(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
27 April 2006 (27.04.2006)

PCT

(10) International Publication Number
WO 2006/045049 A1

(51) International Patent Classification:
C07K 16/22 (2006.01) *A61K 39/395* (2006.01)
C12N 15/13 (2006.01) *A61P 35/00* (2006.01)
C07K 16/46 (2006.01) *A61P 9/00* (2006.01)
C12P 21/08 (2006.01) *G01N 33/68* (2006.01)
C12N 15/20 (2006.01) *G01N 33/577* (2006.01)
A61K 39/385 (2006.01)

(21) International Application Number:
PCT/US2005/037911

(22) International Filing Date: 19 October 2005 (19.10.2005)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

60/620,161	19 October 2004 (19.10.2004)	US
10/982,440	4 November 2004 (04.11.2004)	US

(63) Related by continuation (CON) or continuation-in-part (CIP) to earlier applications:

US	10/982,440 (CON)
Filed on	4 November 2004 (04.11.2004)
US	60/620,161 (CON)
Filed on	19 October 2004 (19.10.2004)

(71) Applicant *(for all designated States except US)*: **AMGEN INC.** [US/US]; One Amgen Center Drive, Thousand Oaks, California 91320 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **OLINER, Jonathon, Daniel** [US/US]; 959 Golden Crest Avenue, Newbury Park, California 91320 (US). **GRAHAM, Kevin** [CA/US]; 1627 Charterwood Court, Thousand Oaks, California 91362 (US).

(74) Agent: **SINGER, Christopher, P.**; McDONNELL BOEHNEN HULBERT & BERGHOFF LLP, 300 South Wacker Drive, Chicago, Illinois 60606 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US (patent), UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: ANGIOPOIETIN-2 SPECIFIC BINDING AGENTS

(57) Abstract: Disclosed are specific binding agents, such as fully human antibodies, that bind to angiopoietin-2. Also disclosed are heavy chain fragments, light chain fragments, and CDRs of the antibodies, as well as methods of making and using the antibodies.

WO 2006/045049 A1

ANGIOPOIETIN-2 SPECIFIC BINDING AGENTS

This is a continuation-in-part application of U.S. Application No. 10/269,805, filed on October 10, 2002, and PCT Application No. PCT/US02/32613, filed October 11, 2002, which claim benefit of U.S. Provisional Application Serial No. 60/328,604, filed October 11, 2001, all of which are incorporated herein by reference. This application also claims benefit of U.S. Provisional Application Serial No. 60/620,161, filed October 19, 2004, and U.S. Application No. 10/982,440, filed November 4, 2004, which are incorporated herein by reference.

FIELD OF THE INVENTION

The present invention relates to specific binding agents that recognize and bind to angiopoietin-2 (Ang-2). More specifically, the invention relates to the production, diagnostic use, and therapeutic use of monoclonal and polyclonal antibodies, and fragments thereof, which specifically bind Ang-2.

BACKGROUND OF THE INVENTION

Angiogenesis, the formation of new blood vessels from existing ones, is essential to many physiological and pathological processes. Normally, angiogenesis is tightly regulated by pro- and anti-angiogenic factors, but in the case of diseases such as cancer, ocular neovascular diseases, arthritis, and psoriasis, the process can go awry. Folkman, *J., Nat. Med.*, 1:27-31 (1995).

There are a number of diseases known to be associated with deregulated or undesired angiogenesis. Such diseases include, but are not limited to, ocular neovascularisation, such as retinopathies (including diabetic retinopathy), age-related macular degeneration, psoriasis, hemangioblastoma, hemangioma, arteriosclerosis, inflammatory disease, such as a rheumatoid or rheumatic inflammatory disease, especially arthritis (including rheumatoid arthritis), or other chronic inflammatory disorders, such as chronic asthma, arterial or post-

transplantational atherosclerosis, endometriosis, and neoplastic diseases, for example so-called solid tumors and liquid (or hematopoietic) tumors (such as leukemias and lymphomas). Other diseases associated with undesired angiogenesis will be apparent to those skilled in the art.

Although many signal transduction systems have been implicated in the regulation of angiogenesis, one of the best-characterized and most endothelial cell-selective systems involves the Tie-2 receptor tyrosine kinase (referred to as "Tie-2" or "Tie-2R" (also referred to as "ORK"); murine Tie-2 is also referred to as "tek") and its ligands, the angiopoietins (Gale, N. W. and Yancopoulos, G. D., *Genes Dev.* 13:1055-1066 (1999)). There are 4 known angiopoietins; angiopoietin-1 ("Ang-1") through angiopoietin-4 ("Ang-4"). These angiopoietins are also referred to as "Tie-2 ligands". (Davis, S., *et al.*, *Cell*, *87*:1161-1169 (1996); Grosios, K., *et al.*, *Cytogenet Cell Genet*, *84*:118-120 (1999); Holash, J., *et al.*, *Investigative Ophthalmology & Visual Science*, *42*:1617-1625 (1999); Koblizek, T. I., *et al.*, *Current Biology*, *8*:529-532 (1998); Lin, P., *et al.*, *Proc Natl Acad Sci USA*, *95*:8829-8834 (1998); Maisonpierre, P. C., *et al.*, *Science*, *277*:55-60 (1997); Papapetropoulos, A., *et al.*, *Lab Invest*, *79*:213-223 (1999); Sato, T. N., *et al.*, *Nature*, *375*:70-74 (1998); Shyu, K. G., *et al.*, *Circulation*, *98*:2081-2087 (1998); Suri, C., *et al.*, *Cell*, *87*:1171-1180 (1996); Suri, C., *et al.*, *Science*, *282*:468-471 (1998); Valenzuela, D. M., *et al.*, *Proceedings of the National Academy of Sciences of the USA*, *96*:1904-1909 (1999); Witzenbichler, B., *et al.*, *J Biol Chem*, *273*:18514-18521 (1998)). Whereas Ang-1 binding to Tie-2 stimulates receptor phosphorylation in cultured endothelial cells, Ang-2 has been observed to both agonize and antagonize Tie-2 receptor phosphorylation (Davis, S., *et al.*, (1996), *supra*; Maisonpierre, P.C., *et al.*, (1997), *supra*; Kim, I., J.H. Kim, *et al.*, Oncogene **19**(39): 4549-4552 (2000); Teichert-Kuliszewska, K., P.C. Maisonpierre, *et al.*, Cardiovascular Research **49**(3): 659-70 (2001)).

The phenotypes of mouse Tie-2 and Ang-1 knockouts are similar and suggest that Ang-1-stimulated Tie-2 phosphorylation mediates remodeling and stabilization of developing vessels *in utero* through maintenance of endothelial cell-support cell adhesion (Dumont, D. J., *et al.*, *Genes & Development*, *8*:1897-

1909 (1994); Sato, T. N., *et al.*, *Nature*, *376*:70-74 (1995); Suri, C., *et al.*, (1996), *supra*). The role of Ang-1 in vessel stabilization is thought to be conserved in the adult, where it is expressed widely and constitutively (Hanahan, D., *Science*, *277*:48-50 (1997); Zagzag, D., *et al.*, *Experimental Neurology*, *159*:391-400 (1999)). In contrast, Ang-2 expression is primarily limited to sites of vascular remodeling, where it is thought to block Ang-1 function, thereby inducing a state of vascular plasticity conducive to angiogenesis (Hanahan, D., (1997), *supra*; Holash, J., *et al.*, *Science*, *284*:1994-1998 (1999); Maisonpierre, P. C., *et al.*, (1997), *supra*).

Numerous published studies have purportedly demonstrated vessel-selective Ang-2 expression in disease states associated with angiogenesis. These pathological conditions include, for example, psoriasis, macular degeneration, and cancer (Bunone, G., *et al.*, *American Journal of Pathology*, *155*:1967-1976 (1999); Etoh, T., *et al.*, *Cancer Research*, *61*:2145-2153 (2001); Hangai, M., *et al.*, *Investigative Ophthalmology & Visual Science*, *42*:1617-1625 (2001); Holash, J., *et al.*, (1999) *supra*; Kuroda, K., *et al.*, *Journal of Investigative Dermatology*, *116*:713-720 (2001); Otani, A., *et al.*, *Investigative Ophthalmology & Visual Science*, *40*:1912-1920 (1999); Stratmann, A., *et al.*, *American Journal of Pathology*, *153*:1459-1466 (1998); Tanaka, S., *et al.*, *J Clin Invest*, *103*:34-345 (1999); Yoshida, Y., *et al.*, *International Journal of Oncology*, *15*:1221-1225 (1999); Yuan, K., *et al.*, *Journal of Periodontal Research*, *35*:165-171 (2000); Zagzag, D., *et al.*, (1999) *supra*). Most of these studies have focused on cancer, in which many tumor types appear to display vascular Ang-2 expression. In contrast with its expression in pathological angiogenesis, Ang-2 expression in normal tissues is extremely limited (Maisonpierre, P. C., *et al.*, (1997), *supra*; Mezquita, J., *et al.*, *Biochemical and Biophysical Research Communications*, *260*:492-498 (1999)). In the normal adult, the three main sites of angiogenesis are the ovary, placenta, and uterus; these are the primary tissues in normal (*i.e.*, non-cancerous) tissues in which Ang-2 mRNA has been detected.

Certain functional studies suggest that Ang-2 may be involved in tumor angiogenesis. Ahmad *et al.* (*Cancer Res.*, 61:1255-1259 (2001)) describe Ang-2

over-expression and show that it is purportedly associated with an increase in tumor growth in a mouse xenograft model. See also Etoh *et al., supra*, and Tanaka *et al., supra,* wherein data is presented purportedly associating Ang-2 over expression with tumor hypervascularity. However, in contrast, Yu *et al.* (*Am. J. Path.*, 158:563-570 (2001)) report data to show that overexpression of Ang-2 in Lewis lung carcinoma and TA3 mammary carcinoma cells purportedly prolonged the survival of mice injected with the corresponding transfectants.

In the past few years, various publications have suggested Ang-1, Ang-2 and/or Tie-2 as a possible target for anti-cancer therapy. For example, U.S. Patent Nos. 6,166,185, 5,650,490, and 5,814,464 each disclose the concept of anti-Tie-2 ligand antibodies and receptor bodies. Lin *et al.* (*Proc. Natl. Acad. Sci USA*, 95:8829-8834 (1998)) injected an adenovirus expressing soluble Tie-2 into mice; the soluble Tie-2 purportedly decreased the number and size of the tumors developed by the mice. In a related study, Lin *et al* (*J. Clin. Invest.*, 100:2072-2078 (1997)) injected a soluble form of Tie-2 into rats; this compound purportedly reduced tumor size in the rats. Siemeister *et al.* (*Cancer Res.*, 59:3185-3189 (1999)) generated human melanoma cell lines expressing the extracellular domain of Tie-2, injected these cell lines into nude mice, and concluded that soluble Tie-2 purportedly resulted in a "significant inhibition" of tumor growth and tumor angiogenesis. In view of this information, and given that both Ang-1 and Ang-2 bind to Tie-2, it is not clear from these studies whether Ang-1, Ang-2, or Tie-2 would be an attractive target for anti-cancer therapy.

The fusion of certain peptides to a stable plasma protein such as an Ig constant region to improve the half-life of these molecules has been described in, for example, PCT publication WO 00/24782, published May 4, 2000.

The fusion of a protein or fragment thereof to a stable plasma protein such as an Ig constant region to improve the half-life of these molecules has been variously described (see, for example, U.S. Patent 5,480,981; Zheng *et al., J. Immunol.*, 154:5590-5600, (1995); Fisher *et al., N. Engl. J. Med.*, 334:1697-1702, (1996); Van Zee, K. *et al., J. Immunol.*, 156:2221-2230, (1996); U.S. Patent 5,808,029, issued September 15, 1998; Capon *et al., Nature*, 337:525-531, (1989);

Harvill *et al.*, *Immunotech.*, 1:95-105, (1995); WO 97/23614, published July 3, 1997; PCT/US 97/23183, filed December 11, 1997; Linsley, *J. Exp. Med.*, 174:561-569, (1991); WO 95/21258, published August 10, 1995).

An effective anti-Ang-2 therapy might benefit a vast population of cancer patients because most solid tumors require neovascularization to grow beyond 1-2 millimeters in diameter. Such therapy might have wider application in other angiogenesis-associated diseases as well, such as retinopathies, arthritis, and psoriasis.

There is an undeveloped need to identify new agents that specifically recognize and bind Ang-2. Such agents would be useful for diagnostic screening and therapeutic intervention in disease states that are associated with Ang-2 activity.

Accordingly, it is an object of the present invention to provide specific binding agents of Ang-2 that modulate Ang-2 activity.

SUMMARY OF THE INVENTION

The present invention provides an antibody comprising a heavy chain and a light chain, wherein said heavy chain comprises a heavy chain variable region selected from the group consisting of 526 HC (SEQ ID NO: 1); 528 HC (SEQ ID NO: 3); 531 HC (SEQ ID NO: 5); 533 HC (SEQ ID NO: 7); 535 HC (SEQ ID NO: 9); 536 HC (SEQ ID NO: 11); 537 HC (SEQ ID NO: 13); 540 HC (SEQ ID NO: 15); 543 HC (SEQ ID NO: 17); 544 HC (SEQ ID NO: 19); 545 HC (SEQ ID NO: 21); 546 HC (SEQ ID NO: 23); 551 HC (SEQ ID NO: 25); 553 HC (SEQ ID NO: 27); 555 HC (SEQ ID NO: 29); 558 HC (SEQ ID NO: 31); 559 HC (SEQ ID NO: 33); 565 HC (SEQ ID NO: 35); F1-C6 HC (SEQ ID NO: 37); FB1-A7 HC (SEQ ID NO: 39); FD-B2 HC (SEQ ID NO: 41); FE-B7 HC (SEQ ID NO: 43); FJ-G11 HC (SEQ ID NO: 45); FK-E3 HC (SEQ ID NO: 47); G1D4 HC (SEQ ID NO: 49); GC1E8 HC (SEQ ID NO: 51); H1C12 HC (SEQ ID NO: 53); IA1-1E7 HC (SEQ ID NO: 55); IF-1C10 HC (SEQ ID NO: 57); IK-2E2 HC (SEQ ID NO: 59); IP-2C11 HC (SEQ ID NO: 61); and antigen binding fragments thereof; and

said light chain comprises a light chain variable region selected from the group consisting of: 526 kappa (SEQ ID NO: 2); 536 (THW) kappa (SEQ ID NO: 12); 536 (LQT) kappa (SEQ ID NO: 210); 543 kappa (SEQ ID NO: 18); 544 kappa (SEQ ID NO: 20); 551 kappa (SEQ ID NO: 26); 553 kappa (SEQ ID NO: 28); 555 kappa (SEQ ID NO: 30); 558 kappa (SEQ ID NO: 32); 565 kappa (SEQ ID NO: 36); FE-B7 kappa (SEQ ID NO: 44); FJ-G11 kappa (SEQ ID NO: 46); FK-E3 kappa (SEQ ID NO: 48); IA1-1E7 kappa (SEQ ID NO: 56); IP-2C11 kappa (SEQ ID NO: 62); 528 lambda (SEQ ID NO: 4); 531 lambda (SEQ ID NO: 6); 533 lambda (SEQ ID NO: 8); 535 lambda (SEQ ID NO: 10); 537 lambda (SEQ ID NO: 14); 540 lambda (SEQ ID NO: 16); 545 lambda (SEQ ID NO: 22); 546 lambda (SEQ ID NO: 24); 559 lambda (SEQ ID NO: 34); F1-C6 lambda (SEQ ID NO: 38); FB1-A7 lambda (SEQ ID NO: 40); FD-B2 lambda (SEQ ID NO: 42); G1D4 lambda (SEQ ID NO: 50); GC1E8 lambda (SEQ ID NO: 52); H1C12 lambda (SEQ ID NO: 54); IF-1C10 lambda (SEQ ID NO: 58); IK-2E2 lambda (SEQ ID NO: 60); and antigen binding fragments thereof.

The invention also provides a specific binding agent comprising at least one peptide selected from the group consisting of:

SEQ ID NO: 1; SEQ ID NO: 3; SEQ ID NO: 5; SEQ ID NO: 7; SEQ ID NO: 9; SEQ ID NO: 11; SEQ ID NO: 13; SEQ ID NO: 15; SEQ ID NO: 17; SEQ ID NO: 19; SEQ ID NO: 21; SEQ ID NO: 23; SEQ ID NO: 25; SEQ ID NO: 27; SEQ ID NO: 29; SEQ ID NO: 31; SEQ ID NO: 33; SEQ ID NO: 35; SEQ ID NO: 37; SEQ ID NO: 39; SEQ ID NO: 41; SEQ ID NO: 43; SEQ ID NO: 45; SEQ ID NO: 47; SEQ ID NO: 49; SEQ ID NO: 51; SEQ ID NO: 53; SEQ ID NO: 55; SEQ ID NO: 57; SEQ ID NO: 59; SEQ ID NO: 61; SEQ ID NO: 2; SEQ ID NO: 12; SEQ ID NO: 18; SEQ ID NO: 20; SEQ ID NO: 26; SEQ ID NO: 28; SEQ ID NO: 30; SEQ ID NO: 32; SEQ ID NO: 36; SEQ ID NO: 44; SEQ ID NO: 46; SEQ ID NO: 48; SEQ ID NO: 56; SEQ ID NO: 62; SEQ ID NO: 4; SEQ ID NO: 6; SEQ ID NO: 8; SEQ ID NO: 10; SEQ ID NO: 14; SEQ ID NO: 16; SEQ ID NO: 22; SEQ ID NO: 24; SEQ ID NO: 34; SEQ ID NO: 38; SEQ ID NO: 40;

SEQ ID NO: 42; SEQ ID NO: 50; SEQ ID NO: 52; SEQ ID NO: 54; SEQ ID NO: 58; and SEQ ID NO: 60, and fragments thereof.

It will be appreciated that the specific binding agent can be, for example, an antibody, such as a polyclonal, monoclonal, chimeric, humanized, or a fully human antibody. The antibody may also be a single chain antibody. The invention further relates to a hybridoma that produces a monoclonal antibody according to the invention.

It will also be appreciated that the invention relates to conjugates as described herein. The conjugate can be, for example, a specific binding agent (such as an antibody) of the invention.

The invention further relates to nucleic acid molecules encoding the specific binding agents (such as an antibody) of the invention, as well as a vector comprising such nucleic acid molecule, as well as a host cell containing the vector.

Additionally, the invention provides a method of making a specific binding agent comprising, (a) transforming a host cell with at least one nucleic acid molecule encoding the specific binding agent of Claim 1; (b) expressing the nucleic acid molecule in said host cell; and (c) isolating said specific binding agent. The invention further provides a method of making an antibody comprising: (a) transforming a host cell with at least one nucleic acid molecule encoding the antibody according to the invention; (b) expressing the nucleic acid molecule in said host cell; and (c) isolating said specific binding agent.

Further, the invention relates to a method of inhibiting undesired angiogenesis in a mammal by administering a therapeutically effective amount of a specific binding agent according to the invention. The invention also provides a method of treating cancer in a mammal by administering a therapeutically effective amount of a specific binding agent according to the invention.

The invention also relates to a method of inhibiting undesired angiogenesis in a mammal comprising by administering a therapeutically effective amount of

an antibody according to the invention. The invention additionally provides a method of treating cancer in a mammal comprising administering a therapeutically effective amount of antibody according to the invention.

It will be appreciated that the invention further relates to pharmaceutical compositions comprising the specific binding agent according to the invention and a pharmaceutically acceptable formulation agent. The pharmaceutical composition may comprise an antibody according to the invention and a pharmaceutically acceptable formulation agent.

The invention provides a method of modulating or inhibiting angiopoietin-2 activity by administering one or more specific binding agents of the invention. The invention also provides a method of modulating or inhibiting angiopoietin-2 activity by administering an antibody of the invention.

The invention further relates to a method of modulating at least one of vascular permeability or plasma leakage in a mammal comprising administering a therapeutically effective amount of the specific binding agent according to the invention. The invention also relates to a method of treating at least one of ocular neovascular disease, obesity, hemangioblastoma, hemangioma, arteriosclerosis, inflammatory disease, inflammatory disorders, atherosclerosis, endometriosis, neoplastic disease, bone-related disease, or psoriasis in a mammal comprising administering a therapeutically effective amount of a specific binding agent according to the invention.

The invention further provides a method of modulating at least one of vascular permeability or plasma leakage in a mammal comprising administering a therapeutically effective amount of an antibody according to the invention. The invention also relates to a method of treating at least one of ocular neovascular disease, obesity, hemangioblastoma, hemangioma, arteriosclerosis, inflammatory disease, inflammatory disorders, atherosclerosis, endometriosis, neoplastic disease, bone-related disease, or psoriasis in a mammal comprising administering a therapeutically effective amount of an antibody according to the invention.

Furthermore, the invention relates to a method of treating cancer in a mammal comprising administering a therapeutically effective amount of a specific binding agent according to the invention and a chemotherapeutic agent. It will be appreciated by those in the art that the specific binding agent and chemotherapeutic agent need not be administered simultaneously.

The invention also relates to a method of treating cancer in a mammal comprising administering a therapeutically effective amount of an antibody according to the invention and a chemotherapeutic agent. The specific binding agent and chemotherapeutic agent need not be administered simultaneously.

The invention also provides a specific binding agent comprising complementarity determining region 1 (CDR 1) of any of: 526 HC (SEQ ID NO: 1); 528 HC (SEQ ID NO: 3); 531 HC (SEQ ID NO: 5); 533 HC (SEQ ID NO: 7); 535 HC (SEQ ID NO: 9); 536 HC (SEQ ID NO: 11); 537 HC (SEQ ID NO: 13); 540 HC (SEQ ID NO: 15); 543 HC (SEQ ID NO: 17); 544 HC (SEQ ID NO: 19); 545 HC (SEQ ID NO: 21); 546 HC (SEQ ID NO: 23); 551 HC (SEQ ID NO: 25); 553 HC (SEQ ID NO: 27); 555 HC (SEQ ID NO: 29); 558 HC (SEQ ID NO: 31); 559 HC (SEQ ID NO: 33); 565 HC (SEQ ID NO: 35); F1-C6 HC (SEQ ID NO: 37); FB1-A7 HC (SEQ ID NO: 39); FD-B2 HC (SEQ ID NO: 41); FE-B7 HC (SEQ ID NO: 43); FJ-G11 HC (SEQ ID NO: 45); FK-E3 HC (SEQ ID NO: 47); G1D4 HC (SEQ ID NO: 49); GC1E8 HC (SEQ ID NO: 51); H1C12 HC (SEQ ID NO: 53); IA1-1E7 HC (SEQ ID NO: 55); IF-1C10 HC (SEQ ID NO: 57); IK-2E2 HC (SEQ ID NO: 59); IP-2C11 HC (SEQ ID NO: 61); 526 kappa (SEQ ID NO: 2); 536 (THW) kappa (SEQ ID NO: 12); 536 (LQT) kappa (SEQ ID NO: 210); 543 kappa (SEQ ID NO: 18); 544 kappa (SEQ ID NO: 20); 551 kappa (SEQ ID NO: 26); 553 kappa (SEQ ID NO: 28); 555 kappa (SEQ ID NO: 30); 558 kappa (SEQ ID NO: 32); 565 kappa (SEQ ID NO: 36); FE-B7 kappa (SEQ ID NO: 44); FJ-G11 kappa (SEQ ID NO: 46); FK-E3 kappa (SEQ ID NO: 48); IA1-1E7 kappa (SEQ ID NO: 56); IP-2C11 kappa (SEQ ID NO: 62); 528 lambda (SEQ ID NO: 4); 531 lambda (SEQ ID NO: 6); 533 lambda (SEQ ID NO: 8); 535 lambda (SEQ ID NO: 10); 537 lambda (SEQ ID NO: 14); 540 lambda (SEQ ID NO: 16); 545

lambda (SEQ ID NO: 22); 546 lambda (SEQ ID NO: 24); 559 lambda (SEQ ID NO: 34); F1-C6 lambda (SEQ ID NO: 38); FB1-A7 lambda (SEQ ID NO: 40); FD-B2 lambda (SEQ ID NO: 42); G1D4 lambda (SEQ ID NO: 50); GC1E8 lambda (SEQ ID NO: 52); H1C12 lambda (SEQ ID NO: 54); IF-1C10 lambda (SEQ ID NO: 58); and IK-2E2 lambda (SEQ ID NO: 60).

The invention further relates to a specific binding agent comprising complementarity determining region 2 (CDR 2) of any of: 526 HC (SEQ ID NO: 1); 528 HC (SEQ ID NO: 3); 531 HC (SEQ ID NO: 5); 533 HC (SEQ ID NO: 7); 535 HC (SEQ ID NO: 9); 536 HC (SEQ ID NO: 11); 537 HC (SEQ ID NO: 13); 540 HC (SEQ ID NO: 15); 543 HC (SEQ ID NO: 17); 544 HC (SEQ ID NO: 19); 545 HC (SEQ ID NO: 21); 546 HC (SEQ ID NO: 23); 551 HC (SEQ ID NO: 25); 553 HC (SEQ ID NO: 27); 555 HC (SEQ ID NO: 29); 558 HC (SEQ ID NO: 31); 559 HC (SEQ ID NO: 33); 565 HC (SEQ ID NO: 35); F1-C6 HC (SEQ ID NO: 37); FB1-A7 HC (SEQ ID NO: 39); FD-B2 HC (SEQ ID NO: 41); FE-B7 HC (SEQ ID NO: 43); FJ-G11 HC (SEQ ID NO: 45); FK-E3 HC (SEQ ID NO: 47); G1D4 HC (SEQ ID NO: 49); GC1E8 HC (SEQ ID NO: 51); H1C12 HC (SEQ ID NO: 53); IA1-1E7 HC (SEQ ID NO: 55); IF-1C10 HC (SEQ ID NO: 57); IK-2E2 HC (SEQ ID NO: 59); IP-2C11 HC (SEQ ID NO: 61); 526 kappa (SEQ ID NO: 2); 536 (THW) kappa (SEQ ID NO: 12); 536 (LQT) kappa (SEQ ID NO: 210); 543 kappa (SEQ ID NO: 18); 544 kappa (SEQ ID NO: 20); 551 kappa (SEQ ID NO: 26); 553 kappa (SEQ ID NO: 28); 555 kappa (SEQ ID NO: 30); 558 kappa (SEQ ID NO: 32); 565 kappa (SEQ ID NO: 36); FE-B7 kappa (SEQ ID NO: 44); FJ-G11 kappa (SEQ ID NO: 46); FK-E3 kappa (SEQ ID NO: 48); IA1-1E7 kappa (SEQ ID NO: 56); IP-2C11 kappa (SEQ ID NO: 62); 528 lambda (SEQ ID NO: 4); 531 lambda (SEQ ID NO: 6); 533 lambda (SEQ ID NO: 8); 535 lambda (SEQ ID NO: 10); 537 lambda (SEQ ID NO: 14); 540 lambda (SEQ ID NO: 16); 545 lambda (SEQ ID NO: 22); 546 lambda (SEQ ID NO: 24); 559 lambda (SEQ ID NO: 34); F1-C6 lambda (SEQ ID NO: 38); FB1-A7 lambda (SEQ ID NO: 40); FD-B2 lambda (SEQ ID NO: 42); G1D4 lambda (SEQ ID NO: 50); GC1E8 lambda (SEQ ID NO: 52); H1C12 lambda (SEQ ID NO: 54); IF-1C10 lambda (SEQ ID NO: 58); and IK-2E2 lambda (SEQ ID NO: 60).

The invention also relates to a specific binding agent comprising complementarity determining region 3 (CDR 3) of any of: 526 HC (SEQ ID NO: 1); 528 HC (SEQ ID NO: 3); 531 HC (SEQ ID NO: 5); 533 HC (SEQ ID NO: 7); 535 HC (SEQ ID NO: 9); 536 HC (SEQ ID NO: 11); 537 HC (SEQ ID NO: 13); 540 HC (SEQ ID NO: 15); 543 HC (SEQ ID NO: 17); 544 HC (SEQ ID NO: 19); 545 HC (SEQ ID NO: 21); 546 HC (SEQ ID NO: 23); 551 HC (SEQ ID NO: 25); 553 HC (SEQ ID NO: 27); 555 HC (SEQ ID NO: 29); 558 HC (SEQ ID NO: 31); 559 HC (SEQ ID NO: 33); 565 HC (SEQ ID NO: 35); F1-C6 HC (SEQ ID NO: 37); FB1-A7 HC (SEQ ID NO: 39); FD-B2 HC (SEQ ID NO: 41); FE-B7 HC (SEQ ID NO: 43); FJ-G11 HC (SEQ ID NO: 45); FK-E3 HC (SEQ ID NO: 47); G1D4 HC (SEQ ID NO: 49); GC1E8 HC (SEQ ID NO: 51); H1C12 HC (SEQ ID NO: 53); IA1-1E7 HC (SEQ ID NO: 55); IF-1C10 HC (SEQ ID NO: 57); IK-2E2 HC (SEQ ID NO: 59); IP-2C11 HC (SEQ ID NO: 61); 526 kappa (SEQ ID NO: 2); 536 (THW) kappa (SEQ ID NO: 12); 536 (LQT) kappa (SEQ ID NO: 210) 543 kappa (SEQ ID NO: 18); 544 kappa (SEQ ID NO: 20); 551 kappa (SEQ ID NO: 26); 553 kappa (SEQ ID NO: 28); 555 kappa (SEQ ID NO: 30); 558 kappa (SEQ ID NO: 32); 565 kappa (SEQ ID NO: 36); FE-B7 kappa (SEQ ID NO: 44); FJ-G11 kappa (SEQ ID NO: 46); FK-E3 kappa (SEQ ID NO: 48); IA1-1E7 kappa (SEQ ID NO: 56); IP-2C11 kappa (SEQ ID NO: 62); 528 lambda (SEQ ID NO: 4); 531 lambda (SEQ ID NO: 6); 533 lambda (SEQ ID NO: 8); 535 lambda (SEQ ID NO: 10); 537 lambda (SEQ ID NO: 14); 540 lambda (SEQ ID NO: 16); 545 lambda (SEQ ID NO: 22); 546 lambda (SEQ ID NO: 24); 559 lambda (SEQ ID NO: 34); F1-C6 lambda (SEQ ID NO: 38); FB1-A7 lambda (SEQ ID NO: 40); FD-B2 lambda (SEQ ID NO: 42); G1D4 lambda (SEQ ID NO: 50); GC1E8 lambda (SEQ ID NO: 52); H1C12 lambda (SEQ ID NO: 54); IF-1C10 lambda (SEQ ID NO: 58); and IK-2E2 lambda (SEQ ID NO: 60).

The invention further provides a nucleic acid molecule encoding a specific binding agent according to the invention.

Moreover, the invention relates to a method of detecting the level of angiopoietin-2 in a biological sample by (a) contacting a specific binding agent of

the invention with the sample; and (b) determining the extent of binding of the specific binding agent to the sample. The invention also relates to a method of detecting the level of angiopoietin-2 in a biological sample by (a) contacting an antibody of the invention with the sample; and (b) determining the extent of binding of the antibody to the sample.

The invention also relates to a method of inhibiting undesired angiogenesis in a mammal comprising administering a therapeutically effective amount of a polypeptide or composition as described herein. The invention also relates to a method of modulating angiogenesis in a mammal comprising administering a therapeutically effective amount of a polypeptide or composition as described herein. The invention further relates to a method of inhibiting tumor growth characterized by undesired angiogenesis in a mammal comprising administering a therapeutically effective amount of a polypeptide or composition as described herein. Additionally, the invention relates to a method of treating cancer in a mammal comprising administering a therapeutically effective amount of a polypeptide or composition as described herein, and a chemotherapeutic agent. In a preferred embodiment, the chemotherapeutic agent is at least one of 5-FU, CPT-11, and Taxotere. It will be appreciated, however, that other suitable chemotherapeutic agents and other cancer therapies can be used.

It will be appreciated that the specific binding agents of the invention can be used to treat a number of diseases associated with deregulated or undesired angiogenesis. Such diseases include, but are not limited to, ocular neovascularisation, such as retinopathies (including diabetic retinopathy and age-related macular degeneration) psoriasis, hemangioblastoma, hemangioma, arteriosclerosis, inflammatory disease, such as a rheumatoid or rheumatic inflammatory disease, especially arthritis (including rheumatoid arthritis), or other chronic inflammatory disorders, such as chronic asthma, arterial or post-transplantational atherosclerosis, endometriosis, and neoplastic diseases, for example so-called solid tumors and liquid tumors (such as leukemias). Additional diseases which can be treated by administration of the specific binding agents will

be apparent to those skilled in the art. Such additional diseases include, but are not limited to, obesity, vascular permeability, plasma leakage, and bone-related disorders, including osteoporosis. Thus, the invention further relates to methods of treating these diseases associated with deregulated or undesired angiogenesis.

Other embodiments of this invention will be readily apparent from the disclosure provided herewith.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 depicts a graph of tumor size (y-axis) versus time (x-axis) in tumor bearing mice treated with either an anti-Ang-2 antibody (clone 533, 537 or 544) of the invention, with a control antibody, or with phosphate buffered saline (PBS). Details are described in the Examples.

Figures 2A, 2B, and 2C depict epitope mapping data (O.D. 370) for full-length human Ang-2 (hAng-2), to the N-terminus of hAng-2, and to the C-terminus of hAng-2, respectively, for peptibodies TN8-Con4-C, L1-7-N, and 12-9-3-C according to the invention, as well as for control peptibody, Tie2-Fc, C2B8, or 5B12. Details are described in the Examples.

DETAILED DESCRIPTION OF INVENTION

The section headings are used herein for organizational purposes only, and are not to be construed as in any way limiting the subject matter described.

Standard techniques may be used for recombinant DNA molecule, protein, and antibody production, as well as for tissue culture and cell transformation. Enzymatic reactions and purification techniques are typically performed according to the manufacturer's specifications or as commonly accomplished in the art using conventional procedures such as those set forth in Sambrook *et al.* (Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1989)), or as described herein. Unless specific definitions are provided, the nomenclature utilized in connection with, and the laboratory procedures and techniques of analytical chemistry, synthetic organic chemistry,

and medicinal and pharmaceutical chemistry described herein are those well known and commonly used in the art. Standard techniques may be used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients.

Definitions

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

The term "Ang-2" refers to the polypeptide set forth in Figure 6 of U.S. Patent No. 6,166,185 ("Tie-2 ligand-2") or fragments thereof as well as related polypeptides which include allelic variants, splice variants, derivatives, substitution, deletions, and/or insertion variants, fusion peptides and polypeptides, and interspecies homologs. The Ang-2 polypeptide may or may not include additional terminal residues, e.g., leader sequences, targeting sequences, amino terminal methionine, amino terminal methionine and lysine residues, and/or tag or fusion proteins sequences, depending on the manner in which it is prepared.

The term "biologically active" when used in relation to Ang-2 or an Ang-2 specific binding agent refers to a peptide or polypeptide having at least one activity characteristic of Ang-2 or of an Ang-2 specific binding agent. A specific binding agent of Ang-2 may have agonist, antagonist, or neutralizing or blocking activity with respect to at least one biological activity of Ang-2.

The term "specific binding agent" refers to a molecule, preferably a proteinaceous molecule, that binds Ang-2 (and variants and derivatives thereof as defined herein) with a greater affinity than other angiopoietins. A specific binding agent may be a protein, peptide, nucleic acid, carbohydrate, lipid, or small molecular weight compound which binds preferentially to Ang-2. In a preferred embodiment, the specific binding agent according to the present invention is an antibody, such as a polyclonal antibody, a monoclonal antibody (mAb), a chimeric antibody, a CDR-grafted antibody, a multi-specific antibody, a bi-specific antibody, a catalytic antibody, a humanized antibody, a human antibody, an anti-

idiotypic (anti-Id) antibody, and antibodies that can be labeled in soluble or bound form, as well as fragments, variants or derivatives thereof, either alone or in combination with other amino acid sequences, provided by known techniques. Such techniques include, but are not limited to enzymatic cleavage, chemical cleavage, peptide synthesis or recombinant techniques. The anti-Ang-2 specific binding agents of the present invention are capable of binding portions of Ang-2 that modulate, e.g., inhibit or promote, the biological activity of Ang-2 and/or other Ang-2-associated activities.

The term "polyclonal antibody" refers to a heterogeneous mixture of antibodies that recognize and bind to different epitopes on the same antigen. Polyclonal antibodies may be obtained from crude serum preparations or may be purified using, for example, antigen affinity chromatography, or Protein A/Protein G affinity chromatography.

The term "monoclonal antibodies" refers to a collection of antibodies encoded by the same nucleic acid molecule which are optionally produced by a single hybridoma or other cell line, or by a transgenic mammal such that each monoclonal antibody will typically recognize the same epitope on the antigen. The term "monoclonal" is not limited to any particular method for making the antibody, nor is the term limited to antibodies produced in a particular species, *e.g.,* mouse, rat, etc.

The term "chimeric antibodies" refers to antibodies in which a portion of the heavy and/or light chain is identical with or homologous to a corresponding sequence in an antibody derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is/are identical with or homologous to a corresponding sequence in antibodies derived from another species or belonging to another antibody class or subclass. Also included are fragments of such antibodies, that exhibit the desired biological activity (i.e., the ability to specifically bind Ang-2). *See*, U.S. Patent No. 4,816,567 and Morrison *et al.*, *Proc Natl Acad Sci (USA),* 81:6851-6855 (1985).

The term "CDR grafted antibody" refers to an antibody in which the CDR from one antibody of a particular species or isotype is recombinantly inserted into the framework of another antibody of the same or different species or isotype.

The term "multi-specific antibody" refers to an antibody having variable regions that recognize more than one epitope on one or more antigens. A subclass of this type of antibody is a "bi-specific antibody" which recognizes two distinct epitopes on the same or different antigens.

"Catalytic" antibodies refers to antibodies wherein one or more cytotoxic, or more generally one or more biologically active, moieties are attached to the targeting binding agent.

The term "humanized antibody" refers to a specific type of CDR-grafted antibody in which the antibody framework region is derived from a human but each CDR is replaced with that derived from another species, such as a murine CDR. The term "CDR" is defined *infra*.

The term "fully human" antibody refers to an antibody in which both the CDR and the framework are derived from one or more human DNA molecules.

The term "anti-idiotype" antibody refers to any antibody that specifically binds to another antibody that recognizes an antigen. Production of anti-idiotype antibodies can be performed by any of the methods described herein for production of an Ang-2-specific antibodies except that these antibodies arise from *e.g.,* immunization of an animal with an Ang-2-specific antibody or Ang-2-binding fragment thereof, rather than Ang-2 polypeptide itself or a fragment thereof.

The term "variants," as used herein, include those polypeptides wherein amino acid residues are inserted into, deleted from and/or substituted into the naturally occurring (or at least a known) amino acid sequence for the binding agent. Variants of the invention include fusion proteins as described below.

"Derivatives" include those binding agents that have been chemically modified in some manner distinct from insertion, deletion, or substitution variants.

"Specifically binds Ang-2" refers to the ability of a specific binding agent (such as an antibody or fragment thereof) of the present invention to recognize

and bind mature, full-length or partial-length human Ang-2 polypeptide, or an ortholog thereof, such that its affinity (as determined by, *e.g.,* Affinity ELISA or BIAcore assays as described herein) or its neutralization capability (as determined by *e.g.,* Neutralization ELISA assays described herein, or similar assays) is at least 10 times as great, but optionally 50 times as great, 100, 250 or 500 times as great, or even at least 1000 times as great as the affinity or neutralization capability of the same for any other angiopoietin or other peptide or polypeptide.

The term "antigen binding domain" or "antigen binding region" refers to that portion of the specific binding agent (such as an antibody molecule) which contains the specific binding agent amino acid residues (or other moieties) that interact with an antigen and confer on the binding agent its specificity and affinity for the antigen. In an antibody, the antigen-binding domain is commonly referred to as the "complementarity-determining region, or CDR."

The term "epitope" refers to that portion of any molecule capable of being recognized by and bound by a specific binding agent, *e.g.* an antibody, at one or more of the binding agent's antigen binding regions. Epitopes usually consist of chemically active surface groupings of molecules, such as for example, amino acids or carbohydrate side chains, and have specific three-dimensional structural characteristics as well as specific charge characteristics. Epitopes as used herein may be contiguous or non-contiguous. Moreover, epitopes may be mimetic in that they comprise a three dimensional structure that is identical to the epitope used to generate the antibody, yet comprise none or only some of the amino acid residues found in the Ang-2 used to stimulate the antibody immune response.

The term "inhibiting and/or neutralizing epitope" is an epitope, which when bound by a specific binding agent such as an antibody, results in the loss of (or at least the decrease in) biological activity of the molecule, cell, or organism containing such epitope, *in vivo*, *in vitro*, or *in situ*. In the context of the present invention, the neutralizing epitope is located on or is associated with a biologically active region of Ang-2. Alternatively, the term "activating epitope" is an epitope, which when bound by a specific binding agent of the invention, such

as an antibody, results in activation, or at least maintenance of a biologically active conformation, of Ang-2.

The term "antibody fragment" refers to a peptide or polypeptide which comprises less than a complete, intact antibody. Complete antibodies comprise two functionally independent parts or fragments: an antigen binding fragment known as "Fab," and a carboxy terminal crystallizable fragment known as the "Fc" fragment. The Fab fragment includes the first constant domain from both the heavy and light chain (CH1 and CL1) together with the variable regions from both the heavy and light chains that bind the specific antigen. Each of the heavy and light chain variable regions includes three complementarity determining regions (CDRs) and framework amino acid residues which separate the individual CDRs. The Fc region comprises the second and third heavy chain constant regions (CH2 and CH3) and is involved in effector functions such as complement activation and attack by phagocytic cells. In some antibodies, the Fc and Fab regions are separated by an antibody "hinge region," and depending on how the full length antibody is proteolytically cleaved, the hinge region may be associated with either the Fab or Fc fragment. For example, cleavage of an antibody with the protease papain results in the hinge region being associated with the resulting Fc fragment, while cleavage with the protease pepsin provides a fragment wherein the hinge is associated with both Fab fragment simultaneously. Because the two Fab fragments are in fact covalently linked following pepsin cleavage, the resulting fragment is termed the F(ab')2 fragment.

An Fc domain may have a relatively long serum half-life, whereas a Fab is short-lived. (Capon *et al.*, Nature, 337: 525-31 (1989)) When expressed as part of a fusion protein, an Fc domain can impart longer half-life or incorporate such functions as Fc receptor binding, Protein A binding, complement fixation and perhaps even placental transfer into the protein to which it is fused. The Fc region may be a naturally occurring Fc region, or may be altered to improve certain qualities, such as therapeutic qualities or circulation time.

The term "variable region" or "variable domain" refers to a portion of the light and/or heavy chains of an antibody, typically including approximately the

amino-terminal 120 to 130 amino acids in the heavy chain and about 100 to 110 amino terminal amino acids in the light chain. The variable regions typically differ extensively in amino acid sequence even among antibodies of the same species. The variable region of an antibody typically determines the binding and specificity of each particular antibody for its particular antigen. The variability in sequence is concentrated in those regions referred to as complementarity-determining regions (CDRs), while the more highly conserved regions in the variable domain are called framework regions (FR). The CDRs of the light and heavy chains contain within them the amino acids which are largely responsible for the direct interaction of the antibody with antigen, however, amino acids in the FRs can significantly affect antigen binding/recognition as discussed herein *infra.*

The term "light chain" when used in reference to an antibody collectively refers to two distinct types, called kappa (k) or lambda (l) based on the amino acid sequence of the constant domains.

The term "heavy chain" when used in reference to an antibody collectively refers to five distinct types, called alpha, delta, epsilon, gamma and mu, based on the amino acid sequence of the heavy chain constant domain. The combination of heavy and light chains give rise to five known classes of antibodies: IgA, IgD, IgE, IgG and IgM, respectively, including four known subclasses of IgG, designated as IgG_1, IgG_2, IgG_3 and IgG_4.

The term "naturally occurring" when used in connection with biological materials such as nucleic acid molecules, polypeptides, host cells, and the like, refers to those which are found in nature and not modified by a human being.

The term "isolated" when used in relation to Ang-2 or to a specific binding agent of Ang-2 refers to a compound that is free from at least one contaminating polypeptide or compound that is found in its natural environment, and preferably substantially free from any other contaminating mammalian polypeptides that would interfere with its therapeutic or diagnostic use.

The term "mature" when used in relation to Ang-2, anti-Ang-2 antibody, or to any other proteinaceous specific binding agent of Ang-2 refers to a peptide or a polypeptide lacking a leader or signal sequence. When a binding agent of the

invention is expressed, for example, in a prokaryotic host cell, the "mature" peptide or polypeptide may also include additional amino acid residues (but still lack a leader sequence) such as an amino terminal methionine, or one or more methionine and lysine residues. A peptide or polypeptide produced in this manner may be utilized with or without these additional amino acid residues having been removed.

The terms "effective amount" and "therapeutically effective amount" when used in relation to a specific binding agent of Ang-2 refers to an amount of a specific binding agent that is useful or necessary to support an observable change in the level of one or more biological activities of Ang-2. The change may be either an increase or decrease in the level of Ang-2 activity. Preferably, the change is a decrease in Ang-2 activity.

Specific binding agents and Antibodies

As used herein, the term "specific binding agent" refers to a molecule that has specificity for recognizing and binding Ang-2 as described herein. Suitable specific binding agents include, but are not limited to, antibodies and derivatives thereof, polypeptides, and small molecules. Suitable specific binding agents may be prepared using methods known in the art. An exemplary Ang-2 polypeptide specific binding agent of the present invention is capable of binding a certain portion of the Ang-2 polypeptide, and preferably modulating the activity or function of Ang-2 polypeptide.

Specific binding agents such as antibodies and antibody fragments that specifically bind Ang-2 polypeptides are within the scope of the present invention. The antibodies may be polyclonal including mono-specific polyclonal, monoclonal (mAbs), recombinant, chimeric, humanized such as CDR-grafted, human, single chain, catalytic, multi-specific and/or bi-specific, as well as fragments, variants, and/or derivatives thereof.

