1M724N	0.531 ± 0.103	37	336 ± 125	54
H2M742N	0.576 ± 0.057	32	427 ± 92	42
H2M744N	0.491 ± 0.051	42	409 ± 162	44
H1M749N	0.603 ± 0.142	29	449 ± 169	39

[0142] A similar experiment was carried out to assess the effect of H1H685P, in particular, on COLO 205 tumor growth. Briefly, $2x10^6$ COLO 205 cells in 100 µl of serum-free medium were implanted subcutaneously into the right hind flank of 9-11 week-old male SCID CB17 mice. When the tumors reached ~ 125 mm^3, mice were randomized into 5 groups (n = 7-8 mice/group) and treated twice per week with Fc protein (15 mg/kg), H1H685P (5 or 25 mg/kg) or Control II (5 or 25 mg/kg) for a period of 19 days. Tumor volumes were measured twice per week over the course of the experiment and tumor weights were measured upon excisionof tumors at the end of the experiment. Averages of tumor weight and growth from the beginning of treatment were calculated for each group. Percent decrease of tumor weight and growth are calculated from comparison to the Fc control group. The results are shown in Table 29.

Table 29

Antibody	Antibody Concentration	Avg Tumor Weight (g)	% Decrease in Tumor Weight	Avg Tumor Growth (mm^3)	% Decrease in Tumor Growth
Fc protein	25 mg/kg	0.800 ± 0.108	-	675 ± 93	-
Control II (Pb)	5 mg/kg	0.481 ± 0.091	40	288 ± 85	57
Control II (Pb)	25 mg/kg	0.393 ± 0.136	51	267 ± 155	60
H1H685P	5 mg/kg	0.458 ± 0.125	43	370 ± 114	45
H1H685P	25 mg/kg	0.430 ± 0.139	46	295 ± 160	56

[0143] As with the PC3 mouse tumor model, several of the antibodies of the invention, including H1H685P, exhibited substantial anti-tumor activities in the COLO 205 mouse model that were at least equivalent to the anti-tumor activities exhibited by the control molecules.

Example 10. Inhibition of Tumor Growth and Perfusion by A Combination of an Anti-Ang-2 Antibody and a VEGF Inhibitor

[0144] To determine the effect of combining an anti-Ang-2 antibody with a VEGF inhibitor on the growth of COLO 205 xenografts, 2 x 10^6 cells were implanted subcutaneously into the right hind flank of 6-8 week-old female SCID mice. When the tumors reached an average volume of ~350 mm^3, mice were randomized into 4 groups (n = 6 mice/group) and treated with: human Fc protein (7.5 mg/kg), H1H685P (5 mg/kg), VEGF Trap (see US 7,087,411) (2.5 mg/kg) or the combination of H1H685P + VEGF Trap. Mice were given a total of 3 doses over 10 days of treatment. Tumor volumes were measured twice per week over the course of the experiment. Averages of tumor growth from the start of treatment (mean +/- standard deviation) were calculated for each treatment group. Percent decrease of tumor growth was calculated from comparison to the Fc control group. The results are shown in Table 30. Note that in the VEGF Trap and in the H1H685P + VEGF Trap groups the average tumor size was smaller at the end of treatment than at the beginning, i.e., tumor regression was observed.

Table 30

Antibody	Avg Tumor Growth (mm^3)	% Decrease in Tumor Growth
Fc protein	366 ± 65	-
H1H685P	74 ± 77	80
VEGF Trap	- 62 ± 44	117
H1H685P + VEGF Trap	- 221 ± 131	160

[0145] The results of this experiment demonstrate that the combination of H1H685P + VEGF Trap causes a decrease in tumor growth that is greater than the percent decrease in tumor growth caused by either component alone.

[0146] To provide additional evidence of combination efficacy, the effect of the H1H685P + VEGF Trap combination on the growth of MMT tumors was assessed. 0.5 x 10^6 MMT cells were implanted subcutaneously into the right hind flank of 6-8 week-old female SCID mice. When the tumors reached an average volume of ~400 mm^3, mice were randomized into 4 groups (n = 11 mice/group) and treated with: human Fc protein (17.5 mg/kg), H1H685P (12.5 mg/kg), VEGF Trap (5 mg/kg) or the combination of H1H685P + VEGF Trap. The Fc and H1H685P groups were given 3 doses over 9 days. The VEGF Trap and combination groups were given 4 doses over 12 days. Tumor volumes were measured twice per week over the

course of the experiment and tumor weights were measured upon excision of tumors at the end of the experiment (due to their large size, tumors from the Fc and H1H685P groups were collected 3 days before tumors from the VEGF Trap and combination groups). Averages (mean +/- standard deviation) of tumor growth from the beginning of treatment and of tumor weight were calculated for each group. Percent decrease of tumor weight and growth are calculated from comparison to the Fc control group. The results are shown in Table 31.