Polyclonal antibodies directed toward an Ang-2 polypeptide generally are produced in animals (*e.g.*, rabbits, hamsters, goats, sheep, horses, pigs, rats, gerbils, guinea pigs, mice, or any other suitable mammal, as well as other non-

mammal species) by means of multiple subcutaneous or intraperitoneal injections of Ang-2 polypeptide or a fragment thereof with or without an adjuvant. Such adjuvants include, but are not limited to, Freund's complete and incomplete, mineral gels such as aluminum hydroxide, and surface-active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, and dinitrophenol. BCG (bacilli Calmette-Guerin) and Corynebacterium parvum are potentially useful human adjuvants. It may be useful to conjugate an antigen polypeptide to a carrier protein that is immunogenic in the species to be immunized, such as keyhole limpet hemocyanin, serum, albumin, bovine thyroglobulin, or soybean trypsin inhibitor. Also, aggregating agents such as alum are used to enhance the immune response. After immunization, the animals are bled and the serum is assayed for anti-Ang-2 polypeptide antibody titer which can be determined using the assays described herein under "Examples". Polyclonal antibodies may be utilized in the sera from which they were detected, or may be purified from the sera, using, for example, antigen affinity chromatography or Protein A or G affinity chromatography.

Monoclonal antibodies directed toward Ang-2 polypeptides can be produced using, for example but without limitation, the traditional "hybridoma" method or the newer "phage display" technique. For example, monoclonal antibodies of the invention may be made by the hybridoma method as described in Kohler *et al.*, *Nature* 256:495 (1975); the human B-cell hybridoma technique (Kosbor *et al.*, *Immunol Today* 4:72 (1983); Cote *et al.*, *Proc Natl Acad Sci (USA)* 80: 2026-2030 (1983); Brodeur *et al.*, *Monoclonal Antibody Production Techniques and Applications*, pp. 51-63, Marcel Dekker, Inc., New York, (1987)) and the EBV-hybridoma technique (Cole *et al.*, *Monoclonal Antibodies and Cancer Therapy*, Alan R Liss Inc, New York N.Y., pp 77-96, (1985)). Also provided by the invention are hybridoma cell lines that produce monoclonal antibodies reactive with Ang-2 polypeptides.

When the hybridoma technique is employed, myeloma cell lines can be used. Such cell lines suited for use in hybridoma-producing fusion procedures preferably are non-antibody-producing, have high fusion efficiency, and enzyme

deficiencies that render them incapable of growing in certain selective media which support the growth of only the desired fused cells (hybridomas). For example, cell lines used in mouse fusions are Sp-20, P3-X63/Ag8, P3-X63-Ag8.653, NS1/1.Ag 4 1, Sp210-Ag14, FO, NSO/U, MPC-11, MPC11-X45-GTG 1.7 and S194/5XX0 Bul; cell lines used in rat fusions are R210.RCY3, Y3-Ag 1.2.3, IR983F and 4B210. Other cell lines useful for cell fusions are U-266, GM1500-GRG2, LICR-LON-HMy2 and UC729-6. Hybridomas and other cell lines that produce monoclonal antibodies are contemplated to be novel compositions of the present invention.

The phage display technique may also be used to generate monoclonal antibodies from any species. Preferably, this technique is used to produce fully human monoclonal antibodies in which a polynucleotide encoding a single Fab or Fv antibody fragment is expressed on the surface of a phage particle. (Hoogenboom *et al.*, *J Mol Biol* 227: 381 (1991); Marks *et al.*, *J Mol Biol* 222: 581 (1991); *see* also U.S. Patent No. 5,885,793)). Each phage can be "screened" using binding assays described herein to identify those antibody fragments having affinity for Ang-2. Thus, these processes mimic immune selection through the display of antibody fragment repertoires on the surface of filamentous bacteriophage, and subsequent selection of phage by their binding to Ang-2. One such procedure is described in PCT Application No. PCT/US98/17364, filed in the name of Adams *et al.*, which describes the isolation of high affinity and functional agonistic antibody fragments for MPL- and msk-receptors using such an approach. In this approach, a complete repertoire of human antibody genes can be created by cloning naturally rearranged human V genes from peripheral blood lymphocytes as previously described (Mullinax *et al.*, *Proc Natl Acad Sci (USA) 87*: 8095-8099 (1990)).

Once a polynucleotide sequences are identified which encode each chain of the full length monoclonal antibody or the Fab or Fv fragment(s) of the invention, host cells, either eukaryotic or prokaryotic, may be used to express the monoclonal antibody polynucleotides using recombinant techniques well known and routinely practiced in the art. Alternatively, transgenic animals are produced

wherein a polynucleotide encoding the desired specific binding agent is introduced into the genome of a recipient animal, such as, for example, a mouse, rabbit, goat, or cow, in a manner that permits expression of the polynucleotide molecules encoding a monoclonal antibody or other specific binding agent. In one aspect, the polynucleotides encoding the monoclonal antibody or other specific binding agent can be ligated to mammary-specific regulatory sequences, and the chimeric polynucleotides can be introduced into the germline of the target animal. The resulting transgenic animal then produces the desired antibody in its milk (Pollock *et al.*, *J Immunol Meth* 231:147-157 (1999); Little *et al.*, *Immunol Today* 8:364-370 (2000)). In addition, plants may be used to express and produce Ang-2 specific binding agents such as monoclonal antibodies by transfecting suitable plants with the polynucleotides encoding the monoclonal antibodies or other specific binding agents.

In another embodiment of the present invention, a monoclonal or polyclonal antibody or fragment thereof that is derived from other than a human species may be "humanized" or "chimerized". Methods for humanizing non-human antibodies are well known in the art. (*see* U.S. Patent Nos. 5,859,205, 5,585,089, and 5,693,762). Humanization is performed, for example, using methods described in the art (Jones *et al.*, *Nature* 321: 522-525 (1986); Riechmann *et al.*, *Nature*, 332: 323-327 (1988); Verhoeyen *et al.*, *Science* 239:1534-1536 (1988)) by substituting at least a portion of, e.g. a rodent, complementarity-determining region (CDRs) for the corresponding regions of a human antibody. The invention also provides variants and derivatives of these human antibodies as discussed herein and well known in the art.

Also encompassed by the invention are fully human antibodies that bind Ang-2 polypeptides, as well as, fragments, variants and/or derivatives thereof. Such antibodies can be produced using the phage display technique described above. Alternatively, transgenic animals (*e.g.*, mice) that are capable of producing a repertoire of human antibodies in the absence of endogenous immunoglobulin production can be used to generate such antibodies. This can be accomplished by immunization of the animal with an Ang-2 antigen or fragments thereof where the

Ang-2 fragments have an amino acid sequence that is unique to Ang-2. Such immunogens can be optionally conjugated to a carrier. *See*, for example, Jakobovits *et al.*, *Proc Natl Acad Sci (USA)*, 90: 2551-2555 (1993); Jakobovits *et al.*, *Nature* 362: 255-258 (1993); Bruggermann *et al.*, *Year in Immuno*, 7: 33 (1993). In one method, such transgenic animals are produced by incapacitating the endogenous loci encoding the heavy and light immunoglobulin chains therein, and inserting loci encoding human heavy and light chain proteins into the genome thereof. Partially modified animals, that are those having less than the full complement of these modifications, are then crossbred to obtain an animal having all of the desired immune system modifications. When administered an immunogen, these transgenic animals are capable of producing antibodies with human variable regions, including human (rather than *e.g.*, murine) amino acid sequences, that are immuno-specific for the desired antigens. *See* PCT application Nos., PCT/US96/05928 and PCT/US93/06926. Additional methods are described in U.S. Patent No. 5,545,807, PCT application Nos. PCT/US91/245, PCT/GB89/01207, and in EP 546073B1 and EP 546073A1. Human antibodies may also be produced by the expression of recombinant DNA in host cells or by expression in hybridoma cells as described herein.

Transgenesis is achieved in a number of different ways. See, for example, Bruggeman *et al., Immunol Today 17*:391-7 (1996). In one approach, a minilocus is constructed such that gene segments in a germline configuration are brought artificially close to each other. Due to size limitations (i.e., having generally less than 30 kb), the resulting minilocus will contain a limited number of differing gene segments, but is still capable of producing a large repertoire of antibodies. Miniloci containing only human DNA sequences, including promoters and enhancers are fully functional in the transgenic mouse.

When larger number of gene segments are desired in the transgenic animal, yeast artificial chromosomes (YACs) are utilized. YACs can range from several hundred kilobases to 1 Mb and are introduced into the mouse (or other appropriate animal) genome via microinjection directly into an egg or via transfer of the YAC into embryonic stem (ES)-cell lines. In general, YACs are transferred

into ES cells by lipofection of the purified DNA, or yeast spheroplast fusion wherein the purified DNA is carried in micelles and fusion is carried out in manner similar to hybridoma fusion protocols. Selection of desired ES cells following DNA transfer is accomplished by including on the YAC any of the selectable markers known in the art.

As another alternative, bacteriophage P1 vectors are used which are amplified in a bacterial E. coli host. While these vectors generally carry less inserted DNA than a YAC, the clones are readily grown in high enough yield to permit direct microinjection into a mouse egg. Use of a cocktail of different P1 vectors has been shown to lead to high levels of homologous recombination.

Once an appropriate transgenic mouse (or other appropriate animal) has been identified, using any of the techniques known in the art to detect serum levels of a circulating antibody (*e.g.*, ELISA), the transgenic animal is crossed with a mouse in which the endogenous Ig locus has been disrupted. The result provides progeny wherein essentially all B cells express human antibodies.

As still another alternative, the entire animal Ig locus is replaced with the human Ig locus, wherein the resulting animal expresses only human antibodies. In another approach, portions of the animal's locus are replaced with specific and corresponding regions in the human locus. In certain cases, the animals resulting from this procedure may express chimeric antibodies, as opposed to fully human antibodies, depending on the nature of the replacement in the mouse Ig locus.

Human antibodies can also be produced by exposing human splenocytes (B or T cells) to an antigen *in vitro*, then reconstituting the exposed cells in an immunocompromised mouse, *e.g.* SCID or nod/SCID. *See* Brams *et al., J Immunol,* 160: 2051-2058 (1998); Carballido *et al., Nat Med,* 6: 103-106 (2000). In one approach, engraftment of human fetal tissue into SCID mice (SCID-hu) results in long-term hematopoiesis and human T-cell development (McCune *et al., Science 241*:1532-1639 (1988); Ifversen *et al., Sem Immunol* 8:243-248 (1996)). Any humoral immune response in these chimeric mice is completely dependent on co-development of T-cells in the animals (Martensson *et al., Immunol 83*:1271-179 (1994)). In an alternative approach, human peripheral blood lymphocytes are

transplanted intraperitoneally (or otherwise) into SCID mice (Mosier *et al.*, *Nature* 335:256-259 (1988)). When the transplanted cells are treated with either a priming agent, such as Staphylococcal Enterotoxin A (SEA) (Martensson *et al.*, *Immunol 84*: 224-230 (1995)), or anti-human CD40 monoclonal antibodies (Murphy *et al.*, *Blood* 86:1946-1953 (1995)), higher levels of B cell production are detected.

Alternatively, an entirely synthetic human heavy chain repertoire is created from unrearranged V gene segments by assembling each human VH segment with D segments of random nucleotides together with a human J segment (Hoogenboom *et al., J Mol Biol 227*:381-388 (1992)). Likewise, a light chain repertoire is constructed by combining each human V segment with a J segment (Griffiths *et al., EMBO J 13*:3245-3260 (1994)). Nucleotides encoding the complete antibody (i.e., both heavy and light chains) are linked as a single chain Fv fragment and this polynucleotide is ligated to a nucleotide encoding a filamentous phage minor coat protein. When this fusion protein is expressed on the surface of the phage, a polynucleotide encoding a specific antibody is identified by selection using an immobilized antigen.

In still another approach, antibody fragments are assembled as two Fab fragments by fusion of one chain to a phage protein and secretion of the other into bacterial periplasm (Hoogenboom *et al.*, *Nucl Acids Res* 19:4133-4137 (1991); Barbas *et al.*, *Proc Natl Acad Sci (USA)* 88:7978-7982 (1991)).

Large-scale production of chimeric, humanized, CDR-grafted, and fully human antibodies, or fragments thereof, are typically produced by recombinant methods. Polynucleotide molecule(s) encoding the heavy and light chains of each antibody or fragments thereof, can be introduced into host cells and expressed using materials and procedures described herein. In a preferred embodiment, the antibodies are produced in mammalian host cells, such as CHO cells. Details of such production is described below.

Fusion Partners of Specific Binding Agents

In a further embodiment of the invention, the polypeptides comprising the amino acid sequence variable domains of Ang-2 antibodies, such as a heavy chain variable region with an amino acid sequence as described herein or a light chain variable region with an amino acid sequence as described herein, may be fused at either the N-terminus or the C-terminus to one or more domains of an Fc region of human IgG. When constructed together with a therapeutic protein such as the Fab of an Ang-2-specific antibody, an Fc domain can provide longer half-life or incorporate such functions as Fc receptor binding, Protein A binding, complement fixation and perhaps even placental transfer. (Capon *et al.*, Nature, 337: 525-531 (1989)).

In one example, the antibody hinge, CH2 and CH3 regions may be fused at either the N-terminus or C-terminus of the specific binding agent polypeptides such as an anti-Ang-2 Fab or Fv fragment (obtained, e.g., from a phage display library) using methods known to the skilled artisan. The resulting fusion protein may be purified by use of a Protein A or Protein G affinity column. Peptides and proteins fused to an Fc region have been found to exhibit a substantially greater half-life *in vivo* than the unfused counterpart. Also, a fusion to an Fc region allows for dimerization/multimerization of the fusion polypeptide. The Fc region may be a naturally occurring Fc region, or may be altered to improve certain qualities, such as therapeutic qualities, circulation time, decrease aggregation problems, etc. Other examples known in the art include those wherein the Fc region, which may be human or another species, or may be synthetic, is fused to the N-terminus of CD30L to treat Hodgkin's Disease, anaplastic lymphoma and T-cell leukemia (U.S. Patent No. 5,480,981), the Fc region is fused to the TNF receptor to treat septic shock (Fisher *et al.*, N Engl J Med, 334: 1697-1702 (1996)), and the Fc region is fused to the Cd4 receptor to treat AIDS (Capon *et al.*, Nature, 337: 525-31 (1989)).

Catalytic antibodies are another type of fusion molecule and include antibodies to which one or more cytotoxic, or more generally one or more biologically active, moieties are attached to the specific binding agent. See, for example (Rader *et al.*, *Chem Eur J* 12:2091-2095 (2000)). Cytotoxic agents of

this type improve antibody-mediated cytotoxicity, and include such moieties as cytokines that directly or indirectly stimulate cell death, radioisotopes, chemotherapeutic drugs (including prodrugs), bacterial toxins (ex. pseudomonas exotoxin, diphtheria toxin, etc.), plant toxins (ex. ricin, gelonin, etc.), chemical conjugates (e.g., maytansinoid toxins, calechaemicin, etc.), radioconjugates, enzyme conjugates (RNase conjugates, antibody-directed enzyme/prodrug therapy (ADEPT))), and the like. In one aspect, the cytotoxic agent can be "attached" to one component of a bi-specific or multi-specific antibody by binding of this agent to one of the alternative antigen recognition sites on the antibody. As an alternative, protein cytotoxins can be expressed as fusion proteins with the specific binding agent following ligation of a polynucleotide encoding the toxin to a polynucleotide encoding the binding agent. In still another alternative, the specific binding agent can be covalently modified to include the desired cytotoxin.

Examples of such fusion proteins are immunogenic polypeptides, proteins with long circulating half lives, such as immunoglobulin constant regions, marker proteins, proteins or polypeptides that facilitate purification of the desired specific binding agent polypeptide, and polypeptide sequences that promote formation of multimeric proteins (such as leucine zipper motifs that are useful in dimer formation/stability).

This type of insertional variant generally has all or a substantial portion of the native molecule, linked at the N- or C-terminus, to all or a portion of a second polypeptide. For example, fusion proteins typically employ leader sequences from other species to permit the recombinant expression of a protein in a heterologous host. Another useful fusion protein includes the addition of an immunologically active domain, such as an antibody epitope, to facilitate purification of the fusion protein. Inclusion of a cleavage site at or near the fusion junction will facilitate removal of the extraneous polypeptide after purification. Other useful fusions include linking of functional domains, such as active sites from enzymes, glycosylation domains, cellular targeting signals or transmembrane regions.

There are various commercially available fusion protein expression systems that may be used in the present invention. Particularly useful systems include but are not limited to the glutathione-S-transferase (GST) system (Pharmacia), the maltose binding protein system (NEB, Beverley, MA), the FLAG system (IBI, New Haven, CT), and the 6xHis system (Qiagen, Chatsworth, CA). These systems are capable of producing recombinant polypeptides bearing only a small number of additional amino acids, which are unlikely to affect the antigenic ability of the recombinant polypeptide. For example, both the FLAG system and the 6xHis system add only short sequences, both of which are known to be poorly antigenic and which do not adversely affect folding of the polypeptide to its native conformation. Another N-terminal fusion that is contemplated to be useful is the fusion of a Met-Lys dipeptide at the N-terminal region of the protein or peptides. Such a fusion may produce beneficial increases in protein expression or activity.

A particularly useful fusion construct may be one in which a specific binding agent peptide is fused to a hapten to enhance immunogenicity of a specific binding agent fusion construct which is useful, for example, in the production of anti-idiotype antibodies of the invention. Such fusion constructs to increase immunogenicity are well known to those of skill in the art, for example, a fusion of specific binding agent with a helper antigen such as hsp70 or peptide sequences such as from diphtheria toxin chain or a cytokine such as IL-2 will be useful in eliciting an immune response. In other embodiments, fusion construct can be made which will enhance the targeting of the antigen binding agent compositions to a specific site or cell.

Other fusion constructs including heterologous polypeptides with desired properties, e.g., an Ig constant region to prolong serum half-life or an antibody or fragment thereof for targeting also are contemplated. Other fusion systems produce polypeptide hybrids where it is desirable to excise the fusion partner from the desired polypeptide. In one embodiment, the fusion partner is linked to the recombinant specific binding agent polypeptide by a peptide sequence containing a specific recognition sequence for a protease. Examples of suitable sequences

are those recognized by the Tobacco Etch Virus protease (Life Technologies, Gaithersburg, MD) or Factor Xa (New England Biolabs, Beverley, MA.

The invention also provides fusion polypeptides comprising all or part of a variable domain of an Ang-2 antibody, such as a heavy chain variable region with an amino acid sequence as described herein or a light chain variable region with an amino acid sequence as described herein in combination with truncated tissue factor (tTF), a vascular targeting agent consisting of a truncated form of a human coagulation-inducing protein that acts as a tumor blood vessel clotting agent. The fusion of tTF to the anti-Ang-2 antibody, or fragments thereof may facilitate the delivery of anti-Ang-2 to target cells.

Variants of Specific Binding Agents

Variants of Specific Binding Agents of the present invention include insertion, deletion, and/or substitution variants. In one aspect of the invention,, insertion variants are provided wherein one or more amino acid residues supplement a specific binding agent amino acid sequence. Insertions may be located at either or both termini of the protein, or may be positioned within internal regions of the specific binding agent amino acid sequence. Insertional variants with additional residues at either or both termini can include, for example, fusion proteins and proteins including amino acid tags or labels. Insertion variants include specific binding agent polypeptides wherein one or more amino acid residues are added to a specific binding agent amino acid sequence, or fragment thereof.

Variant products of the invention also include mature specific binding agent products. Such specific binding agent products have the leader or signal sequences removed, however the resulting protein has additional amino terminal residues as compared to wild-type Ang-2 polypeptide. The additional amino terminal residues may be derived from another protein, or may include one or more residues that are not identifiable as being derived from a specific protein. Specific binding agent products with an additional methionine residue at position -1 (Met^{-1}-specific binding agent) are contemplated, as are specific binding agent

products with additional methionine and lysine residues at positions -2 and -1 (Met^{-2}-Lys^{-1}-specific binding agent). Variants of specific binding agents having additional Met, Met-Lys, Lys residues (or one or more basic residues in general) are particularly useful for enhanced recombinant protein production in bacterial host cells.

The invention also embraces specific binding agent variants having additional amino acid residues that arise from use of specific expression systems. For example, use of commercially available vectors that express a desired polypeptide as part of glutathione-S-transferase (GST) fusion product provides the desired polypeptide having an additional glycine residue at amino acid position -1 after cleavage of the GST component from the desired polypeptide. Variants which result from expression in other vector systems are also contemplated, including those wherein poly-histidine tags are incorporated into the amino acid sequence, generally at the carboxy and/or amino terminus of the sequence.

Insertional variants also include fusion proteins as described above, wherein the amino and/or carboxy termini of the specific binding agent-polypeptide is fused to another polypeptide, a fragment thereof, or amino acid sequences which are not generally recognized to be part of any specific protein sequence.

In another aspect, the invention provides deletion variants wherein one or more amino acid residues in a specific binding agent polypeptide are removed. Deletions can be effected at one or both termini of the specific binding agent polypeptide, or from removal of one or more residues within the specific binding agent amino acid sequence. Deletion variants necessarily include all fragments of a specific binding agent polypeptide.

Antibody fragments include those portions of the antibody that bind to an epitope on the antigen polypeptide. Examples of such fragments include Fab and $F(ab')_2$ fragments generated, for example, by enzymatic or chemical cleavage of full-length antibodies. Other binding fragments include those generated by recombinant DNA techniques, such as the expression of recombinant plasmids containing nucleic acid sequences encoding antibody variable regions. The

invention also embraces polypeptide fragments of an Ang-2 binding agent wherein the fragments maintain the ability to specifically bind an Ang-2 polypeptide. Fragments comprising at least 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 or more consecutive amino acids of a peptide or polypeptide of the invention are comprehended herein. Preferred polypeptide fragments display immunological properties unique to or specific for the antigen-binding agent so of the invention. Fragments of the invention having the desired immunological properties can be prepared by any of the methods well known and routinely practiced in the art.

In still another aspect, the invention provides substitution variants of specific binding agents of the invention. Substitution variants are generally considered to be "similar" to the original polypeptide or to have a certain "percent identity" to the original polypeptide, and include those polypeptides wherein one or more amino acid residues of a polypeptide are removed and replaced with alternative residues. In one aspect, the substitutions are conservative in nature, however, the invention embraces substitutions that are also non-conservative.

Identity and similarity of related polypeptides can be readily calculated by known methods. Such methods include, but are not limited to, those described in Computational Molecular Biology, Lesk, A.M., ed., Oxford University Press, New York (1988); Biocomputing: Informatics and Genome Projects, Smith, D.W., ed., Academic Press, New York (1993); Computer Analysis of Sequence Data, Part 1, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey (1994); Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press (1987); Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M. Stockton Press, New York (1991); and Carillo *et al.*, *SIAM J. Applied Math.*, 48:1073 (1988).

Preferred methods to determine the relatedness or percent identity of two polypeptides are designed to give the largest match between the sequences tested. Methods to determine identity are described in publicly available computer programs. Preferred computer program methods to determine identity between two sequences include, but are not limited to, the GCG program package, including GAP (Devereux *et al.*, *Nucl. Acid. Res.*, 12:387 (1984); Genetics

Computer Group, University of Wisconsin, Madison, WI, BLASTP, BLASTN, and FASTA (Altschul *et al.*, *J. Mol. Biol.*, 215:403-410 (1990)). The BLASTX program is publicly available from the National Center for Biotechnology Information (NCBI) and other sources (*BLAST Manual,* Altschul *et al.* NCB/NLM/NIH Bethesda, MD 20894; Altschul *et al., supra* (1990)). The well-known Smith Waterman algorithm may also be used to determine identity.

Certain alignment schemes for aligning two amino acid sequences may result in the matching of only a short region of the two sequences, and this small aligned region may have very high sequence identity even though there is no significant relationship between the two full-length sequences. Accordingly, in certain embodiments, the selected alignment method (GAP program) will result in an alignment that spans at least ten percent of the full length of the target polypeptide being compared, i.e., at least 40 contiguous amino acids where sequences of at least 400 amino acids are being compared, 30 contiguous amino acids where sequences of at least 300 to about 400 amino acids are being compared, at least 20 contiguous amino acids where sequences of 200 to about 300 amino acids are being compared, and at least 10 contiguous amino acids where sequences of about 100 to 200 amino acids are being compared.

For example, using the computer algorithm GAP (Genetics Computer Group, University of Wisconsin, Madison, WI), two polypeptides for which the percent sequence identity is to be determined are aligned for optimal matching of their respective amino acids (the "matched span", as determined by the algorithm). In certain embodiments, a gap opening penalty (which is typically calculated as 3X the average diagonal; the "average diagonal" is the average of the diagonal of the comparison matrix being used; the "diagonal" is the score or number assigned to each perfect amino acid match by the particular comparison matrix) and a gap extension penalty (which is usually 1/10 times the gap opening penalty), as well as a comparison matrix such as PAM 250 or BLOSUM 62 are used in conjunction with the algorithm. In certain embodiments, a standard comparison matrix (see Dayhoff *et al.*, *Atlas of Protein Sequence and Structure*,

5(3)(1978) for the PAM 250 comparison matrix; Henikoff *et al.*, *Proc. Natl. Acad. Sci* USA, 89:10915-10919 (1992) for the BLOSUM 62 comparison matrix) is also used by the algorithm.

In certain embodiments, the parameters for a polypeptide sequence comparison include the following:

Algorithm: Needleman *et al.*, *J. Mol. Biol.*, 48:443-453 (1970);

Comparison matrix: BLOSUM 62 from Henikoff *et al.*, *supra* (1992);

Gap Penalty: 12

Gap Length Penalty: 4

Threshold of Similarity: 0

The GAP program may be useful with the above parameters. In certain embodiments, the aforementioned parameters are the default parameters for polypeptide comparisons (along with no penalty for end gaps) using the GAP algorithm.

In certain embodiments, the parameters for polynucleotide molecule sequence comparisons include the following:

Algorithm: Needleman *et al.*, *supra* (1970);

Comparison matrix: matches = +10, mismatch = 0

Gap Penalty: 50

Gap Length Penalty: 3

The GAP program may also be useful with the above parameters. The aforementioned parameters are the default parameters for polynucleotide molecule comparisons.

Other exemplary algorithms, gap opening penalties, gap extension penalties, comparison matrices, thresholds of similarity, etc. may be used, including those set forth in the Program Manual, Wisconsin Package, Version 9,

September, 1997. The particular choices to be made will be apparent to those of skill in the art and will depend on the specific comparison to be made, such as DNA-to-DNA, protein-to-protein, protein-to-DNA; and additionally, whether the comparison is between given pairs of sequences (in which case GAP or BestFit are generally preferred) or between one sequence and a large database of sequences (in which case FASTA or BLASTA are preferred).

As used herein, the twenty conventional amino acids and their abbreviations follow conventional usage. See Immunology--A Synthesis (2nd Edition, E. S. Golub and D. R. Gren, Eds., Sinauer Associates, Sunderland, Mass. (1991)), which is incorporated herein by reference for any purpose.

The amino acids may have either L or D stereochemistry (except for Gly, which is neither L nor D) and the polypeptides and compositions of the present invention may comprise a combination of stereochemistries. However, the L stereochemistry is preferred. The invention also provides reverse molecules wherein the amino terminal to carboxy terminal sequence of the amino acids is reversed. For example, the reverse of a molecule having the normal sequence X_1-X_2-X_3 would be X_3-X_2-X_1. The invention also provides retro-reverse molecules wherein, as above, the amino terminal to carboxy terminal sequence of amino acids is reversed and residues that are normally "L" enantiomers are altered to the "D" stereoisomer form.

Stereoisomers (e.g., D-amino acids) of the twenty conventional amino acids, unnatural amino acids such as α-, α-disubstituted amino acids, N-alkyl amino acids, lactic acid, and other unconventional amino acids may also be suitable components for polypeptides of the present invention. Examples of unconventional amino acids include, without limitation: aminoadipic acid, beta-alanine, beta-aminopropionic acid, aminobutyric acid, piperidinic acid, aminocaprioic acid, aminoheptanoic acid, aminoisobutyric acid, aminopimelic acid, diaminobutyric acid, desmosine, diaminopimelic acid, diaminopropionic acid, N-ethylglycine, N-ethylaspargine, hyroxylysine, allo-hydroxylysine, hydroxyproline, isodesmosine, allo-isoleucine, N-methylglycine, sarcosine, N-

methylisoleucine, N-methylvaline, norvaline, norleucine, orithine, 4-hydroxyproline, γ-carboxyglutamate, ε-N,N,N-trimethyllysine, ε-N-acetyllysine, O-phosphoserine, N-acetylserine, N-formylmethionine, 3-methylhistidine, 5-hydroxylysine, σ-N-methylarginine, and other similar amino acids and amino acids (e.g., 4-hydroxyproline).

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

Conservative amino acid substitutions may encompass non-naturally occurring amino acid residues, which are typically incorporated by chemical peptide synthesis rather than by synthesis in biological systems. These include peptidomimetics and other reversed or inverted forms of amino acid moieties.

Naturally occurring residues may be divided into classes based on common side chain properties:

1) hydrophobic: Met, Ala, Val, Leu, Ile;

2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;

3) acidic: Asp, Glu;

4) basic: His, Lys, Arg;

5) residues that influence chain orientation: Gly, Pro; and

6) aromatic: Trp, Tyr, Phe.

For example, non-conservative substitutions may involve the exchange of a member of one of these classes for a member from another class. Such substituted residues may be introduced into regions of the human antibody that are homologous with non-human antibodies, or into the non-homologous regions of the molecule.

In making such changes, according to certain embodiments, the hydropathic index of amino acids may be considered. Each amino acid has been assigned a hydropathic index on the basis of its hydrophobicity and charge characteristics. They are: isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8); glycine (-0.4); threonine (-0.7); serine (-0.8); tryptophan (-0.9); tyrosine (-1.3); proline (-1.6); histidine (-3.2); glutamate (-3.5); glutamine (-3.5); aspartate (-3.5); asparagine (-3.5); lysine (-3.9); and arginine (-4.5).

The importance of the hydropathic amino acid index in conferring interactive biological function on a protein is understood in the art. Kyte *et al.*, *J. Mol. Biol.*, 157:105-131 (1982). It is known that certain amino acids may be substituted for other amino acids having a similar hydropathic index or score and still retain a similar biological activity. In making changes based upon the hydropathic index, in certain embodiments, the substitution of amino acids whose hydropathic indices are within ±2 is included. In certain embodiments, those which are within ±1 are included, and in certain embodiments, those within ±0.5 are included.

It is also understood in the art that the substitution of like amino acids can be made effectively on the basis of hydrophilicity, particularly where the biologically functional protein or peptide thereby created is intended for use in immunological embodiments, as in the present case. In certain embodiments, the greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with its immunogenicity and antigenicity, i.e., with a biological property of the protein.

The following hydrophilicity values have been assigned to these amino acid residues: arginine (+3.0); lysine (+3.0); aspartate (+3.0 ± 1); glutamate (+3.0 ± 1); serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); threonine (-0.4); proline (-0.5 ± 1); alanine (-0.5); histidine (-0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine (-1.8); isoleucine (-1.8); tyrosine (-2.3); phenylalanine (-2.5) and tryptophan (-3.4). In making changes based upon similar hydrophilicity values, in certain embodiments, the substitution of amino acids whose hydrophilicity values are within ±2 is included, in certain embodiments, those which are within ±1 are included, and in certain embodiments, those within ±0.5 are included. One may also identify epitopes from primary amino acid sequences on the basis of hydrophilicity. These regions are also referred to as "epitopic core regions."

Exemplary amino acid substitutions are set forth in Table 1.

Table 1

Amino Acid Substitutions

Original Residues	Exemplary Substitutions	Preferred Substitutions
Ala	Val, Leu, Ile	Val
Arg	Lys, Gln, Asn	Lys
Asn	Gln, Glu, Asp	Gln
Asp	Glu, Gln, Asn	Glu
Cys	Ser, Ala	Ser
Gln	Asn, Glu, Asp	Asn
Glu	Asp, Asn, Gln	Asp
Gly	Pro, Ala	Ala
His	Asn, Gln, Lys, Arg	Arg
Ile	Leu, Val, Met, Ala, Phe, Norleucine	Leu
Leu	Norleucine, Ile, Val, Met, Ala, Phe	Ile

Lys	Arg, 1,4 Diamino-butyric Acid, Gln, Asn	Arg
Met	Leu, Phe, Ile	Leu
Phe	Leu, Val, Ile, Ala, Tyr	Leu
Pro	Ala	Gly
Ser	Thr, Ala, Cys	Thr
Thr	Ser	Ser
Trp	Tyr, Phe	Tyr
Tyr	Trp, Phe, Thr, Ser	Phe
Val	Ile, Met, Leu, Phe, Ala, Norleucine	Leu

A skilled artisan will be able to determine suitable variants of the polypeptide as set forth herein using well-known techniques. In certain embodiments, one skilled in the art may identify suitable areas of the molecule that may be changed without destroying activity by targeting regions not believed to be important for activity. In certain embodiments, one can identify residues and portions of the molecules that are conserved among similar polypeptides. In certain embodiments, even areas that may be important for biological activity or for structure may be subject to conservative amino acid substitutions without destroying the biological activity or without adversely affecting the polypeptide structure.

Additionally, one skilled in the art can review structure-function studies identifying residues in similar polypeptides that are important for activity or structure. In view of such a comparison, one can predict the importance of amino acid residues in a protein that correspond to amino acid residues which are important for activity or structure in similar proteins. One skilled in the art may opt for chemically similar amino acid substitutions for such predicted important amino acid residues.

One skilled in the art can also analyze the three-dimensional structure and amino acid sequence in relation to that structure in similar polypeptides. In view of such information, one skilled in the art may predict the alignment of amino acid residues of an antibody with respect to its three dimensional structure. In certain embodiments, one skilled in the art may choose not to make radical changes to amino acid residues predicted to be on the surface of the protein, since such residues may be involved in important interactions with other molecules. Moreover, one skilled in the art may generate test variants containing a single amino acid substitution at each desired amino acid residue. The variants can then be screened using activity assays known to those skilled in the art. Such variants could be used to gather information about suitable variants. For example, if one discovered that a change to a particular amino acid residue resulted in destroyed, undesirably reduced, or unsuitable activity, variants with such a change may be avoided. In other words, based on information gathered from such routine experiments, one skilled in the art can readily determine the amino acids where further substitutions should be avoided either alone or in combination with other mutations.

A number of scientific publications have been devoted to the prediction of secondary structure. See Moult J., *Curr. Op. in Biotech.*, 7(4):422-427 (1996), Chou *et al., Biochemistry*, 13(2):222-245 (1974); Chou *et al.*, *Biochemistry*, 113(2):211-222 (1974); Chou *et al.*, *Adv. Enzymol. Relat. Areas Mol. Biol.*, 47:45-148 (1978); Chou *et al.*, *Ann. Rev. Biochem.*, 47:251-276 and Chou *et al.*, *Biophys. J.*, 26:367-384 (1979). Moreover, computer programs are currently available to assist with predicting secondary structure. One method of predicting secondary structure is based upon homology modeling. For example, two polypeptides or proteins which have a sequence identity of greater than 30%, or similarity greater than 40% often have similar structural topologies. The recent growth of the protein structural database (PDB) has provided enhanced predictability of secondary structure, including the potential number of folds within a polypeptide's *or* protein's structure. *See* Holm *et al.*, *Nucl. Acid. Res.*, 27(1):244-247 (1999). It has been suggested (Brenner *et al., Curr. Op. Struct.*

Biol., 7(3):369-376 (1997)) that there are a limited number of folds in a given polypeptide or protein and that once a critical number of structures have been resolved, structural prediction will become dramatically more accurate.

Additional methods of predicting secondary structure include "threading" (Jones, D., *Curr. Opin. Struct. Biol.*, 7(3):377-87 (1997); Sippl *et al.*, *Structure*, 4(1):15-19 (1996)), "profile analysis" (Bowie *et al., Science*, 253:164-170 (1991); Gribskov *et al.*, *Meth. Enzym.*, 183:146-159 (1990); Gribskov *et al.*, *Proc. Nat. Acad. Sci.*, 84(13):4355-4358 (1987)), and "evolutionary linkage" (See Holm, *supra* (1999), and Brenner, *supra* (1997)).

In certain embodiments, antibody variants include glycosylation variants wherein the number and/or type of glycosylation site has been altered compared to the amino acid sequences of the parent polypeptide. In certain embodiments, protein variants comprise a greater or a lesser number of N-linked glycosylation sites than the native protein. An N-linked glycosylation site is characterized by the sequence: Asn-X-Ser or Asn-X-Thr, wherein the amino acid residue designated as X may be any amino acid residue except proline. The substitution of amino acid residues to create this sequence provides a potential new site for the addition of an N-linked carbohydrate chain. Alternatively, substitutions which eliminate this sequence will remove an existing N-linked carbohydrate chain. Also provided is a rearrangement of N-linked carbohydrate chains wherein one or more N-linked glycosylation sites (typically those that are naturally occurring) are eliminated and one or more new N-linked sites are created. Additional preferred antibody variants include cysteine variants wherein one or more cysteine residues are deleted from or substituted for another amino acid (e.g., serine) as compared to the parent amino acid sequence. Cysteine variants may be useful when antibodies must be refolded into a biologically active conformation such as after the isolation of insoluble inclusion bodies. Cysteine variants generally have fewer cysteine residues than the native protein, and typically have an even number to minimize interactions resulting from unpaired cysteines.

According to certain embodiments, amino acid substitutions are those which: (1) reduce susceptibility to proteolysis, (2) reduce susceptibility to oxidation, (3) alter binding affinity for forming protein complexes, (4) alter binding affinities, and/or (5) confer or modify other functional properties on such polypeptides. According to certain embodiments, single or multiple amino acid substitutions (in certain embodiments, conservative amino acid substitutions) may be made in the naturally-occurring sequence (in certain embodiments, in the portion of the polypeptide outside the domain(s) forming intermolecular contacts). In certain embodiments, a conservative amino acid substitution typically may not substantially change the structural characteristics of the parent sequence (e.g., a replacement amino acid should not tend to break a helix that occurs in the parent sequence, or disrupt other types of secondary structure that characterizes the parent sequence). Examples of art-recognized polypeptide secondary and tertiary structures are described in Proteins, Structures and Molecular Principles (Creighton, Ed., W. H. Freeman and Company, New York (1984)); Introduction to Protein Structure (C. Branden and J. Tooze, eds., Garland Publishing, New York, N.Y. (1991)); and Thornton et at. Nature 354:105 (1991).

The specific binding agent molecules of this invention that are polypeptide or peptide substitution variants may have up to about ten to twelve percent of the original amino acid sequence replaced. For antibody variants, the heavy chain may have 50, 49, 48, 47, 46, 45, 44, 43, 42, 41, 40, 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid replaced, while the light chain may have 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid replaced.

Derivatives of Specific Binding Agents

The invention also provides derivatives of specific binding agent polypeptides. Derivatives include specific binding agent polypeptides bearing modifications other than insertion, deletion, or substitution of amino acid residues. Preferably, the modifications are covalent in nature, and include for example,

chemical bonding with polymers, lipids, other organic, and inorganic moieties. Derivatives of the invention may be prepared to increase circulating half-life of a specific binding agent polypeptide, or may be designed to improve targeting capacity for the polypeptide to desired cells, tissues, or organs.

The invention further embraces derivative binding agents covalently modified to include one or more water soluble polymer attachments such as polyethylene glycol, polyoxyethylene glycol, or polypropylene glycol as described U.S. Patent Nos: 4,640,835, 4,496,689, 4,301,144, 4,670,417, 4,791,192 and 4,179,337. Still other useful polymers known in the art include monomethoxy-polyethylene glycol, dextran, cellulose, or other carbohydrate based polymers, poly-(N-vinyl pyrrolidone)-polyethylene glycol, propylene glycol homopolymers, a polypropylene oxide/ethylene oxide co-polymer, polyoxyethylated polyols (e.g., glycerol) and polyvinyl alcohol, as well as mixtures of these polymers. Particularly preferred are specific binding agent products covalently modified with polyethylene glycol (PEG) subunits. Water-soluble polymers may be bonded at specific positions, for example at the amino terminus of the specific binding agent products, or randomly attached to one or more side chains of the polypeptide. The use of PEG for improving the therapeutic capacity for specific binding agent, and for humanized antibodies in particular, is described in US Patent 6, 133, 426 to Gonzales *et al.*, issued October 17, 2000.

Target Sites for Antibody Mutagenesis

Certain strategies can be employed to manipulate inherent properties of an Ang-2-specific antibody, such as the affinity of the antibody for its target. These strategies include the use of site-specific or random mutagenesis of the polynucleotide molecule encoding the antibody to generate antibody variants, followed by a screening step designed to recover antibody variants that exhibit the desired change, e.g. increased or decreased affinity.

The amino acid residues most commonly targeted in mutagenic strategies are those in the CDRs. As described *supra*, these regions contain the residues that

actually interact with Ang-2 and other amino acids that affect the spatial arrangement of these residues. However, amino acids in the framework regions of the variable domains outside the CDR regions have also been shown to make substantial contributions to the antigen-binding properties of the antibody, and can be targeted to manipulate such properties. See Hudson, *Curr Opin Biotech*, 9:395-402 (1999) and references therein.

Smaller and more effectively screened libraries of antibody variants can be produced by restricting random or site-directed mutagenesis to sites in the CDRs that correspond to areas prone to "hyper-mutation" during the somatic affinity maturation process. See Chowdhury and Pastan, *Nature Biotech*, 17: 568-572 (1999) and references therein. The types of DNA elements known to define hyper-mutation sites in this manner include direct and inverted repeats, certain consensus sequences, secondary structures, and palindromes. The consensus DNA sequences include the tetrabase sequence Purine-G-Pyrimidine-A/T (i.e. A or G - G - C or T – A or T) and the serine codon AGY (wherein Y can be a C or a T).

Thus, an embodiment of the present invention includes mutagenic strategies with the goal of increasing the affinity of an antibody for its target. These strategies include mutagenesis of the entire variable heavy and light chain, mutagenesis of the CDR regions only, mutagenesis of the consensus hypermutation sites within the CDRs, mutagenesis of framework regions, or any combination of these approaches ("mutagenesis" in this context could be random or site-directed). Definitive delineation of the CDR regions and identification of residues comprising the binding site of an antibody can be accomplished though solving the structure of the antibody in question, and the antibody-ligand complex, through techniques known to those skilled in the art, such as X-ray crystallography. Various methods based on analysis and characterization of such antibody crystal structures are known to those of skill in the art and can be employed, although not definitive, to approximate the CDR regions. Examples of such commonly used methods include the Kabat, Chothia, AbM and contact definitions.

The Kabat definition is based on the sequence variability and is the most commonly used definition to predict CDR regions. (Johnson and Wu, *Nucleic Acids Res*, 28: 214-8 (2000)). The Chothia definition is based on the location of the structural loop regions. (Chothia *et al.*, *J Mol Biol*, 196: 901-17 (1986); Chothia *et al.*, *Nature*, 342: 877-83 (1989)). The AbM definition is a compromise between the Kabat and Chothia definition. AbM is an integral suite of programs for antibody structure modeling produced by Oxford Molecular Group (Martin *et al.*, *Proc Natl Acad Sci (USA)* 86:9268-9272 (1989); Rees, *et al.*, ABM™, a computer program for modeling variable regions of antibodies, Oxford, UK; Oxford Molecular, Ltd.). The AbM suite models the tertiary structure of an antibody from primary sequencing using a combination of knowledge databases and ab initio methods. An additional definition, known as the contact definition, has been recently introduced. (MacCallum *et al.*, *J Mol Biol*, 5:732-45 (1996)). This definition is based on an analysis of the available complex crystal structures.

By convention, the CDR regions in the heavy chain are typically referred to as H1, H2 and H3 and are numbered sequentially in order counting from the amino terminus to the carboxy terminus. The CDR regions in the light chain are typically referred to as L1, L2 and L3 and are numbered sequentially in order counting from the amino terminus to the carboxy terminus.

The CDR-H1 is approximately 10 to 12 residues in length and typically starts 4 residues after a Cys according to the Chothia and AbM definitions or typically 5 residues later according to the Kabat definition. The H1 is typically followed by a Trp, typically Trp-Val, but also Trp-Ile, or Trp-Ala. The length of H1 is approximately 10 to 12 residues according to the AbM definition while the Chothia definition excludes the last 4 residues.

The CDR-H2 typically starts 15 residues after the end of H1 according to the Kabat and AbM definition. The residues preceding H2 are typically Leu-Glu-Trp-Ile-Gly but there are a number of variations. H2 is typically followed by the amino acid sequence Lys/Arg-Leu/Ile/Val/Phe/Thr/Ala-Thr/Ser/Ile/Ala. According to the Kabat definition, the length of the H2 is approximately 16 to 19

residues where the AbM definition predicts the length to be typically 9 to 12 residues.

The CDR-H3 typically starts 33 residues after the end of H2 and is typically preceded by the amino acid sequence (typically Cys-Ala-Arg). The H3 is typically followed by the amino acid sequence-Gly. The length of H3 can be anywhere between 3 to 25 residues.

The CDR-L1 typically starts at approximately residue 24 and will typically follow a Cys. The residue after the CDR-L1 is always a Trp and will typically begin the sequence Trp-Tyr-Gln, Trp-Leu-Gln, Trp-Phe-Gln, or Trp-Tyr-Leu. The length of CDR-L1 is approximately 10 to 17 residues. The punitive CDR-L1 for the antibodies of the invention follows this pattern exactly with a Cys residue followed by 15 amino acids then Trp-Tyr-Gln.

The CDR-L2 starts approximately 16 residues after the end of L1. It will generally follow residues Ile-Tyr, Val-Tyr, Ile-Lys or Ile-Phe. The length of CDR-L2 is approximately 7 residues.

The CDR-L3 typically starts 33 residues after the end of L2 and typically follows a Cys. L3 is typically followed by the amino acid sequence Phe-Gly-XXX-Gly. The length of L3 is approximately 7 to 11 residues.

Various methods for modifying antibodies have been described in the art. For example, US Patent 5,530,101 (to Queen *et al.*, June 25, 1996) describes methods to produce humanized antibodies wherein the sequence of the humanized immunoglobulin heavy chain variable region framework is 65% to 95% identical to the sequence of the donor immunoglobulin heavy chain variable region framework. Each humanized immunoglobulin chain will usually comprise, in addition to the CDRs, amino acids from the donor immunoglobulin framework that are, e.g., capable of interacting with the CDRs to affect binding affinity, such as one or more amino acids which are immediately adjacent to a CDR in the donor immunoglobulin or those within about 3 angstroms as predicted by molecular modeling. The heavy and light chains may each be designed by using any one or all of various position criteria. When combined into an intact antibody, the humanized immunoglobulins of the present invention will be substantially non-

immunogenic in humans and retain substantially the same affinity as the donor immunoglobulin to the antigen, such as a protein or other compound containing an epitope. See also, related methods in US Patent 5,693,761 to Queen, *et al.*, issued December 2, 1997 ("Polynucleotides encoding improved humanized immunoglobulins"); US Patent 5,693,762 to Queen, *et al.*, issued December 2, 1997 ("Humanized Immunoglobulins"); US Patent 5,585,089 to Queen, *et al.* issued December 17, 1996 ("Humanized Immunoglobulins").

In one example, US Patent 5,565,332 to Hoogenboom *et al.* issued October 15, 1996 ("Production of chimeric antibodies - a combinatorial approach") describes methods for the production of antibodies, and antibody fragments which have similar binding specificity as a parent antibody but which have increased human characteristics. Humanized antibodies are obtained by chain shuffling, using, for example, phage display technology, and a polypeptide comprising a heavy or light chain variable domain of a non-human antibody specific for an antigen of interest is combined with a repertoire of human complementary (light or heavy) chain variable domains. Hybrid pairings that are specific for the antigen of interest are identified and human chains from the selected pairings are combined with a repertoire of human complementary variable domains (heavy or light). In another embodiment, a component of a CDR from a non-human antibody is combined with a repertoire of component parts of CDRs from human antibodies. From the resulting library of antibody polypeptide dimers, hybrids are selected and used in a second humanizing shuffling step. Alternatively, this second step is eliminated if the hybrid is already of sufficient human character to be of therapeutic value. Methods of modification to increase human character are also described. See also Winter, *FEBS Letts* 430:92-92 (1998).

As another example, United States Patent 6,054,297 to Carter *et al.*, issued April 25, 2000 describes a method for making humanized antibodies by substituting a CDR amino acid sequence for the corresponding human CDR amino acid sequence and/or substituting a FR amino acid sequence for the corresponding human FR amino acid sequences.

As another example, US Patent 5,766,886 to Studnicka *et al.*, issued June 16, 1998 ("Modified antibody variable domains") describes methods for identifying the amino acid residues of an antibody variable domain which may be modified without diminishing the native affinity of the antigen binding domain while reducing its immunogenicity with respect to a heterologous species and methods for preparing these modified antibody variable domains which are useful for administration to heterologous species. See also US Patent 5,869,619 to Studnicka issued February 9, 1999.

As discussed, modification of an antibody by any of the methods known in the art is typically designed to achieve increased binding affinity for an antigen and/or reduce immunogenicity of the antibody in the recipient. In one approach, humanized antibodies can be modified to eliminate glycosylation sites in order to increase affinity of the antibody for its cognate antigen (Co *et al.*, *Mol Immunol* 30:1361-1367 (1993)). Techniques such as "reshaping," "hyperchimerization," and "veneering/resurfacing" have produced humanized antibodies with greater therapeutic potential. (Vaswami *et al.*, *Annals of Allergy, Asthma, & Immunol* 81:105 (1998); Roguska *et al.*, *Prot Engineer* 9:895-904 (1996)). See also US Patent 6,072,035 to Hardman *et al.*, issued June 6, 2000, which describes methods for reshaping antibodies. While these techniques diminish antibody immunogenicity by reducing the number of foreign residues, they do not prevent anti-idiotypic and anti-allotypic responses following repeated administration of the antibodies. Alternatives to these methods for reducing immunogenicity are described in Gilliland *et al.*, *J Immunol* 62(6): 3663-71 (1999).

In many instances, humanizing antibodies results in a loss of antigen binding capacity. It is therefore preferable to "back mutate" the humanized antibody to include one or more of the amino acid residues found in the original (most often rodent) antibody in an attempt to restore binding affinity of the antibody. See, for example, Saldanha *et al.*, *Mol Immunol* 36:709-19 (1999).

Non-Peptide Specific Binding Agent Analogs/Protein Mimetics

Furthermore, nonpeptide specific binding agent analogs of peptides that provide a stabilized structure or lessened biodegradation, are also contemplated. Specific binding agent peptide mimetic analogs can be prepared based on a selected inhibitory peptide by replacement of one or more residues by nonpeptide moieties. Preferably, the nonpeptide moieties permit the peptide to retain its natural confirmation, or stabilize a preferred, e.g., bioactive, confirmation which retains the ability to recognize and bind Ang-2. In one aspect, the resulting analog/mimetic exhibits increased binding affinity for Ang-2. One example of methods for preparation of nonpeptide mimetic analogs from specific binding agent peptides is described in Nachman *et al.*, *Regul Pept* 57:359-370 (1995). If desired, the specific binding agent peptides of the invention can be modified, for instance, by glycosylation, amidation, carboxylation, or phosphorylation, or by the creation of acid addition salts, amides, esters, in particular C-terminal esters, and N-acyl derivatives of the peptides of the invention. The specific binding agent peptides also can be modified to create peptide derivatives by forming covalent or noncovalent complexes with other moieties. Covalently-bound complexes can be prepared by linking the chemical moieties to functional groups on the side chains of amino acids comprising the specific binding agent peptides, or at the N- or C-terminus.

In particular, it is anticipated that the specific binding agent peptides can be conjugated to a reporter group, including, but not limited to a radiolabel, a fluorescent label, an enzyme (e.g., that catalyzes a colorimetric or fluorometric reaction), a substrate, a solid matrix, or a carrier (e.g., biotin or avidin). The invention accordingly provides a molecule comprising an antibody molecule, wherein the molecule preferably further comprises a reporter group selected from the group consisting of a radiolabel, a fluorescent label, an enzyme, a substrate, a solid matrix, and a carrier. Such labels are well known to those of skill in the art, e.g., biotin labels are particularly contemplated. The use of such labels is well known to those of skill in the art and is described in, e.g., U.S. No. Patent 3,817,837; U.S. Patent No. 3,850,752; U.S. Patent No. 3,996,345 and U.S. Patent

No. 4,277,437. Other labels that will be useful include but are not limited to radioactive labels, fluorescent labels and chemiluminescent labels. U.S. Patents concerning use of such labels include for example U.S. Patent No. 3,817,837; U.S. Patent No. 3,850,752; U.S. Patent No. 3,939,350 and U.S. Patent No. 3,996,345. Any of the peptides of the present invention may comprise one, two, or more of any of these labels.

Methods of Making Specific Binding Agents

Specific binding agents of the present invention that are proteins can be prepared by chemical synthesis in solution or on a solid support in accordance with conventional techniques. The current limit for solid phase synthesis is about 85-100 amino acids in length. However, chemical synthesis techniques can often be used to chemically ligate a series of smaller peptides to generate full length polypeptides. Various automatic synthesizers are commercially available and can be used in accordance with known protocols. See, for example, Stewart and Young, Solid Phase Peptide Synthesis, 2d. ed., Pierce Chemical Co., (1984); Tam *et al.*, *J Am Chem Soc*, 105:6442, (1983); Merrifield, Science, 232:341-347, (1986); and Barany and Merrifield, The Peptides, Gross and Meienhofer, eds, Academic Press, New York, 1-284; Barany *et al.*, Int. J. Peptide Protein Res., 30, 705-739 (1987); and U.S. Pat. No. 5,424,398), each incorporated herein by reference.

Solid phase peptide synthesis methods use a copoly(styrene-divinylbenzene) containing 0.1-1.0 mM amines/g polymer. These methods for peptide synthesis use butyloxycarbonyl (t-BOC) or 9-fluorenylmethyloxy-carbonyl(FMOC) protection of alpha-amino groups. Both methods involve stepwise syntheses whereby a single amino acid is added at each step starting from the C-terminus of the peptide (See, Coligan *et al.*, Current Protocols in Immunology, Wiley Interscience, 1991, Unit 9). On completion of chemical synthesis, the synthetic peptide can be deprotected to remove the t-BOC or FMOC amino acid blocking groups and cleaved from the polymer by treatment with acid at reduced temperature (e.g., liquid HF-10% anisole for about 0.25 to about 1

hours at 0°C). After evaporation of the reagents, the specific binding agent peptides are extracted from the polymer with 1% acetic acid solution that is then lyophilized to yield the crude material. This can normally be purified by such techniques as gel filtration on Sephadex G-15 using 5% acetic acid as a solvent. Lyophilization of appropriate fractions of the column will yield the homogeneous specific binding agent peptide or peptide derivatives, which can then be characterized by such standard techniques as amino acid analysis, thin layer chromatography, high performance liquid chromatography, ultraviolet absorption spectroscopy, molar rotation, solubility, and quantitated by the solid phase Edman degradation.

Chemical synthesis of anti-Ang-2 antibodies, derivatives, variants, and fragments thereof, as well as other protein-based Ang-2 binding agents permits incorporation of non-naturally occurring amino acids into the agent.

Recombinant DNA techniques are a convenient method for preparing full length antibodies and other large proteinaceous specific binding agents of the present invention, or fragments thereof. A cDNA molecule encoding the antibody or fragment may be inserted into an expression vector, which can in turn be inserted into a host cell for production of the antibody or fragment. It is understood that the cDNAs encoding such antibodies may be modified to vary from the "original" cDNA (translated from the mRNA) to provide for codon degeneracy or to permit codon preference usage in various host cells.

Generally, a DNA molecule encoding an antibody can be obtained using procedures described herein in the Examples. Where it is desirable to obtain Fab molecules or CDRs that are related to the original antibody molecule, one can screen a suitable library (phage display library; lymphocyte library, etc.) using standard techniques to identify and clone related Fabs/CDRs. Probes used for such screening may be full length or truncated Fab probes encoding the Fab portion of the original antibody, probes against one or more CDRs from the Fab portion of the original antibody, or other suitable probes. Where DNA fragments are used as probes, typical hybridization conditions are those such as set forth in Ausubel et. al. (Current Protocols in Molecular Biology, Current Protocols Press

(1994)). After hybridization, the probed blot can be washed at a suitable stringency, depending on such factors as probe size, expected homology of probe to clone, the type of library being screened, and the number of clones being screened. Examples of high stringency screening are 0.1 X SSC, and 0.1 percent SDS at a temperature between 50-65°C.

A variety of expression vector/host systems may be utilized to contain and express the polynucleotide molecules encoding the specific binding agent polypeptides of the invention. These systems include but are not limited to microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with virus expression vectors (e.g., baculovirus); plant cell systems transfected with virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with bacterial expression vectors (e.g., Ti or pBR322 plasmid); or animal cell systems.

Mammalian cells that are useful in recombinant specific binding agent protein productions include but are not limited to VERO cells, HeLa cells, Chinese hamster ovary (CHO) cell lines, COS cells (such as COS-7), W138, BHK, HepG2, 3T3, RIN, MDCK, A549, PC12, K562 and 293 cells, as well as hybridoma cell lines as described herein. Mammalian cells are preferred for preparation of those specific binding agents such as antibodies and antibody fragments that are typically glycosylated and require proper refolding for activity. Preferred mammalian cells include CHO cells, hybridoma cells, and myeloid cells.

Some exemplary protocols for the recombinant expression of the specific binding agent proteins are described herein below.

The term "expression vector" refers to a plasmid, phage, virus or vector, for expressing a polypeptide from a DNA (RNA) sequence. An expression vector can comprise a transcriptional unit comprising an assembly of (1) a genetic element or elements having a regulatory role in gene expression, for example, promoters or enhancers, (2) a structural or sequence that encodes the binding

agent which is transcribed into mRNA and translated into protein, and (3) appropriate transcription initiation and termination sequences. Structural units intended for use in yeast or eukaryotic expression systems preferably include a leader sequence enabling extracellular secretion of translated protein by a host cell. Alternatively, where recombinant specific binding agent protein is expressed without a leader or transport sequence, it may include an amino terminal methionine residue. This residue may or may not be subsequently cleaved from the expressed recombinant protein to provide a final specific binding agent product.

For example, the specific binding agents may be recombinantly expressed in yeast using a commercially available expression system, e.g., the Pichia Expression System (Invitrogen, San Diego, CA), following the manufacturer's instructions. This system also relies on the pre-pro-alpha sequence to direct secretion, but transcription of the insert is driven by the alcohol oxidase (AOX1) promoter upon induction by methanol.

The secreted specific binding agent peptide is purified from the yeast growth medium by, e.g., the methods used to purify the peptide from bacterial and mammalian cell supernatants.

Alternatively, the cDNA encoding the specific binding agent peptide may be cloned into the baculovirus expression vector pVL1393 (PharMingen, San Diego, CA). This vector can be used according to the manufacturer's directions (PharMingen) to infect Spodoptera frugiperda cells in sF9 protein-free media and to produce recombinant protein. The specific binding agent protein can be purified and concentrated from the media using a heparin-Sepharose column (Pharmacia).

Alternatively, the peptide may be expressed in an insect system. Insect systems for protein expression are well known to those of skill in the art. In one such system, Autographa californica nuclear polyhedrosis virus (AcNPV) can be used as a vector to express foreign genes in Spodoptera frugiperda cells or in Trichoplusia larvae. The specific binding agent peptide coding sequence can be cloned into a nonessential region of the virus, such as the polyhedrin gene, and

placed under control of the polyhedrin promoter. Successful insertion of the specific binding agent peptide will render the polyhedrin gene inactive and produce recombinant virus lacking coat protein coat. The recombinant viruses can be used to infect S. frugiperda cells or Trichoplusia larvae in which peptide is expressed (Smith *et al.*, *J Virol* 46: 584 (1983); Engelhard *et al.*, *Proc Nat Acad Sci (USA)* 91: 3224-7 (1994)).

In another example, the DNA sequence encoding the specific binding agent peptide can be amplified by PCR and cloned into an appropriate vector for example, pGEX-3X (Pharmacia). The pGEX vector is designed to produce a fusion protein comprising glutathione-S-transferase (GST), encoded by the vector, and a specific binding agent protein encoded by a DNA fragment inserted into the vector's cloning site. The primers for the PCR can be generated to include for example, an appropriate cleavage site. Where the specific binding agent fusion moiety is used solely to facilitate expression or is otherwise not desirable as an attachment to the peptide of interest, the recombinant specific binding agent fusion protein may then be cleaved from the GST portion of the fusion protein. The pGEX-3X/specific binding agent peptide construct is transformed into E. coli XL-1 Blue cells (Stratagene, La Jolla CA), and individual transformants isolated and grown. Plasmid DNA from individual transformants can be purified and partially sequenced using an automated sequencer to confirm the presence of the desired specific binding agent encoding nucleic acid insert in the proper orientation.

Expression of polynucleotides encoding anti-Ang-2 antibodies and fragments thereof using the recombinant systems described above may result in production of antibodies or fragments thereof that must be "re-folded" (to properly create various disulphide bridges)in order to be biologically active. Typical refolding procedures for such antibodies are set forth in the Examples herein and in the following section.

Specific binding agents made in bacterial cells may be produced as an insoluble inclusion body in the bacteria, can be purified as follows. Host cells can be sacrificed by centrifugation; washed in 0.15 M NaCl, 10 mM Tris, pH 8, 1 mM

EDTA; and treated with 0.1 mg/ml lysozyme (Sigma, St. Louis, MO) for 15 minutes at room temperature. The lysate can be cleared by sonication, and cell debris can be pelleted by centrifugation for 10 minutes at 12,000 X g. The specific binding agent-containing pellet can be resuspended in 50 mM Tris, pH 8, and 10 mM EDTA, layered over 50% glycerol, and centrifuged for 30 min. at 6000 X g. The pellet can be resuspended in standard phosphate buffered saline solution (PBS) free of Mg^{++} and Ca^{++}. The specific binding agent can be further purified by fractionating the resuspended pellet in a denaturing SDS polyacrylamide gel (Sambrook *et al.*, *supra*). The gel can be soaked in 0.4 M KCl to visualize the protein, which can be excised and electroeluted in gel-running buffer lacking SDS. If the GST fusion protein is produced in bacteria, as a soluble protein, it can be purified using the GST Purification Module (Pharmacia).

Mammalian host systems for the expression of the recombinant protein are well known to those of skill in the art. Host cell strains can be chosen for a particular ability to process the expressed protein or produce certain post-translation modifications that will be useful in providing protein activity. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation and acylation. Different host cells such as CHO, HeLa, MDCK, 293, WI38, as well as hybridoma cell lines, and the like have specific cellular machinery and characteristic mechanisms for such post-translational activities and can be chosen to ensure the correct modification and processing of the introduced, foreign protein.

A number of selection systems can be used to recover the cells that have been transformed for recombinant protein production. Such selection systems include, but are not limited to, HSV thymidine kinase, hypoxanthine-guanine phosphoribosyltransferase and adenine phosphoribosyltransferase genes, in tk-, hgprt- or aprt- cells, respectively. Also, anti-metabolite resistance can be used as the basis of selection for DHFR which confers resistance to methotrexate; gpt which confers resistance to mycophenolic acid; neo which confers resistance to the aminoglycoside G418 and confers resistance to chlorsulfuron; and hygro which that confers resistance to hygromycin. Additional selectable genes that

may be useful include trpB, which allows cells to utilize indole in place of tryptophan, or hisD, which allows cells to utilize histinol in place of histidine. Markers that give a visual indication for identification of transformants include anthocyanins, β-glucuronidase and its substrate, GUS, and luciferase and its substrate, luciferin.

Purification and Refolding of Specific Binding Agents

In some cases, the specific binding agents produced using procedures described above may need to be "refolded" and oxidized into a proper tertiary structure and generating di-sulfide linkages in order to be biologically active. Refolding can be accomplished using a number of procedures well known in the art. Such methods include, for example, exposing the solubilized polypeptide agent to a pH usually above 7 in the presence of a chaotropic agent. The selection of chaotrope is similar to the choices used for inclusion body solubilization, however a chaotrope is typically used at a lower concentration. An exemplary chaotropic agent is guanidine. In most cases, the refolding/oxidation solution will also contain a reducing agent plus its oxidized form in a specific ratio to generate a particular redox potential which allows for dusykfide shuffling to occur for the formation of cysteine bridges. Some commonly used redox couples include cysteine/cystamine, glutathione/dithiobisGSH, cupric chloride, dithiothreitol DTT/dithiane DTT, and 2-mercaptoethanol (bME)/dithio-bME. In many instances, a co-solvent may be used to increase the efficiency of the refolding. Commonly used cosolvents include glycerol, polyethylene glycol of various molecular weights, and arginine.

It will be desirable to purify specific binding agent proteins or variants thereof of the present invention. Protein purification techniques are well known to those of skill in the art. These techniques involve, at one level, the crude fractionation of the polypeptide and non-polypeptide fractions. Having separated the specific binding agent polypeptide from other proteins, the polypeptide of interest can be further purified using chromatographic and electrophoretic techniques to achieve partial or complete purification (or purification to

homogeneity). Analytical methods particularly suited to the preparation of a pure specific binding agent peptide are ion-exchange chromatography, exclusion chromatography; polyacrylamide gel electrophoresis; isoelectric focusing. A particularly efficient method of purifying peptides is fast protein liquid chromatography or even HPLC.

Certain aspects of the present invention concern the purification, and in particular embodiments, the substantial purification, of an encoded specific binding agent protein or peptide. The term "purified specific binding agent protein or peptide" as used herein, is intended to refer to a composition, isolatable from other components, wherein the specific binding agent protein or peptide is purified to any degree relative to its naturally-obtainable state. A purified specific binding agent protein or peptide therefore also refers to a specific binding agent protein or peptide, free from the environment in which it may naturally occur.

Generally, "purified" will refer to a specific binding agent composition that has been subjected to fractionation to remove various other components, and which composition substantially retains its expressed biological activity. Where the term "substantially purified" is used, this designation will refer to a specific binding agent composition in which the specific binding agent protein or peptide forms the major component of the composition, such as constituting about 50%, about 60%, about 70%, about 80%, about 90%, about 95% or more of the proteins in the composition.

Various methods for quantifying the degree of purification of the specific binding agent will be known to those of skill in the art in light of the present disclosure. These include, for example, determining the specific binding activity of an active fraction, or assessing the amount of specific binding agent polypeptides within a fraction by SDS/PAGE analysis. A preferred method for assessing the purity of a specific binding agent fraction is to calculate the binding activity of the fraction, to compare it to the binding activity of the initial extract, and to thus calculate the degree of purification, herein assessed by a "-fold purification number." The actual units used to represent the amount of binding activity will, of course, be dependent upon the particular assay technique chosen

to follow the purification and whether or not the expressed specific binding agent protein or peptide exhibits a detectable binding activity.

Various techniques suitable for use in specific binding agent protein purification will be well known to those of skill in the art. These include, for example, precipitation with ammonium sulphate, PEG, antibodies (immunoprecipitation) and the like or by heat denaturation, followed by centrifugation; chromatography steps such as affinity chromatography (e.g., Protein-A-Sepharose), ion exchange, gel filtration, reverse phase, hydroxylapatite and affinity chromatography; isoelectric focusing; gel electrophoresis; and combinations of such and other techniques. As is generally known in the art, it is believed that the order of conducting the various purification steps may be changed, or that certain steps may be omitted, and still result in a suitable method for the preparation of a substantially purified specific binding agent.

There is no general requirement that the specific binding agent always be provided in its most purified state. Indeed, it is contemplated that less substantially specific binding agent products will have utility in certain embodiments. Partial purification may be accomplished by using fewer purification steps in combination, or by utilizing different forms of the same general purification scheme. For example, it is appreciated that a cation-exchange column chromatography performed utilizing an HPLC apparatus will generally result in a greater "-fold" purification than the same technique utilizing a low-pressure chromatography system. Methods exhibiting a lower degree of relative purification may have advantages in total recovery of specific binding agent protein product, or in maintaining binding activity of an expressed specific binding agent protein.

It is known that the migration of a polypeptide can vary, sometimes significantly, with different conditions of SDS/PAGE (Capaldi *et al.*, *Biochem Biophys \ Res Comm*, 76: 425 (1977)). It will therefore be appreciated that under differing electrophoresis conditions, the apparent molecular weights of purified or partially purified specific binding agent expression products may vary.

Binding Assays

Immunological binding assays typically utilize a capture agent to bind specifically to and often immobilize the analyte target antigen. The capture agent is a moiety that specifically binds to the analyte. In one embodiment of the present invention, the capture agent is an antibody or fragment thereof that specifically binds Ang-2. These immunological binding assays are well known in the art (see, Asai, ed., Methods in Cell Biology, Vol. 37, Antibodies in Cell Biology, Academic Press, Inc., New York (1993)).

Immunological binding assays frequently utilize a labeling agent that will signal the existence of the bound complex formed by the capture agent and antigen. The labeling agent can be one of the molecules comprising the bound complex; i.e. it can be labeled specific binding agent or a labeled anti-specific binding agent antibody. Alternatively, the labeling agent can be a third molecule, commonly another antibody, which binds to the bound complex. The labeling agent can be, for example, an anti-specific binding agent antibody bearing a label. The second antibody, specific for the bound complex, may lack a label, but can be bound by a fourth molecule specific to the species of antibodies which the second antibody is a member of. For example, the second antibody can be modified with a detectable moiety, such as biotin, which can then be bound by a fourth molecule, such as enzyme-labeled streptavidin. Other proteins capable of specifically binding immunoglobulin constant regions, such as protein A or protein G may also be used as the labeling agent. These binding proteins are normal constituents of the cell walls of streptococcal bacteria and exhibit a strong non-immunogenic reactivity with immunoglobulin constant regions from a variety of species (see, generally Akerstrom, *J Immunol*, 135:2589-2542 (1985); and Chaubert, *Mod Pathol*, 10:585-591 (1997)).

Throughout the assays, incubation and/or washing steps may be required after each combination of reagents. Incubation steps can vary from about 5 seconds to several hours, preferably from about 5 minutes to about 24 hours. However, the incubation time will depend upon the assay format, analyte, volume

of solution, concentrations, and the like. Usually, the assays will be carried out at ambient temperature, although they can be conducted over a range of temperatures.

A. Non-competitive binding assays:

Immunological binding assays can be of the non-competitive type. These assays have an amount of captured analyte that is directly measured. For example, in one preferred "sandwich" assay, the capture agent (antibody) can be bound directly to a solid substrate where it is immobilized. These immobilized antibodies then capture (bind to) antigen present in the test sample. The protein thus immobilized is then bound to a labeling agent, such as a second antibody having a label. In another preferred "sandwich" assay, the second antibody lacks a label, but can be bound by a labeled antibody specific for antibodies of the species from which the second antibody is derived. The second antibody also can be modified with a detectable moiety, such as biotin, to which a third labeled molecule can specifically bind, such as streptavidin. (See, Harlow and Lane, Antibodies, A Laboratory Manual, Ch 14, Cold Spring Harbor Laboratory, NY (1988), incorporated herein by reference).

B. Competitive Binding Assays:

Immunological binding assays can be of the competitive type. The amount of analyte present in the sample is measure indirectly by measuring the amount of an added analyte displaced, or competed away, from a capture agent by the analyte present in the sample. In one preferred competitive binding assay, a known amount of analyte, usually labeled, is added to the sample and the sample is then contacted with an antibody (the capture agent). The amount of labeled analyze bound to the antibody is inversely proportional to the concentration of analyte present in the sample. (See, Harlow and Lane, Antibodies, A Laboratory Manual, Ch 14, pp. 579-583, *supra*).

In another preferred competitive binding assay, the antibody is immobilized on a solid substrate. The amount of protein bound to the antibody

may be determined either by measuring the amount of protein present in a protein/antibody complex, or alternatively by measuring the amount of remaining uncomplexed protein. The amount of protein may be detected by providing a labeled protein. See, Harlow and Lane, Antibodies, A Laboratory Manual, Ch 14, *supra*).

Yet another preferred competitive binding assay, hapten inhibition is utilized. Here, a known analyte is immobilized on a solid substrate. A known amount of antibody is added to the sample, and the sample is contacted with the immobilized analyte. The amount of antibody bound to the immobilized analyte is inversely proportional to the amount of analyte present in the sample. The amount of immobilized antibody may be detected by detecting either the immobilized fraction of antibody or the fraction that remains in solution. Detection may be direct where the antibody is labeled or indirect by the subsequent addition of a labeled moiety that specifically binds to the antibody as described above.

C. Utilization of Competitive Binding Assays:

The competitive binding assays can be used for cross-reactivity determinations to permit a skilled artisan to determine if a protein or enzyme complex which is recognized by a specific binding agent of the invention is the desired protein and not a cross-reacting molecule or to determine whether the antibody to is specific for the antigen and does not bind unrelated antigens. In assays of this type, antigen can be immobilized to a solid support and an unknown protein mixture is added to the assay, which will compete with the binding of the specific binding agents to the immobilized protein. The competing molecule also binds one or more antigens unrelated to the antigen. The ability of the proteins to compete with the binding of the specific binding agents antibodies to the immobilized antigen is compared to the binding by the same protein that was immobilized to the solid support to determine the cross-reactivity of the protein mix.

D. Other Binding Assays:

The present invention also provides Western blot methods to detect or quantify the presence of Ang-2 in a sample. The technique generally comprises separating sample proteins by gel electrophoresis on the basis of molecular weight and transferring the proteins to a suitable solid support, such as nitrocellulose filter, a nylon filter, or derivatized nylon filter. The sample is incubated with antibodies or fragments thereof that specifically bind Ang-2 and the resulting complex is detected. These antibodies may be directly labeled or alternatively may be subsequently detected using labeled antibodies that specifically bind to the antibody.

Binding assays to detect those Ang-2 specific binding agents that disrupt Ang-2 binding to its receptor are set forth in the Examples herein.

Diagnostic Assays

The antibodies or fragments thereof of present invention are useful for the diagnosis of conditions or diseases characterized by expression of Ang-2 or subunits, or in assays to monitor patients being treated with inducers of Ang-2, its fragments, agonists or inhibitors of Ang-2 activity. Diagnostic assays for Ang-2 include methods utilizing a specific binding agent and a label to detect Ang-2 in human body fluids or extracts of cells or tissues. The specific binding agents of the present invention can be used with or without modification. In a preferred diagnostic assay, the specific binding agents will be labeled by attaching, e.g., a label or a reporter molecule. A wide variety of labels and reporter molecules are known, some of which have been already described herein. In particular, the present invention is useful for diagnosis of human disease.

A variety of protocols for measuring Ang-2 proteins using either polyclonal or monoclonal antibodies specific for the respective protein are known in the art. Examples include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA) and fluorescence activated cell sorting (FACS). A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on Ang-2 is preferred, but a competitive binding

assay can be employed. These assays are described, for example, in Maddox *et al., J Exp Med*, 158:1211 (1983).

In order to provide a basis for diagnosis, normal or standard values for human Ang-2 expression are usually established. This determination can be accomplished by combining body fluids or cell extracts from normal subjects, preferably human, with a specific binding agent, for example, an antibody, to Ang-2, under conditions suitable for complex formation that are well known in the art. The amount of standard complex formation can be quantified by comparing the binding of the specific binding agents to known quantities of Ang-2 protein, with both control and disease samples. Then, standard values obtained from normal samples can be compared with values obtained from samples from subjects potentially affected by disease. Deviation between standard and subject values suggests a role for Ang-2 in the disease state.

For diagnostic applications, in certain embodiments, specific binding agents typically will be labeled with a detectable moiety. The detectable moiety can be any one that is capable of producing, either directly or indirectly, a detectable signal. For example, the detectable moiety may be a radioisotope, such as ^{3}H, ^{14}C, ^{32}P, ^{35}S, or ^{125}I, a fluorescent or chemiluminescent compound, such as fluorescein isothiocyanate, rhodamine, or luciferin; or an enzyme, such as alkaline phosphatase, β-galactosidase, or horseradish peroxidase (Bayer *et al., Meth Enz*, 184: 138-163, (1990)).

Diseases

The present invention provides a specific binding agent that binds to Ang-2 that is useful for the treatment of human diseases and pathological conditions. Agents that modulate Ang-2 binding activity, or other cellular activity, may be used in combination with other therapeutic agents to enhance their therapeutic effects or decrease potential side effects.

In one aspect, the present invention provides reagents and methods useful for treating diseases and conditions characterized by undesirable or aberrant levels of Ang-2 activity in a cell. These diseases include cancers, and other

hyperproliferative conditions, such as hyperplasia, psoriasis, contact dermatitis, immunological disorders, and infertility.

The present invention also provides methods of treating cancer in an animal, including humans, comprising administering to the animal an effective amount of a specific binding agent that inhibits or decreases Ang-2 activity. The invention is further directed to methods of inhibiting cancer cell growth, including processes of cellular proliferation, invasiveness, and metastasis in biological systems. Methods include use of a compound of the invention as an inhibitor of cancer cell growth. Preferably, the methods are employed to inhibit or reduce cancer cell growth, invasiveness, metastasis, or tumor incidence in living animals, such as mammals. Methods of the invention are also readily adaptable for use in assay systems, *e.g.*, assaying cancer cell growth and properties thereof, as well as identifying compounds that affect cancer cell growth.

The cancers treatable by methods of the present invention preferably occur in mammals. Mammals include, for example, humans and other primates, as well as pet or companion animals such as dogs and cats, laboratory animals such as rats, mice and rabbits, and farm animals such as horses, pigs, sheep, and cattle.

Tumors or neoplasms include growths of tissue cells in which the multiplication of the cells is uncontrolled and progressive. Some such growths are benign, but others are termed malignant and may lead to death of the organism. Malignant neoplasms or cancers are distinguished from benign growths in that, in addition to exhibiting aggressive cellular proliferation, they may invade surrounding tissues and metastasize. Moreover, malignant neoplasms are characterized in that they show a greater loss of differentiation (greater dedifferentiation), and of their organization relative to one another and their surrounding tissues. This property is also called "anaplasia."

Neoplasms treatable by the present invention also include solid tumors, *i.e.*, carcinomas and sarcomas. Carcinomas include those malignant neoplasms derived from epithelial cells that infiltrate (invade) the surrounding tissues and give rise to metastases. Adenocarcinomas are carcinomas derived from glandular tissue, or which form recognizable glandular structures. Another broad category

or cancers includes sarcomas, which are tumors whose cells are embedded in a fibrillar or homogeneous substance like embryonic connective tissue. The invention also enables treatment of cancers of the myeloid or lymphoid systems, including leukemias, lymphomas and other cancers that typically do not present as a tumor mass, but are distributed in the vascular or lymphoreticular systems.

The type of cancer or tumor cells amenable to treatment according to the invention include, for example, ACTH-producing tumor, acute lymphocytic leukemia, acute nonlymphocytic leukemia, cancer of the adrenal cortex, bladder cancer, brain cancer, breast cancer, cervical cancer, chronic lymphocytic leukemia, chronic myelocytic leukemia, colorectal cancer, cutaneous T-cell lymphoma, endometrial cancer, esophageal cancer, Ewing's sarcoma, gallbladder cancer, hairy cell leukemia, head and neck cancer, Hodgkin's lymphoma, Kaposi's sarcoma, kidney cancer, liver cancer, lung cancer (small and non-small cell), malignant peritoneal effusion, malignant pleural effusion, melanoma, mesothelioma, multiple myeloma, neuroblastoma, glioma, non-Hodgkin's lymphoma, osteosarcoma, ovarian cancer, ovarian (germ cell) cancer, pancreatic cancer, penile cancer, prostate cancer, retinoblastoma, skin cancer, soft tissue sarcoma, squamous cell carcinomas, stomach cancer, testicular cancer, thyroid cancer, trophoblastic neoplasms, uterine cancer, vaginal cancer, cancer of the vulva, and Wilms' tumor.

The invention is particularly illustrated herein in reference to treatment of certain types of experimentally defined cancers. In these illustrative treatments, standard state-of-the-art *in vitro* and *in vivo* models have been used. These methods can be used to identify agents that can be expected to be efficacious in *in vivo* treatment regimens. However, it will be understood that the method of the invention is not limited to the treatment of these tumor types, but extends to any solid tumor derived from any organ system. Cancers whose invasiveness or metastasis is associated with Ang-2 expression or activity are especially susceptible to being inhibited or even induced to regress by means of the invention.

The invention can also be practiced by including with a specific binding agent of the invention, such as an antibody, in combination with another anti-cancer chemotherapeutic agent, such as any conventional chemotherapeutic agent. The combination of a specific binding agent with such other agents can potentiate the chemotherapeutic protocol. Numerous chemotherapeutic protocols will present themselves in the mind of the skilled practitioner as being capable of incorporation into the method of the invention. Any chemotherapeutic agent can be used, including alkylating agents, antimetabolites, hormones and antagonists, radioisotopes, as well as natural products. For example, the compound of the invention can be administered with antibiotics such as doxorubicin and other anthracycline analogs, nitrogen mustards such as cyclophosphamide, pyrimidine analogs such as 5-fluorouracil, cisplatin, hydroxyurea, taxol and its natural and synthetic derivatives, and the like. As another example, in the case of mixed tumors, such as adenocarcinoma of the breast, where the tumors include gonadotropin-dependent and gonadotropin-independent cells, the compound can be administered in conjunction with leuprolide or goserelin (synthetic peptide analogs of LH-RH). Other antineoplastic protocols include the use of a tetracycline compound with another treatment modality, *e.g.*, surgery, radiation, etc., also referred to herein as "adjunct antineoplastic modalities." Thus, the method of the invention can be employed with such conventional regimens with the benefit of reducing side effects and enhancing efficacy.

The present invention thus provides compositions and methods useful for the treatment of a wide variety of cancers, including solid tumors and leukemias. Types of cancer that may be treated include, but are not limited to: adenocarcinoma of the breast, prostate, and colon; all forms of bronchogenic carcinoma of the lung; myeloid; melanoma; hepatoma; neuroblastoma; papilloma; apudoma; choristoma; branchioma; malignant carcinoid syndrome; carcinoid heart disease; carcinoma (*e.g.*, Walker, basal cell, basosquamous, Brown-Pearce, ductal, Ehrlich tumor, Krebs 2, merkel cell, mucinous, non-small cell lung, oat cell, papillary, scirrhous, bronchiolar, bronchogenic, squamous cell, and transitional cell); histiocytic disorders; leukemia; histiocytosis malignant; Hodgkin's disease;

immunoproliferative small lung cell carcinoma; non-Hodgkin's lymphoma; plasmacytoma; reticuloendotheliosis; melanoma; chondroblastoma; chondroma; chondrosarcoma; fibroma; fibrosarcoma; giant cell tumors; histiocytoma; lipoma; liposarcoma; mesothelioma; myxoma; myxosarcoma; osteoma; osteosarcoma; chordoma; craniopharyngioma; dysgerminoma; hamartoma; mesenchymoma; mesonephroma; myosarcoma; ameloblastoma; cementoma; odontoma; teratoma; thymoma; tophoblastic tumor. Further, the following types of cancers may also be treated: adenoma; cholangioma; cholesteatoma; cyclindroma; cystadenocarcinoma; cystadenoma; granulosa cell tumor; gynandroblastoma; hepatoma; hidradenoma; islet cell tumor; Leydig cell tumor; papilloma; Sertoli cell tumor; theca cell tumor; leiomyoma; leiomyosarcoma; myoblastoma; myoma; myosarcoma; rhabdomyoma; rhabdomyosarcoma; ependymoma; ganglioneuroma; glioma; medulloblastoma; meningioma; neurilemmoma; neuroblastoma; neuroepithelioma; neurofibroma; neuroma; paraganglioma; paraganglioma nonchromaffin; angiokeratoma; angiolymphoid hyperplasia with eosinophilia; angioma sclerosing; angiomatosis; glomangioma; hemangioendothelioma; hemangioma; hemangiopericytoma; hemangiosarcoma; lymphangioma; lymphangiomyoma; lymphangiosarcoma; pinealoma; carcinosarcoma; chondrosarcoma; cystosarcoma phyllodes; fibrosarcoma; hemangiosarcoma; leiomyosarcoma; leukosarcoma; liposarcoma; lymphangiosarcoma; myosarcoma; myxosarcoma; ovarian carcinoma; rhabdomyosarcoma; sarcoma; neoplasms; nerofibromatosis; and cervical dysplasia.

Another aspect of the present invention is using the materials and methods of the present invention to prevent and/or treat any hyperproliferative condition of the skin including psoriasis and contact dermatitis or other hyperproliferative diseases. It has been demonstrated that patients with psoriasis and contact dermatitis have elevated Ang-2 activity within these lesions (Ogoshi *et al., J. Inv. Dermatol.*, 110:818-23 (1998)). Preferably, specific binding agents specific for Ang-2 will be used in combination with other pharmaceutical agents to treat humans that express these clinical symptoms. The specific binding agents can be

delivered using any of the various carriers through routes of administration described herein and others that are well known to those of skill in the art.

Other aspects of the present invention include treating various retinopathies (including diabetic retinopathy and age-related macular degeneration) in which angiogenesis is involved, as well as disorders/diseases of the female reproductive tract such as endometriosis, uterine fibroids, and other such conditions associated with dysfunctional vascular proliferation (including endometrial microvascular growth) during the female reproductive cycle.

Still another aspect of the present invention relates to treating abnormal vascular growth including cerebral arteriovenous malformations (AVMs) gastrointestinal mucosal injury and repair, ulceration of the gastroduodenal mucosa in patients with a history of peptic ulcer disease, including ischemia resulting from stroke, a wide spectrum of pulmonary vascular disorders in liver disease and portal hypertension in patients with nonhepatic portal hypertension.

Another aspect of present invention is the prevention of cancers utilizing the compositions and methods provided by the present invention. Such reagents will include specific binding agents against Ang-2.

Pharmaceutical Compositions

Pharmaceutical compositions of Ang-2 specific binding agents are within the scope of the present invention. Pharmaceutical compositions comprising antibodies are described in detail in, for example, US Patent 6,171,586, to Lam *et al.*, issued January 9, 2001. Such compositions comprise a therapeutically or prophylactically effective amount of a specific binding agent, such as an antibody, or a fragment, variant, derivative or fusion thereof as described herein, in admixture with a pharmaceutically acceptable agent. In a preferred embodiment, pharmaceutical compositions comprise antagonist specific binding agents that modulate partially or completely at least one biological activity of Ang-2 in admixture with a pharmaceutically acceptable agent. Typically, the specific binding agents will be sufficiently purified for administration to an animal.

The pharmaceutical composition may contain formulation materials for modifying, maintaining or preserving, for example, the pH, osmolarity, viscosity, clarity, color, isotonicity, odor, sterility, stability, rate of dissolution or release, adsorption or penetration of the composition. Suitable formulation materials include, but are not limited to, amino acids (such as glycine, glutamine, asparagine, arginine or lysine); antimicrobials; antioxidants (such as ascorbic acid, sodium sulfite or sodium hydrogen-sulfite); buffers (such as borate, bicarbonate, Tris-HCl, citrates, phosphates, other organic acids); bulking agents (such as mannitol or glycine), chelating agents (such as ethylenediamine tetraacetic acid (EDTA)); complexing agents (such as caffeine, polyvinylpyrrolidone, beta-cyclodextrin or hydroxypropyl-beta-cyclodextrin); fillers; monosaccharides; disaccharides and other carbohydrates (such as glucose, mannose, or dextrins); proteins (such as serum albumin, gelatin or immunoglobulins); coloring; flavoring and diluting agents; emulsifying agents; hydrophilic polymers (such as polyvinylpyrrolidone); low molecular weight polypeptides; salt-forming counter ions (such as sodium); preservatives (such as benzalkonium chloride, benzoic acid, salicylic acid, thimerosal, phenethyl alcohol, methylparaben, propylparaben, chlorhexidine, sorbic acid or hydrogen peroxide); solvents (such as glycerin, propylene glycol or polyethylene glycol); sugar alcohols (such as mannitol or sorbitol); suspending agents; surfactants or wetting agents (such as pluronics, PEG, sorbitan esters, polysorbates such as polysorbate 20, polysorbate 80, triton, tromethamine, lecithin, cholesterol, tyloxapal); stability enhancing agents (sucrose or sorbitol); tonicity enhancing agents (such as alkali metal halides (preferably sodium or potassium chloride, mannitol sorbitol); delivery vehicles; diluents; excipients and/or pharmaceutical adjuvants. (Remington's Pharmaceutical Sciences, 18th Edition, A.R. Gennaro, ed., Mack Publishing Company, 1990).

The optimal pharmaceutical composition will be determined by one skilled in the art depending upon, for example, the intended route of administration, delivery format, and desired dosage. See for example, Remington's Pharmaceutical Sciences, *supra*. Such compositions may influence the physical

state, stability, rate of *in vivo* release, and rate of *in vivo* clearance of the specific binding agent.

The primary vehicle or carrier in a pharmaceutical composition may be either aqueous or non-aqueous in nature. For example, a suitable vehicle or carrier may be water for injection, physiological saline solution or artificial cerebrospinal fluid, possibly supplemented with other materials common in compositions for parenteral administration. Neutral buffered saline or saline mixed with serum albumin are further exemplary vehicles. Other exemplary pharmaceutical compositions comprise Tris buffer of about pH 7.0-8.5, or acetate buffer of about pH 4.0-5.5, which may further include sorbitol or a suitable substitute therefore. In one embodiment of the present invention, binding agent compositions may be prepared for storage by mixing the selected composition having the desired degree of purity with optional formulation agents (Remington's Pharmaceutical Sciences, *supra*) in the form of a lyophilized cake or an aqueous solution. Further, the binding agent product may be formulated as a lyophilizate using appropriate excipients such as sucrose.

The pharmaceutical compositions can be selected for parenteral delivery. Alternatively, the compositions may be selected for inhalation or for enteral delivery such as orally, aurally, opthalmically, rectally, or vaginally. The preparation of such pharmaceutically acceptable compositions is within the skill of the art.

The formulation components are present in concentrations that are acceptable to the site of administration. For example, buffers are used to maintain the composition at physiological pH or at slightly lower pH, typically within a pH range of from about 5 to about 8.

When parenteral administration is contemplated, the therapeutic compositions for use in this invention may be in the form of a pyrogen-free, parenterally acceptable aqueous solution comprising the desired specific binding agent in a pharmaceutically acceptable vehicle. A particularly suitable vehicle for parenteral injection is sterile distilled water in which a binding agent is formulated as a sterile, isotonic solution, properly preserved. Yet another preparation can

involve the formulation of the desired molecule with an agent, such as injectable microspheres, bio-erodible particles, polymeric compounds (polylactic acid, polyglycolic acid), beads, or liposomes, that provides for the controlled or sustained release of the product which may then be delivered via a depot injection. Hyaluronic acid may also be used, and this may have the effect of promoting sustained duration in the circulation. Other suitable means for the introduction of the desired molecule include implantable drug delivery devices.

In another aspect, pharmaceutical formulations suitable for parenteral administration may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks' solution, ringer's solution, or physiologically buffered saline. Aqueous injection suspensions may contain substances that increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils, such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate, triglycerides, or liposomes. Non-lipid polycationic amino polymers may also be used for delivery. Optionally, the suspension may also contain suitable stabilizers or agents to increase the solubility of the compounds and allow for the preparation of highly concentrated solutions.

In another embodiment, a pharmaceutical composition may be formulated for inhalation. For example, a binding agent may be formulated as a dry powder for inhalation. Polypeptide or nucleic acid molecule inhalation solutions may also be formulated with a propellant for aerosol delivery. In yet another embodiment, solutions may be nebulized. Pulmonary administration is further described in PCT Application No. PCT/US94/001875, which describes pulmonary delivery of chemically modified proteins.

It is also contemplated that certain formulations may be administered orally. In one embodiment of the present invention, binding agent molecules that are administered in this fashion can be formulated with or without those carriers customarily used in the compounding of solid dosage forms such as tablets and capsules. For example, a capsule may be designed to release the active portion of

the formulation at the point in the gastrointestinal tract when bioavailability is maximized and pre-systemic degradation is minimized. Additional agents can be included to facilitate absorption of the binding agent molecule. Diluents, flavorings, low melting point waxes, vegetable oils, lubricants, suspending agents, tablet disintegrating agents, and binders may also be employed.

Pharmaceutical compositions for oral administration can also be formulated using pharmaceutically acceptable carriers well known in the art in dosages suitable for oral administration. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for ingestion by the patient.