Table 31

Antibody	Avg Tumor Weight (g)	% Decrease in Tumor Weight	Avg Tumor Growth (mm^3)	% Decrease in Tumor Growth
Fc protein	1.591 ± 0.265	-	1337 ± 273	-
H1H685P	1.409 ± 0.314	11	1135 ± 306	15
VEGF Trap	0.889 ± 0.141	44	536 ± 179	60
H1H685P + VEGF Trap	0.599 ± 0.066	62	215 ± 92	84

[0147] These results confirm the enhanced tumor inhibiting effect of H1H685P + VEGF Trap relative to the single agent treatments.

[0148] To determine whether the combination of H1H685P + VEGF Trap has a greater effect on tumor vessel function than the single agents, a micro-ultrasound (VisualSonics' Vevo 770 imaging system) was used to assess changes in tumor perfusion. COLO 205 tumors were grown to ~125 mm^3 and mice were then treated for 24 hrs with H1H685P, VEGF Trap or the combination of both agents. Following treatment, tumor vessel perfusion was determined based on contrast-enhanced micro-ultrasound 2D image acquisition and analysis of a "wash-in" curve, which represents the amount of contrast agent entering the tumor. Average (mean +/- standard deviation) tumor perfusion was calculated for each group. Percent decrease was calculated from comparison to the Fc control group. The results are shown in Table 32.

Table 32

Antibody	Relative Tumor Perfusion	% Decrease in Tumor Perfusion
Fc protein	8.09 ± 2.16	-
H1H685P	6.32 ± 2.81	22
VEGF Trap	6.99 ± 1.36	14
H1H685P + VEGF Trap	2.46 ± 0.34	70

[0149] Consistent with the enhanced effect of the combination treatment on perfusion, anti-CD31 staining of tumor sections demonstrated a more potent effect of the combination on tumor blood vessel density (data not shown). The increased effect of the H1H685P + VEGF Trap

combination on the function of the tumor vasculature provides a potential explanation for the enhanced effects of the combination therapy on tumor growth.

Example 11. Inhibition of Tumor Growth by A Combination of an Anti-Ang-2 Antibody and a Chemotherapeutic Agent

[0150] To test the effect of H1H685P in combination with a chemotherapeutic agent on tumor growth, 2.5 x 10^6 COLO 205 tumor cells were implanted subcutaneously into the right hind flank of 8-9 week-old male SCID mice. When the tumors reached an average volume of ~150 mm^3 (day 17 after implantation), mice were randomized into 4 groups (n = 5 mice/group) and treated as follows: the first group was treated sc with 15 mg/kg hFc and intraperitoneally (ip) with 5-FU vehicle; the second group was treated sc with 15 mg/kg of H1H685P; the third group was treated ip with 75mg/kg of 5-FU; the fourth group was treated with the combination of 15 mg/kg H1H685P sc plus 75 mg/kg 5-FU ip. Mice received a total of three treatments, administered every 3-4 days. Tumor volumes were measured twice per week over the course of the experiment. Average (mean +/- standard deviation) tumor growth from the beginning of treatment until day 38 was calculated for each group. Percent decrease of tumor growth was calculated from comparison to the control group. The results are shown in Table 33.

Table 33

Treatment	Avg Tumor Growth (mm^3)	% Decrease in Tumor Growth
Fc protein + 5-FU vehicle	574 ± 110	-
H1H685P	405 ± 80	29
5-FU	313 ± 60	45
H1H685P + 5-FU	175 ± 78	70

[0151] The results of this experiment show that the combination of H1H685P and 5-FU caused a greater decrease in tumor growth than either agent administered separately.

Example 12. Anti-Ang-2 Antibodies Attenuate Ocular Angiogenesis In Vivo

[0152] In this Example, the effects of selected anti-Ang-2 antibodies on retinal vascularization in a mouse model was assessed.