Pharmaceutical preparations for oral use can be obtained through combining active compounds with solid excipient and processing the resultant mixture of granules (optionally, after grinding) to obtain tablets or dragee cores. Suitable auxiliaries can be added, if desired. Suitable excipients include carbohydrate or protein fillers, such as sugars, including lactose, sucrose, mannitol, and sorbitol; starch from corn, wheat, rice, potato, or other plants; cellulose, such as methyl cellulose, hydroxypropylmethyl-cellulose, or sodium carboxymethylcellulose; gums, including arabic and tragacanth; and proteins, such as gelatin and collagen. If desired, disintegrating or solubilizing agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, and alginic acid or a salt thereof, such as sodium alginate.

Dragee cores may be used in conjunction with suitable coatings, such as concentrated sugar solutions, which may also contain gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for product identification or to characterize the quantity of active compound, i.e., dosage.

Pharmaceutical preparations that can be used orally also include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a coating, such as glycerol or sorbitol. Push-fit capsules can contain active ingredients mixed with fillers or binders, such as lactose or starches, lubricants,

such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid, or liquid polyethylene glycol with or without stabilizers.

Another pharmaceutical composition may involve an effective quantity of binding agent in a mixture with non-toxic excipients that are suitable for the manufacture of tablets. By dissolving the tablets in sterile water, or other appropriate vehicle, solutions can be prepared in unit dose form. Suitable excipients include, but are not limited to, inert diluents, such as calcium carbonate, sodium carbonate or bicarbonate, lactose, or calcium phosphate; or binding agents, such as starch, gelatin, or acacia; or lubricating agents such as magnesium stearate, stearic acid, or talc.

Additional pharmaceutical compositions will be evident to those skilled in the art, including formulations involving binding agent molecules in sustained- or controlled-delivery formulations. Techniques for formulating a variety of other sustained- or controlled-delivery means, such as liposome carriers, bio-erodible microparticles or porous beads and depot injections, are also known to those skilled in the art. See for example, PCT/US93/00829 that describes controlled release of porous polymeric microparticles for the delivery of pharmaceutical compositions. Additional examples of sustained-release preparations include semipermeable polymer matrices in the form of shaped articles, e.g. films, or microcapsules. Sustained release matrices may include polyesters, hydrogels, polylactides (U.S. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma ethyl-L-glutamate (Sidman *et al., Biopolymers*, 22:547-556 (1983)), poly (2-hydroxyethyl-methacrylate) (Langer *et al., J Biomed Mater Res*, 15:167-277, (1981)) and (Langer *et al., Chem Tech*, 12:98-105(1982)), ethylene vinyl acetate (Langer *et al., supra*) or poly-D(-)-3-hydroxybutyric acid (EP 133,988). Sustained-release compositions also include liposomes, which can be prepared by any of several methods known in the art. See e.g., Eppstein *et al., Proc Natl Acad Sci (USA)*, 82:3688-3692 (1985); EP 36,676; EP 88,046; EP 143,949.

The pharmaceutical composition to be used for *in vivo* administration typically must be sterile. This may be accomplished by filtration through sterile filtration membranes. Where the composition is lyophilized, sterilization using this method may be conducted either prior to or following lyophilization and reconstitution. The composition for parenteral administration may be stored in lyophilized form or in solution. In addition, parenteral compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

Once the pharmaceutical composition has been formulated, it may be stored in sterile vials as a solution, suspension, gel, emulsion, solid, or a dehydrated or lyophilized powder. Such formulations may be stored either in a ready-to-use form or in a form (e.g., lyophilized) requiring reconstitution prior to administration.

In a specific embodiment, the present invention is directed to kits for producing a single-dose administration unit. The kits may each contain both a first container having a dried protein and a second container having an aqueous formulation. Also included within the scope of this invention are kits containing single and multi-chambered pre-filled syringes (e.g., liquid syringes and lyosyringes).

An effective amount of a pharmaceutical composition to be employed therapeutically will depend, for example, upon the therapeutic context and objectives. One skilled in the art will appreciate that the appropriate dosage levels for treatment will thus vary depending, in part, upon the molecule delivered, the indication for which the binding agent molecule is being used, the route of administration, and the size (body weight, body surface or organ size) and condition (the age and general health) of the patient. Accordingly, the clinician may titer the dosage and modify the route of administration to obtain the optimal therapeutic effect. A typical dosage may range from about 0.1 mg/kg to up to about 100 mg/kg or more, depending on the factors mentioned above. In other embodiments, the dosage may range from 0.1 mg/kg up to about 100 mg/kg; or 1 mg/kg up to about 100 mg/kg; or 5 mg/kg up to about 100 mg/kg.

For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays or in animal models such as mice, rats, rabbits, dogs, or pigs. An animal model may also be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

The exact dosage will be determined in light of factors related to the subject requiring treatment. Dosage and administration are adjusted to provide sufficient levels of the active compound or to maintain the desired effect. Factors that may be taken into account include the severity of the disease state, the general health of the subject, the age, weight, and gender of the subject, time and frequency of administration, drug combination(s), reaction sensitivities, and response to therapy. Long-acting pharmaceutical compositions may be administered every 3 to 4 days, every week, or biweekly depending on the half-life and clearance rate of the particular formulation.

The frequency of dosing will depend upon the pharmacokinetic parameters of the binding agent molecule in the formulation used. Typically, a composition is administered until a dosage is reached that achieves the desired effect. The composition may therefore be administered as a single dose, or as multiple doses (at the same or different concentrations/dosages) over time, or as a continuous infusion. Further refinement of the appropriate dosage is routinely made. Appropriate dosages may be ascertained through use of appropriate dose-response data.

The route of administration of the pharmaceutical composition is in accord with known methods, e.g. orally, through injection by intravenous, intraperitoneal, intracerebral (intra-parenchymal), intracerebroventricular, intramuscular, intra-ocular, intraarterial, intraportal, intralesional routes, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual, urethral, vaginal, or rectal means, by sustained release systems

or by implantation devices. Where desired, the compositions may be administered by bolus injection or continuously by infusion, or by implantation device.

Alternatively or additionally, the composition may be administered locally via implantation of a membrane, sponge, or another appropriate material on to which the desired molecule has been absorbed or encapsulated. Where an implantation device is used, the device may be implanted into any suitable tissue or organ, and delivery of the desired molecule may be via diffusion, timed-release bolus, or continuous administration.

In some cases, it may be desirable to use pharmaceutical compositions in an *ex vivo* manner. In such instances, cells, tissues, or organs that have been removed from the patient are exposed to the pharmaceutical compositions after which the cells, tissues and/or organs are subsequently implanted back into the patient.

In other cases, a binding agent which is a polypeptide can be delivered by implanting certain cells that have been genetically engineered, using methods such as those described herein, to express and secrete the polypeptide. Such cells may be animal or human cells, and may be autologous, heterologous, or xenogeneic. Optionally, the cells may be immortalized. In order to decrease the chance of an immunological response, the cells may be encapsulated to avoid infiltration of surrounding tissues. The encapsulation materials are typically biocompatible, semi-permeable polymeric enclosures or membranes that allow the release of the protein product(s) but prevent the destruction of the cells by the patient's immune system or by other detrimental factors from the surrounding tissues.

Combination Therapy

Specific binding agents of the invention can be utilized in combination with other therapeutic in the treatment of Ang-2 pathologies. These other therapeutics include, for example radiation treatment, chemotherapy, and targeted therapies as described herein below. Additional combination therapies not specifically listed herein are also within the scope of the present invention.

The invention thus includes administration of one or more specific binding agent of the invention administered to the same patient in combination with one or more additionally suitable agent(s), each being administered according to a regimen suitable for that medicament. This includes concurrent administration of a specific binding agent of the invention and one or more suitable agents. As used herein, the terms "concurrently administered" and "concurrent administration" encompass substantially simultaneous administration of one or more specific binding agent(s) according to the invention and one or more additionally suitable agents(s).

As used herein, the term, "non-concurrent" administration encompasses administering one or more selective binding agent according to the invention and one or more additionally suitable agent(s), at different times, in any order, whether overlapping or not. This includes, but is not limited to, sequential treatment (such as pretreatment, post-treatment, or overlapping treatment) with the components of the combination, as well as regimens in which the drugs are alternated, or wherein one component is administered long-term and the other(s) are administered intermittently. Components may be administered in the same or in separate compositions, and by the same or different routes of administration.

In certain embodiments, the combination therapy comprises a specific binding agent capable of binding Ang-2, in combination with at least one additional anti-angiogenic agent. Agents include, but are not limited to, *in vitro* synthetically prepared chemical compositions, antibodies, antigen binding regions, radionuclides, and combinations and conjugates thereof. In certain embodiments, an agent may act as an agonist, antagonist, alllosteric modulator, or toxin. In certain embodiments, an agent may act to inhibit or stimulate its target (e.g., receptor or enzyme activation or inhibition), and thereby promote cell death or arrest cell growth.

Chemotherapy treatment can employ anti-neoplastic agents including, for example, alkylating agents including: nitrogen mustards, such as mechlorethamine, cyclophosphamide, ifosfamide, melphalan and chlorambucil; nitrosoureas, such as carmustine (BCNU), lomustine (CCNU), and semustine

(methyl-CCNU); ethylenimines/methylmelamine such as thriethylenemelamine (TEM), triethylene, thiophosphoramide (thiotepa), hexamethylmelamine (HMM, altretamine); alkyl sulfonates such as busulfan; triazines such as dacarbazine (DTIC); antimetabolites including folic acid analogs such as methotrexate and trimetrexate, pyrimidine analogs such as 5-fluorouracil, fluorodeoxyuridine, gemcitabine, cytosine arabinoside (AraC, cytarabine), 5-azacytidine, 2,2′-difluorodeoxycytidine, purine analogs such as 6-mercaptopurine, 6-thioguanine, azathioprine, 2'-deoxycoformycin (pentostatin), erythrohydroxynonyladenine (EHNA), fludarabine phosphate, and 2-chlorodeoxyadenosine (cladribine, 2-CdA); natural products including antimitotic drugs such as paclitaxel, vinca alkaloids including vinblastine (VLB), vincristine, and vinorelbine, taxotere, estramustine, and estramustine phosphate; ppipodophylotoxins such as etoposide and teniposide; antibiotics such as actimomycin D, daunomycin (rubidomycin), doxorubicin, mitoxantrone, idarubicin, bleomycins, plicamycin (mithramycin), mitomycinC, and actinomycin; enzymes such as L-asparaginase; biological response modifiers such as interferon-alpha, IL-2, G-CSF and GM-CSF; miscellaneous agents including platinium coordination complexes such as cisplatin and carboplatin, anthracenediones such as mitoxantrone, substituted urea such as hydroxyurea, methylhydrazine derivatives including N-methylhydrazine (MIH) and procarbazine, adrenocortical suppressants such as mitotane (o,p′-DDD) and aminoglutethimide; hormones and antagonists including adrenocorticosteroid antagonists such as prednisone and equivalents, dexamethasone and aminoglutethimide; progestin such as hydroxyprogesterone caproate, medroxyprogesterone acetate and megestrol acetate; estrogen such as diethylstilbestrol and ethinyl estradiol equivalents; antiestrogen such as tamoxifen; androgens including testosterone propionate and fluoxymesterone/equivalents; antiandrogens such as flutamide, gonadotropin-releasing hormone analogs and leuprolide; and non-steroidal antiandrogens such as flutamide.

Cancer therapies, which may be administered with a specific binding agent to Ang-2, also include, but are not limited to, targeted therapies as described herein. Examples of targeted therapies include, but are not limited to, use of

therapeutic antibodies. Exemplary therapeutic antibodies, include, but are not limited to, mouse, mouse-human chimeric, CDR-grafted, humanized and fully human antibodies, and synthetic antibodies, including, but not limited to, those selected by screening antibody libraries. Exemplary antibodies include, but are not limited to, those which bind to cell surface proteins Her2, CDC20, CDC33, mucin-like glycoprotein, and epidermal growth factor receptor (EGFR) present on tumor cells, and optionally induce a cytostatic and/or cytotoxic effect on tumor cells displaying these proteins. Exemplary antibodies also include HERCEPTIN™ (trastuzumab), which may be used to treat breast cancer and other forms of cancer, and RITUXAN™ (rituximab), ZEVALIN™ (ibritumomab tiuxetan), GLEEVEC™, and LYMPHOCIDE™ (epratuzumab), which may be used to treat non-Hodgkin's lymphoma and other forms of cancer. Certain exemplary antibodies also include ERBITUX™ (IMC-C225); IRESSA™ (ertinolib) ; BEXXAR™ (iodine 131 tositumomab); KDR (kinase domain receptor) inhibitors; anti-VEGF antibodies and antagonists (e.g., AVASTIN™ and VEGAF-TRAP); anti-VEGF receptor antibodies and antigen binding regions; anti-Ang-1 antibodies and antigen binding regions; antibodies to Tie-2 and other Ang-1 and Ang-2 receptors; Tie-2 ligands; antibodies against Tie-2 kinase inhibitors; and Campath® (Alemtuzumab). In certain embodiments, cancer therapy agents are polypeptides which selectively induce apoptosis in tumor cells, including, but not limited to, TNF-related polypeptides, such as TRAIL (TNF Receptor Apoptosis-Inducing Ligand).

In certain embodiments, suitable cancer therapy agents are known to be anti-angiogenic. Certain such agents include, but are not limited to, IL-8; Campath™, B-FGF; FGF antagonists; Tek antagonists (Cerretti *et al.*, U.S. Publication No. 2003/0162712; Cerretti et al., U.S. Patent No. 6,413,932, and Cerretti et al., U.S. Patent No. 6,521,424, each of which is incorporated herein by reference for any purpose); anti-TWEAK agents (which include, but are not limited to, antibodies and antigen binding regions); soluble TWEAK receptor antagonists (Wiley, U.S. Patent No. 6,727,225); ADAM distintegrins (or domains thereof to antagonize the binding of integrin to its ligands (Fanslow et al., U.S.

Publication No. 2002/0042368); anti-eph receptor and anti-ephrin antibodies; antigen binding regions, or antagonists (U.S. Patent Nos. 5,981,245; 5,728,813; 5,969,110; 6,596,852; 6,232,447; 6,057,124 and patent family members thereof); anti-VEGF agents as described herein (*e.g.*, antibodies or antigen binding regions that specifically bind VEGF, or soluble VEGF receptors or a ligand binding regions thereof) such as AVASTIN™ or VEGF-TRAP™, and anti-VEGF receptor agents (*e.g.*, antibodies or antigen binding regions that specifically bind thereto), EGFR inhibitory agents (*e.g.*, antibodies or antigen binding regions that specifically bind thereto) such as panitumumab, IRESSA™ (gefitinib), TARCEVA™ (erlotinib), anti-Ang-1 and anti-Ang-2 agents (e.g., antibodies or antigen binding regions specifically binding thereto or to their receptors, e.g., Tie-2/TEK), and anti-Tie-2 kinase inhibitory agents (e.g., antibodies or antigen binding regions that specifically bind and inhibit the activity of growth factors, such as antagonists of hepatocyte growth factor (HGF, also known as Scatter Factor), and antibodies or antigen binding regions that specifically bind its receptor "c-met"; anti-PDGF-BB antagonists; antibodies and antigen binding regions to PDGF-BB ligands; and PDGFR kinase inhibitors.

In certain embodiments, cancer therapy agents are angiogenesis inhibitors. Certain such inhibitors include, but are not limited to, SD-7784 (Pfizer, USA); cilengitide.(Merck KGaA, Germany, EPO 770622); pegaptanib octasodium, (Gilead Sciences, USA); Alphastatin, (BioActa, UK); M-PGA, (Celgene, USA, US 5712291); ilomastat, (Arriva, USA, US 5892112); semaxanib, (Pfizer, USA, US 5792783); vatalanib, (Novartis, Switzerland); 2-methoxyestradiol, (EntreMed, USA); TLC ELL-12, (Elan, Ireland); anecortave acetate, (Alcon, USA); alpha-D148 Mab, (Amgen, USA); CEP-7055,(Cephalon, USA); anti-Vn Mab, (Crucell, Netherlands) DAC:antiangiogenic, (ConjuChem, Canada); Angiocidin, (InKine Pharmaceutical, USA); KM-2550, (Kyowa Hakko, Japan); SU-0879, (Pfizer, USA); CGP-79787, (Novartis, Switzerland, EP 970070); ARGENT technology, (Ariad, USA); YIGSR-Stealth, (Johnson & Johnson, USA); fibrinogen-E fragment, (BioActa, UK); angiogenesis inhibitor, (Trigen, UK); TBC-1635, (Encysive Pharmaceuticals, USA); SC-236, (Pfizer, USA); ABT-567, (Abbott,

USA); Metastatin, (EntreMed, USA); angiogenesis inhibitor, (Tripep, Sweden); maspin, (Sosei, Japan); 2-methoxyestradiol, (Oncology Sciences Corporation, USA); ER-68203-00, (IVAX, USA); Benefin, (Lane Labs, USA); Tz-93, (Tsumura, Japan); TAN-1120, (Takeda, Japan); FR-111142, (Fujisawa, Japan, JP 02233610); platelet factor 4, (RepliGen, USA, EP 407122); vascular endothelial growth factor antagonist, (Borean, Denmark); cancer therapy, (University of South Carolina, USA); bevacizumab (pINN), (Genentech, USA); angiogenesis inhibitors, (SUGEN, USA); XL 784, (Exelixis, USA); XL 647, (Exelixis, USA); MAb, alpha5beta3 integrin, second generation, (Applied Molecular Evolution, USA and MedImmune, USA); gene therapy, retinopathy, (Oxford BioMedica, UK); enzastaurin hydrochloride (USAN), (Lilly, USA); CEP 7055, (Cephalon, USA and Sanofi-Synthelabo, France); BC 1, (Genoa Institute of Cancer Research, Italy); angiogenesis inhibitor, (Alchemia, Australia); VEGF antagonist, (Regeneron, USA); rBPI 21 and BPI-derived antiangiogenic, (XOMA, USA); PI 88, (Progen, Australia); cilengitide (pINN), (Merck KGaA; Munich Technical University, Germany, Scripps Clinic and Research Foundation, USA); cetuximab (INN), (Aventis, France); AVE 8062, (Ajinomoto, Japan); AS 1404, (Cancer Research Laboratory, New Zealand); SG 292 (Telios, USA); Endostatin, (Boston Childrens Hospital, USA); ATN 161, (Attenuon, USA); ANGIOSTATIN, (Boston Childrens Hospital, USA); 2-methoxyestradiol, (Boston Childrens Hospital, USA); ZD 6474, (AstraZeneca, UK); ZD 6126, (Angiogene Pharmaceuticals, UK); PPI 2458, (Praecis, USA); AZD 9935, (AstraZeneca, UK); AZD 2171, (AstraZeneca, UK); vatalanib (pINN), (Novartis, Switzerland and Schering AG, Germany); tissue factor pathway inhibitors, (EntreMed, USA); pegaptanib (Pinn), (Gilead Sciences, USA); xanthorrhizol, (Yonsei University, South Korea); vaccine, gene-based, VEGF-2, (Scripps Clinic and Research Foundation, USA); SPV5.2, (Supratek, Canada); SDX 103, (University of California at San Diego, USA); PX 478, (ProlX, USA); METASTATIN, (EntreMed, USA); troponin I, (Harvard University, USA); SU 6668, (SUGEN, USA); OXI 4503, (OXiGENE, USA); o-guanidines, (Dimensional Pharmaceuticals, USA); motuporamine C, (British Columbia University, Canada); CDP 791, (Celltech Group, UK);

atiprimod (pINN), (GlaxoSmithKline, UK); E 7820, (Eisai, Japan); CYC 381, (Harvard University, USA); AE 941, (Aeterna, Canada); vaccine, angiogenesis, (EntreMed, USA); urokinase plasminogen activator inhibitor, (Dendreon, USA); oglufanide (pINN), (Melmotte, USA); HIF-1alfa inhibitors, (Xenova, UK); CEP 5214, (Cephalon, USA); BAY RES 2622, (Bayer, Germany); Angiocidin, (InKine, USA); A6, (Angstrom, USA); KR 31372, (Korea Research Institute of Chemical Technology, South Korea); GW 2286, (GlaxoSmithKline, UK); EHT 0101, (ExonHit, France); CP 868596, (Pfizer, USA); CP 564959, (OSI, USA); CP 547632, (Pfizer, USA); 786034, (GlaxoSmithKline, UK); KRN 633, (Kirin Brewery, Japan); drug delivery system, intraocular, 2-methoxyestradiol, (EntreMed, USA); anginex, (Maastricht University, Netherlands, and Minnesota University, USA); ABT 510, (Abbott, USA); AAL 993, (Novartis, Switzerland); VEGI, (ProteomTech, USA); tumor necrosis factor-alpha inhibitors, (National Institute on Aging, USA); SU 11248, (Pfizer, USA and SUGEN USA); ABT 518, (Abbott, USA); YH16, (Yantai Rongchang, China); S-3APG , (Boston Childrens Hospital, USA and EntreMed, USA); MAb, KDR, (ImClone Systems, USA); MAb, alpha5 beta1, (Protein Design, USA); KDR kinase inhibitor, (Celltech Group, UK, and Johnson & Johnson, USA); GFB 116, (South Florida University, USA and Yale University, USA); CS 706, (Sankyo, Japan); combretastatin A4 prodrug, (Arizona State University, USA); chondroitinase AC, (IBEX, Canada); BAY RES 2690, (Bayer, Germany); AGM 1470, (Harvard University, USA, Takeda, Japan, and TAP, USA); AG 13925, (Agouron, USA); Tetrathiomolybdate, (University of Michigan, USA); GCS 100, (Wayne State University, USA) CV 247, (Ivy Medical, UK); CKD 732, (Chong Kun Dang, South Korea); MAb, vascular endothelium growth factor, (Xenova, UK); irsogladine (INN), (Nippon Shinyaku, Japan); RG 13577, (Aventis, France); WX 360, (Wilex, Germany); squalamine (pINN), (Genaera, USA); RPI 4610, (Sirna, USA); cancer therapy, (Marinova, Australia); heparanase inhibitors, (InSight, Israel); KL 3106, (Kolon, South Korea); Honokiol, (Emory University, USA); ZK CDK, (Schering AG, Germany); ZK Angio, (Schering AG, Germany); ZK 229561, (Novartis, Switzerland, and Schering AG, Germany); XMP 300,

(XOMA, USA); VGA 1102, (Taisho, Japan); VEGF receptor modulators, (Pharmacopeia, USA); VE-cadherin-2 antagonists , (ImClone Systems, USA); Vasostatin, (National Institutes of Health, USA);vaccine, Flk-1, (ImClone Systems, USA); TZ 93, (Tsumura, Japan); TumStatin, (Beth Israel Hospital, USA); truncated soluble FLT 1 (vascular endothelial growth factor receptor 1), (Merck & Co, USA); Tie-2 ligands, (Regeneron, USA); thrombospondin 1 inhibitor, (Allegheny Health, Education and Research Foundation, USA); ; 2-Benzenesulfonamide,4-(5-(4-chlorophenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl)- ; Arriva; and C-Met. AVE 8062 ((2S)-2-amino-3-hydroxy-N-[2-methoxy-5-[(1Z)-2-(3,4,5-trimethoxyphenyl)ethenyl]p henyl]propanamide monohydrochloride); metelimumab (pINN)(immunoglobulin G4, anti-(human transforming growth factor .beta.1 (human monoclonal CAT 192 .gamma.4-chain)), disulfide with human monoclonal CAT 192 .kappa.-chain dimer); Flt3 ligand; CD40 ligand; interleukin-2; interleukin-12; 4-1BB ligand; anti-4-1BB antibodies; TNF antagonists and TNF receptor antagonists including TNFR/Fc, TWEAK antagonists and TWEAK-R antagonists including TWEAK-R/Fc; TRAIL; VEGF antagonists including anti-VEGF antibodies; VEGF receptor (including VEGF-R1 and VEGF-R2, also known as Flt1 and Flk1 or KDR) antagonists; CD148 (also referred to as DEP-1, ECRTP, and PTPRJ, see Takahashi et al., J. Am. Soc. Nephrol. 10: 2135-45 (1999), hereby incorporated by reference for any purpose) agonists; thrombospondin 1 inhibitor, and inhibitors of one or both of Tie-2 or Tie-2 ligands (such as Ang-2). A number of inhibitors of Ang-2 are known in the art, including certain anti-Ang-2 antibodies described in published U.S. Patent Application No. 20030124129 (corresponding to PCT Application No. WO03/030833), and U.S. Patent No. 6,166,185, the contents of which are hereby incorporated by reference in their entirety. Additionally, Ang-2 peptibodies are also known in the art, and can be found in, for example, published U.S. Patent Application No. 20030229023 (corresponding to PCT Application No. WO03/057134), and published U.S. Patent Application No. 20030236193, the contents of which are hereby incorporated by reference in their entirety.

Certain cancer therapy agents include, but are not limited to: thalidomide and thalidomide analogues (N-(2,6-dioxo-3-piperidyl)phthalimide); tecogalan sodium (sulfated polysaccharide peptidoglycan); TAN 1120 (8-acetyl-7,8,9,10-tetrahydro-6,8,11-trihydroxy-1-methoxy-10-[[octahydro-5-hydroxy-2-(2-hydroxypropyl)-4,10-dimethylpyrano[3,4-d]-1,3,6-dioxazocin-8-yl]oxy]-5,12-naphthacenedione); suradista (7,7'-[carbonylbis[imino(1-methyl-1H-pyrrole-4,2-diyl)carbonylimino(1-methyl-1H- pyrrole-4,2-diyl)carbonylimino]]bis-1,3-naphthalenedisulfonic acid tetrasodium salt); SU 302; SU 301; SU 1498 ((E)-2-cyano-3-[4-hydroxy-3,5-bis(1-methylethyl)phenyl]-N-(3-phenylpropyl)-2-propenamide); SU 1433 (4-(6,7-dimethyl-2-quinoxalinyl)-1,2-benzenediol); ST 1514; SR 25989;soluble Tie-2; SERM derivatives, Pharmos; semaxanib (pINN)(3-[(3,5-dimethyl-1H-pyrrol-2-yl)methylene]-1,3-dihydro-2H-indol-2-one); S 836; RG 8803; RESTIN; R 440 (3-(1-methyl-1H-indol-3-yl)-4-(1-methyl-6-nitro-1H-indol-3-yl)-1H-pyrrole-2,5-dione); R 123942 (1-[6-(1,2,4-thiadiazol-5-yl)-3-pyridazinyl]-N-[3-(trifluoromethyl)phenyl]-4-piperidinamine); prolyl hydroxylase inhibitor; progression elevated genes; prinomastat (INN) ((S)-2,2-dimethyl-4-[[p-(4-pyridyloxy)phenyl]sulphonyl]-3-thiomorpholinecarbohyd roxamic acid); NV 1030; NM 3 (8-hydroxy-6-methoxy-alpha-methyl-1-oxo-1H-2-benzopyran-3-acetic acid); NF 681; NF 050; MIG; METH 2; METH 1; manassantin B (alpha-[1-[4-[5-[4-[2-(3,4-dimethoxyphenyl)-2-hydroxy-1-methylethoxy]-3-methoxyphenyl]tetrahydro-3,4-dimethyl-2-furanyl]-2-methoxyphenoxy]ethyl]-1,3-benzodioxole-5-methanol); KDR monoclonal antibody; alpha5beta3 integrin monoclonal antibody; LY 290293 (2-amino-4-(3-pyridinyl)-4H-naphtho[1,2-b]pyran-3-carbonitrile); KP 0201448; KM 2550; integrin-specific peptides; INGN 401; GYKI 66475; GYKI 66462; greenstatin (101-354-plasminogen (human)); gene therapy for rheumatoid arthritis, prostate cancer, ovarian cancer, glioma, endostatin, colorectal cancer, ATF BTPI, antiangiogenesis genes, angiogenesis inhibitor, or angiogenesis; gelatinase inhibitor, FR 111142 (4,5-dihydroxy-2-hexenoic acid 5-methoxy-4-[2-methyl-3-(3-methyl-2-butenyl)oxiranyl]-1-oxaspiro[2.5]oct-6-yl ester); forfenimex (pINN) (S)-alpha-amino-3-hydroxy-4-(hydroxymethyl)benzeneacetic acid); fibronectin antagonist (1-acetyl-L-prolyl-L-

histidyl-L-seryl-L-cysteinyl-L-aspartamide); fibroblast growth factor receptor inhibitor; fibroblast growth factor antagonist; FCE 27164 (7,7'-[carbonylbis[imino(1-methyl-1H-pyrrole-4,2-diyl)carbonylimino(1-methyl-1H-pyrrole-4,2-diyl)carbonylimino]]bis-1,3,5-naphthalenetrisulfonic acid hexasodium salt); FCE 26752 (8,8'-[carbonylbis[imino(1-methyl-1H-pyrrole-4,2-diyl)carbonylimino(1-methyl-1H-pyrrole-4,2-diyl)carbonylimino]]bis-1,3,6-naphthalenetrisulfonic acid); endothelial monocyte activating polypeptide II; VEGFR antisense oligonucleotide; anti-angiogenic and trophic factors; ANCHOR angiostatic agent; endostatin; Del-1 angiogenic protein; CT 3577; contortrostatin; CM 101; chondroitinase AC; CDP 845; CanStatin; BST 2002; BST 2001; BLS 0597; BIBF 1000; ARRESTIN; apomigren (1304-1388-type XV collagen (human gene COL15A1 alpha1-chain precursor)); angioinhibin; aaATIII; A 36; 9alpha-fluoromedroxyprogesterone acetate ((6-alpha)-17-(acetyloxy)-9-fluoro-6-methyl-pregn-4-ene-3,20-dione); 2-methyl-2-phthalimidino-glutaric acid (2-(1,3-dihydro-1-oxo-2H-isoindol-2-yl)-2-methylpentanedioic acid); Yttrium 90 labelled monoclonal antibody BC-1; Semaxanib (3-(4,5-Dimethylpyrrol-2-ylmethylene)indolin-2-one)(C15 H14 N2 O); PI 88 (phosphomannopentaose sulfate); Alvocidib (4H-1-Benzopyran-4-one, 2-(2-chlorophenyl)-5,7-dihydroxy-8-(3-hydroxy-1- methyl-4-piperidinyl)- cis-(-)-) (C21 H20 Cl N O5); E 7820; SU 11248 (5-[3-Fluoro-2-oxo-1,2-dihydroindol-(3Z)-ylidenemethyl]-2,4-dimethyl-1H- pyrrole-3-carboxylic acid (2-diethylaminoethyl)amide) (C22 H27 F N4 O2); Squalamine (Cholestane-7,24-diol, 3-[[3-[(4-aminobutyl)aminopropyl]amino]-, 24-(hydrogen sulfate), (3.beta.,5.alpha.,7.alpha.)-) (C34 H65 N3 O5 S); Eriochrome Black T; AGM 1470 (Carbamic acid, (chloroacetyl)-, 5-methoxy-4-[2-methyl-3-(3-methyl-2- butenyl)oxiranyl] -1-oxaspiro[2,5] oct-6-yl ester, [3R-[3alpha, 4alpha(2R, 3R), 5beta, 6beta]]) (C19 H28 Cl N O6); AZD 9935; BIBF 1000; AZD 2171; ABT 828; KS-interleukin-2; Uteroglobin; A 6; NSC 639366 (1-[3-(Diethylamino)-2-hydroxypropylamino]-4-(oxyran-2-ylmethylamino)anthraquinone fumerate) (C24 H29 N3 O4 . C4 H4 O4); ISV 616; anti-ED-B fusion proteins; HUI 77; Troponin I; BC-1 monoclonal antibody; SPV 5.2; ER 68203; CKD 731 (3-(3,4,5-Trimethoxyphenyl)-2(E)-propenoic acid

(3R,4S,5S,6R)-4-[2(R)- methyl-3(R)-3(R)-(3-methyl-2-butenyl)oxiran-2-yl]-5-methoxy-1- oxaspiro[2.5]oct-6-yl ester) (C28 H38 O8); IMC-1C11; aaATIII; SC 7; CM 101; Angiocol; Kringle 5; CKD 732 (3-[4-[2-(Dimethylamino)ethoxy]phenyl]-2(E)-propenoic acid)(C29 H41 N O6); U 995; Canstatin; SQ 885; CT 2584 (1-[11-(Dodecylamino)-10-hydroxyundecyl]-3,7-dimethylxanthine)(C30 H55 N5 O3); Salmosin; EMAP II; TX 1920 (1-(4-Methylpiperazino)-2-(2-nitro-1H-1-imidazoyl)-1-ethanone) (C10 H15 N5 O3); Alpha-v Beta-x inhibitor; CHIR 11509 (N-(1-Propynyl)glycyl-[N-(2-naphthyl)]glycyl-[N-(carbamoylmethyl)]glycine bis(4-methoxyphenyl)methylamide)(C36 H37 N5 O6); BST 2002; BST 2001; B 0829; FR 111142; 4,5-Dihydroxy-2(E)-hexenoic acid (3R,4S, 5S, 6R)-4-[1(R),2(R)-epoxy-1,5- dimethyl-4-hexenyl]-5-methoxy-1-oxaspiro[2.5]octan-6-yl ester (C22 H34 O7); and kinase inhibitors including, but not limited to, N-(4-chlorophenyl)-4-(4-pyridinylmethyl)-1-phthalazinamine; 4-[4-[[[[4-chloro-3-(trifluoromethyl)phenyl]amino]carbonyl]amino]phenoxy]-N-methyl-2-pyridinecarboxamide; N-[2-(diethylamino)ethyl]-5-[(5-fluoro-1,2-dihydro-2-oxo-3H-indol-3-ylidene)methyl]-2,4-dimethyl-1H-pyrrole-3-carboxamide; 3-[(4-bromo-2,6-difluorophenyl)methoxy]-5-[[[[4-(1-pyrrolidinyl)butyl]amino]carbonyl]amino]-4-isothiazolecarboxamide; N-(4-bromo-2-fluorophenyl)-6-methoxy-7-[(1-methyl-4-piperidinyl)methoxy]-4-quinazolinamine; 3-[5,6,7,13-tetrahydro-9-[(1-methylethoxy)methyl]-5-oxo-12H-indeno[2,1-a]pyrrolo[3,4-c]carbazol-12-yl]propyl ester N,N-dimethyl-glycine; N-[5-[[[5-(1,1-dimethylethyl)-2-oxazolyl]methyl]thio]-2-thiazolyl]-4-piperidinecarboxamide; *N*-[3-chloro-4-[(3-fluorophenyl)methoxy]phenyl]-6-[5-[[[2-(methylsulfonyl)ethyl]amino]methyl]-2-furanyl]-4-quinazolinamine; 4-[(4-Methyl-1-piperazinyl)methyl]-N-[4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]-phenyl]benzamide; *N*-(3-chloro-4-fluorophenyl)-7-methoxy-6-[3-(4-morpholinyl)propoxy]-4-quinazolinamine; *N*-(3-ethynylphenyl)-6,7-bis(2-methoxyethoxy)-4-quinazolinamine; N-(3-((((2R)-1-methyl-2-pyrrolidinyl)methyl)oxy)-5-(trifluoromethyl)phenyl)-2-((3-(1,3-oxazol-5-yl)phenyl)amino)-3-pyridinecarboxamide; 2-(((4-fluorophenyl)methyl)amino)-N-

(3-((((2R)-1-methyl-2-pyrrolidinyl)methyl)oxy)-5-(trifluoromethyl)phenyl)-3-pyridinecarboxamide; N-[3-(Azetidin-3-ylmethoxy)-5-trifluoromethyl-phenyl]-2-(4-fluoro-benzylamino)-nicotinamide; 6-fluoro-N-(4-(1-methylethyl)phenyl)-2-((4-pyridinylmethyl)amino)-3-pyridinecarboxamide; 2-((4-pyridinylmethyl)amino)-N-(3-(((2S)-2-pyrrolidinylmethyl)oxy)-5-(trifluoromethyl)phenyl)-3-pyridinecarboxamide; N-(3-(1,1-dimethylethyl)-1H-pyrazol-5-yl)-2-((4-pyridinylmethyl)amino)-3-pyridinecarboxamide; N-(3,3-dimethyl-2,3-dihydro-1-benzofuran-6-yl)-2-((4-pyridinylmethyl)amino)-3-pyridinecarboxamide; N-(3-((((2S)-1-methyl-2-pyrrolidinyl)methyl)oxy)-5-(trifluoromethyl)phenyl)-2-((4-pyridinylmethyl)amino)-3-pyridinecarboxamide; 2-((4-pyridinylmethyl)amino)-N-(3-((2-(1-pyrrolidinyl)ethyl)oxy)-4-(trifluoromethyl)phenyl)-3-pyridinecarboxamide; N-(3,3-dimethyl-2,3-dihydro-1H-indol-6-yl)-2-((4-pyridinylmethyl)amino)-3-pyridinecarboxamide; N-(4-(pentafluoroethyl)-3-(((2S)-2-pyrrolidinylmethyl)oxy)phenyl)-2-((4-pyridinylmethyl)amino)-3-pyridinecarboxamide; N-(3-((3-azetidinylmethyl)oxy)-5-(trifluoromethyl)phenyl)-2-((4-pyridinylmethyl)amino)-3-pyridinecarboxamide; N-(3-(4-piperidinyloxy)-5-(trifluoromethyl)phenyl)-2-((2-(3-pyridinyl)ethyl)amino)-3-pyridinecarboxamide; N-(4,4-dimethyl-1,2,3,4-tetrahydro-isoquinolin-7-yl)-2-(1H-indazol-6-ylamino)-nicotinamide; 2-(1H-indazol-6-ylamino)-N-[3-(1-methylpyrrolidin-2-ylmethoxy)-5-trifluoromethyl-phenyl]-nicotinamide; N-[1-(2-dimethylamino-acetyl)-3,3-dimethyl-2,3-dihydro-1H-indol-6-yl]-2-(1H-indazol-6-ylamino)-nicotinamide; 2-(1H-indazol-6-ylamino)-N-[3-(pyrrolidin-2-ylmethoxy)-5-trifluoromethyl-phenyl]-nicotinamide; N-(1-acetyl-3,3-dimethyl-2,3-dihydro-1H-indol-6-yl)-2-(1H-indazol-6-ylamino)-nicotinamide; N-(4,4-dimethyl-1-oxo-1,2,3,4-tetrahydro-isoquinolin-7-yl)-2-(1H-indazol-6-ylamino)-nicotinamide; N-[4-(tert-butyl)-3-(3-piperidylpropyl)phenyl][2-(1H-indazol-6-ylamino)(3-pyridyl)]carboxamide; N-[5-(tert-butyl)isoxazol-3-yl][2-(1H-indazol-6-ylamino)(3-pyridyl)]carboxamide; and N-[4-(tert-butyl)phenyl][2-(1H-indazol-6-ylamino)(3-pyridyl)]carboxamide, and kinase inhibitors disclosed in U.S. Patent Nos. 6,258,812; 6,235,764; 6,630,500; 6,515,004; 6,713,485; 5,521,184; 5,770,599; 5,747,498; 5,990,141; U.S.

Publication No. US20030105091; and Patent Cooperation Treaty publication nos. WO01/37820; WO01/32651; WO02/68406; WO02/66470; WO02/55501; WO04/05279; WO04/07481; WO04/07458; WO04/09784; WO02/59110; WO99/45009; WO98/35958; WO00/59509; WO99/61422; WO00/12089; and WO00/02871, each of which publications are hereby incorporated by reference for any purpose.

Combination therapy with growth factors can include cytokines, lymphokines, growth factors, or other hematopoietic factors such as M-CSF, GM-CSF, TNF, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IFN, TNF0, TNF1, TNF2, G-CSF, Meg-CSF, GM-CSF, thrombopoietin, stem cell factor, and erythropoietin. Other compositions can include known angiopoietins, for example Ang-1, -2, -4, -Y, and/or the human Ang-like polypeptide, and/or vascular endothelial growth factor (VEGF). Growth factors include angiogenin, bone morphogenic protein-1, bone morphogenic protein-2, bone morphogenic protein-3, bone morphogenic protein-4, bone morphogenic protein-5, bone morphogenic protein-6, bone morphogenic protein-7, bone morphogenic protein-8, bone morphogenic protein-9, bone morphogenic protein-10, bone morphogenic protein-11, bone morphogenic protein-12, bone morphogenic protein-13, bone morphogenic protein-14, bone morphogenic protein-15, bone morphogenic protein receptor-IA, bone morphogenic protein receptor IB, brain derived neurotrophic factor, ciliary neutrophic factor, ciliary neutrophic factor receptor, cytokine-induced neutrophil chemotactic factor-1, cytokine-induced neutrophil, chemotactic factor-2, cytokine-induced neutrophil chemotactic factor-2, endothelial cell growth factor, endothelin-1, epidermal growth factor, epithelial-derived neutrophil attractant, fibroblast growth factor-4, fibroblast growth factor-5, fibroblast growth factor-6, fibroblast growth factor-7, fibroblast growth factor-8, fibroblast growth factor-8b, fibroblast growth factor-8c, fibroblast growth factor-9, fibroblast growth factor-10, fibroblast growth factor acidic, fibroblast growth factor basic, glial cell line-derived neutrophic factor receptor-1, glial cell line-derived neutrophic factor receptor-2, growth related protein, growth related protein-2, growth related protein

-2, growth related protein-3, heparin binding epidermal growth factor, hepatocyte growth factor, hepatocyte growth factor receptor, insulin-like growth factor I, insulin-like growth factor receptor, insulin-like growth factor II, insulin-like growth factor binding protein, keratinocyte growth factor, leukemia inhibitory factor, leukemia inhibitory factor receptor-1, nerve growth factor nerve growth factor receptor, neurotrophin-3, neurotrophin-4, placenta growth factor, placenta growth factor-2, platelet-derived endothelial cell growth factor, platelet derived growth factor, platelet derived growth factor A chain, platelet derived growth factor AA, platelet derived growth factor AB, platelet derived growth factor B chain, platelet derived growth factor BB, platelet derived growth factor receptor-1, platelet derived growth factor receptor-2, pre-B cell growth stimulating factor, stem cell factor, stem cell factor receptor, transforming growth factor-1, transforming growth factor-2, transforming growth factor-3, transforming growth factor-1.2, transforming growth factor-4, transforming growth factor-5, latent transforming growth factor-1, transforming growth factor-1 binding protein I, transforming growth factor-1 binding protein II, transforming growth factor-1 binding protein III, tumor necrosis factor receptor type I (TNF-R1), tumor necrosis factor receptor type II (TNF-R2), urokinase-type plasminogen activator receptor, vascular endothelial growth factor, and chimeric proteins and biologically or immunologically active fragments thereof.

It will be appreciated that the specific binding agents of the invention may be administered with one or more anti-inflammatory agents. As used herein, the term "anti-inflammatory agent" refers generally to any agent that reduces inflammation or swelling in a patient. A number of exemplary anti-inflammatory agents are recited herein, but it will be appreciated that there may be additional suitable anti-inflammatory agents not specifically recited herein, but which are encompassed by the present invention.

The anti-inflammatory agent can be, for example, a compound that inhibits the interaction of inflammatory cytokines with their receptors. Examples of cytokine inhibitors useful in combination with the specific binding agents of the

invention include, for example, antagonists (such as antibodies) of TGF-β, as well as antagonists (such as antibodies) directed against interleukins involved in inflammation. Such interleukins are described herein and preferably include, but are not limited to, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, IL-9, IL-11, IL-12, IL-13, IL-17, and IL-18. See Feghali, et al., *Frontiers in Biosci.*, 2:12-26 (1997).

Specific binding agents of the invention also may be administered in combination with inhibitors of Protein Kinase A Type 1 to enhance T cell proliferation in HIV-infected patients who are receiving anti-retroviral therapy.

Nerve growth factors (NGFs) also can be combined with the specific binding agents of the invention to treat certain conditions. Such conditions include neurodegenerative diseases, spinal cord injury and multiple sclerosis. Other conditions treatable with this combination are glaucoma and diabetes.

A preferred combination therapy relates to a specific binding agent of the invention administered to a patient in combination with one or more suitable IL-1 inhibitor. Inhibitors of IL-1 include, but are not limited to, receptor-binding peptide fragments of IL-1, antibodies directed against IL-1 or IL-1 beta or IL-1 receptor type I, and recombinant proteins comprising all or portions of receptors for IL-1 or modified variants thereof, including genetically-modified muteins, multimeric forms and sustained-release formulations. Specific antagonists include IL-1ra polypeptides, IL-1 beta converting enzyme (ICE) inhibitors, antagonistic type I IL-1 receptor antibodies, IL-1 binding forms of type I IL-1 receptor and type II IL-1 receptor, antibodies to IL-1, including IL-1 alpha and IL-1 beta and other IL-1 family members, and a therapeutic known as IL-1 Trap (Regeneron). IL-1ra polypeptides include the forms of IL-1ra described in US Patent No. 5,075,222 and modified forms and variants including those described in U.S. 5,922,573, WO 91/17184, WO 92 16221, and WO 96 09323. IL-1 beta converting enzyme (ICE) inhibitors include peptidyl and small molecule ICE inhibitors including those described in PCT patent applications WO 91/15577; WO 93/05071; WO 93/09135; WO 93/14777 and WO 93/16710; and European patent application 0 547 699. Non-peptidyl compounds include those described in

PCT patent application WO 95/26958, U.S. Patent No. 5,552,400, U.S. Patent No. 6,121,266, and Dolle *et al.*, *J. Med. Chem.*, 39, pp. 2438-2440 (1996). Additional ICE inhibitors are described in U.S. Pat. Nos. 6,162,790, 6,204,261, 6,136,787, 6,103,711, 6,025,147, 6,008,217, 5,973,111, 5,874,424, 5,847,135, 5,843,904, 5,756,466, 5,656,627, 5,716,929. IL-1 binding forms of Type I IL-1 receptor and type II IL-1 receptor are described in U.S. Patent Nos. 4,968,607, 4,968,607, 5,081,228, Re 35,450, 5,319,071, and 5,350,683. Other suitable IL-1 antagonists include, but are not limited to, peptides derived from IL-1 that are capable of binding competitively to the IL-1 signaling receptor, IL-1 R type I. Additional guidance regarding certain IL-1 (and other cytokine) antagonists can be found in U.S. Patent No. 6,472,179.

Additionally, TNF inhibitors are suitable, and include, but are not limited to, receptor-binding peptide fragments of TNFα, antisense oligonucleotides or ribozymes that inhibit TNFα production, antibodies directed against TNFα, and recombinant proteins comprising all or portions of receptors for TNFα or modified variants thereof, including genetically-modified muteins, multimeric forms and sustained-release formulations. Also suitable are TACE (Tumor Necrosis Factor-α Converting Enzyme) inhibitors, such as TAPI (Immunex Corp.) and GW-3333X (Glaxo Wellcome Inc.). Also suitable are molecules that inhibit the formation of the IgA-α_1AT complex, such as the peptides disclosed in EP 0 614 464 B, or antibodies against this complex. Additionally suitable molecules include, but are not limited to, TNFα-inhibiting disaccharides, sulfated derivatives of glucosamine, or other similar carbohydrates described in U.S. Patent No. 6,020,323. Further suitable molecules include peptide TNFα inhibitors disclosed in U.S. Patent Nos. 5,641,751 and 5,519,000, and the D-amino acid-containing peptides described in U.S. Patent No. 5,753,628. In addition, inhibitors of TNFα converting enzyme are also suitable. WO 01/03719 describes further additional agents which can be used in combination in accordance with the invention.

Still further suitable compounds include, but are not limited to, small molecules such as thalidomide or thalidomide analogs, pentoxifylline, or matrix metalloproteinase (MMP) inhibitors or other small molecules. Suitable MMP inhibitors for this purpose include, for example, those described in U.S. Patent Nos. 5,883,131, 5,863,949 and 5,861,510 as well as mercapto alkyl peptidyl compounds as described in U.S. Patent No. 5,872,146. Other small molecules capable of reducing TNFα production, include, for example, the molecules described in U.S. Patent Nos. 5,508,300, 5,596,013, and 5,563,143. Additional suitable small molecules include, but are not limited to, MMP inhibitors as described in U.S. Patent Nos. 5,747,514, and 5,691,382, as well as hydroxamic acid derivatives such as those described in U.S. Patent No. 5,821,262. Further suitable molecules include, for example, small molecules that inhibit phosphodiesterase IV and TNFα production, such as substituted oxime derivatives (WO 96/00215), quinoline sulfonamides (U.S. Patent No. 5,834,485), aryl furan derivatives (WO 99/18095) and heterobicyclic derivatives (WO 96/01825; GB 2 291 422 A). Also useful are thiazole derivatives that suppress TNFα and IFNγ (WO 99/15524), as well as xanthine derivatives that suppress TNFα and other proinflammatory cytokines (see, for example, U.S. Patent Nos. 5,118,500, 5,096,906 and 5,196,430). Additional small molecules useful for treating the hereindescribed conditions include those disclosed in U.S. Patent No. 5,547,979.

Further examples of drugs and drug types which can be administered by combination therapy include, but are not limited to, antivirals, antibiotics, analgesics (*e.g.*, acetaminophen, codeine, propoxyphene napsylate, oxycodone hydrochloride, hydrocodone bitartrate, tramadol), corticosteroids, antagonists of inflammatory cytokines, Disease-Modifying Anti-Rheumatic Drugs (DMARDs), Non-Steroidal Anti-Inflammatory drugs (NSAIDs), and Slow-Acting Anti-Rheumatic Drugs (SAARDs).

Exemplary Disease-Modifying Anti-Rheumatic Drugs (DMARDs) include, but are not limited to: Rheumatrex™ (methotrexate); Enbrel® (etanercept); Remicade® (infliximab); Humira™ (adalimumab); Segard®

(afelimomab); Arava™ (leflunomide); Kineret™ (anakinra); Arava™ (leflunomide); D-penicillamine; Myochrysine; Plaquenil; Ridaura™ (auranofin); Solganal; lenercept (Hoffman-La Roche); CDP870 (Celltech); CDP571 (Celltech), as well as the antibodies described in EP 0 516 785 B1, U.S. Patent No. 5,656,272, EP 0 492 448 A1; onercept (Serono; CAS reg. no. 199685-57-9); MRA (Chugai); Imuran™ (azathioprine); NFKB inhibitors; Cytoxan™ (cyclophosphamide); cyclosporine; hydroxychloroquine sulfate; minocycline; sulfasalazine; and gold compounds such as oral gold, gold sodium thiomalate and aurothioglucose.

Further suitable molecules include, for example, soluble TNFRs derived from the extracellular regions of TNFα receptor molecules other than the p55 and p75 TNFRs, such as for example the TNFR described in WO 99/04001, including TNFR-Ig's derived from this TNFR. Additional suitable TNFα inhibitors are suitable for use as described herein. These include the use not only of an antibody against TNFα or TNFR as described herein, but also a TNFα-derived peptide that can act as a competitive inhibitor of TNFα (such as those described in U.S. Patent No. 5,795,859 or U.S. Patent No. 6,107,273), TNFR-IgG fusion proteins, such as one containing the extracellular portion of the p55 TNFα receptor, a soluble TNFR other than an IgG fusion protein, or other molecules that reduce endogenous TNFα levels, such as inhibitors of the TNFα converting enzyme (see *e.g.*, U.S. 5,594,106), or small molecules or TNFα inhibitors, a number of which are described herein.

With respect to antibodies to TNF, although dose will optimally be determined by an experienced healthcare provider in accordance with the specific needs of the patient in mind, one exemplary preferred dose range for an antibody against TNFα is 0.1 to 20 mg/kg, and more preferably is 1-10 mg/kg. Another preferred dose range for anti-TNFα antibody is 0.75 to 7.5 mg/kg of body weight.

The present invention can also utilize a specific binding agent and any of one or more Non-Steroidal Anti-Inflammatory Drugs (NSAIDs). NSAIDs owe

their anti-inflammatory action, at least in part, to the inhibition of prostaglandin synthesis. Goodman and Gilman, *The Pharmacological Basis of Therapeutics*, MacMillan 7th Edition (1985). NSAIDs can be characterized into nine groups: (1) salicylic acid derivatives; (2) propionic acid derivatives; (3) acetic acid derivatives; (4) fenamic acid derivatives; (5) carboxylic acid derivatives; (6) butyric acid derivatives; (7) oxicams; (8) pyrazoles and (9) pyrazolones. Examples of NSAIDs include, but are not limited to: Anaprox™, Anaprox DS™ (naproxen sodium); Ansaid™ (flurbiprofen); Arthrotec™ (diclofenac sodium + misoprostil); Cataflam™/Voltaren™ (diclofenac potassium); Clinoril™ (sulindac); Daypro™ (oxaprozin); Disalcid™ (salsalate); Dolobid™ (diflunisal); EC Naprosyn™ (naproxen sodium); Feldene™ (piroxicam); Indocin™, Indocin SR™ (indomethacin); Lodine™, Lodine XL™ (etodolac); Motrin™ (ibuprofen); Naprelan™ (naproxen); Naprosyn™ (naproxen); Orudis™, (ketoprofen); Oruvail™ (ketoprofen); Relafen™ (nabumetone); Tolectin™, (tolmetin sodium); Trilisate™ (choline magnesium trisalicylate); Cox-1 inhibitors; Cox-2 Inhibitors such as Vioxx™ (rofecoxib); Arcoxia™ (etoricoxib), Celebrex™ (celecoxib); Mobic™ (meloxicam); Bextra™ (valdecoxib), Dynastat™ paracoxib sodium; Prexige™ (lumiracoxib), and nambumetone. Additional suitable NSAIDs, include, but are not limited to, the following: ε-acetamidocaproic acid, S-adenosylmethionine, 3-amino-4-hydroxybutyric acid, amixetrine, anitrazafen, antrafenine, bendazac, bendazac lysinate, benzydamine, beprozin, broperamole, bucolome, bufezolac, ciproquazone, cloximate, dazidamine, deboxamet, detomidine, difenpiramide, difenpyramide, difisalamine, ditazol, emorfazone, fanetizole mesylate, fenflumizole, floctafenine, flumizole, flunixin, fluproquazone, fopirtoline, fosfosal, guaimesal, guaiazolene, isonixirn, lefetamine HCl, leflunomide, lofemizole, lotifazole, lysin clonixinate, meseclazone, nabumetone, nictindole, nimesulide, orgotein, orpanoxin, oxaceprolm, oxapadol, paranyline, perisoxal, perisoxal citrate, pifoxime, piproxen, pirazolac, pirfenidone, proquazone, proxazole, thielavin B, tiflamizole, timegadine, tolectin, tolpadol, tryptamid and those designated by company code number such as 480156S, AA861, AD1590, AFP802, AFP860, AI77B, AP504, AU8001, BPPC, BW540C, CHINOIN 127,

CN100, EB382, EL508, F1044, FK-506, GV3658, ITF182, KCNTEI6090, KME4, LA2851, MR714, MR897, MY309, ONO3144, PR823, PV102, PV108, R830, RS2131, SCR152, SH440, SIR133, SPAS510, SQ27239, ST281, SY6001, TA60, TAI-901 (4-benzoyl-1-indancarboxylic acid), TVX2706, U60257, UR2301 and WY41770. Structurally related NSAIDs having similar analgesic and anti-inflammatory properties to the NSAIDs are also encompassed by this group.

Suitable SAARDs or DMARDS include, but are not limited to: allocupreide sodium, auranofin, aurothioglucose, aurothioglycanide, azathioprine, brequinar sodium, bucillamine, calcium 3-aurothio-2-propanol-1-sulfonate, chlorambucil, chloroquine, clobuzarit, cuproxoline, cyclophosphamide, cyclosporin, dapsone, 15-deoxyspergualin, diacerein, glucosamine, gold salts (*e.g.*, cycloquine gold salt, gold sodium thiomalate, gold sodium thiosulfate), hydroxychloroquine, hydroxyurea, kebuzone, levamisole, lobenzarit, melittin, 6-mercaptopurine, methotrexate, mizoribine, mycophenolate mofetil, myoral, nitrogen mustard, D-penicillamine, pyridinol imidazoles such as SKNF86002 and SB203580, rapamycin, thiols, thymopoietin and vincristine. Structurally related SAARDs or DMARDs having similar analgesic and anti-inflammatory properties are also intended to be encompassed by this group.

Inhibitors of kinases in signaling cascades are also suitable agents for combination with the specific binding agents of the invention. These include, but are not limited to, agents which are capable of inhibiting P-38 (*a.k.a.*, "RK" or "SAPK-2", Lee *et al., Nature,* 372:739 (1994). P-38 is described as a serine/threonine kinase (see Han *et al., Biochimica Biophysica Acta,* 1265:224-227 (1995). Inhibitors of P-38 have been shown to intervene between the extracellular stimulus and the secretion of IL-1 and TNFα from the cell involves blocking signal transduction through inhibition of a kinase which lies on the signal pathway.

Additionally suitable are MK2 inhibitors, and tpl-2 inhibitors. Additionally, T-cell inhibitors are also suitable, including, for example, ctla-4, CsA, Fk-506, OX40, OX40R-Fc, OX40 antibody, OX40 ligand, OX40 ligand

antibody, lck, and ZAP70. Also suitable are retinoids, including oral retinoids, as well as antagonists of TGF-β.

Further suitable agents for combination with the specific binding agents of the invention include, for example, any of one or more salicylic acid derivatives, prodrug esters or pharmaceutically acceptable salts thereof. Such salicylic acid derivatives, prodrug esters and pharmaceutically acceptable salts thereof comprise: acetaminosalol, aloxiprin, aspirin, benorylate, bromosaligenin, calcium acetylsalicylate, choline magnesium trisalicylate diflusinal, etersalate, fendosal, gentisic acid, glycol salicylate, imidazole salicylate, lysine acetylsalicylate, mesalamine, morpholine salicylate, 1-naphthyl salicylate, olsalazine, parsalmide, phenyl acetylsalicylate, phenyl salicylate, salacetamide, salicylamide O-acetic acid, salsalate and sulfasalazine. Structurally related salicylic acid derivatives having similar analgesic and anti-inflammatory properties are also intended to be encompassed by this group. Additionally suitable agents include, for example propionic acid derivatives, prodrug esters or pharmaceutically acceptable salts thereof. The propionic acid derivatives, prodrug esters and pharma-ceutically acceptable salts thereof comprise: alminoprofen, benoxaprofen, bucloxic acid, carprofen, dexindoprofen, fenoprofen, flunoxaprofen, fluprofen, flurbiprofen, furcloprofen, ibuprofen, ibuprofen aluminum, ibuproxam, indoprofen, isoprofen, ketoprofen, loxoprofen, miroprofen, naproxen, oxaprozin, piketoprofen, pimeprofen, pirprofen, pranoprofen, protizinic acid, pyridoxiprofen, suprofen, tiaprofenic acid and tioxaprofen. Structurally related propionic acid derivatives having similar analgesic and anti-inflammatory properties are also intended to be encompassed by this group. Also suitable for use are acetic acid derivatives, prodrug esters or pharmaceutically acceptable salts thereof. The acetic acid derivatives, prodrug esters and pharmaceutically acceptable salts thereof comprise: acemetacin, alclofenac, amfenac, bufexamac, cinmetacin, clopirac, delmetacin, diclofenac sodium, etodolac, felbinac, fenclofenac, fenclorac, fenclozic acid, fentiazac, furofenac, glucametacin, ibufenac, indomethacin, isofezolac, isoxepac, lonazolac, metiazinic acid, oxametacin, oxpinac, pimetacin, proglumetacin, sulindac, talmetacin, tiaramide, tiopinac, tolmetin, zidometacin

and zomepirac. Structurally related acetic acid derivatives having similar analgesic and anti-inflammatory properties are also intended to be encompassed by this group. Further suitable for use as described herein are fenamic acid derivatives, prodrug esters or pharmaceutically acceptable salts thereof. The fenamic acid derivatives, prodrug esters and pharmaceutically acceptable salts thereof comprise: enfenamic acid, etofenamate, flufenamic acid, isonixin, meclofenamic acid, meclofenamate sodium, medofenamic acid, mefanamic acid, niflumic acid, talniflumate, terofenamate, tolfenamic acid and ufenamate. Structurally related fenamic acid derivatives having similar analgesic and anti-inflammatory properties are also intended to be encompassed by this group.

Also suitable are carboxylic acid derivatives, prodrug esters and pharmaceutically acceptable salts thereof which can be used comprise: clidanac, diflunisal, flufenisal, inoridine, ketorolac and tinoridine. Structurally related carboxylic acid derivatives having similar analgesic and anti-inflammatory properties are also intended to be encompassed by this group. Additionally suitable are butyric acid derivatives, prodrug esters or pharmaceutically acceptable salts thereof. The butyric acid derivatives, prodrug esters and pharmaceutically acceptable salts thereof comprise: bumadizon, butibufen, fenbufen and xenbucin. Structurally related butyric acid derivatives having similar analgesic and anti-inflammatory properties are also intended to be encompassed by this group. Oxicams, prodrug esters or pharmaceutically acceptable salts thereof are also suitable. Oxicams, prodrug esters and pharmaceutically acceptable salts thereof comprise: droxicam, enolicam, isoxicam, piroxicam, sudoxicam, tenoxicam and 4-hydroxyl-1,2-benzothiazine 1,1-dioxide 4-(N-phenyl)-carboxamide. Structurally related oxicams having similar analgesic and anti-inflammatory properties are also intended to be encompassed by this group. Pyrazoles, prodrug esters or pharmaceutically acceptable salts thereof are also suitable. The pyrazoles, prodrug esters and pharmaceutically acceptable salts thereof which may be used comprise: difenamizole and epirizole. Structurally related pyrazoles having similar analgesic and anti-inflammatory properties are also intended to be encompassed by this group. Furthermore, pyrazolones, prodrug esters or

pharmaceutically acceptable salts thereof are suitable. The pyrazolones, prodrug esters and pharmaceutically acceptable salts thereof which may be used comprise: apazone, azapropazone, benzpiperylon, feprazone, mofebutazone, morazone, oxyphenbutazone, phenylbutazone, pipebuzone, propylphenazone, ramifenazone, suxibuzone and thiazolinobutazone. Structurally related pyrazalones having similar analgesic and anti-inflammatory properties are also intended to be encompassed by this group.

Also suitable are prodrug esters or pharmaceutically acceptable salts thereof for the treatment of TNF-mediated diseases. Corticosteroids, prodrug esters and pharmaceutically acceptable salts thereof include hydrocortisone and compounds which are derived from hydrocortisone, such as 21-acetoxy-pregnenolone, alclomerasone, algestone, amcinonide, beclomethasone, beta-methasone, betamethasone valerate, budesonide, chloroprednisone, clobetasol, clobetasol propionate, clobetasone, clobetasone butyrate, clocortolone, cloprednol, corticosterone, cortisone, cortivazol, deflazacon, desonide, desoximerasone, dexamethasone, diflorasone, diflucortolone, difluprednate, enoxolone, fluazacort, flucloronide, flumethasone, flumethasone pivalate, flunisolide, flucinolone acetonide, fluocinonide, fluorocinolone acetonide, fluocortin butyl, fluocortolone, fluorocortolone hexanoate, diflucortolone valerate, fluorometholone, fluperolone acetate, fluprednidene acetate, fluprednisolone, flurandenolide, formocortal, halcinonide, halometasone, halopredone acetate, hydrocortamate, hydrocortisone, hydrocortisone acetate, hydro-cortisone butyrate, hydrocortisone phosphate, hydrocortisone 21-sodium succinate, hydrocortisone tebutate, mazipredone, medrysone, meprednisone, methylprednicolone, mometasone furoate, paramethasone, prednicarbate, prednisolone, prednisolone 21-diedryaminoacetate, prednisolone sodium phosphate, prednisolone sodium succinate, prednisolone sodium 21-*m*-sulfobenzoate, prednisolone sodium 21-stearoglycolate, prednisolone tebutate, prednisolone 21-trimethylacetate, prednisone, prednival, prednylidene, prednylidene 21-diethylaminoacetate, tixocortol, triamcinolone, triamcinolone acetonide, triamcinolone benetonide and triamcinolone

hexacetonide. Structurally related corticosteroids having similar analgesic and anti-inflammatory properties are also intended to be encompassed by this group.

Antimicrobials (and prodrug esters or pharmaceutically acceptable salts thereof) are also suitable for combination use as described herein. Suitable antimicrobials include, for example, ampicillin, amoxycillin, aureomicin, bacitracin, ceftazidime, ceftriaxone, cefotaxime, cephachlor, cephalexin, cephradine, ciprofloxacin, clavulanic acid, cloxacillin, dicloxacillan, erythromycin, flucloxacillan, gentamicin, gramicidin, methicillan, neomycin, oxacillan, penicillin and vancomycin. Structurally related antimicrobials having similar analgesic and anti-inflammatory properties are also intended to be encompassed by this group.

Additional suitable compounds include, but are not limited to: BN 50730; tenidap; E 5531; tiapafant PCA 4248; nimesulide; panavir; rolipram; RP 73401; peptide T; MDL 201,449A; (1R,3S)-Cis-1-[9-(2,6-diaminopurinyl)]-3-hydroxy-4-cyclopentene hydrochloride; (1R,3R)-trans-1-[9-(2,6-diamino)purine]-3-acetoxycyclopentane; (1R,3R)-trans-1-[9-adenyl)-3-azidocyclopentane hydrochloride and (1R,3R)-trans-1-[6-hydroxy-purin-9-yl)-3-azidocyclopentane.

It has been found that IL-4 can induce an inflammatory effect in some instances, such as in asthma, in which over-expression of IL-4 in the lungs causes epithelial cell hypertrophy and an accumulation of lymphocytes, eosinophils, and neutrophils. This response is representative of the main features of the proinflammatory response induced by other Th2 cytokines. As noted above, therefore, inhibitors of IL-4 are also useful in accordance with the invention. Additionally, it will be appreciated that certain immunosuppressant drugs can also be used in the treatment of arthritis, including, but not limited to, iNOS inhibitors, and 5-lipoxygenase inhibitors.

Ginger has been shown to have certain anti-inflammatory properties, and is therefore suitable for use as an anti-inflammatory agent in accordance with the invention, as is chondroitin.

In certain embodiments, a specific binding agent to Ang-2 may be administered prior to, concurrent with, and subsequent to treatment with a cancer therapy agent. Exemplary cancers include, but are not limited to, breast cancer, colorectal cancer, gastric carcinoma, glioma, head and neck squamous cell carcinoma, hereditary and sporadic papillary renal carcinoma, leukemia, lymphoma, Li-Fraumeni syndrome, malignant pleural mesothelioma, melanoma, multiple myeloma, non-small cell lung carcinoma, osteosarcoma, ovarian cancer, pancreatic cancer, prostate cancer, small cell lung cancer, synovial sarcoma, thyroid carcinoma, and transitional cell carcinoma of urinary bladder.

In certain embodiments, a specific binding agent to Ang-2 may be used alone or with at least one additional therapeutic agents for the treatment of cancer. In certain embodiments, a specific binding agent to Ang-2 is used in conjunction with a therapeutically effective amount of an additional therapeutic agent. Exemplary therapeutic agents that may be administered with a specific binding agent to Ang-2 include, but are not limited to, a member of the geldanamycin family of anisamycin antibiotics; a Pro-HGF; NK2; a c-Met peptide inhibitor; an antagonist of Grb2 Src homology 2; a Gab1 modulator; dominant-negative Src; a von-Hippel-Landau inhibitor, including, but not limited to, wortmannin; P13 kinase inhibitors, other anti-receptor therapies, anti EGFR, a COX-2 inhibitor, Celebrex™, Vioxx™; a vascular endothelial growth factor (VEGF), a VEGF modulator, a fibroblast growth factor (FGF), an FGF modulator, an epidermal growth factor (EGF); an EGF modulator; a keratinocyte growth factor (KGF), a KGF-related molecule, a KGF modulator; a matrix metalloproteinase (MMP) modulator.

In certain embodiments, the present invention is directed to therapies comprising a specific binding agent to Ang-2 and at least one serine protease inhibitor, and methods of treatment using such therapies. In certain embodiments, a therapy comprises a specific binding agent to Ang-2 and a serine protease inhibitor and at least one additional molecule described herein.

In certain instances, a disturbance of the protease/protease inhibitor balance can lead to protease-mediated tissue destruction, including, but not limited to, tumor invasion of normal tissue leading to metastasis.

In certain embodiments, a specific binding agent to Ang-2 may be used with at least one therapeutic agent for inflammation. In certain embodiments, a specific binding agent to Ang-2 may be used with at least one therapeutic agent for an immune disorder. Exemplary therapeutic agents for inflammation and immune disorders include, but are not limited to cyclooxygenase type 1 (COX-1) and cyclooxygenase type 2 (COX-2) inhibitors small molecule modulators of 38 kDa mitogen-activated protein kinase (p38-MAPK); small molecule modulators of intracellular molecules involved in inflammation pathways, wherein such intracellular molecules include, but are not limited to, jnk, IKK, NF-κB, ZAP70, and lck. Certain exemplary therapeutic agents for inflammation are described, e.g., in C.A. Dinarello and L.L. Moldawer *Proinflammatory and Anti-Inflammatory Cytokines in Rheumatoid Arthritis: A Primer for Clinicians* Third Edition (2001) Amgen Inc. Thousand Oaks, CA.

In certain embodiments, pharmaceutical compositions will include more than one different a specific binding agent to Ang-2. In certain embodiments, pharmaceutical compositions will include more than one a specific binding agent to Ang-2 wherein the specific binding agents to Ang-2 bind more than one epitope.

Immunotherapeutics

Immunotherapeutics generally rely on the use of immune effector cells and molecules to target and destroy cancer cells. The immune effectors may be, for example an antibody of the present invention that recognizes some marker on the surface of a target cell. The antibody alone may serve as an effector of therapy or it may recruit other cells to actually affect cell killing. The antibody may also be conjugated to a drug or toxin (chemotherapeutic, radionuclide, ricin A chain, cholera toxin, pertussis toxin, etc.) and thus may merely serve as a targeting agent.

According to the present invention, mutant forms of Ang-2 may be targeted by immunotherapy either antibodies or antibody conjugates of the invention. It is particularly contemplated that the antibody compositions of the invention may be used in a combined therapy approach in conjunction with Ang-2 targeted therapy.

Passive immunotherapy has proved to be particularly effective against a number of cancers. See, for example, WO 98/39027.

The following examples are intended for illustration purposes only, and should not be construed as limiting the scope of the invention in any way.

EXAMPLE 1

Ang-2 Expression in Pathological and Normal Tissue

Ang-2 expression was examined in normal and pathological tissue using *in situ* hybridization. Fragments of the human (Genbank Accession Number: AF004327, nucleotides 1274-1726) and murine (Genbank Accession Number: AF004326, nucleotides 1135-1588) Ang-2 sequences were amplified by reverse transcriptase-PCR from human or murine fetal lung cDNA, cloned into the pGEM-T plasmid and verified by sequencing. ^{33}P-labeled antisense RNA probes were transcribed from linearized plasmid templates using ^{33}P –UTP and RNA polymerase. Blocks of formaldehyde-fixed, paraffin-embedded tissues were sectioned at 5 μm and collected on charged slides. Prior to *in situ* hybridization, tissues were permeabilized with 0.2M HCL, followed by digestion with Proteinase K, and acetylation with triethanolamine and acetic anhydride. Sections were hybridized with the radio labeled probe overnight at 55°C then subjected to RNase digestion and a high stringency wash in about 0.1X SSC at 55°C. Slides were dipped in Kodak NTB2 emulsion, exposed at 4°C for 2-3 weeks, developed, and counterstained. Sections were examined with dark field and standard illumination to allow simultaneous evaluation of tissue morphology and hybridization signal.

The results indicated that in the normal postnatal human, Ang-2 expression is restricted to the few tissues containing angiogenic vasculature, such as the

ovary, placenta, and uterus. No Ang-2 expression was detectable in normal adult human heart, brain, kidney, liver, lung, pancreas, spleen, muscle, tonsil, thymus, appendix, lymph node, gall bladder, prostate or testis. In five-week-old mouse (but not adult monkey or human), kidneys displayed prominent Ang-2 expression in the vasa recta. To determine whether this expression was a remnant of embryonic development, this experiment was repeated on kidneys derived from mice ranging in age up to one-year-old using the murine Ang-2 probe and conditions described above. Ang-2 expression was observed to decrease during post-natal development, but was still evident in kidneys of one-year-old mice.

Ang-2 expression was also detected in virtually all tumor types tested, including, primary human tumors such as colon carcinoma (5 cases), breast carcinoma (10 cases), lung carcinoma (8 cases), glioblastoma (1 case), metastatic human tumors such as breast carcinoma (2 cases), lung carcinoma (2 cases) and ovarian carcinoma (2 cases) which had metastized to brain, and rodent tumor models such as C6 (rat glioma), HT29 (human colon carcinoma), Colo-205 (human colon carcinoma), HCT116 (human colon carcinoma), A431 (human epidermoid carcinoma), A673 (human rhabdomyosarcoma), HT1080 (human fibrosarcoma), PC-3 (human prostate carcinoma), B16F10 (murine melanoma), MethA (murine sarcoma), and Lewis lung carcinoma mets. Additionally, Ang-2 expression was detected in neovessels growing into a Matrigel plug in response to VEGF and in a mouse hypoxia model of retinopathy of prematurity.

EXAMPLE 2

Production of Recombinant mAng-2 Protein and Rabbit Polyclonal anti-Ang-2 Antiserum

Full length, His-tagged murine Ang-2 cDNA was obtained by PCR (Clontech Advantage PCR Kit, Cat. # K1905-01) from a murine 15-day embryo cDNA library (Marathon-Ready-cDNA, Cat.# 7459-1, Clonetech, Inc.) using PCR primers for full length human Ang-2. The PCR product was ligated into a CMV promoter expression vector, and the resultant plasmid was transfected into

HT1080 human fibrosarcoma cells (obtained from ATCC) using FuGENE6 Transfection Reagent (Roche, Cat. #1814443). Stable clones were isolated by G418 selection. Anti-His tag ELISAs and Western blotting were used to screen for mAng-2-his expressing clones.

Recombinant mAng-2 polypeptide was purified from conditioned media (C.M.) of these cells. The C.M. containing mAng-2-His was purified by a two-step chromatography protocol. Briefly, the conditioned media was titrated to pH 8.9 by adding Tris buffer pH 9.5 to about 20 mM final concentration. Additionally, the detergent CHAPS was added to about 5 mM final concentration. The C.M. was then applied directly to an anion exchange column Q-sepharose ff (Pharmacia). The column was then washed with about 10 mM Tris pH 8.0 containing about 50 mM NaCl. Recombinant mAng-2-His was eluted in a single step using 10 mM Tris pH 8.0 containing about 350 mM NaCl and about 5 mM CHAPS.

The eluate from the Q-sepharose column was adjusted to about 4 mM imidazole, and applied to an immobilized metal affinity column (Ni-NTA superflow (Qiagen)). The bound protein was eluted with PBS containing about 5 mM CHAPS and about 100 mM imidazole. The eluate was then concentrated to approximately 1.0 mg/ml, followed by dialysis against PBS. The purity of mAng-2-His was greater than 90 percent as measured by SDS-PAGE Coomassie staining.

Rabbits were immunized with about 0.2 mg mAng-2/injection in an attempt to produce antibodies. Rabbits were injected with about 1 mL Hunter's TiterMax® (Sigma) and mAng-2 at a ratio of 1:1. Four weeks later, each rabbit received a repeat injection or booster; two weeks after that, they received their next booster, and at week seven, sera were drawn and evaluated for titer against mAng-2. If the serum titer was high, 50 mL production bleeds were drawn on a weekly basis for six consecutive weeks. However, if serum titer was low, rabbits were given an additional booster, and 50 mL production bleeds were drawn on a weekly basis for six consecutive weeks, beginning at week 9. After six consecutive production bleeds, rabbits were allowed to rest for six weeks. If more

sera were required, the rabbits were boosted again one month after the last production bleed.

Using the Neutralization ELISA (described *infra*), anti-mAng-2 rabbit polyclonal antisera from two rabbits, 5254 and 5255, were observed to neutralize the mAng-2:Tie2 interaction.

EXAMPLE 3

Molecular Assays to Evaluate Ang-2 Antibodies

Molecular assays (Affinity ELISA, Neutralization ELISA and BIAcore) were developed to assess direct antibody binding to Ang-2 and related family members, and the effect of antibodies on the Ang-2:Tie2 interaction. These *in vitro* and cell-based assays are described as follows.

A. Affinity ELISA

For the initial screening of candidate anti-Ang-2 antibodies, purified human Ang-2 (R and D Systems, Inc; catalog number 623-AN; Ang-2 is provided as a mixture of 2 truncated versions) or murine Ang-2 polypeptide (prepared as described above) were used. For confirmatory binding assays, human Ang-2 was obtained from conditioned media of human 293T cells transfected with full length human Ang-2 DNA and cultured in serum free DMEM containing about 50 micrograms per ml of bovine serum albumin (BSA).

Using microtiter plates, approximately 100 microliters per well of Ang-2 was added to each well and the plates were incubated about 2 hours, after which the plates were washed with phosphate buffered saline (PBS) containing about 0.1 percent Tween-20 four times. The wells were then blocked using about 250 microliters per well of about 5 percent BSA in PBS, and the plates were incubated at room temperature for about 2 hours. After incubation, excess blocking solution was discarded, and about 100 microliters of candidate anti-Ang-2 antibody was added to each well in a dilution series starting at a concentration of about 40 nanomolar and then serially diluting 4-fold in PBS containing about 1 percent BSA. The plates were then incubated overnight at room temperature. After

incubation, plates were washed with PBS containing about 0.1 percent Tween-20. Washing was repeated four additional times, after which about 100 microliters per well of goat anti-human IgG(Fc)-HRP (Pierce Chemical Co., catalog # 31416) previously diluted 1:5000 in PBS containing 1 percent BSA (bovine serum albumin) was added. Plates were incubated approximately 1 hour at room temperature. Plates were then washed five times in PBS containing about 0.1 percent Tween-20, after which about 100 microliters per well of TMB (3,3',5,5'-Tetramethylbenzidine Liquid Substrate System; Sigma chemical Company, St. Louis, MO, catalog number T8665) substrate was added and plates were incubated about 5-15 minutes until blue color developed. Absorbance was then read in a spectrophotomer at about 370 nm.

B. Neutralization ELISA

Microtiter plates to which human Ang-2 polypeptide was bound were prepared as described for the Affinity ELISA. Candidate anti-Ang-2 antibodies were prepared in serial dilutions as described for the Affinity ELISA above in a solution of PBS containing about 1 percent BSA and about 1 nM Tie2 (provided as a Tie2-Fc molecule where the Tie2 portion contains only the soluble extracellular portion of the molecule; R and D Systems, catalog number 313-TI). After about 100 microliters of the antibody/Tie2 solution was added to each well, the plates were incubated overnight at room temperature, and then washed five times in PBS containing about 0.1 percent Tween-20. After washing, about 100 microliters per well of anti-Tie2 antibody (Pharmingen Inc., catalog # 557039) was added to a final concentration of about 1 microgram per ml and the plates were incubated about 1 hour at room temperature. Next, about 100 microliters per well of goat anti-mouse-IgG-HRP (Pierce Chemical CO., catalog # 31432) was added at a dilution of 1:10,000 in PBS containing about 1 percent BSA. Plates were incubated at room temperature for about 1 hour, after which they were washed five times with PBS containing about 0.1 percent Tween-20. About 100 microliters per well of TMB substrate (described above) was then added and color

was allowed to develop. Absorbance was then read in a spectrophotomer at 370 nm.

C. Affinity BIAcore

An affinity analysis of each candidate Ang-2 antibody was performed on a BIAcore®2000 (Biacore, Inc., Piscataway, NJ) with PBS and 0.005 percent P20 surfactant (BIAcore, Inc.) as running buffer. Recombinant Protein G (Repligen, Needham, MA) was immobilized to a research grade CM5 sensor chip (Biacore, Inc.) via primary amine groups using the Amine Coupling Kit (Biacore, Inc.) according to the manufacturer's suggested protocol.

Binding assays were carried out by first attaching about 100 Ru of each candidate anti-Ang-2 antibody to the immobilized Protein G, after which various concentrations (0 – 100 nM) of huAng-2 or mAng-2 were then injected over the bound antibody surface at a flow rate of about 50 ul/min for about 3 minutes. Antibody binding kinetics including k_a (association rate constant), k_d (dissociation rate constant) and K_D (dissociation equilibrium constant) were determined using the BIA evaluation 3.1 computer program (BIAcore, Inc.). Lower dissociation equilibrium constants indicated greater affinity of the antibody for Ang-2.

EXAMPLE 4

Production of Fully Human Ang-2 Antibodies by Phage Display

Fully human Ang-2 antibodies were generated by panning a Target Quest Phage Display Fab library (Target Quest, Inc.) against a human Ang-2 polypeptide (R and D Systems Inc., catalog 623-AN), according to the following protocol.

Human Ang-2 was immobilized on the surface of polystyrene magnetic beads by two methods: (1) direct coating of Ang-2 at 50 ug/ml at 4C overnight; and (2) indirect capture of Ang-2 by goat anti-Ang-2 antibody at 50 ug/ml at 4C overnight. The bead surface was blocked by 2% milk in PBS (MPBS). The human Fab phage library was pre-selected to remove phage clones reactive to uncoated magnetic beads or the goat anti-Ang-2 antibody. Ang-2-coated

magnetic beads were then incubated with library phage at room temperature for 1.5 hours. After the phage binding step, the surface was washed 6 times with MPBS containing about 0.1 percent Tween 20, followed by washing 6 times with PBS containing about 0.1 percent Tween 20, followed 2 times with PBS. The bound phage were eluted first with about 100 ug/ml human Tie2-Fc (R and D Systems, Minneapolis, MN), and then with about 100 mM triethanolamine. Eluted phage were infected into *E. coli* TG1 cells, amplified, and rescued for the next round of screening. Selection pressure was increased in successive rounds by incorporating more stringent washes and reducing the number of input phage. After 3 rounds of selection, 18 unique, Ang-2-binding Fab clones were identified, virtually all of which recognized human Ang-2, mouse Ang-2, and rat Ang-2 as measured using the ELISA affinity assay described above. Approximately ten percent of these phage also bound human Ang-1. These clones were converted into IgG1 antibodies as described below.

To obtain additional unique phage, a second round of screening was conducted using the same library but a slightly different protocol. In this protocol, human Ang-2 was plated in NaHCO3 buffer at pH 9.6 in Nunc maxisorp immunotubes at about 4C overnight. Ang-2 was plated at about 1.5, 0.74, and 0.3 ug/ml for panning rounds 1, 2, and 3, respectively. The immunotube surface was blocked using about 2 percent milk in PBS (MPBS), before it was incubated with about 2 trillion phage particles (about 50 copies of each unique phage in the library) from the same phage display library referred to above (Target Quest) in about 4ml of 2% MPBS. After the phage incubation step, the surface was washed 20 times with PBS plus about 0.1 percent Tween 20, followed 20 times with PBS. The bound phage were eluted using 1uM hAng-2 or 1uM human Tie2 (R and D Systems, described above). Eluted phage were infected into *E. coli* TG1 cells (provided with the phage library), amplified, and rescued for the next round of screening. Sixteen unique, Ang-2-binding Fab clones were identified by PCR amplification of all phage to which hAng-2 or Tie2 bound, and these clones were analyzed by restriction digestion. The DNA of each clone was sequenced.

The sequence coding for the variable region of each heavy chain from each phage was amplified with complementary primers. The primers were designed to incorporate a HindIII site, XbaI site, Kozak sequence and a signal sequence (translated peptide is MDMRVPAQLLGLLLLWLRGARC; SEQ ID NO: 202) onto the 5' end of the variable region, while a BsmBI site was added onto the 3' end of the PCR product. As an example of how heavy chains were cloned, the template phage DNA for clone 544 (SEQ ID NO: 19) was amplified using primers 2248-21 (GTG GTT GAG AGG TGC CAG ATG TCA GGT CCA GCT GGT GCA G; SEQ ID NO: 203) which added the last 7 amino acids of the signal sequence and 2502-31 (ATT ACG TCT CAC AGT TCG TTT GAT CTC CAC; SEQ ID NO: 204) which added the BsmBI site onto the end of the variable region. The resulting product was amplified by primers 2148-98 (CCG CTC AGC TCC TGG GGC TCC TGC TAT TGT GGT TGA GAG GTG CCA GAT; SEQ ID NO: 205) which added nine amino acids to the signal peptide (AQLLGLLLL; SEQ ID NO: 206) and 2502-31, and then 2489-36 (CAG CAG AAG CTT CTA GAC CAC CAT GGA CAT GAG GGT CCC CGC TCA GCT CCT GGG; SEQ ID NO: 207) and 2502-31. Primer 2489-36 added, from 5' to 3', the HindIII site, XbaI site, Kozak sequence, and the first 6 amino acids of the signal sequence. The PCR products were digested with XbaI and BsmBI, and then cloned into a mammalian expression vector containing the human IgG1 constant region. This vector contains an SV40 promoter and DHFR selection.

Light chains from each phage were either kappa or lambda class. For each light chain, complementary primers were designed to add, from 5' to 3', a HindIII site, an XbaI site, Kozak sequence and signal sequence (set forth above). Those chains which had error-free coding regions were cloned as full-length products. As an example, the light chains from phage clone 536 (SEQ ID NO: 11 and SEQ ID NO: 210) was amplified as a full length coding region using primers 2627-69 (GTG GTT GAG AGG TGC CAG ATG TGA CAT TGT GAT GAC TCA GTC TCC; SEQ ID NO: 208), which added the last seven amino acids of the signal sequence, and primer 2458-54 (CTT GTC GAC TTA TTA ACA CTC TCC CCT GTT G; SEQ ID NO: 209), which added a SalI site after the stop codon. This

PCR product was then amplified as previously stated with additional 5' primers, 2148-98 and 2489-36 respectively, paired with primer 2458-54, to finish addition of the signal sequence and cloning sites. The full-length light chains were cloned as XbaI-SalI fragments into the mammalian expression vector described above.

Certain lambda clones had errors in their constant regions when compared to natural human constant region sequence. To correct for these discrepancies, overlap PCR was performed using DNA coding for a perfect lambda constant region and the phage derived variable region. These clones were also cloned as XbaI-SalI fragments as described above.

Where kappa variable regions were cloned separately from their constant regions, a BsmBI site was added to the 3' end of the PCR product. After digestion of the PCR product with XbaI and BsmBI, the kappa chain variable region was cloned into an expression vector containing the human kappa constant region.

The paired light and heavy chain constructs from each converted phage were co-transfected into CHO cells using the Calcium Phosphate Transfection Kit (Invitrogen Corp.) generally according to the manufacturer's suggested protocol. Media was changed 14-16 hours post transfection, and the cells were passaged into tissue culture dishes for selection after about 48 hours per the manufacturer's recommendations. Transfected cells were isolated by HT selection for approximately 3 weeks, at which time transfected CHO cell colonies were trypsinzed and combined into a "pool" of transfected cells.

Small-scale conditioned media was collected after 48 hours and assayed for antibody production by Western blot analysis using either anti-human Fc antibody, anti-human kappa antibody, or anti-human lambda antibody. The selected cell populations were then passaged under selective pressure using standard tissue culture sterile technique until enough cells were obtained to seed four 850 cm^2 roller bottles with $2x10^7$ viable cells each, and to prepare frozen stock cell lines using DMSO. After seeding, the cells were maintained in roller bottles with about 10 percent serum containing DMEM medium (Gibco/BRL, Inc) supplemented with glutamine and non-essential amino acids. Cells were maintained for two to three days until a cell confluency of approximately 80%

was reached. At this point the media in the roller bottles was switched to a serum free media mixture (50 percent DMEM, 50 percent F12, Gibco) supplemented with glutamine and non-essential amino acids. Conditioned media was harvested after seven days, with fresh serum-free medium being added for one or two additional harvests.

Antibodies were purified by Protein G affinity chromatography directly from conditioned medium, using standard procedures. Elution from the Protein G column was accomplished using low pH (about pH 3) buffer, after which the eluted antibody protein was neutralized using 1M Tris, pH 8.5, and then concentrated using 10 kD molecular weight cutoff centrifugal concentrators. The concentrated antibody stock was then buffer exchanged into PBS.

Thirty-one antibodies have been created, and each consists of two heavy chains and 2 light (kappa or lambda) chains as designated in the following Table 2.

Table 2

Antibody Heavy Chain	Antibody Light Chain#
526 HC*	526 kappa
528 HC*	528 lambda C1
531 HC*	531 lambda C3
533 HC*	533 -kappa
535 HC*	535 lambda C3
536 HC*	536 kappa
537 HC*	537 lambda C3 (G 107A R)
540 HC*	540 lambda C3
543 HC*	543 kappa
544 HC*	544 -lambda C3
545 HC*	545 lambda C2

546 HC*	546 lambda C1 (G 107A S, N 112 A, T114 S)
551 HC*	551 kappa
553 HC*	553 kappa
555 HC*	555 kappa
558 HC	558 kappa
559 HC	559 lambda C1 (N 112 A, T 114 S)
565 HC*	565 kappa
F1-C6 HC	F1-C6 lambda C2
FB1-A7 HC	FB1-A7 lambda C2 (G 107A S)
FD-B2 HC	FD-B2 lambda C3 (G 107A S)
FE-B7 HC	FE-B7 kappa
FJ-G11 HC	FJ-G11 kappa
FK-E3 HC	FK-E3 kappa
G1D4 HC*	G1D4 lambda C2
GC1E8 HC	GC1E8 lambda C3 (K 149 R)
H1C12 HC	H1C12 lambda C2
IA1-1E7 HC	IA1-1E7 kappa
IF-1C10 HC	IF-1C10 lambda C3 (T 212 A)
IK-2E2 HC	IK-2E2 lambda C2 (T 212 A)
IP-2C11 HC	IP-2C11 kappa

* Tested for binding to hAng-2, mAng-2, and hAng-1 as described herein.

Some lambda light chain constant regions appear to be chimers of more than one germline lambda constant region gene. The closest lambda constant region germline gene is indicated along with the amino acids differing from that germline gene, numbered using the Kabat system.

The following four tables set forth the sequences and SEQ ID NOs. of the heavy and light (kappa and lambda) chains of the 31 anti-Ang-2 antibodies converted from phage to full length IgG1 antibodies. The complementarity-determining regions (CDRs) of the monoclonal antibodies were predicted using the VBASE database which uses the technique described by Kabat et al in: *Sequences of Proteins of Immunological Interest* (NIH Publication No. 91-3242; U.S. Dept. Health and Human Services, 5th ed.). Fab regions were aligned to sequences in the database with the -closest germline sequence using tools available from MRC Centre for Protein Engineering, Cambridge, UK, and then visually compared with such sequences. The CDRs for each variable region (heavy or light chain) are set forth in Table 7.