[0153] In one set of experiments wild-type mice were used. In another set, mice expressing a human Ang-2 in place of the wild-type mouse Ang-2 (designated "hu-Ang-2 mice") were used. The mice at two days of age (P2) were injected subcutaneously with either control Fc or with selected anti-Ang-2 antibodies at a dose of 12.5 mg/kg. Three days later (at P5), pups were euthanized, and eyeballs were enucleated and fixed in 4% PFA for 30 minutes. Retinas were dissected, stained with *Griffonia simplicifolia* lectin-1 for 3 hours or overnight at 4°C to visualize

the vasculature, and flat-mounted on microscope slides. Images were taken using a Nikon Eclipse 80i microscope camera and analyzed using Adobe Photoshop CS3, Fovea 4.0, and Scion 1.63 software.

[0154] Areas of the retina covered with superficial vasculature were measured and used as a readout of antibody activity. The reduction in the size of the vascular areas in mice treated with antibody compared to Fc-treated controls is presented in Table 34. The percent reduction in vascular area reflects the anti-angiogenic potency of the antibody. (N/D = not determined)

Table 34

Antibody	% Reduction in Vascular Area Relative to Fc Control	
	Wild-Type Mice	hAng-2 Mice
H1H685P	39.7	N/D
H1H690P	30.7	41.5
H1H691P	30.4	N/D
H1H696P	31.1	N/D
H1H724N	32.2	33.2
H1H744N	35.8	50.5
Control I (Ab)	26.9	35.6

[0155] As shown in this Example, the selected anti-Ang-2 antibodies of the present invention substantially inhibited ocular angiogenesis *in vivo*, thus reflecting the likely anti-angiogenic potential of these antibodies in other therapeutic contexts.

Example 13. Amino Acids of Ang-2 Important for Antibody Binding

[0156] To further characterize binding between hAng2 and anti-hAng2 mAbs of the invention, seven variant hAng2-FD-mFc proteins were generated, each containing a single point mutation. Amino acids selected for mutation were based on the difference in sequence between hAng-2 and hAng-1 in the region that interacts with hTie-2 (Figure 1). In particular, amino acids within the fibrinogen-like domain (FD) of Ang-2 which are believed to interact with Tie-2 based on crystal structure analysis, but which differ from the corresponding amino acid in Ang-1, were individually mutated to the corresponding hAng-1 residue. The results of this example indicate the amino acid residues of hAng-2 with which the Ang-2 preferential binding antibodies interact. That is, if a particular residue (or residues) of hAng-2 is/are changed to the corresponding residue of hAng-1, and the binding of an Ang-2 preferential binding antibody is substantially reduced, then it can be concluded that the antibody interacts with that particular residue(s) of hAng-2.

[0157] In this experiment, each of the seven hAng-2FD-mFc mutant proteins were captured (~147-283 RU) on an anti-mouse-Fc surface created through direct chemical coupling to a

BIACORE™ chip. Then each Ang-2 antibody (or peptibody, as the case may be) at 100 nM was injected over the captured mFc-tagged hAng-2FD protein surface at a flowrate of 50 μl/min for 180 sec, and the dissociation of variant hAng2-FD-mFc and antibody was monitored in real time for 20 min at 25°C. Results are summarized in Tables 35a – 35d and Figure 3.

Table 35a

Mutated hAng-2 Amino Acid(s)[1]	H1H685P			H1H744N		
	RU	K_D (M)	T ½ (min)	RU	K_D (M)	T ½ (min)
WT[2]	210.70	2.23E-11	1988	213	3.98E-11	904
S-417-I	127.65	3.05E-11	1809	127	5.12E-11	1590
K-432-N	152.68	1.40E-11	4468	137	4.87E-11	1690
I-434-M	235.95	1.79E-11	3600	213	3.18E-11	2589
N-467-G	152.25	9.38E-12	6762	139	7.72E-11	1011
F-469-L	101.16	1.38E-08	4	180	1.95E-10	237
Y-475-H	181.53	1.96E-10	289	247	3.06E-10	136
S-480-P	161.13	2.05E-10	289	228	2.25E-11	2129

Table 35b

Mutated hAng-2 Amino Acid(s)[1]	Control I (Ab)			Control II (Pb)		
	RU	K_D (M)	T ½ (min)	RU	K_D (M)	T ½ (min)
WT[2]	195.25	4.69E-10	54.33	67.44	4.29E-10	39.86
S-417-I	142.96	5.79E-10	32.81	49.99	1.88E-10	36.38
K-432-N	189.69	3.49E-10	51.75	63.21	1.39E-10	42.34
I-434-M	282.10	4.64E-10	48.80	89.15	1.36E-10	57.09
N-467-G	180.90	4.61E-10	44.66	60.94	1.54E-10	46.97
F-469-L	173.01	1.05E-09	25.13	46.73	2.40E-10	36.20
Y-475-H	170.05	1.15E-08	1.85	74.79	1.40E-10	54.12
S-480-P	181.32	2.98E-09	13.36	71.90	1.79E-10	45.45