Table 3

Heavy Chain Variable Regions

Antibody HC	Sequence
526 HC (SEQ ID NO: 1)	EVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGK GLEWVSAISGSGGSTYYADSVKGRFTISRDNAKNSLYLQMNSLR AEDTAVYYCARDLLDYDILTGPYAYWGQGTLVTVSS
528 HC (SEQ ID NO: 3)	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQ GLEWMGGIIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRS EDTAVYYCARGVVGDFDWLSFFDYWGQGTLVTVSS
531 HC (SEQ ID NO: 5)	EVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQ GLEWMGGIIPILGIANYAQKFQGRVTITADKSTNTAYMELTSLTS DDTAVYYCARDREDTAMVFNYWGQGTLVTVSS
533 HC (SEQ ID NO: 7)	EVQLVQSGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGK GLEWVSYISSSGSTIYYADSVKGRFTISRDNAKNSLYLQMNSLRA EDTAVYYCARDLLDYDILTGYGYWGQGTLVTVSS
535 HC (SEQ ID NO: 9)	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQ GLEWMGGIIPIFGTANYAQKFQGRVTITADKSTSTAYMELSSLRS EDTAVYYCAAFSPFTETDAFDIWGQGTMVTVSS
536 HC (SEQ ID NO: 11)	EVQLVQSGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGK GLEWVSYISSSGSTIYYADSVKGRFTISRDNAKNSLYLQMNSLRA EDTAVYYCARDLLDYDILTGYGYWGQGTLVTVSS
537 HC (SEQ ID NO: 13)	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQ GLEWMGGIIPILGIANYAQKFQGRVTITADKSTSTAYMELSGLGS EDTAVYYCARGSSDAAVAGMWGQGTLVTVSS
540 HC (SEQ ID NO: 15)	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQ GLEWMGGIIPILGIANYAQKFQGRVTITADKFTSTAYMELSSLGS EDTAVYYCARAVPGTEDAFDIWGQGTMVTVSS
543 HC (SEQ ID NO: 17)	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQ GLEWMGRIIPILGIANYAQKFQGRVTITADKSTSTAYMELSSLRS EDTAVYYCARPYYDFWSGPGGMDVWGQGTTVTVSS
544 HC (SEQ ID NO: 19)	QVQLVQSGAEVKKPGASVKVSCKASGGTFSSYAISWVRQAPGQ GLEWMGGIIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRS EDTAVYYCARFESGYWGDAFDIWGQGTMVTVSS
545 HC (SEQ ID NO: 21)	QVQLQESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGK GLEWVAVISYDGSNKYYADSVKGRFTISRDNSKNTLYLQMNSL RAEDTAVYYCAKGPVDFDYGDYAIDYWGQGTLVTVSS
546 HC (SEQ ID NO: 23)	EVQLVDSGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGK GLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLR AEDTAVYYCAKETISFSTFSGYFDYWAQGTLVTVSS

551 HC (SEQ ID NO: 25)	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQ GLEWMGGIIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRS EDTAVYYCARGYDFWSGYSLDAFDIWGQGTMVTVSS
553 HC (SEQ ID NO: 27)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYAMHWVRQAPG QRLEWMGWINAGNGNTKYSQKFQGRVTITRDTSASTAYMELSG LRSEDTAVYYCARGVDDYGGNSWAFDIWGQGTMVTVSS
555 HC (SEQ ID NO: 29)	QVQLQESGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGK GLEWVAVISYDGSNKYYADSVKGRFTISRDNSKNTLYLQMNSL RAEDTAVYYCARSASDHYYDSSGYYSDAFDIWGQGTMVTVSS
558 HC (SEQ ID NO: 31)	QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWSWIRQSPGK GLEWIGEINHSGSTNFNPSLKSRITISVDTSNNQFSLKLSSVTAAD TAAYYCARGHDWGMGIGGAAYDIWGQGTMVTVSS
559 HC (SEQ ID NO: 33)	QVQLVQSGAEVKKPGASVKVSCKVSGYTLTESSMHWVRQAPG KGLEWMGGFDPEHGETIYAQKFQGRLTMTEDTSTDTAYMELSS LRSEDTAVYFCARGVQVTSGYHYFDHWGQGTLVTVSS
565 HC (SEQ ID NO: 35)	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQ GLEWMGGIIPIFGTANYAQKFQGRVTITADESTSTAYMELSSLRS EDTAVYYCARSPIYYDILTGIDAFDIWGQGTMVTVSS
F1-C6 HC (SEQ ID NO: 37)	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQ GLEWMGRIIPILGIANYAQKFQGRVTITADKSTSTAYMELSSLRS EDTAVYYCARDPIPSGWYFDLWGRGTLVTVSS
FB1-A7 HC (SEQ ID NO: 39)	QVQLVESGGGLVKPGRSLRLSCAASGFTFSSYGMHWVRQAPGK GLEWVAVIWYDGSNKYYADSVKGRFTISRDNSKNTLYLQMNSL RAEDTAVYYCAREVGNYYDSSGYGYWGQGTLVTVSS
FD-B2 HC (SEQ ID NO: 41)	QVQLQQSGPGLVKPSQTLSLTCAISGDTVSSNSAAWNWIRQSPS RGLEWLGRTYYRSKWYSDYAVSLRGRITINLDTDTSKNQFSLQL NSVTPEDTAVYYCARDRGGYIDSWGQGTLVTVSS
FE-B7 HC (SEQ ID NO: 43)	EVQLVESGGGLGQPGGSLRLSCAATGFSLDDYEMNWVRQAPGR GLEWVSYIIGSGKTIFYADSVKGRFTISRDNGKNSVYLQMNSLR AEDTAIYYCARGGGSAYYLNTSDIWGQGTMVTVSS
FJ-G11 HC (SEQ ID NO: 45)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYGISWVRQAPGQ GLEWMGWISAYNGNTNYAQKLQGRVTMTTDTSTSTAYMELRS LRSDDTAVYYCARDRGIAARSAYYYGMDVWGQGTTVTVSS
FK-E3 HC (SEQ ID NO: 47)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYDLNWVRQASG QGLEWMGWMNPTSGNTGYAQKFQGRITMTRNTSISTAYMELRS LRSDDTAVYYCARDPPSGGWEFDYWGQGTLVTVSS
G1D4 HC (SEQ ID NO: 49)	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSHAISWVRQAPGQ GLEWMGRIIPILGIANYAQKFQGRVTITADESTSTAYMELSSLRS EDTAVYYCATSRLEWLLYLDYWGQGTLVTVSS
GC1E8 HC (SEQ ID NO: 51)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYGISWVRQAPGQ GLEWMGWISAYNGNTNYAQKLQGRVTMTTDTSTSTAYMEVRS LRSDDTAVYYCARGGSPYGGYAYPFDYWGQGTLVTVSS

H1C12 HC (SEQ ID NO: 53)	EVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGK GLEWVSYISSSGSTIYYADSVKGRFTISRDNAKNSLYLQMNSLRA EDTAVYYCARDLLDYDILTGYGYWGQGTLVTVSS
IA1-1E7 HC (SEQ ID NO: 55)	QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWSWIRQSPGK GLEWIGEINHSGSTNFNPSLKSRITISVDTSNNQFSLKLSSVTAAD TAVYYCARGHDWGMGIGGAAYDIWGQGTMVTVSS
IF-1C10 HC (SEQ ID NO: 57)	QVQLVESGGGLVQPGGSLRLSCAASGFTFFSTYAMTWVRQAPG KGLEWVSVIRSNGGTDYADFVKGRFTISRDNSKNTLYLQMNGL RAEDTAVYYCMTDYYWGQGTLVTVSS
IK-2E2 HC (SEQ ID NO: 59)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGK GLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLR AEDTAVYYCAKETISFSTFSGYFDYWGQGTLVTVSS
IP-2C11 HC (SEQ ID NO: 61)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYDINWVRQATGQ GLEWMGWMNPNSGNTGYAQKFQGRVTMTRNTSISTAYMELSS LRSEDTAVYYCAKEIAVAGTRYGMDVWGQGTTVTVSS

Table 4

Kappa Chain Variable Regions

Antibody LC	**Sequence**
526 kappa (SEQ ID NO: 2)	DIVMTQSPLSLPVTPGEPASISCRSSQSLLHSNGYNYLDWYLQKP GQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGV YYCMQALQTPPTFGGGTKVEIK
533 kappa (SEQ ID NO: 8)	DIVMTQSPLSLPVTPGEPASISCRSSQSLLHSNGYNYLNWYLQKP GQSPQILIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGV YYCMQGLQTPPTFGQGTKLEIK
536 kappa (THW) (SEQ ID NO: 12)	DIVMTQSPLSLPVTPGEPASISCRSSQSLLHSNGYNYLDWYLQKP GQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGV YYCMQGTHWPPTFGQGTKLEIK
536 kappa (LQT) (SEQ ID NO: 210)	DIVMTQSPLSLPVTPGEPASISCRSSQSLLHSNGYNYLDWYLQKP GQSPQLLIYLGSNRASGVPDRFSGSGSGTDF TLKISRVEAEDVGVYYCMQGLQTPPTFGQGTKLEIK
543 kappa (SEQ ID NO: 18)	DIVMTQSPLSLPVTPGEPASISCRSSQSLLHSNGYNYLDWYLQKP GQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGV YYCMQALQTPLTFGGGTKVEIK
551 kappa (SEQ ID NO: 26)	DIVMTQSPLSLPVTPGEPASISCRSSQSLLHSNGYNYLDWYLQKP GQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGV YYCMQALQTPLTFGGGTKVEIK

553 kappa (SEQ ID NO: 28)	DIVMTQSPLSLPVTPGEPASISCRSSQSLLHSNGYNYLDWYLQKP GQSPQLLIYLGSNRASGVPDRFTGSGSATDFTLRISRVEAEDVGV YYCMQALQTPLTFGGGTKVEIK
555 kappa (SEQ ID NO: 30)	DIVMTQSPLSLPVTPGEPASISCRSSQSLLHSNGYNYLDWYLQKP GQSPQLLIYLASNRASGVPDRFSGSGSGTDFTLRISRVEAEDVGV YYCMQTLQIPITFGPGTKVDIK
558 kappa (SEQ ID NO: 32)	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSSLAWYQQKPGQAP RLLVYAASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQ HYGSSPRTFGQGTKVEIK
565 kappa (SEQ ID NO: 36)	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSSLAWYQQKPGQAP RLLVYAASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQ HYGSSPRTFGQGTKVEIK
565 kappa (2) (SEQ ID NO: 211)	DIVMTQSPLSLPVTPGEPASISCRSSQSLLHSNGYNYLDWYLQKP GQSPQLLIFLGSSRASGVPDRFSGSGSGTDFTLKISKVEADDVGIY YCMQALDTPPTFGPGTKVEIK
FE-B7 kappa (SEQ ID NO: 44)	DIVMTQSPLSLPVTPGEPASISCRSSQSLLHSKGDNYLDWYLQKP GQSPQLLIYLGSHRASGVPDRFSGSGSGTDFTLKISRVEAEDVGV YYCMQALQTPLTFGGGTKVEIK
FJ-G11 kappa (SEQ ID NO: 46)	DIVMTQTPLSLPVTPGEPASISCRSSQSLLDSDDGKTYLDWYLQR PGQSPQLLMYTTSSRASGVPDRFSGSGSGTDFTLKISRVEAEDVG VYYCMQATQFPYTFGQGTKLEIK
FK-E3 kappa (SEQ ID NO: 48)	DIVMTQTPLSSTVTLGQPASISCRSSQSLVHEDGNTYLNWLHQRP GQPPRLLIYKISKRFSGVPDRFSGSGAGTDFTLKISRVEPEDVGVY YCMQSTRFPRTFGQGTKLEIK
IA1-1E7 kappa (SEQ ID NO: 56)	EIVLTQSPATLSLSPGERATLSCRASQSVSSSFLAWYQQKAGQAP RLLIYDTSTRATGIADRFSGSGSGTDFTLTISRLEAEDSAVYYCQQ YDFSPLTFGGGTKVEIK
IP-2C11 kappa (SEQ ID NO: 62)	EIVLTQSPGTLSLSPGERATLSCRASQSISTFLAWYQQKPGQAPRL LIYDASNRATGIPGRFSGSGSGTDFTLTISNLEPEDFAVYYCQHRI NWPLTFGGGTKVEIK

Table 5

Lambda Chain Variable Regions

Antibody LC	**Sequence**
528 lambda (SEQ ID NO: 4)	SYELTQPPSVSVSPGQTASITCSGDKLGYTYTSWFQQKPGQSPVL VIFQDFKRPSGIPERFSGSNSGNTATLTISGTQAMDEADYYCQAW DSTTAVVFGTGTKVTVL
531 lambda (SEQ ID NO: 6)	QSVLTQPPSVSAAPGQKVTVSCSGSSSNIGNNYVSWYQQLPGTA PKLLIYDNNKRPSGIPDRFSGSKSGTSATLGITGLQTGDEADYYC GTWDSSLSAFWVFGGGTKLTVL

535 lambda (SEQ ID NO: 10)	QSVLTQPPSVSAAPGQKVTISCSGSNSNIGNNFVSWYQQLPGTAP KLLVYDNNKRPSGIPDRFSGSKSGTSATLGITGLQTGDEADYYC GTWDSSLSAAEVVFGGGTKLTVL
537 lambda (SEQ ID NO: 14)	QSVLTQPPSVSAAPGQDVTISCSGNNSNIGNNYVSWYQQVPGTA PKLLVYDNHKRPSGISDRFSGSKSDTSATLDITGLQPGDEADYYC GTWDTSLSANWVFGGGTKLTVL
540 lambda (SEQ ID NO: 16)	QSVLTQPPSVSAAPGQKVTISCSGSSSNIGANYVSWYQQLPGTAP KLLIYNNNKRPSGIPDRFSGSKSDTSATLGITGLQTGDEADYYCG AWDSSLSASWVFGGGTKLTVL
544 lambda (SEQ ID NO: 20)	SYELTQPPSVSVSPGQTARITCSGDALPKQYAYWYQQKPGQAPV LVIYKDSERPSGIPERFSGSSSGTTVTLTISGVQAEDEADYYCQSA DSSHVVFGGGTKLTVL
545 lambda (SEQ ID NO: 22)	QSVLTQPSSVSGAPGQRVTISCTGQSSNIGAGYDVHWYQQFPGR APKLLIYGNSNRPSGVPDRFSGSKSGTSASLAITGLQPEDEADYY CQSYDSRLSGSVFGGGTKLTVL
546 lambda (SEQ ID NO: 24)	QSVLTQPSSVSEAPRQRVTISCSGSASNIGANGVSWYHQVPGKA PRLLLSHDGLVTSGVPDRLSVSKSGTSASLAISGLHSDDEGDYYC AVWDDSLNAVVFGGGTKLTVL
559 lambda (SEQ ID NO: 34)	QSALTQPPSASGSPGQSITISCTGTNSDIGSYPFVSWYQRHPGKAP KLLIYDVSNRPSGVSDRFSGSKSGNTASLTISGLQAEDEGDYYCS SFTMNSFVIFGGGTKLTVL
F1-C6 lambda (SEQ ID NO: 38)	QSVLTQPPSVSEAPRQRVTISCSGSSSNIGNNAVNWYQQLPGKAP KLLIYYDDLLPSGVSDRFSGSKSGTSASLAISGLRSEDEADYYCA TWDDSLSGWVFGGGTKLTVL
FB1-A7 lambda (SEQ ID NO: 40)	NFMLTQPHSVSESPGKTVTISCTRSGGGIGSSFVHWFQQRPGSSP TTVIFDDNQRPTGVPDRFSAAIDTSSSSASLTISGLTAEDEADYYC QSSHSTAVVFGGGTKLTVL
FD-B2 lambda (SEQ ID NO: 42)	NFMLTQPHSVSESPGKTVTISCTRSSGSIATNYVQWYQQRPGSSP ATVIYEDNQRPSGVPDRFSGSIDTSSNSASLTISGLTTEDEADYFC QSYGDNNWVFGGGTKLTVL
G1D4 lambda (SEQ ID NO: 50)	NFMLTQPHSVSESPGKTVIIPCTRSSGSIASNYVQWYQKRPGSAP SIVIYEDKQRPSGVPDRFSGSIDSSSNSASLTISGLKTEDEADYYC QSYNSRGVMFGGGTKLTVL
GC1E8 lambda (SEQ ID NO: 52)	NFMLTQPHSVLESAGKTVTISCTRSSGSIASNYVQWYQQRPGTSP TNVIFEDNQRPSGVPDRFSGSIDSSSNSASLTISGLKTEDEADYFC QSYDSNIWVFGGGTKLTVL
H1C12 lambda (SEQ ID NO: 54)	QSVLTQPPSVSAAPGQKVTISCSGSSSNIGNNYVSWYQHLPGTAP KLLIYGNTNRPSGVPDRFSGSKSGTSASLAIAGLQAEDEADYYC QSYDSSLSGSLVFGGGTKLTVL
IF-1C10 lambda (SEQ ID NO: 58)	NFMLTQPHSVSESPGKTVTISCTGSGGSIASNYVQWYQQRPGSA PTTVIYEDNQRPSGVPDRFSGSIDSSSNSASLTISGLKTEDEADYY CQSYDSSTWVFGGGTKLTVL

IK-2E2 lambda (SEQ ID NO: 60)	QSALTQPASVSGSPGQSITISCTGTSSDVGGYNYVSWFQQHPGK APKLMIYKVNNRPSGLSNRFSGSQSGNTASLTISGLQAEDEADY YCSSYTSSSTLGFGGGTKLTVL

Table 6

Human Constant Regions (CR)

Antibody CR	**Sequence**
Human lambda constant region 1 (C1) (SEQ ID NO: 63)	GQPKANPTVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWK ADGSPVKAGVETTKPSKQSNNKYAASSYLSLTPEQWKSHRSY SCQVTHEGSTVEKTVAPTECS
Human lambda constant region 2 (C2) (SEQ ID NO: 64)	GQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWK ADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSHRSYS CQVTHEGSTVEKTVAPTECS
Human lambda constant region 3 (C3) (SEQ ID NO: 65)	GQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWK ADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSHKSYS CQVTHEGSTVEKTVAPTECS
Human lambda constant region 7 (C7) (SEQ ID NO: 66)	GQPKAAPSVTLFPPSSEELQANKATLVCLVSDFYPGAVTVAW KADGSPVKVGVETTKPSKQSNNKYAASSYLSLTPEQWKSHRS YSCRVTHEGSTVEKTVAPAECS
Human kappa constant region (SEQ ID NO: 67)	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKV DNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVY ACEVTHQGLSSPVTKSFNRGEC
Human IgG1 constant region (SEQ ID NO: 68)	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNH KPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPK DTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPA PIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYP SDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Table 7

Complementarity-Determining Regions (CDRs) of Heavy Chains (HC) and Light Chains (LC) of Ang-2 Antibodies

	CDR 1	CDR 2	CDR 3

Antibody	Residues	Residues	Residues
Ab 526 HC	SYGMH (SEQ ID NO: 69)	AISGSGGSTYYADSVKG (SEQ ID NO: 105)	DLLDYDILTGPYAY (SEQ ID NO: 144)
Ab 526 KC	RSSQSLLHSNGYNYLD (SEQ ID NO: 70)	LGSNRAS - (SEQ ID NO: 106)	MQALQTPPT (SEQ ID NO: 145)
Ab 528 HC	SYAIS (SEQ ID NO: 71)	GIIPIFGTANYAQKFQG (SEQ ID NO: 107)	GVVGDFDWLSFFDY (SEQ ID NO: 146)
Ab 528 LC	SGDKLGYTYTS (SEQ ID NO: 72)	QDFKRPS (SEQ ID NO: 108)	QAWDSTTAVV (SEQ ID NO: 147)
Ab 531 HC	SYAIS (SEQ ID NO: 71)	GIIPILGIANYAQKFQG (SEQ ID NO: 109)	DREDTAMVFNY (SEQ ID NO: 148)
Ab 531 LC	SGSSSNIGNNYVS (SEQ ID NO: 73)	DNNKRPS (SEQ ID NO: 110)	GTWDSSLSAFWV (SEQ ID NO: 149)
Ab 533 HC	SYGMH (SEQ ID NO: 69)	YISSSGSTIYYADSVKG (SEQ ID NO: 111)	DLLDYDILTGYGY (SEQ ID NO: 150)
Ab 533 KC	RSSQSLLHSNGYNYLN (SEQ ID NO: 74)	LGSNRAS (SEQ ID NO: 106)	MQGLQTPPT (SEQ ID NO: 151)
Ab 535 HC	SYAIS (SEQ ID NO: 71)	GIIPIFGTANYAQKFQG (SEQ ID NO: 107)	FSPFTETDAFDI (SEQ ID NO: 152)
Ab 535 LC	SGSNSNIGNNFVS (SEQ ID NO: 75)	DNNKRPS (SEQ ID NO: 110)	GTWDSSLSAAEVV (SEQ ID NO: 153)

Ab 536 HC	SYGMH (SEQ ID NO: 69)	YISSSGSTIYYADSVKG (SEQ ID NO: 111)	DLLDYDILTGYGY (SEQ ID NO: 150)
Ab 536 (THW) KC	RSSQSLLHSNGYNYLD (SEQ ID NO: 70)	LGSNRAS (SEQ ID NO: 106)	MQGTHWPPT (SEQ ID NO: 154)
Ab 536 (LQT) KC	RSSQSLLHSNGYNYLD (SEQ ID NO: 70)	LGSNRAS (SEQ ID NO: 106)	MQGLQTPPT (SEQ ID NO: 212)
Ab 537 HC	SYAIS (SEQ ID NO: 71)	GIIPILGIANYAQKFQG (SEQ ID NO: 109)	GSSDAAVAGM (SEQ ID NO: 155)
Ab 537 LC	SGNNSNIGNNYVS (SEQ ID NO: 76)	DNHKRPS (SEQ ID NO: 112)	GTWDTSLSANWV (SEQ ID NO: 156)
Ab 540 HC	SYAIS (SEQ ID NO: 71)	GIIPILGIANYAQKFQG (SEQ ID NO: 109)	AVPGTEDAFDI (SEQ ID NO: 157)
Ab 540 LC	SGSSSNIGANYVS (SEQ ID NO: 77)	NNNKRPS (SEQ ID NO: 113)	GAWDSSLSASWV (SEQ ID NO: 158)
Ab 543 HC	SYAIS (SEQ ID NO: 71)	RIIPILGIANYAQKFQG (SEQ ID NO: 114)	PYYDFWSGPGGMDV (SEQ ID NO: 159)
Ab 543 KC	RSSQSLLHSNGYNYLD (SEQ ID NO: 70)	LGSNRAS (SEQ ID NO: 106)	MQALQTPLT (SEQ ID NO: 160)
Ab 544 HC	SYAIS (SEQ ID NO: 71)	GIIPIFGTANYAQKFQG (SEQ ID NO: 107)	FESGYWGDAFDI (SEQ ID NO: 161)
Ab 544			

LC	SGDALPKQYAY (SEQ ID NO: 78)	KDSERPS (SEQ ID NO: 115)	QSADSSHVV (SEQ ID NO: 162)
Ab 545 HC	SYGMH (SEQ ID NO: 69)	VISYDGSNKYYADSVKG (SEQ ID NO: 116)	GPVDFDYGDYAIDY (SEQ ID NO: 163)
Ab 545 LC	TGQSSNIGAGYDVH (SEQ ID NO: 79)	GNSNRPS (SEQ ID NO: 117)	QSYDSRLSGSV (SEQ ID NO: 164)
Ab 546 HC	SYAMS (SEQ ID NO: 80)	AISGSGGSTYYADSVKG (SEQ ID NO: 105)	ETISFSTFSGYFDY (SEQ ID NO: 165)
Ab 546 LC	SGSASNIGANGVS (SEQ ID NO: 81)	HDGLVTS (SEQ ID NO: 118)	AVWDDSLNAVV (SEQ ID NO: 166)
Ab 551 HC	SYAIS (SEQ ID NO: 71)	GIIPIFGTANYAQKFQG (SEQ ID NO: 107)	GYDFWSGYSLDAFDI (SEQ ID NO: 167)
Ab 551 KC	RSSQSLLHSNGYNYLD (SEQ ID NO: 70)	LGSNRAS (SEQ ID NO: 106)	MQALQTPLT (SEQ ID NO: 160)
Ab 553 HC	SYAMH (SEQ ID NO: 82)	WINAGNGNTKYSQKFQG (SEQ ID NO: 119)	GVDDYGGNSWAFDI (SEQ ID NO: 168)
Ab 553 KC	RSSQSLLHSNGYNYLD (SEQ ID NO: 70)	LGSNRAS (SEQ ID NO: 106)	MQALQTPLT (SEQ ID NO: 160)
Ab 555 HC	SYAMH (SEQ ID NO: 82)	VISYDGSNKYYADSVKG (SEQ ID NO: 116)	SASDHYYDSSGYYSDAFDI (SEQ ID NO: 169)
Ab 555 KC	RSSQSLLHSNGYNYLD	LASNRAS (SEQ ID NO:	MQTLQIPIT (SEQ ID NO:

	(SEQ ID NO: 70)	120)	170)
Ab 558 HC	GYYWS (SEQ ID NO: 83)	EINHSGSTNFNPSLKS (SEQ ID NO: 121)	GHDWGMGIGGAAYDI (SEQ ID NO: 171)
Ab 558 KC	RASQSVSSSSLA (SEQ ID NO: 84)	AASSRAT (SEQ ID NO: 122)	QHYGSSPRT (SEQ ID NO: 172)
Ab 559 HC	ESSMH (SEQ ID NO: 85)	GFDPEHGETIYAQKFQG (SEQ ID NO: 123)	GVQVTSGYHYFDH (SEQ ID NO: 173)
Ab 559 LC	TGTNSDIGSYPFVS (SEQ ID NO: 86)	DVSNRPS (SEQ ID NO: 124)	SSFTMNSFVI (SEQ ID NO: 174)
Ab 565 HC	SYAIS (SEQ ID NO: 71)	GIIPIFGTANYAQKFQG (SEQ ID NO: 107)	SPIYYDILTGIDAFDI (SEQ ID NO: 175)
Ab 565 KC	RASQSVSSSSLA (SEQ ID NO: 213)	AASSRAT (SEQ ID NO: 214)	QHYGSSPRT (SEQ ID NO: 215)
Ab 565 (2) KC	RSSQSLLHSNGYNYLD (SEQ ID NO: 70)	LGSSRAS (SEQ ID NO: 125)	MQALDTPPT (SEQ ID NO: 176)
Ab F1-C6 HC	SYAIS (SEQ ID NO: 71)	RIIPILGIANYAQKFQG (SEQ ID NO: 114)	DPIPSGWYFDL (SEQ ID NO: 177)
Ab F1-C6 LC	SGSSSNIGNNAVN (SEQ ID NO: 87)	YDDLLPS (SEQ ID NO: 126)	ATWDDSLSGWV (SEQ ID NO: 178)
Ab FB1-A7 HC	SYGMH (SEQ ID NO: 69)	VIWYDGSNKYYADSVKG (SEQ ID NO: 127)	EVGNYYDSSGYGY (SEQ ID NO: 179)
Ab FB1-			

A7 LC	TRSGGGIGSSFVH (SEQ ID NO: 88)	DDNQRPT (SEQ ID NO: 128)	QSSHSTAVV (SEQ ID NO: 180)
Ab FD-B2 HC	SNSAAWN (SEQ ID NO: 89)	RTYYRSKWYSDYAVSLRG (SEQ ID NO: 129)	DRGGYIDS (SEQ ID NO: 181)
Ab FD-B2 LC	TRSSGSIATNYVQ (SEQ ID NO: 90)	EDNQRPS (SEQ ID NO: 130)	QSYGDNNWV (SEQ ID NO: 182)
Ab FE-B7 HC	DYEMN (SEQ ID NO: 91)	YIIGSGKTIFYADSVKG (SEQ ID NO: 131)	GGGSAYYLNTSDI (SEQ ID NO: 183)
Ab FE-B7 KC	RSSQSLLHSKGDNYLD (SEQ ID NO: 92)	LGSHRAS (SEQ ID NO: 132)	MQALQTPLT (SEQ ID NO: 160)
Ab FJ-G11 HC	SYGIS (SEQ ID NO: 93)	WISAYNGNTNYAQKLQG (SEQ ID NO: 133)	DRGIAARSAYYYGMDV (SEQ ID NO: 184)
Ab FJ-G11 KC	RSSQSLLDSDDGKTYLD (SEQ ID NO: 94)	TTSSRAS (SEQ ID NO: 134)	MQATQFPYT (SEQ ID NO: 185)
Ab FK-E3 HC	SYDLN (SEQ ID NO: 95)	WMNPTSGNTGYAQKFQG (SEQ ID NO: 135)	DPPSGGWEFDY (SEQ ID NO: 186)
Ab FK-E3 KC	RSSQSLVHEDGNTYLN (SEQ ID NO: 96)	KISKRFS (SEQ ID NO: 136)	MQSTRFPRT (SEQ ID NO: 187)
Ab G1D4 HC	SHAIS (SEQ ID NO: 97)	RIIPILGIANYAQKFQG (SEQ ID NO: 114)	SRLEWLLYLDY (SEQ ID NO: 188)
Ab G1D4	TRSSGSIASNYVQ (SEQ	EDKQRPS (SEQ ID NO:	QSYNSRGVM

LC	ID NO: 98)	137)	(SEQ ID NO: 189)
Ab GC1E8 HC	SYGIS (SEQ ID NO: 93)	WISAYNGNTNYAQKLQG (SEQ ID NO: 133)	GGSPYGGYAYPFDY (SEQ ID NO: 190)
Ab GC1E8 LC	TRSSGSIASNYVQ (SEQ ID NO: 98)	EDNQRPS (SEQ ID NO: 130)	QSYDSNIWV (SEQ ID NO: 191)
Ab H1C12 HC	SYGMH (SEQ ID NO: 69)	YISSSGSTIYYADSVKG (SEQ ID NO: 111)	DLLDYDILTGYGY (SEQ ID NO: 150)
Ab H1C12 LC	SGSSSNIGNNYVS (SEQ ID NO: 73)	GNTNRPS (SEQ ID NO: 138)	QSYDSSLSGSLV (SEQ ID NO: 192)
Ab IA1-1E7 HC	GYYWS (SEQ ID NO: 83)	EINHSGSTNFNPSLKS (SEQ ID NO: 121)	GHDWGMGIGGAAYDI (SEQ ID NO: 171)
Ab IA1-1E7 KC	RASQSVSSSFLA (SEQ ID NO: 99)	DTSTRAT (SEQ ID NO: 139)	QQYDFSPLT (SEQ ID NO: 193)
Ab IF-1C10 HC	STYAMT (SEQ ID NO: 100)	VIRSNGGTDYADFVKG (SEQ ID NO: 140)	DYY (SEQ ID NO: 194)
Ab IF-1C10 LC	TGSGGSIASNYVQ (SEQ ID NO: 101)	EDNQRPS (SEQ ID NO: 130)	QSYDSSTWV (SEQ ID NO: 195)
Ab IK-2E2 HC	SYAMS (SEQ ID NO: 80)	AISGSGGSTYYADSVKG (SEQ ID NO: 105)	ETISFSTFSGYFDY (SEQ ID NO: 165)
Ab IK-2E2 LC	TGTSSDVGGYNYVS (SEQ ID NO: 102)	KVNNRPS (SEQ ID NO: 141)	SSYTSSSTLG (SEQ ID NO: 196)

Ab IP-2C11 HC	SYDIN (SEQ ID NO: 103)	WMNPNSGNTGYAQKFQG (SEQ ID NO: 142)	EIAVAGTRYGMDV (SEQ ID NO: 197)
Ab IP-2C11 KC	RASQSISTFLA (SEQ ID NO: 104)	DASNRAT (SEQ ID NO: 143)	QHRINWPLT (SEQ ID NO: 198)

Seventeen of the antibodies and a negative control IgG1 (referred to as RDB1) were tested using affinity and neutralization ELISA (as described in Example 3 above) as well as the BIAcore neutralization assay to determine their affinity, neutralization, and specificity capabilities. The results are set forth below (Table 8) and were calculated using standard procedures.

Table 8

Ang-2 Antibody EC50s and IC50s

	hAng-2		mAng-2		hAng-1	
Antibody	**IC50 (nM)**	**EC50 (nM)**	**IC50 (nM)**	**EC50 (nM)**	**IC50 (nM)**	**EC50 (nM)**
Ab 536 (THW/LQT mixture)	0.08	0.005	0.05	0.01	114.65	30
Ab 565	0.26		0.26		No inhibition	
Ab 546	0.37		1.09		No inhibition	
Ab 543	0.51		0.24		No inhibition	
Ab 533	0.3		0.08		No inhibition	
Ab 537	0.56		0.62		No inhibition	
Ab 540	0.70		1.53		No inhibition	
Ab 544	0.97		1.82		23.32	

Ab 545	1.04	0.02	1.30	0.05	8.31	2
Ab 528	1.37		0.73		No inhibition	
Ab G1D4	1.39		0.60		69.48	
Ab 551	1.41		2.88		No inhibition	
Ab 553	1.47		1.41		No inhibition	
Ab 526	1.83		0.27		243.15	
Ab 531	2.15		1.67		No inhibition	
Ab 555	2.21		1.76		No inhibition	
Ab 535	2.81		2.45		No inhibition	
RDB1	No inhibition	No binding	No inhibition	No binding	No inhibition	No binding

Two antibodies, clone 536 and clone 545, were evaluated using the BIAcore analysis described above. Antibody binding was determined as described above for the BIAcore assay, with lower K_Ds indicating greater affinities, and results are reported in the following Table 9.

Table 9

Antibody Affinities for hAng-2 and mAng-2

	hAng-2			**mAng-2**		
Ab	**K_D (nM)**	**k_a(1/Ms)**	**k_d(1/s)**	**K_D (nM)**	**k_a(1/Ms)**	**k_d(1/s)**
Ab 536 (THW/LQT mixture)	0.12	3.2×10^5	3.8×10^{-5}	0.15	6.2×10^5	9.5×10^{-5}
Ab 545	1.2	3.3×10^5	3.9×10^{-4}	0.9	5.9×10^5	5.3×10^{-4}

The clone 536 analyzed above comprised a mixture of two antibody variants, which are shown in Table 4 as SEQ ID NO: 12 (536 kappa THW) and SEQ ID NO: 210 (536 kappa LQT). These two 536 variants were separated and analyzed separately for potency using ELISA and HTRF assays.

For the ELISA assay, ninety-six-well microtiter plates were coated with recombinant angiopoietins in 293T cell conditioned media (DMEM/50 ug/ml BSA) at 37°C for 1 hour. The conditioned media were used at angiopoietin concentrations that conferred 80% of maximally-achievable binding to 1 nM hTie2-Fc (R&D Systems, catalog # 313-TI). Plates were washed with PBS/0.1% Tween-20 and then blocked for 2 hours at room temperature with PBS/5% BSA. Angiopoietin-neutralizing agents titrated from 100 nM to 0.4 pM in a solution of PBS/1% BSA/1 nM Tie2 were added to the angiopoietin-coated plates, which were incubated overnight at room temperature and then washed with PBS/0.1 percent Tween-20. Mouse-derived anti-Tie2 antibody (BD Pharmingen Inc., catalog # 557039) was added to each plate at a final concentration of 1 ug/ml (1 hour incubation at room temperature), after which plates were washed in PBS/0.1% Tween-20. Goat anti-mouse-IgG-HRP (Pierce, catalog # 31432) was added at a dilution of 1:10,000 in PBS/1% BSA (1 hour incubated at room temperature), after which plates were washed several times with PBS/0.1 % Tween-20. TMB substrate (Sigma, catalog # T8665) was added, O.D. 370 nm absorbance was measured, and degree of angiopoietin:Tie2 neutralization was determined by comparison against a Tie2 standard curve.

For HTRF assays, a ninety-six-well microtiter "mix plate" was prepared by adding 50 ul of HTRF buffer (50 mM Tris-HCl, pH 7.5, 100 mM NaCl, 0.05% Tween 20, 0.1% BSA) containing 0.8 nM Europium-conjugated streptavidin (PERKIN ELMER LIFE SCIENCES INC., catalog # AD0062) and 4.0 nM biotinylated human angiopoietin 1 (R & D Systems) or angiopoietin 2 (Amgen Inc.) to each well. In a separate microtiter plate, angiopoietin-neutralizing agents were titrated from 400 nM to 20 pM in HTRF buffer, and 50 ul of each serially-diluted angiopoietin-neutralizing agent were then transferred and mixed with the "mix plate" containing the streptavidin-Europium/angiopoietin. The plate was

then incubated on a shaker at room temperature for one hour. Next, 20 ul from each well of the "mix plate" were transferred to an "assay plate" containing 20 ul of 10 nM human allophycocyanin-conjugated human Tie2-Fc in HTRF buffer in each of the ninety-six-wells. The final "assay plate" was incubated at room temperature with shaking for two hours. The final concentrations of the "assay plate" were: 1.0 nM angiopoietin, 5.0 nM human Tie2-Fc, and 100 nM to 5.0 pM for the serially-diluted angiopoietin-neutralizing agents. Assay plates were analyzed using a Rubystar plate reader (BMG Labtechnologies, Offenberg, Germany). Degree of angiopoietin:Tie2 neutralization was determined by calculating the percentage inhibition of each angiopoietin-neutralizing agent dilution using a "no-angiopoietin-neutralizing agent" control (to represent zero inhibition) and a "no-angiopoietin" control (representing complete inhibition). IC50 values were then calculated by analyzing percentage inhibition using the GRAFIT 5.0 program (Erithacus Software Ltd.).

All results were expressed as IC50 curves calculated from samples that were tested in duplicate using the formula below. IC50 results fit inhibition data to a 2 parameter equation, where the lower data limit was 0, (*i.e.* the data were background corrected, and the upper data limit was 100, (*i.e.* the data were range corrected).

$$y = \frac{100\%}{1 + \left(\frac{x}{IC_{50}}\right)^{s}}$$

In this equation, s is a slope factor. The equation assumes that y falls with increasing x. This equation was utilized in the software program GRAFIT 5.0 (Erithacus Software Limited).

The results are shown in Tables 10 and 11.

Table 10
Ab536 Variant ELISA IC50 Results

Sample	Human Ang1 IC50 (nM)	Human Ang2 IC50 (nM)	Mouse Ang2 IC50 (nM)

Ab536 LQT	>100	0.35	0.10
Ab536 THW	>100	0.31	0.088

Table 11
Ab536 Variant HTRF IC50 Results

Sample	Human Ang1 IC50 (nM)	Human Ang2 IC50 (nM)
Ab536 LQT	>100	0.072
Ab536 THW	>100	0.071

EXAMPLE 5

Therapeutic Efficacy Studies using Anti-Ang-2 Antibodies

The pharmacokinetics of Protein-G purified rabbit anti-Ang-2 polyclonal antibodies were examined in mice. Twenty-four mice were treated with polyclonal anti-Ang-2 rabbit antibody (1 mg per mouse). Four treated animals were sacrificed at each of the following time points post-injection of antibody: 1 hour, 6 hours, 1 day, 3 days, 7 days, and 14 days.

The results indicated that total rabbit IgG had a circulatory half-life in serum of approximately 19 days, while the anti-Ang-2 IgG component of the total IgG had a half-life of approximately eight days.

To assess therapeutic efficacy, mice (10 animals/group) bearing A431 tumor xenografts were given 10 doses (about 10 mg IgG per mouse per dose) intraperitoneally of Protein G purified anti-mAng-2 polyclonal antibody on days 1, 5, 6, 7, 8, 12, 13, 14, 15, and 18 after xenografting. Tumor size was measured on days 7, 12, 15, 19, and 21. Body weight was measured on days 0, 7, 15, and 21, and was unaffected by treatment. Results indicated that the anti-Ang-2 polyclonal antibody inhibited the A431 tumor xenograft growth by about 50 percent with p=0.008 versus controls of non-immune purified polyclonal

antiserum (10 mg IgG per mouse per dose) and vehicle (PBS) by repeated measures ANOVA.

To test the efficacy of the fully human monoclonal anti-Ang-2 antibodies *in vivo*, mice (10 animals/group) bearing A431 tumor xenografts were treated intraperitoneally with either anti-Ang-2 antibody clone 533, 537, or 544, or with negative controls of PBS or human IgG1-kappa. Dosing was about 420 ug protein per mouse for the first dose, about 140 ug protein per mouse for each of the next three doses, and about 55 ug protein per mouse for each of the next four doses, for a total of 8 doses per mouse. Tumor volumes and body weights were recorded twice weekly. At the end of the study, animals were sacrificed and their serum was collected for measuring antibody levels by ELISA. Tumors and a panel of normal tissues were collected from all groups.

Remarkable differences in tumor growth between the anti-Ang-2-treated and control groups were found as shown in Figure 1. All three anti-Ang-2 treatments inhibited tumor growth as compared to controls ($p < .005$ vs. hIgG1 control in all treatments using repeated measure ANOVA for all 3 antibodies). In contrast, tumors in control groups continued to grow at a much greater rate.

Example 6

Epitope Mapping

Full-length (amino acids 1-495), N-terminal (amino acids 1-254) and C-terminal (amino acids 255-495) human Ang-2 (hAng-2) proteins were cloned into a CMV-driven mammalian expression vector with C-terminal 6xHis tags. The three resultant constructs plus a vector control were transiently expressed into 293T cells. Conditioned media were then collected from the transfected cells, and the expression level of Ang-2 in the media was estimated by anti-6xhis ELISA and Western blotting.

The binding epitope of anti-Ang-2 antibodies and peptibodies was determined by their ability to bind the three versions of human hAng-2 by ELISA according to the following protocol: a high-binding 96-well assay plate was coated with 100 µl of conditioned media per well, and incubated at 37°C for 1

hour. Conditioned media was aspirated, and the plate was blocked with 200 µl per well of 5% BSA in PBS at room temperature for 1 hour. The blocking solution was then aspirated. 100 µl per well of antibody, peptibody, or Tie2-Fc was added at 1µg/ml in 1% BSA in PBS, and incubated at room temperature for 1 hour. The wells were washed 4 times with 200 µl of 0.1% Tween in PBS. 100 µl per well of HRP-conjugated goat anti-human IgG or goat anti-mouse IgG were added, and incubated at room temperature for 45 minutes. The wells were then washed with 200 µl of 0.1% Tween in PBS 4 times. 100 µl per well of TMB substrate was then added. O.D. was read at 370nm.

The results are set forth in Figure 2A, Figure 2B, and Figure 2C.

What is Claimed

We Claim:

1. An isolated polypeptide that specifically binds angiopoietin-2, wherein the polypeptide comprises at least one complementarity determining region (CDR), wherein the CDR is:

a) a CDR1 region comprising an amino acid sequence of the formula:

$X^1X^2X^3X^4X^5X^6X^7X^8X^9X^{10}X^{11}X^{12}X^{13}X^{14}X^{15}X^{16}X^{17}$ (SEQ ID NO: 199), wherein

X^1 is R, S, T, G, E, D;

X^2 is S, G, A, R, N, T, Y, H;

X^3 is S, D, N, Q, T, Y, A, G, E;

X^4 is Q, K, S, N, A, G, I, M, W, L;

X^5 is S, L, G, A, M, H, N;

X^6 is L, G, N, P, V, D, W, S, T, I, or absent;

X^7 is L, Y, I, K, S, N, V, or absent;

X^8 is H, T, G, Q, S, A, D, or absent;

X^9 is S, Y, N, A, T, E, G, F, or absent;

X^{10} is N, T, A, G, S, Y, K, D, F, L, or absent;

X^{11} is G, S, Y, F, L, P, A, F, D, N, or absent;

X^{12} is Y, V, D, A, F, G, N, or absent;

X^{13} is N, S, V, H, Q, K, T, or absent;

X^{14} is Y, H, S, T, or absent;

X^{15} is L, Y, or absent;

X^{16} is D, N, L, or absent;

X^{17} is D, or absent;

b) a CDR2 region comprising an amino acid sequence of the formula:

$X^1X^2X^3X^4X^5X^6X^7X^8X^9X^{10}X^{11}X^{12}X^{13}X^{14}X^{15}X^{16}X^{17}X^{18}$ (SEQ ID NO: 200), wherein

X^1 is L, Q, D, N, K, G, H, A, Y, E, T, R, V, W;

X^2 is G, D, N, A, V, T, I, F, M;

X^3 is S, F, N, H, G, D, K, T, I, W, Y, R;

X^4 is N, K, E, L, S, Q, H, T, G, P, Y, A;

X^5 is R, V, L, S, I, D, G, E, Y, T, N;

X^6 is A, P, T, F, G, L, N, H, S;

X^7 is S, T, G, K;

X^8 is S, T, I, N, E, W, or absent;

X^9 is T, A, I, K, N, Y, D, or absent;

X^{10} is Y, N, K, F, I, S, G, or absent;

X^{11} is Y, N, D, A, or absent;

X^{12} is A, S, P, Y, D, or absent;

X^{13} is D, Q, S, A, F, or absent;

X^{14} is S, K, L, V, or absent;

X^{15} is V, F, K, S, L, or absent;

X^{16} is K, Q, S, L, F, G, or absent;

X^{17} is G, R, or absent;

X^{18} is G, or absent; or

c) a CDR3 region comprising an amino acid sequence of the formula:

$X^1X^2X^3X^4X^5X^6X^7X^8X^9X^{10}X^{11}X^{12}X^{13}X^{14}X^{15}X^{16}X^{17}X^{18}X^{19}$ (SEQ ID NO: 201), wherein

X^1 is D, M, G, Q, F, A, P, E, S;

X^2 is L, Q, V, A, R, T, S, Y, E, P, H, G, I;

X^3 is L, A, V, W, E, P, G, S, Y, I, D, T, Q, F, R;

X^4 is D, L, G, F, T, S, W, V, Y, P, N, H, I, E;

X^5 is Y, Q, D, S, T, H, A, F, W, G, M, R, N;

X^6 is D, T, F, A, S, E, W, R, G, Y, I, M, N, L;

X^7 is I, P, D, T, M, L, S, V, G, H, Y, W, A, N, R;

X^8 is L, P, W, A, V, S, D, G, F, N, Y, I, R, E, T;

X^9 is T, L, V, F, A, G, P, D, S, H, N, M, Y, or absent;

X^{10} is G, S, V, N, F, A, M, D, Y, L, W, I, T, or absent;

X^{11} is P, F, Y, W, D, E, I, G, A, V, L, S, M, or absent;

X^{12} is Y, F, V, G, I, V, M, A, D, or absent;

X^{13} is A, D, Y, V, F, H, I, G, or absent;

X^{14} is Y, V, D, I, S, F, M, or absent;

X^{15} is I, D, or absent;

X^{16} is A, I, V, or absent;

X^{17} is F, or absent;

X^{18} is D, or absent; and

X^{19} is I, or absent.

2. The polypeptide of claim 1, comprising at least one amino acid sequence selected from SEQ ID NO: 69-104 and SEQ ID NO: 213.

3. The polypeptide of claim 1, comprising at least one amino acid sequence selected from SEQ ID NO: 105-143 and SEQ ID NO: 214.

4. The polypeptide of claim 1, comprising at least one amino acid sequence selected from SEQ ID NO: 144-198, SEQ ID NO: 212, and SEQ ID NO: 215.

5. The isolated polypeptide of Claim 1 that is an antibody.

6. The antibody of Claim 5 that is a polyclonal, monoclonal, chimeric, humanized, or fully human antibody.

7. The antibody of Claim 6 that is a single chain antibody.

8. A hybridoma that produces the monoclonal antibody according to Claim 6.

9. A nucleic acid molecule encoding the polypeptide of Claim 1.

10. A vector comprising the nucleic acid molecule of Claim 9.

11. A host cell containing the vector of Claim 10.

12. A nucleic acid molecule encoding the antibody of Claim 5, 6, or 7.

13. A vector comprising the nucleic acid molecule of Claim 12.

14. A host cell containing the vector of Claim 13.

15. A method of making an antibody comprising:

(a) transforming a host cell with at least one nucleic acid molecule encoding the antibody of Claim 5, 6, or 7;

(b) expressing the nucleic acid molecule in said host cell; and

(c) isolating said specific binding agent.

16. A method of inhibiting undesired angiogenesis in a mammal comprising administering a therapeutically effective amount of an isolated polypeptide of Claim 1.

17. A method of treating cancer in a mammal comprising administering a therapeutically effective amount of an isolated polypeptide of Claim 1.