Table 35c

Mutated hAng-2 Amino Acid(s)[1]	Control III (Pb)			Control V (Ab)		
	RU	K_D (M)	T ½ (min)	RU	K_D (M)	T ½ (min)
WT[2]	80.33	2.07E-11	170.03	214.48	7.97E-10	48.43
S-417-I	57.13	5.31E-11	114.81	126.45	2.40E-09	29.17
K-432-N	79.22	2.88E-11	200.94	149.14	8.48E-10	75.59
I-434-M	116.22	2.15E-10	62.77	214.75	2.23E-09	31.76

N-467-G	74.64	8.90E-11	109.07	146.77	1.11E-09	55.66
F-469-L	72.66	2.74E-10	66.11	131.96	1.37E-08	1.46
Y-475-H	76.21	6.87E-09	4.11	260.93	2.66E-10	93.22
S-480-P	77.93	2.78E-09	11.69	177.10	3.47E-09	10.33

Table 35d

Mutated hAng-2 Amino Acid(s)[1]	Negative Control (irrelevant antibody)		
	RU	K_D (M)	T ½ (min)
WT[2]	0.81	N/B	N/B
S-417-I	-1.21	N/B	N/B
K-432-N	-0.38	N/B	N/B
I-434-M	-1.31	N/B	N/B
N-467-G	-1.09	N/B	N/B
F-469-L	0.32	N/B	N/B
Y-475-H	-0.20	N/B	N/B
S-480-P	-0.52	N/B	N/B

[1] Amino acid numbering is based on the amino acid numbering of SEQ ID NO:518.
[2] WT = wild-type Ang-2FD-mFc construct.
N/B = no binding observed.

[0158] For purposes of the present invention, an anti-Ang-2 antibody is deemed to interact with a particular Ang-2 amino acid residue if, when the residue is mutated to the corresponding residue of Ang-1, the T½ of dissociation is at least 5-fold less than the T½ of dissociation observed for the wild-type construct under the experimental conditions used in this Example. In view of this definition, antibody H1H685P appears to be unique among the antibodies tested in that it interacts with F469, Y475 and S480. Since H1H685P is also unique because of its strong preferential binding to Ang-2 over Ang-1, it can be concluded that F469, Y475 and S480 comprise an epitope that enables the immunological distinction of Ang-2 from Ang-1. The other antibodies/peptibodies tested in this experiment appear to interact with at most one or two of these residues; *i.e.*, H1H744N and Control I interact with Y475; Control III interacts with Y475 and S480; and Control V interacts with F469. Interestingly, Control II, which was shown to block both Ang-1 and Ang-2 binding to Tie-2 with equal potency, does not interact with any of the Ang-2-specific amino acids identified in this experiment.

[0159] The present invention is not to be limited in scope by the specific embodiments describe herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description. Such modifications are intended to fall within the scope of the appended claims.

What is claimed is:

1. An isolated human antibody or antigen-binding fragment thereof that specifically binds human angiopoietin-2 (hAng-2) but does not substantially bind hAng-1.

2. The isolated antibody or antigen-binding fragment of claim 1, wherein the antibody or antigen-binding fragment binds an epitope on hAng-2 (SEQ ID NO:518) comprising at least one amino acid selected from F-469, Y-475, and S-480.

3. The isolated antibody or antigen binding fragment of claim 2, wherein the antibody or antigen-binding fragment binds an epitope on hAng-2 comprising amino acids F-469, Y-475, and S-480.

4. The isolated antibody or antigen-binding fragment of any one of claims 1 to 3, wherein the antibody or antigen-binding fragment binds hAng-2 with a K_D of less than about 80 pM, less than about 40 pM, less than about 20 pM, or less than about 10 pM, as measured in a surface plasmon resonance assay.

5. The isolated antibody or antigen-binding fragment of any one of claims 1 to 4, wherein the antibody or antigen-binding fragment binds hAng-1 with a K_D of greater than about 1 nM, greater than about 10 nM, greater than about 50 nM, or greater than about 100 nM, as measured in a surface plasmon resonance assay.

6. The isolated antibody or antigen-binding fragment of any one of claims 1 to 4, wherein the antibody or antigen-binding fragment does not exhibit any binding to hAng-1 when tested in a surface plasmon resonance assay.