18. A method of inhibiting undesired angiogenesis in a mammal comprising administering a therapeutically effective amount of an antibody of Claim 5, 6, or 7.

19. A method of treating cancer in a mammal comprising administering a therapeutically effective amount of antibody according to Claim 5, 6, or 7.

20. A pharmaceutical composition comprising the isolated polypeptide of Claim 1 and a pharmaceutically acceptable formulation agent.

21. A pharmaceutical composition comprising the antibody of Claim 5, 6, or 7 and a pharmaceutically acceptable formulation agent.

22. A method of modulating or inhibiting angiopoietin-2 activity comprising administering to a patient the isolated polypeptide of Claim 1.

23. A method of modulating or inhibiting angiopoietin-2 activity comprising administering to a patient the antibody of Claim 5, 6, or 7.

24. A method of modulating at least one of vascular permeability or plasma leakage in a mammal comprising administering to a mammal a therapeutically effective amount of the isolated polypeptide of Claim 1.

25. A method of treating at least one of ocular neovascular disease, obesity, hemangioblastoma, hemangioma, arteriosclerosis, inflammatory disease, inflammatory disorders, atherosclerosis, endometriosis, neoplastic disease, bone-related disease, or psoriasis in a mammal comprising administering a therapeutically effective amount of an isolated polypeptide of Claim 1.

26. A method of modulating at least one of vascular permeability or plasma leakage in a mammal comprising administering a therapeutically effective amount of the antibody of Claim 5, 6, or 7.

27. A method of treating at least one of ocular neovascular disease, obesity, hemangioblastoma, hemangioma, arteriosclerosis, inflammatory disease, inflammatory disorders, atherosclerosis, endometriosis, neoplastic disease,

bone-related disease, or psoriasis in a mammal comprising administering a therapeutically effective amount of an antibody of Claim 5, 6, or 7.

28. A method of treating cancer in a mammal comprising administering a therapeutically effective amount of an isolated polypeptide of Claim 1 and a chemotherapeutic agent.

29. The method according to Claim 28 wherein the isolated polypeptide and chemotherapeutic agent are administered simultaneously.

30. The method according to Claim 28 wherein the isolated polypeptide and chemotherapeutic agent are not administered simultaneously.

31. A method of treating cancer in a mammal comprising administering a therapeutically effective amount of an antibody of Claim 5, 6, or 7 and a chemotherapeutic agent.

32. An isolated antibody that binds angiopoietin-2 comprising a CDR1 that comprises an amino acid sequence as shown in any of SEQ ID NO: 69-104 or SEQ ID NO: 213.

33. An isolated antibody that binds angiopoietin-2 comprising a CDR2 that comprises an amino acid sequence as shown in any of SEQ ID NO: 105-143 or SEQ ID NO: 214.

34. An isolated antibody that binds angiopoietin-2 comprising a CDR3 that comprises an amino acid sequence as shown in any of SEQ ID NO: 144-198, SEQ ID NO: 212, or SEQ ID NO: 215.

35. A nucleic acid molecule encoding the antibody of Claim 32, 33, or 34.

36. A vector comprising the nucleic acid molecule of Claim 35.

37. A host cell containing the vector of Claim 36.

38. A method of detecting the level of angiopoietin-2 in a biological sample comprising:

(a) contacting an isolated polypeptide of Claim 1 with said sample; and

(b) determining the extent of binding of the specific binding agent to said sample.

39. A method of detecting the level of angiopoietin-2 in a biological sample comprising:

(a) contacting an antibody of Claim 32, 33, or 34 with said sample; and

(b) determining the extent of binding of the antibody to said sample.

40. An antibody comprising a heavy chain and a light chain, said heavy chain comprising a heavy chain variable region selected from the group consisting of:

SEQ ID NO: 1; SEQ ID NO: 3; SEQ ID NO: 5; SEQ ID NO: 7; SEQ ID NO: 9; SEQ ID NO: 11; SEQ ID NO: 13; SEQ ID NO: 15; SEQ ID NO: 17; SEQ ID NO: 19; SEQ ID NO: 21; SEQ ID NO: 23; SEQ ID NO: 25; SEQ ID NO: 27; SEQ ID NO: 29; SEQ ID NO: 31; SEQ ID NO: 33; SEQ ID NO: 35; SEQ ID NO: 37; SEQ ID NO: 39; SEQ ID NO: 41; SEQ ID NO: 43; SEQ ID NO: 45; SEQ ID NO: 47; SEQ ID NO: 49; SEQ ID NO: 51; SEQ ID NO: 53; SEQ ID NO: 55; SEQ ID NO: 57; SEQ ID NO: 59; SEQ ID NO: 61; and antigen binding fragments thereof;

and said light chain comprising a light chain variable region comprising the amino acid sequence of SEQ ID NO: 12, SEQ ID NO: 210, or SEQ ID NO: 211, or an antigen binding fragment thereof.

41. A nucleic acid molecule encoding the antibody according to Claim 40.

42. A vector comprising the nucleic acid molecule of Claim 41.

43. A host cell containing the vector of Claim 42.

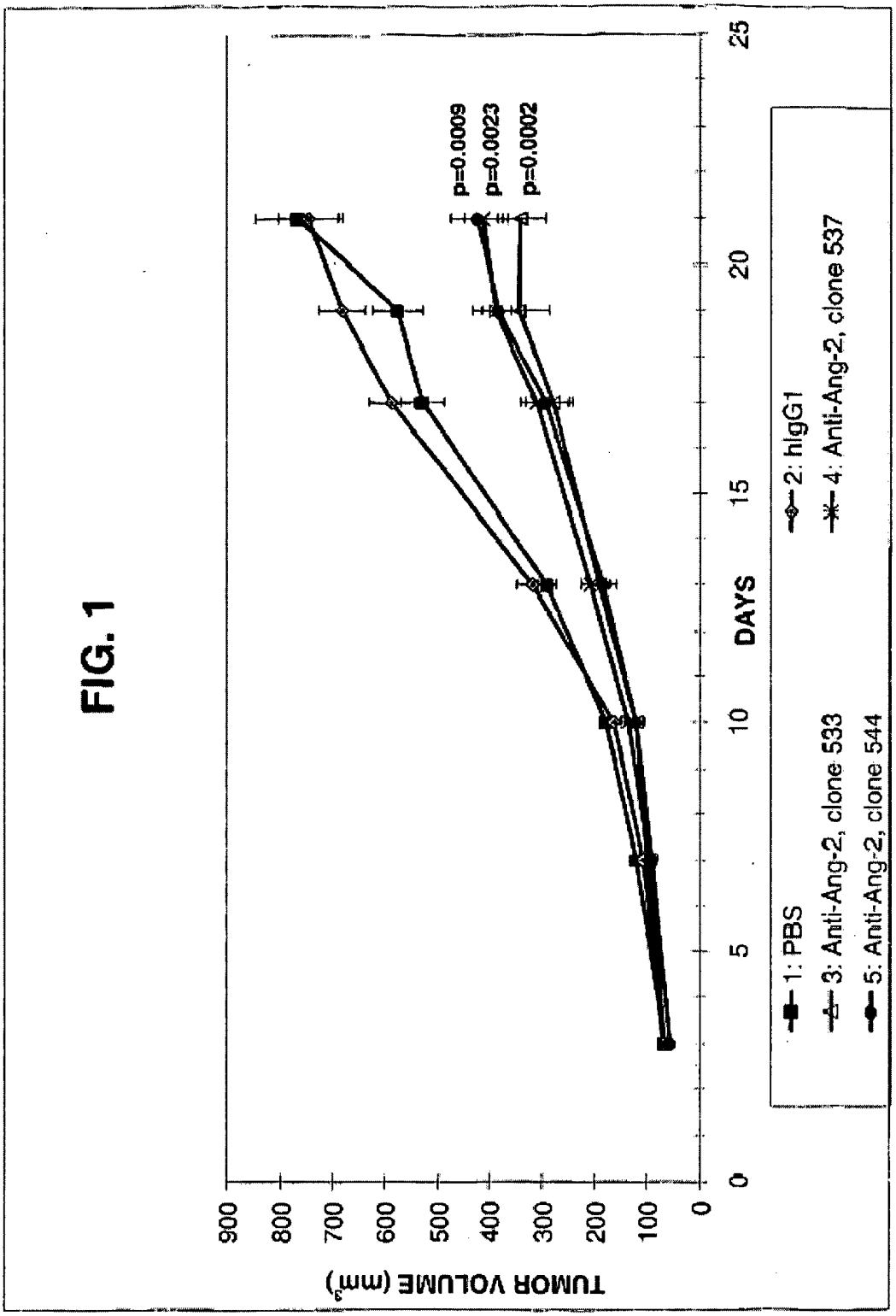
44. An antibody that specifically binds angiopoietin-2 comprising a heavy chain and a light chain, wherein the light chain comprises a light chain variable region comprising the amino acid sequence of SEQ ID NO: 12, SEQ ID NO: 210, or SEQ ID NO: 211, or an antigen binding fragment thereof.

45. A nucleic acid molecule encoding the antibody according to Claim 44.

46. A vector comprising the nucleic acid molecule of Claim 45.

47. A host cell containing the vector of Claim 46.

FIG. 1


TUMOR VOLUME (mm³)
900
800
700
600
500
400
300
200
100
0
0
5
10
15
20
25
DAYS
p=0.0009
p=0.0023
p=0.0002
1: PBS
2: hIgG1
3: Anti-Ang-2, clone 533
4: Anti-Ang-2, clone 537
5: Anti-Ang-2, clone 544

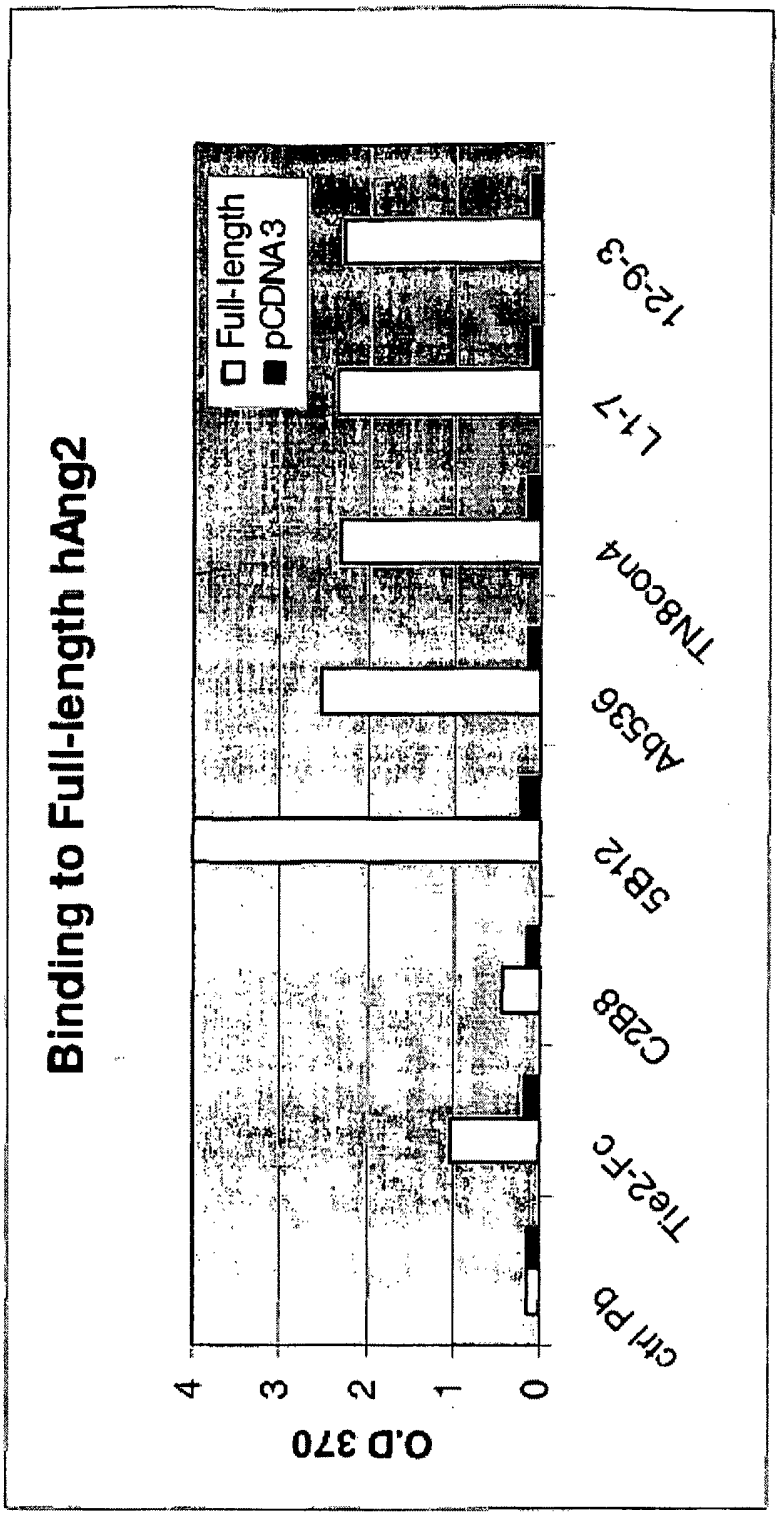


Figure 2a
Epitope Mapping

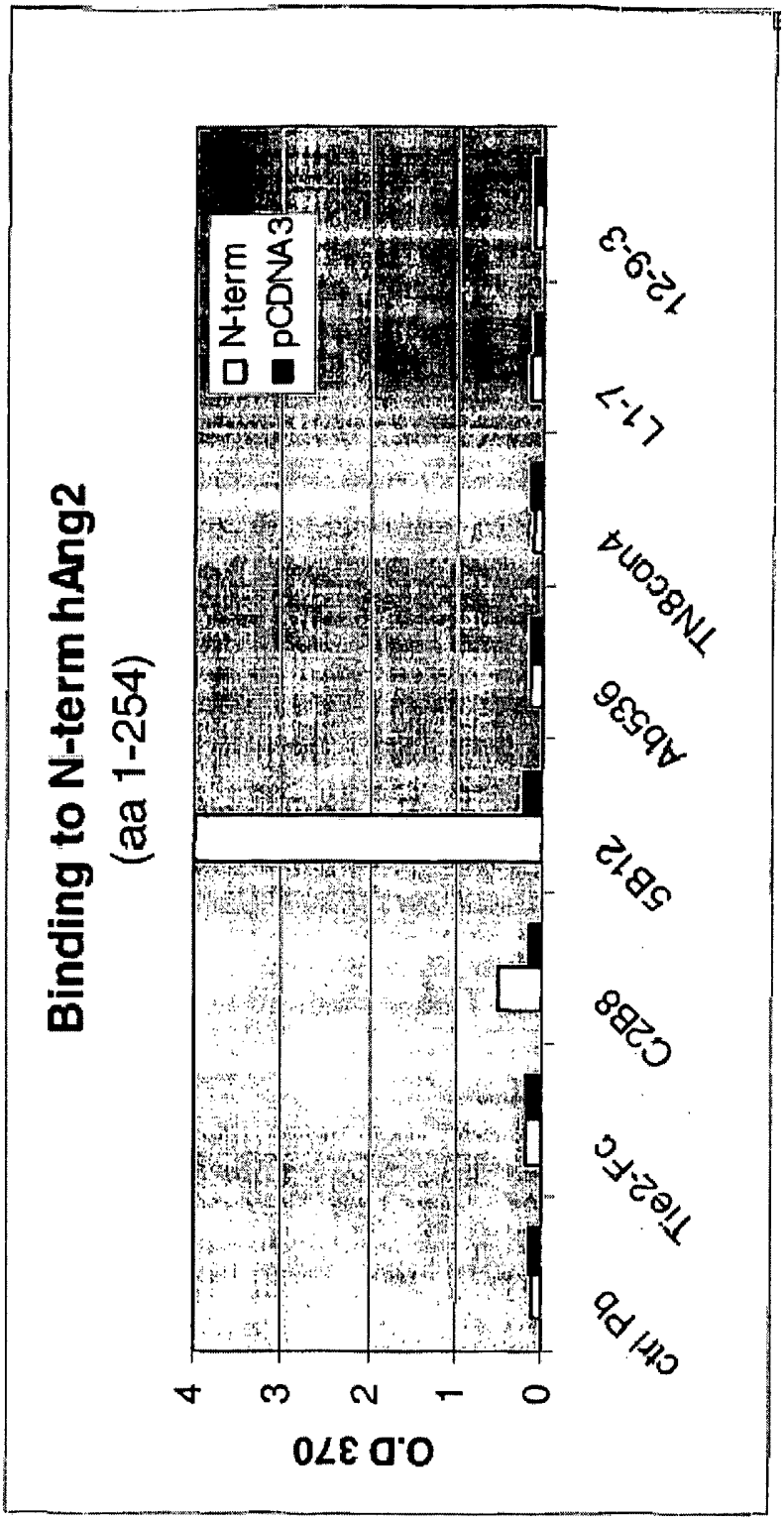


Figure 2b
Epitope Mapping

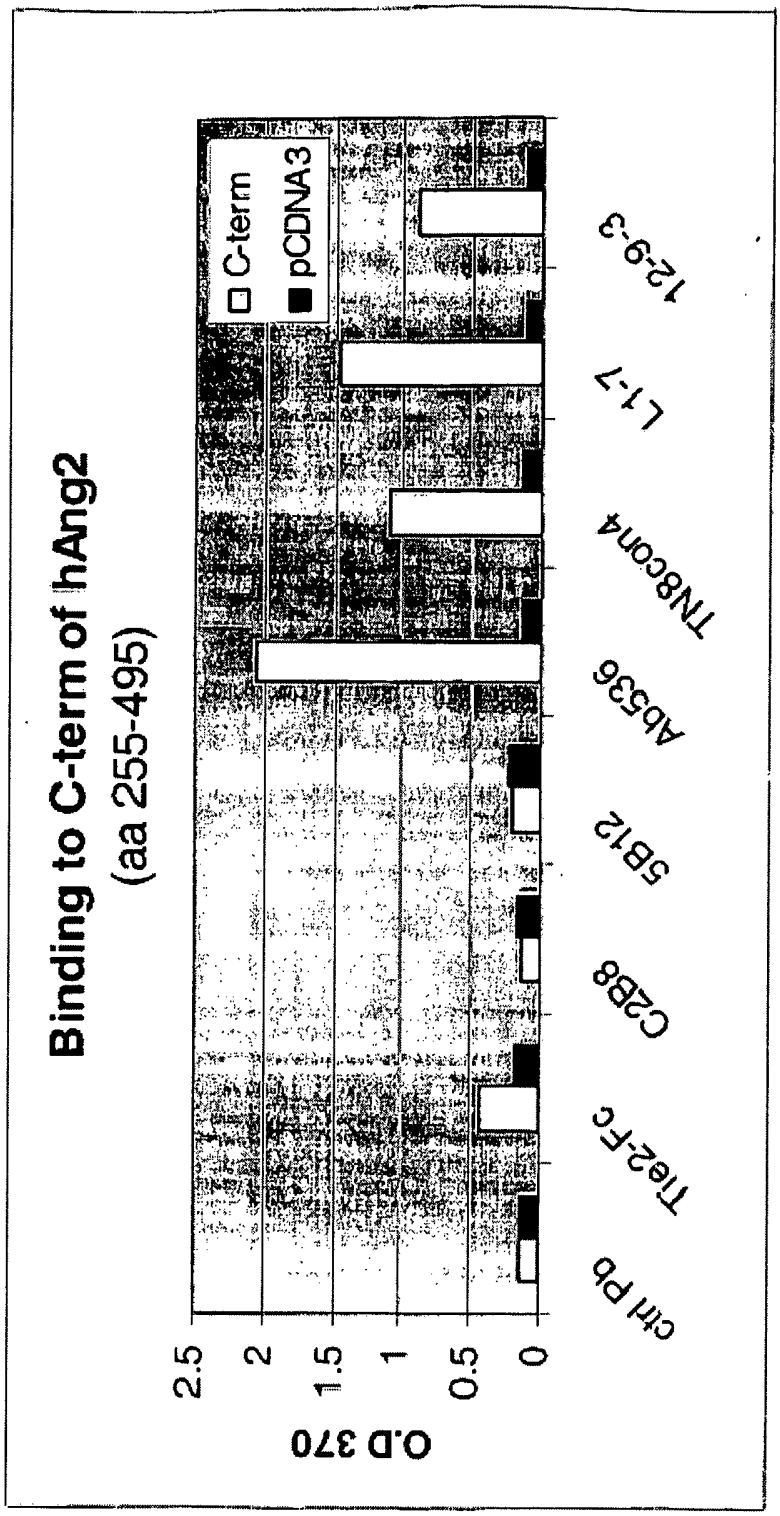


Figure 2c
Epitope Mapping

SEQUENCE LISTING

<110> Oliner, John
Graham, Kevin

<120> Angiopoietin-2 Specific Binding Agents

<130> 04-881-T

<140> TBD
<141> 2005-10-19

<150> 10/982,440
<151> 2004-11-04

<150> 60/620,161
<151> 2004-10-19

<160> 215

<170> PatentIn version 3.3

<210> 1
<211> 123
<212> PRT
<213> Homo sapiens

<400> 1

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asp Leu Leu Asp Tyr Asp Ile Leu Thr Gly Pro Tyr Ala Tyr
100 105 110

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 2
<211> 112
<212> PRT
<213> Homo sapiens

<400> 2

Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu His Ser
20 25 30

Asn Gly Tyr Asn Tyr Leu Asp Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Leu Gly Ser Asn Arg Ala Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln Ala
85 90 95

Leu Gln Thr Pro Pro Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110

<210> 3
<211> 123
<212> PRT
<213> Homo sapiens

<400> 3

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr
20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Glu Ser Thr Ser Thr Ala Tyr
65 70 75 80

```
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Gly Val Val Gly Asp Phe Asp Trp Leu Ser Phe Phe Asp Tyr
            100                 105                 110

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
        115                 120
```

<210> 4
<211> 107
<212> PRT
<213> Homo sapiens

<400> 4

```
Ser Tyr Glu Leu Thr Gln Pro Pro Ser Val Ser Val Ser Pro Gly Gln
1               5                   10                  15

Thr Ala Ser Ile Thr Cys Ser Gly Asp Lys Leu Gly Tyr Thr Tyr Thr
            20                  25                  30

Ser Trp Phe Gln Gln Lys Pro Gly Gln Ser Pro Val Leu Val Ile Phe
        35                  40                  45

Gln Asp Phe Lys Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
    50                  55                  60

Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Gly Thr Gln Ala Met
65                  70                  75                  80

Asp Glu Ala Asp Tyr Tyr Cys Gln Ala Trp Asp Ser Thr Thr Ala Val
                85                  90                  95

Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu
            100                 105
```

<210> 5
<211> 120
<212> PRT
<213> Homo sapiens

<400> 5

```
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1               5                   10                  15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr
            20                  25                  30
```

```
Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45

Gly Gly Ile Ile Pro Ile Leu Gly Ile Ala Asn Tyr Ala Gln Lys Phe
    50                  55                  60

Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Asn Thr Ala Tyr
65                  70                  75                  80

Met Glu Leu Thr Ser Leu Thr Ser Asp Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Asp Arg Glu Asp Thr Ala Met Val Phe Asn Tyr Trp Gly Gln
            100                 105                 110

Gly Thr Leu Val Thr Val Ser Ser
        115                 120
```

<210> 6
<211> 111
<212> PRT
<213> Homo sapiens

<400> 6

```
Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Ala Ala Pro Gly Gln
1               5                   10                  15

Lys Val Thr Val Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Asn Asn
            20                  25                  30

Tyr Val Ser Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
        35                  40                  45

Ile Tyr Asp Asn Asn Lys Arg Pro Ser Gly Ile Pro Asp Arg Phe Ser
    50                  55                  60

Gly Ser Lys Ser Gly Thr Ser Ala Thr Leu Gly Ile Thr Gly Leu Gln
65                  70                  75                  80

Thr Gly Asp Glu Ala Asp Tyr Tyr Cys Gly Thr Trp Asp Ser Ser Leu
                85                  90                  95

Ser Ala Phe Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
            100                 105                 110
```

<210> 7
<211> 122
<212> PRT
<213> Homo sapiens

<400> 7

Glu Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Tyr Ile Ser Ser Ser Gly Ser Thr Ile Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asp Leu Leu Asp Tyr Asp Ile Leu Thr Gly Tyr Gly Tyr Trp
100 105 110

Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 8
<211> 112
<212> PRT
<213> Homo sapiens

<400> 8

Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu His Ser
20 25 30

Asn Gly Tyr Asn Tyr Leu Asn Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Ile Leu Ile Tyr Leu Gly Ser Asn Arg Ala Ser Gly Val Pro
50 55 60

```
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65                  70                  75                  80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln Gly
                85                  90                  95

Leu Gln Thr Pro Pro Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
            100                 105                 110
```

<210> 9
<211> 121
<212> PRT
<213> Homo sapiens

<400> 9

```
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1               5                   10                  15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr
            20                  25                  30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45

Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe
    50                  55                  60

Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
65                  70                  75                  80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Ala Phe Ser Pro Phe Thr Glu Thr Asp Ala Phe Asp Ile Trp Gly
            100                 105                 110

Gln Gly Thr Met Val Thr Val Ser Ser
        115                 120
```

<210> 10
<211> 112
<212> PRT
<213> Homo sapiens

<400> 10

```
Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Ala Ala Pro Gly Gln
1               5                   10                  15
```

```
Lys Val Thr Ile Ser Cys Ser Gly Ser Asn Ser Asn Ile Gly Asn Asn
            20                  25                  30

Phe Val Ser Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
        35                  40                  45

Val Tyr Asp Asn Asn Lys Arg Pro Ser Gly Ile Pro Asp Arg Phe Ser
    50                  55                  60

Gly Ser Lys Ser Gly Thr Ser Ala Thr Leu Gly Ile Thr Gly Leu Gln
65                  70                  75                  80

Thr Gly Asp Glu Ala Asp Tyr Tyr Cys Gly Thr Trp Asp Ser Ser Leu
                85                  90                  95

Ser Ala Ala Glu Val Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
            100                 105                 110
```

<210> 11
<211> 122
<212> PRT
<213> Homo sapiens

<400> 11

```
Glu Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1               5                   10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
            20                  25                  30

Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Ser Tyr Ile Ser Ser Ser Gly Ser Thr Ile Tyr Tyr Ala Asp Ser Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Asp Leu Leu Asp Tyr Asp Ile Leu Thr Gly Tyr Gly Tyr Trp
            100                 105                 110
```

```
Gly Gln Gly Thr Leu Val Thr Val Ser Ser
        115                 120


<210>  12
<211>  112
<212>  PRT
<213>  Homo sapiens

<400>  12

Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
1               5                   10                  15


Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu His Ser
            20                  25                  30


Asn Gly Tyr Asn Tyr Leu Asp Trp Tyr Leu Gln Lys Pro Gly Gln Ser
        35                  40                  45


Pro Gln Leu Leu Ile Tyr Leu Gly Ser Asn Arg Ala Ser Gly Val Pro
    50                  55                  60


Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65                  70                  75                  80


Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln Gly
                85                  90                  95


Thr His Trp Pro Pro Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
            100                 105                 110


<210>  13
<211>  119
<212>  PRT
<213>  Homo sapiens

<400>  13

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1               5                   10                  15


Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr
            20                  25                  30


Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45


Gly Gly Ile Ile Pro Ile Leu Gly Ile Ala Asn Tyr Ala Gln Lys Phe
    50                  55                  60
```

```
Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
65                  70                  75                  80

Met Glu Leu Ser Gly Leu Gly Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Gly Ser Ser Asp Ala Ala Val Ala Gly Met Trp Gly Gln Gly
            100                 105                 110

Thr Leu Val Thr Val Ser Ser
        115
```

<210> 14
<211> 111
<212> PRT
<213> Homo sapiens

<400> 14

```
Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Ala Ala Pro Gly Gln
1               5                   10                  15

Asp Val Thr Ile Ser Cys Ser Gly Asn Asn Ser Asn Ile Gly Asn Asn
            20                  25                  30

Tyr Val Ser Trp Tyr Gln Gln Val Pro Gly Thr Ala Pro Lys Leu Leu
        35                  40                  45

Val Tyr Asp Asn His Lys Arg Pro Ser Gly Ile Ser Asp Arg Phe Ser
    50                  55                  60

Gly Ser Lys Ser Asp Thr Ser Ala Thr Leu Asp Ile Thr Gly Leu Gln
65                  70                  75                  80

Pro Gly Asp Glu Ala Asp Tyr Tyr Cys Gly Thr Trp Asp Thr Ser Leu
                85                  90                  95

Ser Ala Asn Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
            100                 105                 110
```

<210> 15
<211> 120
<212> PRT
<213> Homo sapiens

<400> 15

```
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1               5                   10                  15
```

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr
20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Gly Ile Ile Pro Ile Leu Gly Ile Ala Asn Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Phe Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Gly Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ala Val Pro Gly Thr Glu Asp Ala Phe Asp Ile Trp Gly Gln
100 105 110

Gly Thr Met Val Thr Val Ser Ser
115 120

<210> 16
<211> 111
<212> PRT
<213> Homo sapiens

<400> 16

Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Ala Ala Pro Gly Gln
1 5 10 15

Lys Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Ala Asn
20 25 30

Tyr Val Ser Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
35 40 45

Ile Tyr Asn Asn Asn Lys Arg Pro Ser Gly Ile Pro Asp Arg Phe Ser
50 55 60

Gly Ser Lys Ser Asp Thr Ser Ala Thr Leu Gly Ile Thr Gly Leu Gln
65 70 75 80

Thr Gly Asp Glu Ala Asp Tyr Tyr Cys Gly Ala Trp Asp Ser Ser Leu
85 90 95

Ser Ala Ser Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
100 105 110

<210> 17
<211> 123
<212> PRT
<213> Homo sapiens

<400> 17

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr
20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Arg Ile Ile Pro Ile Leu Gly Ile Ala Asn Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Pro Tyr Tyr Asp Phe Trp Ser Gly Pro Gly Gly Met Asp Val
100 105 110

Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
115 120

<210> 18
<211> 112
<212> PRT
<213> Homo sapiens

<400> 18

Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu His Ser
20 25 30

Asn Gly Tyr Asn Tyr Leu Asp Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

```
Pro Gln Leu Leu Ile Tyr Leu Gly Ser Asn Arg Ala Ser Gly Val Pro
    50                  55                  60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65                  70                  75                  80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln Ala
                85                  90                  95

Leu Gln Thr Pro Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
            100                 105                 110
```

<210> 19
<211> 121
<212> PRT
<213> Homo sapiens

<400> 19

```
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1               5                   10                  15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr
            20                  25                  30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45

Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe
    50                  55                  60

Gln Gly Arg Val Thr Ile Thr Ala Asp Glu Ser Thr Ser Thr Ala Tyr
65                  70                  75                  80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Phe Glu Ser Gly Tyr Trp Gly Asp Ala Phe Asp Ile Trp Gly
            100                 105                 110

Gln Gly Thr Met Val Thr Val Ser Ser
        115                 120
```

<210> 20
<211> 106
<212> PRT
<213> Homo sapiens

<400> 20

Ser Tyr Glu Leu Thr Gln Pro Pro Ser Val Ser Val Ser Pro Gly Gln
1 5 10 15

Thr Ala Arg Ile Thr Cys Ser Gly Asp Ala Leu Pro Lys Gln Tyr Ala
20 25 30

Tyr Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Ile Tyr
35 40 45

Lys Asp Ser Glu Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
50 55 60

Ser Ser Gly Thr Thr Val Thr Leu Thr Ile Ser Gly Val Gln Ala Glu
65 70 75 80

Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Ala Asp Ser Ser His Val Val
85 90 95

Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
100 105

<210> 21
<211> 123
<212> PRT
<213> Homo sapiens

<400> 21

Gln Val Gln Leu Gln Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Val Ile Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

```
Ala Lys Gly Pro Val Asp Phe Asp Tyr Gly Asp Tyr Ala Ile Asp Tyr
            100                 105                 110

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
        115                 120

<210>  22
<211>  111
<212>  PRT
<213>  Homo sapiens

<400>  22

Gln Ser Val Leu Thr Gln Pro Ser Ser Val Ser Gly Ala Pro Gly Gln
1               5                   10                  15

Arg Val Thr Ile Ser Cys Thr Gly Gln Ser Ser Asn Ile Gly Ala Gly
            20                  25                  30

Tyr Asp Val His Trp Tyr Gln Gln Phe Pro Gly Arg Ala Pro Lys Leu
        35                  40                  45

Leu Ile Tyr Gly Asn Ser Asn Arg Pro Ser Gly Val Pro Asp Arg Phe
    50                  55                  60

Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Leu
65                  70                  75                  80

Gln Pro Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Ser Arg
                85                  90                  95

Leu Ser Gly Ser Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
            100                 105                 110

<210>  23
<211>  123
<212>  PRT
<213>  Homo sapiens

<400>  23

Glu Val Gln Leu Val Asp Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                   10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
            20                  25                  30

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45
```

```
Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Lys Glu Thr Ile Ser Phe Ser Thr Phe Ser Gly Tyr Phe Asp Tyr
            100                 105                 110

Trp Ala Gln Gly Thr Leu Val Thr Val Ser Ser
        115                 120
```

<210> 24
<211> 110
<212> PRT
<213> Homo sapiens

<400> 24

```
Gln Ser Val Leu Thr Gln Pro Ser Ser Val Ser Glu Ala Pro Arg Gln
1               5                   10                  15

Arg Val Thr Ile Ser Cys Ser Gly Ser Ala Ser Asn Ile Gly Ala Asn
            20                  25                  30

Gly Val Ser Trp Tyr His Gln Val Pro Gly Lys Ala Pro Arg Leu Leu
        35                  40                  45

Leu Ser His Asp Gly Leu Val Thr Ser Gly Val Pro Asp Arg Leu Ser
    50                  55                  60

Val Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu His
65                  70                  75                  80

Ser Asp Asp Glu Gly Asp Tyr Tyr Cys Ala Val Trp Asp Asp Ser Leu
                85                  90                  95

Asn Ala Val Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
            100                 105                 110
```

<210> 25
<211> 124
<212> PRT
<213> Homo sapiens

<400> 25

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr
20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Glu Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Gly Tyr Asp Phe Trp Ser Gly Tyr Ser Leu Asp Ala Phe Asp
100 105 110

Ile Trp Gly Gln Gly Thr Met Val Thr Val Ser Ser
115 120

<210> 26
<211> 112
<212> PRT
<213> Homo sapiens

<400> 26

Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu His Ser
20 25 30

Asn Gly Tyr Asn Tyr Leu Asp Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Leu Gly Ser Asn Arg Ala Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln Ala
85 90 95

Leu Gln Thr Pro Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110

<210> 27
<211> 123
<212> PRT
<213> Homo sapiens

<400> 27

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
20 25 30

Ala Met His Trp Val Arg Gln Ala Pro Gly Gln Arg Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Ala Gly Asn Gly Asn Thr Lys Tyr Ser Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Ile Thr Arg Asp Thr Ser Ala Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Gly Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Gly Val Asp Asp Tyr Gly Gly Asn Ser Trp Ala Phe Asp Ile
100 105 110

Trp Gly Gln Gly Thr Met Val Thr Val Ser Ser
115 120

<210> 28
<211> 112
<212> PRT
<213> Homo sapiens

<400> 28

Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu His Ser
20 25 30

```
Asn Gly Tyr Asn Tyr Leu Asp Trp Tyr Leu Gln Lys Pro Gly Gln Ser
        35                  40                  45

Pro Gln Leu Leu Ile Tyr Leu Gly Ser Asn Arg Ala Ser Gly Val Pro
    50                  55                  60

Asp Arg Phe Thr Gly Ser Gly Ser Ala Thr Asp Phe Thr Leu Arg Ile
65                  70                  75                  80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln Ala
                85                  90                  95

Leu Gln Thr Pro Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
            100                 105                 110
```

<210> 29
<211> 128
<212> PRT
<213> Homo sapiens

<400> 29

```
Gln Val Gln Leu Gln Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1               5                   10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
            20                  25                  30

Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Ala Val Ile Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Ser Ala Ser Asp His Tyr Tyr Asp Ser Ser Gly Tyr Tyr Ser
            100                 105                 110

Asp Ala Phe Asp Ile Trp Gly Gln Gly Thr Met Val Thr Val Ser Ser
        115                 120                 125
```

<210> 30

<211> 112
<212> PRT
<213> Homo sapiens

<400> 30

Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu His Ser
20 25 30

Asn Gly Tyr Asn Tyr Leu Asp Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Leu Ala Ser Asn Arg Ala Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Arg Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln Thr
85 90 95

Leu Gln Ile Pro Ile Thr Phe Gly Pro Gly Thr Lys Val Asp Ile Lys
100 105 110

<210> 31
<211> 123
<212> PRT
<213> Homo sapiens

<400> 31

Gln Val Gln Leu Gln Gln Trp Gly Ala Gly Leu Leu Lys Pro Ser Glu
1 5 10 15

Thr Leu Ser Leu Thr Cys Ala Val Tyr Gly Gly Ser Phe Ser Gly Tyr
20 25 30

Tyr Trp Ser Trp Ile Arg Gln Ser Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45

Gly Glu Ile Asn His Ser Gly Ser Thr Asn Phe Asn Pro Ser Leu Lys
50 55 60

Ser Arg Ile Thr Ile Ser Val Asp Thr Ser Asn Asn Gln Phe Ser Leu
65 70 75 80

Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Ala Tyr Tyr Cys Ala
85 90 95

Arg Gly His Asp Trp Gly Met Gly Ile Gly Gly Ala Ala Tyr Asp Ile
100 105 110

Trp Gly Gln Gly Thr Met Val Thr Val Ser Ser
115 120

<210> 32
<211> 108
<212> PRT
<213> Homo sapiens

<400> 32

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Ser
20 25 30

Ser Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
35 40 45

Val Tyr Ala Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
50 55 60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65 70 75 80

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln His Tyr Gly Ser Ser Pro
85 90 95

Arg Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 33
<211> 122
<212> PRT
<213> Homo sapiens

<400> 33

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Val Ser Gly Tyr Thr Leu Thr Glu Ser
20 25 30

```
Ser Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
        35                  40                  45

Gly Gly Phe Asp Pro Glu His Gly Glu Thr Ile Tyr Ala Gln Lys Phe
    50                  55                  60

Gln Gly Arg Leu Thr Met Thr Glu Asp Thr Ser Thr Asp Thr Ala Tyr
65                  70                  75                  80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Phe Cys
                85                  90                  95

Ala Arg Gly Val Gln Val Thr Ser Gly Tyr His Tyr Phe Asp His Trp
            100                 105                 110

Gly Gln Gly Thr Leu Val Thr Val Ser Ser
        115                 120
```

<210> 34
<211> 110
<212> PRT
<213> Homo sapiens

<400> 34

```
Gln Ser Ala Leu Thr Gln Pro Pro Ser Ala Ser Gly Ser Pro Gly Gln
1               5                   10                  15

Ser Ile Thr Ile Ser Cys Thr Gly Thr Asn Ser Asp Ile Gly Ser Tyr
            20                  25                  30

Pro Phe Val Ser Trp Tyr Gln Arg His Pro Gly Lys Ala Pro Lys Leu
        35                  40                  45

Leu Ile Tyr Asp Val Ser Asn Arg Pro Ser Gly Val Ser Asp Arg Phe
    50                  55                  60

Ser Gly Ser Lys Ser Gly Asn Thr Ala Ser Leu Thr Ile Ser Gly Leu
65                  70                  75                  80

Gln Ala Glu Asp Glu Gly Asp Tyr Tyr Cys Ser Ser Phe Thr Met Asn
                85                  90                  95

Ser Phe Val Ile Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
            100                 105                 110
```

<210> 35

<211> 125
<212> PRT
<213> Homo sapiens

<400> 35

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr
20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Glu Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Pro Ile Tyr Tyr Asp Ile Leu Thr Gly Ile Asp Ala Phe
100 105 110

Asp Ile Trp Gly Gln Gly Thr Met Val Thr Val Ser Ser
115 120 125

<210> 36
<211> 108
<212> PRT
<213> Homo sapiens

<400> 36

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Ser
20 25 30

Ser Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
35 40 45

Val Tyr Ala Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
50 55 60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65 70 75 80

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln His Tyr Gly Ser Ser Pro
85 90 95

Arg Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 37
<211> 120
<212> PRT
<213> Homo sapiens

<400> 37

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr
20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Arg Ile Ile Pro Ile Leu Gly Ile Ala Asn Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asp Pro Ile Pro Ser Gly Trp Tyr Phe Asp Leu Trp Gly Arg
100 105 110

Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 38
<211> 110
<212> PRT
<213> Homo sapiens

<400> 38

Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Glu Ala Pro Arg Gln
1 5 10 15

```
Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Asn Asn
            20                  25                  30

Ala Val Asn Trp Tyr Gln Gln Leu Pro Gly Lys Ala Pro Lys Leu Leu
        35                  40                  45

Ile Tyr Tyr Asp Asp Leu Leu Pro Ser Gly Val Ser Asp Arg Phe Ser
    50                  55                  60

Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Arg
65                  70                  75                  80

Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Thr Trp Asp Asp Ser Leu
                85                  90                  95

Ser Gly Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
            100                 105                 110
```

<210> 39
<211> 122
<212> PRT
<213> Homo sapiens

<400> 39

```
Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Arg
1               5                   10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
            20                  25                  30

Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Ala Val Ile Trp Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Glu Val Gly Asn Tyr Tyr Asp Ser Ser Gly Tyr Gly Tyr Trp
            100                 105                 110

Gly Gln Gly Thr Leu Val Thr Val Ser Ser
```

115 120

<210> 40
<211> 110
<212> PRT
<213> Homo sapiens

<400> 40

Asn Phe Met Leu Thr Gln Pro His Ser Val Ser Glu Ser Pro Gly Lys
1 5 10 15

Thr Val Thr Ile Ser Cys Thr Arg Ser Gly Gly Gly Ile Gly Ser Ser
20 25 30

Phe Val His Trp Phe Gln Gln Arg Pro Gly Ser Ser Pro Thr Thr Val
35 40 45

Ile Phe Asp Asp Asn Gln Arg Pro Thr Gly Val Pro Asp Arg Phe Ser
50 55 60

Ala Ala Ile Asp Thr Ser Ser Ser Ser Ala Ser Leu Thr Ile Ser Gly
65 70 75 80

Leu Thr Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Ser His Ser
85 90 95

Thr Ala Val Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
100 105 110

<210> 41
<211> 122
<212> PRT
<213> Homo sapiens

<400> 41

Gln Val Gln Leu Gln Gln Ser Gly Pro Gly Leu Val Lys Pro Ser Gln
1 5 10 15

Thr Leu Ser Leu Thr Cys Ala Ile Ser Gly Asp Thr Val Ser Ser Asn
20 25 30

Ser Ala Ala Trp Asn Trp Ile Arg Gln Ser Pro Ser Arg Gly Leu Glu
35 40 45

Trp Leu Gly Arg Thr Tyr Tyr Arg Ser Lys Trp Tyr Ser Asp Tyr Ala
50 55 60

```
Val Ser Leu Arg Gly Arg Ile Thr Ile Asn Leu Asp Thr Asp Thr Ser
65                  70                  75                  80

Lys Asn Gln Phe Ser Leu Gln Leu Asn Ser Val Thr Pro Glu Asp Thr
                85                  90                  95

Ala Val Tyr Tyr Cys Ala Arg Asp Arg Gly Gly Tyr Ile Asp Ser Trp
            100                 105                 110

Gly Gln Gly Thr Leu Val Thr Val Ser Ser
        115                 120
```

<210> 42
<211> 110
<212> PRT
<213> Homo sapiens

<400> 42

```
Asn Phe Met Leu Thr Gln Pro His Ser Val Ser Glu Ser Pro Gly Lys
1               5                   10                  15

Thr Val Thr Ile Ser Cys Thr Arg Ser Ser Gly Ser Ile Ala Thr Asn
            20                  25                  30

Tyr Val Gln Trp Tyr Gln Gln Arg Pro Gly Ser Ser Pro Ala Thr Val
        35                  40                  45

Ile Tyr Glu Asp Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
    50                  55                  60

Gly Ser Ile Asp Thr Ser Ser Asn Ser Ala Ser Leu Thr Ile Ser Gly
65                  70                  75                  80

Leu Thr Thr Glu Asp Glu Ala Asp Tyr Phe Cys Gln Ser Tyr Gly Asp
                85                  90                  95

Asn Asn Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
            100                 105                 110
```

<210> 43
<211> 122
<212> PRT
<213> Homo sapiens

<400> 43

```
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Gly Gln Pro Gly Gly
1               5                   10                  15
```

Ser Leu Arg Leu Ser Cys Ala Ala Thr Gly Phe Ser Leu Asp Asp Tyr
20 25 30

Glu Met Asn Trp Val Arg Gln Ala Pro Gly Arg Gly Leu Glu Trp Val
35 40 45

Ser Tyr Ile Ile Gly Ser Gly Lys Thr Ile Phe Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Gly Lys Asn Ser Val Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Ile Tyr Tyr Cys
85 90 95

Ala Arg Gly Gly Gly Ser Ala Tyr Tyr Leu Asn Thr Ser Asp Ile Trp
100 105 110

Gly Gln Gly Thr Met Val Thr Val Ser Ser
115 120

<210> 44
<211> 112
<212> PRT
<213> Homo sapiens

<400> 44

Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu His Ser
20 25 30

Lys Gly Asp Asn Tyr Leu Asp Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Leu Gly Ser His Arg Ala Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln Ala
85 90 95

Leu Gln Thr Pro Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys

100 105 110

<210> 45
<211> 125
<212> PRT
<213> Homo sapiens

<400> 45

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
20 25 30

Gly Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Ser Ala Tyr Asn Gly Asn Thr Asn Tyr Ala Gln Lys Leu
50 55 60

Gln Gly Arg Val Thr Met Thr Thr Asp Thr Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Arg Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asp Arg Gly Ile Ala Ala Arg Ser Ala Tyr Tyr Tyr Gly Met
100 105 110

Asp Val Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
115 120 125

<210> 46
<211> 113
<212> PRT
<213> Homo sapiens

<400> 46

Asp Ile Val Met Thr Gln Thr Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu Asp Ser
20 25 30

Asp Asp Gly Lys Thr Tyr Leu Asp Trp Tyr Leu Gln Arg Pro Gly Gln
35 40 45

```
Ser Pro Gln Leu Leu Met Tyr Thr Thr Ser Ser Arg Ala Ser Gly Val
    50                  55                  60

Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys
65                  70                  75                  80

Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln
                85                  90                  95

Ala Thr Gln Phe Pro Tyr Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile
            100                 105                 110

Lys
```