7. The isolated antibody or antigen-binding fragment of claim 2, wherein the antibody or antigen-binding fragment: (a) binds an epitope on hAng-2 comprising amino acids F-469, Y-475, and S-480; (b) binds hAng-2 with a K_D of less than about 80 pM as measured in a surface plasmon resonance assay; and (c) does not exhibit any binding to hAng-1 when tested in a surface plasmon resonance assay.

8. The isolated antibody or antigen-binding fragment of any one of claims 1 to 7, wherein the antibody or antigen-binding fragment comprises the complementarity determining regions (CDRs) of a heavy chain variable region (HCVR) having the amino acid sequence of SEQ ID NO:18, and the CDRs of a light chain variable region (LCVR) having the amino acid sequence of SEQ ID NO:20.

9. The isolated antibody or antigen-binding fragment of claim 8, wherein the antibody or antigen-binding fragment comprises a heavy chain CDR-1 (HCDR1) having the amino acid sequence of SEQ ID NO:4, an HCDR-2 having the amino acid sequence of SEQ ID NO:6, an

HCDR-3 having the amino acid sequence of SEQ ID NO:8, a light chain CDR-1 (LCDR-1) having the amino acid sequence of SEQ ID NO:12, an LCDR-2 having the amino acid sequence of SEQ ID NO:14, and an LCDR-3 having the amino acid sequence of SEQ ID NO:16.

10. The isolated antibody or antigen-binding fragment of any one of claims 1 to 9, wherein the antibody or antigen-binding fragment comprises a HCVR having the amino acid sequence of SEQ ID NO:18 and a LCVR having the amino acid sequence of SEQ ID NO:20.

11. The isolated antibody or antigen-binding fragment of any one of claims 1 to 10, wherein the antibody or antigen-binding fragment blocks the binding of hAng-2 to hTie-2 but does not substantially block the binding of hAng-1 to hTie-2.

12. A pharmaceutical composition comprising the antibody or antigen-binding fragment of any one of claims 1 to 11 and a pharmaceutically acceptable carrier or diluent.

13. The pharmaceutical composition of claim 12, wherein the antibody or antigen-binding fragment comprises the complementarity determining regions (CDRs) of a heavy chain variable region (HCVR) having the amino acid sequence of SEQ ID NO:18, and the CDRs of a light chain variable region (LCVR) having the amino acid sequence of SEQ ID NO:20.

14. The pharmaceutical composition of claim 12 or 13, further comprising a VEGF antagonist.

15. The pharmaceutical composition of claim 14, wherein the VEGF antagonist is selected from an anti-VEGF antibody, a small molecule kinase inhibitor of VEGF receptor and a VEGF-inhibiting fusion protein.

16. An isolated human antibody or antigen-binding fragment thereof that binds to the same epitope on hAng-2 as an antibody which comprises the complementarity determining regions (CDRs) of a heavy chain variable region (HCVR) having the amino acid sequence of SEQ ID NO:18, and the CDRs of a light chain variable region (LCVR) having the amino acid sequence of SEQ ID NO:20.

17. An isolated human antibody or antigen-binding fragment thereof that competes for binding to hAng-2 with an antibody which comprises the complementarity determining regions (CDRs) of a heavy chain variable region (HCVR) having the amino acid sequence of SEQ ID NO:18, and the CDRs of a light chain variable region (LCVR) having the amino acid sequence of SEQ ID NO:20.

18. The isolated antibody or antigen-binding fragment of any one of claims 1 to 11 or 16 to 17 for use in treating a patient having a tumor.

19. The isolated antibody or antigen-binding fragment of any one of claims 1 to 11 or 16 to 17 for use in treating a patient having hypertension, diabetes, asthma, sepsis, kidney disease, edema or an eye disease.

20. Use of the isolated antibody or antigen-binding fragment of any one of claims 1 to 11 or 16 to 17 in the manufacture of a medicament for use in treating a patient having a tumor.

21. Use of the isolated antibody or antigen-binding fragment of any one of claims 1 to 11 or 16 to 17 in the manufacture of a medicament for use in treating a patient having hypertension, diabetes, asthma, sepsis, kidney disease, edema or an eye disease.

22. A method for treating a tumor, the method comprising administering to a patient in need of such treatment an isolated antibody or antigen-binding fragment thereof as set forth in any one of claims 1 to 11 or 16 to 17.

23. A method for treating a disease or disorder selected from hypertension, diabetes, asthma, sepsis, kidney disease, edema and eye disease, said method comprising administering to a patient in need of such treatment an isolated antibody or antigen-binding fragment thereof as set forth in any one of claims 1 to 11 or 16 to 17.