<210> 47
<211> 120
<212> PRT
<213> Homo sapiens

<400> 47

```
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1               5                   10                  15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
            20                  25                  30

Asp Leu Asn Trp Val Arg Gln Ala Ser Gly Gln Gly Leu Glu Trp Met
        35                  40                  45

Gly Trp Met Asn Pro Thr Ser Gly Asn Thr Gly Tyr Ala Gln Lys Phe
    50                  55                  60

Gln Gly Arg Ile Thr Met Thr Arg Asn Thr Ser Ile Ser Thr Ala Tyr
65                  70                  75                  80

Met Glu Leu Arg Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Asp Pro Pro Ser Gly Gly Trp Glu Phe Asp Tyr Trp Gly Gln
            100                 105                 110

Gly Thr Leu Val Thr Val Ser Ser
        115                 120
```

<210> 48
<211> 112

<212> PRT
<213> Homo sapiens

<400> 48

Asp Ile Val Met Thr Gln Thr Pro Leu Ser Ser Thr Val Thr Leu Gly
1 5 10 15

Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Glu
20 25 30

Asp Gly Asn Thr Tyr Leu Asn Trp Leu His Gln Arg Pro Gly Gln Pro
35 40 45

Pro Arg Leu Leu Ile Tyr Lys Ile Ser Lys Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ala Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Pro Glu Asp Val Gly Val Tyr Tyr Cys Met Gln Ser
85 90 95

Thr Arg Phe Pro Arg Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 49
<211> 120
<212> PRT
<213> Homo sapiens

<400> 49

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser His
20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Arg Ile Ile Pro Ile Leu Gly Ile Ala Asn Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Glu Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys

85 90 95

Ala Thr Ser Arg Leu Glu Trp Leu Leu Tyr Leu Asp Tyr Trp Gly Gln
100 105 110

Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 50
<211> 110
<212> PRT
<213> Homo sapiens

<400> 50

Asn Phe Met Leu Thr Gln Pro His Ser Val Ser Glu Ser Pro Gly Lys
1 5 10 15

Thr Val Ile Ile Pro Cys Thr Arg Ser Ser Gly Ser Ile Ala Ser Asn
20 25 30

Tyr Val Gln Trp Tyr Gln Lys Arg Pro Gly Ser Ala Pro Ser Ile Val
35 40 45

Ile Tyr Glu Asp Lys Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
50 55 60

Gly Ser Ile Asp Ser Ser Ser Asn Ser Ala Ser Leu Thr Ile Ser Gly
65 70 75 80

Leu Lys Thr Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asn Ser
85 90 95

Arg Gly Val Met Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
100 105 110

<210> 51
<211> 123
<212> PRT
<213> Homo sapiens

<400> 51

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
20 25 30

```
Gly Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45

Gly Trp Ile Ser Ala Tyr Asn Gly Asn Thr Asn Tyr Ala Gln Lys Leu
    50                  55                  60

Gln Gly Arg Val Thr Met Thr Thr Asp Thr Ser Thr Ser Thr Ala Tyr
65                  70                  75                  80

Met Glu Val Arg Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Gly Gly Ser Pro Tyr Gly Gly Tyr Ala Tyr Pro Phe Asp Tyr
            100                 105                 110

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
        115                 120
```

<210> 52
<211> 110
<212> PRT
<213> Homo sapiens

<400> 52

```
Asn Phe Met Leu Thr Gln Pro His Ser Val Leu Glu Ser Ala Gly Lys
1               5                   10                  15

Thr Val Thr Ile Ser Cys Thr Arg Ser Ser Gly Ser Ile Ala Ser Asn
            20                  25                  30

Tyr Val Gln Trp Tyr Gln Gln Arg Pro Gly Thr Ser Pro Thr Asn Val
        35                  40                  45

Ile Phe Glu Asp Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
    50                  55                  60

Gly Ser Ile Asp Ser Ser Ser Asn Ser Ala Ser Leu Thr Ile Ser Gly
65                  70                  75                  80

Leu Lys Thr Glu Asp Glu Ala Asp Tyr Phe Cys Gln Ser Tyr Asp Ser
                85                  90                  95

Asn Ile Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
            100                 105                 110
```

<210> 53
<211> 122

<212> PRT
<213> Homo sapiens

<400> 53

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Tyr Ile Ser Ser Ser Gly Ser Thr Ile Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asp Leu Leu Asp Tyr Asp Ile Leu Thr Gly Tyr Gly Tyr Trp
100 105 110

Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 54
<211> 111
<212> PRT
<213> Homo sapiens

<400> 54

Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Ala Ala Pro Gly Gln
1 5 10 15

Lys Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Asn Asn
20 25 30

Tyr Val Ser Trp Tyr Gln His Leu Pro Gly Thr Ala Pro Lys Leu Leu
35 40 45

Ile Tyr Gly Asn Thr Asn Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
50 55 60

Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ala Gly Leu Gln

65 70 75 80

Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Ser Ser Leu
85 90 95

Ser Gly Ser Leu Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
100 105 110

<210> 55
<211> 123
<212> PRT
<213> Homo sapiens

<400> 55

Gln Val Gln Leu Gln Gln Trp Gly Ala Gly Leu Leu Lys Pro Ser Glu
1 5 10 15

Thr Leu Ser Leu Thr Cys Ala Val Tyr Gly Gly Ser Phe Ser Gly Tyr
20 25 30

Tyr Trp Ser Trp Ile Arg Gln Ser Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45

Gly Glu Ile Asn His Ser Gly Ser Thr Asn Phe Asn Pro Ser Leu Lys
50 55 60

Ser Arg Ile Thr Ile Ser Val Asp Thr Ser Asn Asn Gln Phe Ser Leu
65 70 75 80

Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala
85 90 95

Arg Gly His Asp Trp Gly Met Gly Ile Gly Gly Ala Ala Tyr Asp Ile
100 105 110

Trp Gly Gln Gly Thr Met Val Thr Val Ser Ser
115 120

<210> 56
<211> 108
<212> PRT
<213> Homo sapiens

<400> 56

Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

```
Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Ser
            20                  25                  30

Phe Leu Ala Trp Tyr Gln Gln Lys Ala Gly Gln Ala Pro Arg Leu Leu
        35                  40                  45

Ile Tyr Asp Thr Ser Thr Arg Ala Thr Gly Ile Ala Asp Arg Phe Ser
    50                  55                  60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65                  70                  75                  80

Ala Glu Asp Ser Ala Val Tyr Tyr Cys Gln Gln Tyr Asp Phe Ser Pro
                85                  90                  95

Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
            100                 105
```

<210> 57
<211> 112
<212> PRT
<213> Homo sapiens

<400> 57

```
Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                   10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Phe Ser Thr
            20                  25                  30

Tyr Ala Met Thr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp
        35                  40                  45

Val Ser Val Ile Arg Ser Asn Gly Gly Thr Asp Tyr Ala Asp Phe Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65                  70                  75                  80

Leu Gln Met Asn Gly Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Met Thr Asp Tyr Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
            100                 105                 110
```

<210> 58
<211> 110

<212> PRT
<213> Homo sapiens

<400> 58

Asn Phe Met Leu Thr Gln Pro His Ser Val Ser Glu Ser Pro Gly Lys
1 5 10 15

Thr Val Thr Ile Ser Cys Thr Gly Ser Gly Gly Ser Ile Ala Ser Asn
20 25 30

Tyr Val Gln Trp Tyr Gln Gln Arg Pro Gly Ser Ala Pro Thr Thr Val
35 40 45

Ile Tyr Glu Asp Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
50 55 60

Gly Ser Ile Asp Ser Ser Ser Asn Ser Ala Ser Leu Thr Ile Ser Gly
65 70 75 80

Leu Lys Thr Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Ser
85 90 95

Ser Thr Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
100 105 110

<210> 59
<211> 123
<212> PRT
<213> Homo sapiens

<400> 59

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys

85 90 95

Ala Lys Glu Thr Ile Ser Phe Ser Thr Phe Ser Gly Tyr Phe Asp Tyr
100 105 110

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 60
<211> 110
<212> PRT
<213> Homo sapiens

<400> 60

Gln Ser Ala Leu Thr Gln Pro Ala Ser Val Ser Gly Ser Pro Gly Gln
1 5 10 15

Ser Ile Thr Ile Ser Cys Thr Gly Thr Ser Ser Asp Val Gly Gly Tyr
20 25 30

Asn Tyr Val Ser Trp Phe Gln Gln His Pro Gly Lys Ala Pro Lys Leu
35 40 45

Met Ile Tyr Lys Val Asn Asn Arg Pro Ser Gly Leu Ser Asn Arg Phe
50 55 60

Ser Gly Ser Gln Ser Gly Asn Thr Ala Ser Leu Thr Ile Ser Gly Leu
65 70 75 80

Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Ser Ser Tyr Thr Ser Ser
85 90 95

Ser Thr Leu Gly Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
100 105 110

<210> 61
<211> 122
<212> PRT
<213> Homo sapiens

<400> 61

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
20 25 30

```
Asp Ile Asn Trp Val Arg Gln Ala Thr Gly Gln Gly Leu Glu Trp Met
        35                  40                  45

Gly Trp Met Asn Pro Asn Ser Gly Asn Thr Gly Tyr Ala Gln Lys Phe
    50                  55                  60

Gln Gly Arg Val Thr Met Thr Arg Asn Thr Ser Ile Ser Thr Ala Tyr
65                  70                  75                  80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Lys Glu Ile Ala Val Ala Gly Thr Arg Tyr Gly Met Asp Val Trp
            100                 105                 110

Gly Gln Gly Thr Thr Val Thr Val Ser Ser
        115                 120
```

<210> 62
<211> 107
<212> PRT
<213> Homo sapiens

<400> 62

```
Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1               5                   10                  15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Ile Ser Thr Phe
            20                  25                  30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
        35                  40                  45

Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Gly Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Asn Leu Glu Pro
65                  70                  75                  80

Glu Asp Phe Ala Val Tyr Tyr Cys Gln His Arg Ile Asn Trp Pro Leu
                85                  90                  95

Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
            100                 105
```

<210> 63
<211> 106

<212> PRT
<213> Homo sapiens

<400> 63

Gly Gln Pro Lys Ala Asn Pro Thr Val Thr Leu Phe Pro Pro Ser Ser
1 5 10 15

Glu Glu Leu Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp
20 25 30

Phe Tyr Pro Gly Ala Val Thr Val Ala Trp Lys Ala Asp Gly Ser Pro
35 40 45

Val Lys Ala Gly Val Glu Thr Thr Lys Pro Ser Lys Gln Ser Asn Asn
50 55 60

Lys Tyr Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys
65 70 75 80

Ser His Arg Ser Tyr Ser Cys Gln Val Thr His Glu Gly Ser Thr Val
85 90 95

Glu Lys Thr Val Ala Pro Thr Glu Cys Ser
100 105

<210> 64
<211> 106
<212> PRT
<213> Homo sapiens

<400> 64

Gly Gln Pro Lys Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser
1 5 10 15

Glu Glu Leu Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp
20 25 30

Phe Tyr Pro Gly Ala Val Thr Val Ala Trp Lys Ala Asp Ser Ser Pro
35 40 45

Val Lys Ala Gly Val Glu Thr Thr Thr Pro Ser Lys Gln Ser Asn Asn
50 55 60

Lys Tyr Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys
65 70 75 80

Ser His Arg Ser Tyr Ser Cys Gln Val Thr His Glu Gly Ser Thr Val

85 90 95

Glu Lys Thr Val Ala Pro Thr Glu Cys Ser
100 105

<210> 65
<211> 106
<212> PRT
<213> Homo sapiens

<400> 65

Gly Gln Pro Lys Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser
1 5 10 15

Glu Glu Leu Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp
20 25 30

Phe Tyr Pro Gly Ala Val Thr Val Ala Trp Lys Ala Asp Ser Ser Pro
35 40 45

Val Lys Ala Gly Val Glu Thr Thr Thr Pro Ser Lys Gln Ser Asn Asn
50 55 60

Lys Tyr Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys
65 70 75 80

Ser His Lys Ser Tyr Ser Cys Gln Val Thr His Glu Gly Ser Thr Val
85 90 95

Glu Lys Thr Val Ala Pro Thr Glu Cys Ser
100 105

<210> 66
<211> 106
<212> PRT
<213> Homo sapiens

<400> 66

Gly Gln Pro Lys Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser
1 5 10 15

Glu Glu Leu Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Val Ser Asp
20 25 30

Phe Tyr Pro Gly Ala Val Thr Val Ala Trp Lys Ala Asp Gly Ser Pro
35 40 45

```
Val Lys Val Gly Val Glu Thr Thr Lys Pro Ser Lys Gln Ser Asn Asn
    50                  55                  60

Lys Tyr Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys
65                  70                  75                  80

Ser His Arg Ser Tyr Ser Cys Arg Val Thr His Glu Gly Ser Thr Val
                85                  90                  95

Glu Lys Thr Val Ala Pro Ala Glu Cys Ser
            100                 105
```

<210> 67
<211> 107
<212> PRT
<213> Homo sapiens

<400> 67

```
Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
1               5                   10                  15

Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe
            20                  25                  30

Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
        35                  40                  45

Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
    50                  55                  60

Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
65                  70                  75                  80

Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser
                85                  90                  95

Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
            100                 105
```

<210> 68
<211> 330
<212> PRT
<213> Homo sapiens

<400> 68

```
Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
1               5                   10                  15
```

```
Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
            20                  25                  30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
        35                  40                  45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
    50                  55                  60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
65                  70                  75                  80

Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
                85                  90                  95

Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys
            100                 105                 110

Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro
        115                 120                 125

Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
    130                 135                 140

Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
145                 150                 155                 160

Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
                165                 170                 175

Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
            180                 185                 190

His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
        195                 200                 205

Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
    210                 215                 220

Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu
225                 230                 235                 240

Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
                245                 250                 255
```

```
Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
            260                 265                 270

Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
        275                 280                 285

Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
    290                 295                 300

Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
305                 310                 315                 320

Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
                325                 330
```

<210> 69
<211> 5
<212> PRT
<213> Homo sapiens

<400> 69

```
Ser Tyr Gly Met His
1               5
```

<210> 70
<211> 16
<212> PRT
<213> Homo sapiens

<400> 70

```
Arg Ser Ser Gln Ser Leu Leu His Ser Asn Gly Tyr Asn Tyr Leu Asp
1               5                   10                  15
```

<210> 71
<211> 5
<212> PRT
<213> Homo sapiens

<400> 71

```
Ser Tyr Ala Ile Ser
1               5
```

<210> 72
<211> 11
<212> PRT
<213> Homo sapiens

<400> 72

```
Ser Gly Asp Lys Leu Gly Tyr Thr Tyr Thr Ser
```

1 5 10

<210> 73
<211> 13
<212> PRT
<213> Homo sapiens

<400> 73

Ser Gly Ser Ser Ser Asn Ile Gly Asn Asn Tyr Val Ser
1 5 10

<210> 74
<211> 16
<212> PRT
<213> Homo sapiens

<400> 74

Arg Ser Ser Gln Ser Leu Leu His Ser Asn Gly Tyr Asn Tyr Leu Asn
1 5 10 15

<210> 75
<211> 13
<212> PRT
<213> Homo sapiens

<400> 75

Ser Gly Ser Asn Ser Asn Ile Gly Asn Asn Phe Val Ser
1 5 10

<210> 76
<211> 13
<212> PRT
<213> Homo sapiens

<400> 76

Ser Gly Asn Asn Ser Asn Ile Gly Asn Asn Tyr Val Ser
1 5 10

<210> 77
<211> 13
<212> PRT
<213> Homo sapiens

<400> 77

Ser Gly Ser Ser Ser Asn Ile Gly Ala Asn Tyr Val Ser
1 5 10

<210> 78
<211> 11
<212> PRT

<213> Homo sapiens

<400> 78

Ser Gly Asp Ala Leu Pro Lys Gln Tyr Ala Tyr
1 5 10

<210> 79
<211> 14
<212> PRT
<213> Homo sapiens

<400> 79

Thr Gly Gln Ser Ser Asn Ile Gly Ala Gly Tyr Asp Val His
1 5 10

<210> 80
<211> 5
<212> PRT
<213> Homo sapiens

<400> 80

Ser Tyr Ala Met Ser
1 5

<210> 81
<211> 13
<212> PRT
<213> Homo sapiens

<400> 81

Ser Gly Ser Ala Ser Asn Ile Gly Ala Asn Gly Val Ser
1 5 10

<210> 82
<211> 5
<212> PRT
<213> Homo sapiens

<400> 82

Ser Tyr Ala Met His
1 5

<210> 83
<211> 5
<212> PRT
<213> Homo sapiens

<400> 83

Gly Tyr Tyr Trp Ser
1 5

<210> 84
<211> 12
<212> PRT
<213> Homo sapiens

<400> 84

Arg Ala Ser Gln Ser Val Ser Ser Ser Ser Leu Ala
1 5 10

<210> 85
<211> 5
<212> PRT
<213> Homo sapiens

<400> 85

Glu Ser Ser Met His
1 5

<210> 86
<211> 14
<212> PRT
<213> Homo sapiens

<400> 86

Thr Gly Thr Asn Ser Asp Ile Gly Ser Tyr Pro Phe Val Ser
1 5 10

<210> 87
<211> 13
<212> PRT
<213> Homo sapiens

<400> 87

Ser Gly Ser Ser Ser Asn Ile Gly Asn Asn Ala Val Asn
1 5 10

<210> 88
<211> 13
<212> PRT
<213> Homo sapiens

<400> 88

Thr Arg Ser Gly Gly Gly Ile Gly Ser Ser Phe Val His
1 5 10

<210> 89
<211> 7
<212> PRT
<213> Homo sapiens

<400> 89

Ser Asn Ser Ala Ala Trp Asn
1 5

<210> 90
<211> 13
<212> PRT
<213> Homo sapiens

<400> 90

Thr Arg Ser Ser Gly Ser Ile Ala Thr Asn Tyr Val Gln
1 5 10

<210> 91
<211> 5
<212> PRT
<213> Homo sapiens

<400> 91

Asp Tyr Glu Met Asn
1 5

<210> 92
<211> 16
<212> PRT
<213> Homo sapiens

<400> 92

Arg Ser Ser Gln Ser Leu Leu His Ser Lys Gly Asp Asn Tyr Leu Asp
1 5 10 15

<210> 93
<211> 5
<212> PRT
<213> Homo sapiens

<400> 93

Ser Tyr Gly Ile Ser
1 5

<210> 94
<211> 17
<212> PRT
<213> Homo sapiens

<400> 94

Arg Ser Ser Gln Ser Leu Leu Asp Ser Asp Asp Gly Lys Thr Tyr Leu
1 5 10 15

Asp

<210> 95
<211> 5
<212> PRT
<213> Homo sapiens

<400> 95

Ser Tyr Asp Leu Asn
1 5

<210> 96
<211> 16
<212> PRT
<213> Homo sapiens

<400> 96

Arg Ser Ser Gln Ser Leu Val His Glu Asp Gly Asn Thr Tyr Leu Asn
1 5 10 15

<210> 97
<211> 5
<212> PRT
<213> Homo sapiens

<400> 97

Ser His Ala Ile Ser
1 5

<210> 98
<211> 13
<212> PRT
<213> Homo sapiens

<400> 98

Thr Arg Ser Ser Gly Ser Ile Ala Ser Asn Tyr Val Gln
1 5 10

<210> 99
<211> 12
<212> PRT
<213> Homo sapiens

<400> 99

Arg Ala Ser Gln Ser Val Ser Ser Ser Phe Leu Ala
1 5 10

<210> 100

<211> 6
<212> PRT
<213> Homo sapiens

<400> 100

Ser Thr Tyr Ala Met Thr
1 5

<210> 101
<211> 13
<212> PRT
<213> Homo sapiens

<400> 101

Thr Gly Ser Gly Gly Ser Ile Ala Ser Asn Tyr Val Gln
1 5 10

<210> 102
<211> 14
<212> PRT
<213> Homo sapiens

<400> 102

Thr Gly Thr Ser Ser Asp Val Gly Gly Tyr Asn Tyr Val Ser
1 5 10

<210> 103
<211> 5
<212> PRT
<213> Homo sapiens

<400> 103

Ser Tyr Asp Ile Asn
1 5

<210> 104
<211> 11
<212> PRT
<213> Homo sapiens

<400> 104

Arg Ala Ser Gln Ser Ile Ser Thr Phe Leu Ala
1 5 10

<210> 105
<211> 17
<212> PRT
<213> Homo sapiens

<400> 105

Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 106
<211> 7
<212> PRT
<213> Homo sapiens

<400> 106

Leu Gly Ser Asn Arg Ala Ser
1 5

<210> 107
<211> 17
<212> PRT
<213> Homo sapiens

<400> 107

Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe Gln
1 5 10 15

Gly

<210> 108
<211> 7
<212> PRT
<213> Homo sapiens

<400> 108

Gln Asp Phe Lys Arg Pro Ser
1 5

<210> 109
<211> 17
<212> PRT
<213> Homo sapiens

<400> 109

Gly Ile Ile Pro Ile Leu Gly Ile Ala Asn Tyr Ala Gln Lys Phe Gln
1 5 10 15

Gly

<210> 110

<211> 7
<212> PRT
<213> Homo sapiens

<400> 110

Asp Asn Asn Lys Arg Pro Ser
1 5

<210> 111
<211> 17
<212> PRT
<213> Homo sapiens

<400> 111

Tyr Ile Ser Ser Ser Gly Ser Thr Ile Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 112
<211> 7
<212> PRT
<213> Homo sapiens

<400> 112

Asp Asn His Lys Arg Pro Ser
1 5

<210> 113
<211> 7
<212> PRT
<213> Homo sapiens

<400> 113

Asn Asn Asn Lys Arg Pro Ser
1 5

<210> 114
<211> 17
<212> PRT
<213> Homo sapiens

<400> 114

Arg Ile Ile Pro Ile Leu Gly Ile Ala Asn Tyr Ala Gln Lys Phe Gln
1 5 10 15

Gly

<210> 115
<211> 7
<212> PRT
<213> Homo sapiens

<400> 115

Lys Asp Ser Glu Arg Pro Ser
1 5

<210> 116
<211> 17
<212> PRT
<213> Homo sapiens

<400> 116

Val Ile Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 117
<211> 7
<212> PRT
<213> Homo sapiens

<400> 117

Gly Asn Ser Asn Arg Pro Ser
1 5

<210> 118
<211> 7
<212> PRT
<213> Homo sapiens

<400> 118

His Asp Gly Leu Val Thr Ser
1 5

<210> 119
<211> 17
<212> PRT
<213> Homo sapiens

<400> 119

Trp Ile Asn Ala Gly Asn Gly Asn Thr Lys Tyr Ser Gln Lys Phe Gln
1 5 10 15

Gly

<210> 120
<211> 7
<212> PRT
<213> Homo sapiens

<400> 120

Leu Ala Ser Asn Arg Ala Ser
1 5

<210> 121
<211> 16
<212> PRT
<213> Homo sapiens

<400> 121

Glu Ile Asn His Ser Gly Ser Thr Asn Phe Asn Pro Ser Leu Lys Ser
1 5 10 15

<210> 122
<211> 7
<212> PRT
<213> Homo sapiens

<400> 122

Ala Ala Ser Ser Arg Ala Thr
1 5

<210> 123
<211> 17
<212> PRT
<213> Homo sapiens

<400> 123

Gly Phe Asp Pro Glu His Gly Glu Thr Ile Tyr Ala Gln Lys Phe Gln
1 5 10 15

Gly

<210> 124
<211> 7
<212> PRT
<213> Homo sapiens

<400> 124

Asp Val Ser Asn Arg Pro Ser
1 5

<210> 125
<211> 7
<212> PRT
<213> Homo sapiens

<400> 125

Leu Gly Ser Ser Arg Ala Ser
1 5

<210> 126
<211> 7
<212> PRT
<213> Homo sapiens

<400> 126

Tyr Asp Asp Leu Leu Pro Ser
1 5

<210> 127
<211> 17
<212> PRT
<213> Homo sapiens

<400> 127

Val Ile Trp Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 128
<211> 7
<212> PRT
<213> Homo sapiens

<400> 128

Asp Asp Asn Gln Arg Pro Thr
1 5

<210> 129
<211> 18
<212> PRT
<213> Homo sapiens

<400> 129

Arg Thr Tyr Tyr Arg Ser Lys Trp Tyr Ser Asp Tyr Ala Val Ser Leu
1 5 10 15

Arg Gly

<210> 130
<211> 7
<212> PRT
<213> Homo sapiens

<400> 130

Glu Asp Asn Gln Arg Pro Ser
1 5

<210> 131
<211> 17
<212> PRT
<213> Homo sapiens

<400> 131

Tyr Ile Ile Gly Ser Gly Lys Thr Ile Phe Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 132
<211> 7
<212> PRT
<213> Homo sapiens

<400> 132

Leu Gly Ser His Arg Ala Ser
1 5

<210> 133
<211> 17
<212> PRT
<213> Homo sapiens

<400> 133

Trp Ile Ser Ala Tyr Asn Gly Asn Thr Asn Tyr Ala Gln Lys Leu Gln
1 5 10 15

Gly

<210> 134
<211> 7
<212> PRT
<213> Homo sapiens

<400> 134

Thr Thr Ser Ser Arg Ala Ser
1 5

<210> 135
<211> 17
<212> PRT
<213> Homo sapiens

<400> 135

Trp Met Asn Pro Thr Ser Gly Asn Thr Gly Tyr Ala Gln Lys Phe Gln
1 5 10 15

Gly

<210> 136
<211> 7
<212> PRT
<213> Homo sapiens

<400> 136

Lys Ile Ser Lys Arg Phe Ser
1 5

<210> 137
<211> 7
<212> PRT
<213> Homo sapiens

<400> 137

Glu Asp Lys Gln Arg Pro Ser
1 5

<210> 138
<211> 7
<212> PRT
<213> Homo sapiens

<400> 138

Gly Asn Thr Asn Arg Pro Ser
1 5

<210> 139
<211> 7
<212> PRT
<213> Homo sapiens

<400> 139

Asp Thr Ser Thr Arg Ala Thr

1 5

<210> 140
<211> 16
<212> PRT
<213> Homo sapiens

<400> 140

Val Ile Arg Ser Asn Gly Gly Thr Asp Tyr Ala Asp Phe Val Lys Gly
1 5 10 15

<210> 141
<211> 7
<212> PRT
<213> Homo sapiens

<400> 141

Lys Val Asn Asn Arg Pro Ser
1 5

<210> 142
<211> 17
<212> PRT
<213> Homo sapiens

<400> 142

Trp Met Asn Pro Asn Ser Gly Asn Thr Gly Tyr Ala Gln Lys Phe Gln
1 5 10 15

Gly

<210> 143
<211> 7
<212> PRT
<213> Homo sapiens

<400> 143

Asp Ala Ser Asn Arg Ala Thr
1 5

<210> 144
<211> 14
<212> PRT
<213> Homo sapiens

<400> 144

Asp Leu Leu Asp Tyr Asp Ile Leu Thr Gly Pro Tyr Ala Tyr
1 5 10

<210> 145
<211> 9
<212> PRT
<213> Homo sapiens

<400> 145

Met Gln Ala Leu Gln Thr Pro Pro Thr
1 5

<210> 146
<211> 14
<212> PRT
<213> Homo sapiens

<400> 146

Gly Val Val Gly Asp Phe Asp Trp Leu Ser Phe Phe Asp Tyr
1 5 10

<210> 147
<211> 10
<212> PRT
<213> Homo sapiens

<400> 147

Gln Ala Trp Asp Ser Thr Thr Ala Val Val
1 5 10

<210> 148
<211> 11
<212> PRT
<213> Homo sapiens

<400> 148

Asp Arg Glu Asp Thr Ala Met Val Phe Asn Tyr
1 5 10

<210> 149
<211> 12
<212> PRT
<213> Homo sapiens

<400> 149

Gly Thr Trp Asp Ser Ser Leu Ser Ala Phe Trp Val
1 5 10

<210> 150
<211> 13
<212> PRT
<213> Homo sapiens

<400> 150

Asp Leu Leu Asp Tyr Asp Ile Leu Thr Gly Tyr Gly Tyr
1 5 10

<210> 151
<211> 9
<212> PRT
<213> Homo sapiens

<400> 151

Met Gln Gly Leu Gln Thr Pro Pro Thr
1 5

<210> 152
<211> 12
<212> PRT
<213> Homo sapiens

<400> 152

Phe Ser Pro Phe Thr Glu Thr Asp Ala Phe Asp Ile
1 5 10

<210> 153
<211> 13
<212> PRT
<213> Homo sapiens

<400> 153

Gly Thr Trp Asp Ser Ser Leu Ser Ala Ala Glu Val Val
1 5 10

<210> 154
<211> 9
<212> PRT
<213> Homo sapiens

<400> 154

Met Gln Gly Thr His Trp Pro Pro Thr
1 5

<210> 155
<211> 10
<212> PRT
<213> Homo sapiens

<400> 155

Gly Ser Ser Asp Ala Ala Val Ala Gly Met
1 5 10

<210> 156
<211> 12
<212> PRT
<213> Homo sapiens

<400> 156

Gly Thr Trp Asp Thr Ser Leu Ser Ala Asn Trp Val
1 5 10

<210> 157
<211> 11
<212> PRT
<213> Homo sapiens

<400> 157

Ala Val Pro Gly Thr Glu Asp Ala Phe Asp Ile
1 5 10

<210> 158
<211> 12
<212> PRT
<213> Homo sapiens

<400> 158

Gly Ala Trp Asp Ser Ser Leu Ser Ala Ser Trp Val
1 5 10

<210> 159
<211> 14
<212> PRT
<213> Homo sapiens

<400> 159

Pro Tyr Tyr Asp Phe Trp Ser Gly Pro Gly Gly Met Asp Val
1 5 10

<210> 160
<211> 9
<212> PRT
<213> Homo sapiens

<400> 160

Met Gln Ala Leu Gln Thr Pro Leu Thr
1 5

<210> 161
<211> 12
<212> PRT
<213> Homo sapiens

<400> 161

Phe Glu Ser Gly Tyr Trp Gly Asp Ala Phe Asp Ile
1 5 10

<210> 162
<211> 9
<212> PRT
<213> Homo sapiens

<400> 162

Gln Ser Ala Asp Ser Ser His Val Val
1 5

<210> 163
<211> 14
<212> PRT
<213> Homo sapiens

<400> 163

Gly Pro Val Asp Phe Asp Tyr Gly Asp Tyr Ala Ile Asp Tyr
1 5 10

<210> 164
<211> 11
<212> PRT
<213> Homo sapiens

<400> 164

Gln Ser Tyr Asp Ser Arg Leu Ser Gly Ser Val
1 5 10

<210> 165
<211> 14
<212> PRT
<213> Homo sapiens

<400> 165

Glu Thr Ile Ser Phe Ser Thr Phe Ser Gly Tyr Phe Asp Tyr
1 5 10

<210> 166
<211> 11
<212> PRT
<213> Homo sapiens

<400> 166

Ala Val Trp Asp Asp Ser Leu Asn Ala Val Val
1 5 10

<210> 167

<211> 15
<212> PRT
<213> Homo sapiens

<400> 167

Gly Tyr Asp Phe Trp Ser Gly Tyr Ser Leu Asp Ala Phe Asp Ile
1 5 10 15

<210> 168
<211> 14
<212> PRT
<213> Homo sapiens

<400> 168

Gly Val Asp Asp Tyr Gly Gly Asn Ser Trp Ala Phe Asp Ile
1 5 10

<210> 169
<211> 19
<212> PRT
<213> Homo sapiens

<400> 169

Ser Ala Ser Asp His Tyr Tyr Asp Ser Ser Gly Tyr Tyr Ser Asp Ala
1 5 10 15

Phe Asp Ile

<210> 170
<211> 9
<212> PRT
<213> Homo sapiens

<400> 170

Met Gln Thr Leu Gln Ile Pro Ile Thr
1 5

<210> 171
<211> 15
<212> PRT
<213> Homo sapiens

<400> 171

Gly His Asp Trp Gly Met Gly Ile Gly Gly Ala Ala Tyr Asp Ile
1 5 10 15

<210> 172
<211> 9
<212> PRT

<213> Homo sapiens

<400> 172

Gln His Tyr Gly Ser Ser Pro Arg Thr
1 5

<210> 173
<211> 13
<212> PRT
<213> Homo sapiens

<400> 173

Gly Val Gln Val Thr Ser Gly Tyr His Tyr Phe Asp His
1 5 10

<210> 174
<211> 10
<212> PRT
<213> Homo sapiens

<400> 174

Ser Ser Phe Thr Met Asn Ser Phe Val Ile
1 5 10

<210> 175
<211> 16
<212> PRT
<213> Homo sapiens

<400> 175

Ser Pro Ile Tyr Tyr Asp Ile Leu Thr Gly Ile Asp Ala Phe Asp Ile
1 5 10 15

<210> 176
<211> 9
<212> PRT
<213> Homo sapiens

<400> 176

Met Gln Ala Leu Asp Thr Pro Pro Thr
1 5

<210> 177
<211> 11
<212> PRT
<213> Homo sapiens

<400> 177

Asp Pro Ile Pro Ser Gly Trp Tyr Phe Asp Leu
1 5 10

<210> 178
<211> 11
<212> PRT
<213> Homo sapiens

<400> 178

Ala Thr Trp Asp Asp Ser Leu Ser Gly Trp Val
1 5 10

<210> 179
<211> 13
<212> PRT
<213> Homo sapiens

<400> 179

Glu Val Gly Asn Tyr Tyr Asp Ser Ser Gly Tyr Gly Tyr
1 5 10

<210> 180
<211> 9
<212> PRT
<213> Homo sapiens

<400> 180

Gln Ser Ser His Ser Thr Ala Val Val
1 5

<210> 181
<211> 8
<212> PRT
<213> Homo sapiens

<400> 181

Asp Arg Gly Gly Tyr Ile Asp Ser
1 5

<210> 182
<211> 9
<212> PRT
<213> Homo sapiens

<400> 182

Gln Ser Tyr Gly Asp Asn Asn Trp Val
1 5

<210> 183
<211> 13
<212> PRT
<213> Homo sapiens

<400> 183

Gly Gly Gly Ser Ala Tyr Tyr Leu Asn Thr Ser Asp Ile
1 5 10

<210> 184
<211> 16
<212> PRT
<213> Homo sapiens

<400> 184

Asp Arg Gly Ile Ala Ala Arg Ser Ala Tyr Tyr Tyr Gly Met Asp Val
1 5 10 15

<210> 185
<211> 9
<212> PRT
<213> Homo sapiens

<400> 185

Met Gln Ala Thr Gln Phe Pro Tyr Thr
1 5

<210> 186
<211> 11
<212> PRT
<213> Homo sapiens

<400> 186

Asp Pro Pro Ser Gly Gly Trp Glu Phe Asp Tyr
1 5 10

<210> 187
<211> 9
<212> PRT
<213> Homo sapiens

<400> 187

Met Gln Ser Thr Arg Phe Pro Arg Thr
1 5

<210> 188
<211> 11
<212> PRT
<213> Homo sapiens

<400> 188

Ser Arg Leu Glu Trp Leu Leu Tyr Leu Asp Tyr
1 5 10

<210> 189
<211> 9
<212> PRT
<213> Homo sapiens

<400> 189

Gln Ser Tyr Asn Ser Arg Gly Val Met
1 5

<210> 190
<211> 14
<212> PRT
<213> Homo sapiens

<400> 190

Gly Gly Ser Pro Tyr Gly Gly Tyr Ala Tyr Pro Phe Asp Tyr
1 5 10

<210> 191
<211> 9
<212> PRT
<213> Homo sapiens

<400> 191

Gln Ser Tyr Asp Ser Asn Ile Trp Val
1 5

<210> 192
<211> 12
<212> PRT
<213> Homo sapiens

<400> 192

Gln Ser Tyr Asp Ser Ser Leu Ser Gly Ser Leu Val
1 5 10

<210> 193
<211> 9
<212> PRT
<213> Homo sapiens

<400> 193

Gln Gln Tyr Asp Phe Ser Pro Leu Thr
1 5

<210> 194
<211> 3
<212> PRT
<213> Homo sapiens

<400> 194

Asp Tyr Tyr
1

<210> 195
<211> 9
<212> PRT
<213> Homo sapiens

<400> 195

Gln Ser Tyr Asp Ser Ser Thr Trp Val
1 5

<210> 196
<211> 10
<212> PRT
<213> Homo sapiens

<400> 196

Ser Ser Tyr Thr Ser Ser Ser Thr Leu Gly
1 5 10

<210> 197
<211> 13
<212> PRT
<213> Homo sapiens

<400> 197

Glu Ile Ala Val Ala Gly Thr Arg Tyr Gly Met Asp Val
1 5 10

<210> 198
<211> 9
<212> PRT
<213> Homo sapiens

<400> 198

Gln His Arg Ile Asn Trp Pro Leu Thr
1 5

<210> 199
<211> 17
<212> PRT
<213> Artificial

<220>
<223> CDR1 Region

<220>
<221> misc_feature
<222> (1)..(1)

<223> Xaa is R, S, T, G, E, or D.

<220>
<221> misc_feature
<222> (2)..(2)
<223> Xaa is S, G, A, R, N, T, Y, or H.

<220>
<221> misc_feature
<222> (3)..(3)
<223> Xaa is S, D, N, Q, T, Y, A, G, or E.

<220>
<221> misc_feature
<222> (4)..(4)
<223> Xaa is Q, K, S, N, A , G, I, M, W, or L.

<220>
<221> misc_feature
<222> (5)..(5)
<223> Xaa is S, L, G, A, M, H, or N.

<220>
<221> misc_feature
<222> (6)..(6)
<223> Xaa is L, G, N, P, V, D, W, S, T, I, or absent.

<220>
<221> misc_feature
<222> (7)..(7)
<223> Xaa is L, Y, I, K, S, N, V, or absent.

<220>
<221> misc_feature
<222> (8)..(8)
<223> Xaa is H, T, G, Q, S, A, D, or absent.

<220>
<221> misc_feature
<222> (9)..(9)
<223> Xaa is S, Y, N, A, T, E, G, F, or absent.

<220>
<221> misc_feature
<222> (10)..(10)
<223> Xaa is N, T, A, G, S, Y, K, D, F, L, or absent.

<220>
<221> misc_feature
<222> (11)..(11)
<223> Xaa is G, S, Y, F, L, P, A, F, D, N, or absent.

<220>
<221> misc_feature
<222> (12)..(12)
<223> Xaa is Y, V, D, A, F, G, N, or absent.

<220>
<221> misc_feature
<222> (13)..(13)

<223> Xaa is N, S, V, H, Q, K, T, or absent.

<220>
<221> misc_feature
<222> (14)..(14)
<223> Xaa is Y, H, S, T, or absent.

<220>
<221> misc_feature
<222> (15)..(15)
<223> Xaa is L, Y, or absent.

<220>
<221> misc_feature
<222> (16)..(16)
<223> Xaa is D, N, L, or absent

<220>
<221> misc_feature
<222> (17)..(17)
<223> Xaa is D, or absent.

<400> 199

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
1 5 10 15

Xaa

<210> 200
<211> 18
<212> PRT
<213> Artificial

<220>
<223> CDR2 Region

<220>
<221> misc_feature
<222> (1)..(1)
<223> Xaa is L, Q, D, N, K, G, H, A, Y, E, T, R, V, or W.

<220>
<221> misc_feature
<222> (2)..(2)
<223> Xaa is G, D, N, A, V, T, I, F, or M.

<220>
<221> misc_feature
<222> (3)..(3)
<223> Xaa is S, F, N, H, G, D, K, T, I, W, Y, or R.

<220>
<221> misc_feature
<222> (4)..(4)
<223> Xaa is N, K, E, L, S, Q, H, T, G, P, Y, or A.

<220>
<221> misc_feature
<222> (5)..(5)
<223> Xaa is R, V, L, S, I, D, G, E, Y, T, or N.

<220>
<221> misc_feature
<222> (6)..(6)
<223> Xaa is A, P, T, F, G, L, N, H, or S.

<220>
<221> misc_feature
<222> (7)..(7)
<223> Xaa is S, T, G, or K.

<220>
<221> misc_feature
<222> (8)..(8)
<223> Xaa is S, T, I, N, E, W, or absent.

<220>
<221> misc_feature
<222> (9)..(9)
<223> Xaa is T, A, I, K, N, Y, D, or absent.

<220>
<221> misc_feature
<222> (10)..(10)
<223> Xaa is Y, N, K, F, I, S, G, or absent.

<220>
<221> misc_feature
<222> (11)..(11)
<223> Xaa is Y, N, D, A, or absent.

<220>
<221> misc_feature
<222> (12)..(12)
<223> Xaa is A, S, P, Y, D, or absent.

<220>
<221> misc_feature
<222> (13)..(13)
<223> Xaa is D, Q, S, A, F, or absent.

<220>
<221> misc_feature
<222> (14)..(14)
<223> Xaa is S, K, L, V, or absent.

<220>
<221> misc_feature
<222> (15)..(15)
<223> Xaa is V, F, K, S, L, or absent.

<220>
<221> misc_feature
<222> (16)..(16)
<223> Xaa is K, Q, S, L, F, G, or absent

<220>
<221> misc_feature
<222> (17)..(17)
<223> Xaa is G, R, or absent.

<220>
<221> misc_feature
<222> (18)..(18)
<223> Xaa is G, or absent.

<400> 200

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
1 5 10 15

Xaa Xaa

<210> 201
<211> 19
<212> PRT
<213> Artificial

<220>
<223> CDR3 Region

<220>
<221> misc_feature
<222> (1)..(1)
<223> Xaa is D, M, G, Q, F, A, P, E, or S.

<220>
<221> misc_feature
<222> (2)..(2)
<223> Xaa is L, Q, V, A, R, T, S, Y, E, P, H, G, or I.

<220>
<221> misc_feature
<222> (3)..(3)
<223> Xaa is L, A, V, W, E, P, G, S, Y, I, D, T, Q, F, or R.

<220>
<221> misc_feature
<222> (4)..(4)
<223> Xaa is D, L, G, F, T, S, W, V, Y, P, N, H, I, or E.

<220>
<221> misc_feature
<222> (5)..(5)
<223> Xaa is Y, Q, D, S, T, H, A, F, W, G, M, R, or N.

<220>
<221> misc_feature
<222> (6)..(6)
<223> Xaa is D, T, F, A, S, E, W, R, G, Y, I, M, N, or L.

<220>
<221> misc_feature

<222> (7)..(7)
<223> Xaa is I, P, D, T, M, L, S, V, G, H, Y, W, A, N, or R.

<220>
<221> misc_feature
<222> (8)..(8)
<223> Xaa is

<220>
<221> misc_feature
<222> (9)..(9)
<223> Xaa is

<220>
<221> misc_feature
<222> (10)..(10)
<223> Xaa is

<220>
<221> misc_feature
<222> (11)..(11)
<223> Xaa is

<220>
<221> misc_feature
<222> (12)..(12)
<223> Xaa is

<220>
<221> misc_feature
<222> (13)..(13)
<223> Xaa is

<220>
<221> misc_feature
<222> (14)..(14)
<223> Xaa is

<220>
<221> misc_feature
<222> (15)..(15)
<223> Xaa is I, D, or absent.

<220>
<221> misc_feature
<222> (16)..(16)
<223> Xaa is A, I, V, or absent.

<220>
<221> misc_feature
<222> (17)..(17)
<223> Xaa is F, or absent.

<220>
<221> misc_feature
<222> (18)..(18)
<223> Xaa is D, or absent.

<220>
<221> misc_feature
<222> (19)..(19)

<223> Xaa is I, or absent.

<400> 201

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
1 5 10 15

Xaa Xaa Xaa

<210> 202
<211> 22
<212> PRT
<213> Homo sapiens

<400> 202

Met Asp Met Arg Val Pro Ala Gln Leu Leu Gly Leu Leu Leu Leu Trp
1 5 10 15

Leu Arg Gly Ala Arg Cys
20

<210> 203
<211> 40
<212> DNA
<213> Homo sapiens

<400> 203
gtggttgaga ggtgccagat gtcaggtcca gctggtgcag 40

<210> 204
<211> 30
<212> DNA
<213> Homo sapiens

<400> 204
attacgtctc acagttcgtt tgatctccac 30

<210> 205
<211> 48
<212> DNA
<213> Homo sapiens

<400> 205
ccgctcagct cctggggctc ctgctattgt ggttgagagg tgccagat 48

<210> 206
<211> 9
<212> PRT
<213> Homo sapiens

<400> 206

Ala Gln Leu Leu Gly Leu Leu Leu Leu
1 5

<210> 207
<211> 54
<212> DNA
<213> Homo sapiens

<400> 207
cagcagaagc ttctagacca ccatggacat gagggtcccc gctcagctcc tggg 54

<210> 208
<211> 45
<212> DNA
<213> Homo sapiens

<400> 208
gtggttgaga ggtgccagat gtgacattgt gatgactcag tctcc 45

<210> 209
<211> 31
<212> DNA
<213> Homo sapiens

<400> 209
cttgtcgact tattaacact ctcccctgtt g 31

<210> 210
<211> 112
<212> PRT
<213> Homo sapiens

<400> 210

Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu His Ser
20 25 30

Asn Gly Tyr Asn Tyr Leu Asp Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Leu Gly Ser Asn Arg Ala Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln Gly
85 90 95

```
Leu Gln Thr Pro Pro Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
            100                 105                 110


<210>  211
<211>  112
<212>  PRT
<213>  Homo sapiens

<400>  211

Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
1               5                   10                  15


Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu His Ser
            20                  25                  30


Asn Gly Tyr Asn Tyr Leu Asp Trp Tyr Leu Gln Lys Pro Gly Gln Ser
        35                  40                  45


Pro Gln Leu Leu Ile Phe Leu Gly Ser Ser Arg Ala Ser Gly Val Pro
    50                  55                  60


Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65                  70                  75                  80


Ser Lys Val Glu Ala Asp Asp Val Gly Ile Tyr Tyr Cys Met Gln Ala
                85                  90                  95


Leu Asp Thr Pro Pro Thr Phe Gly Pro Gly Thr Lys Val Glu Ile Lys
            100                 105                 110


<210>  212
<211>  9
<212>  PRT
<213>  Homo sapiens

<400>  212

Met Gln Gly Leu Gln Thr Pro Pro Thr
1               5


<210>  213
<211>  12
<212>  PRT
<213>  Homo sapiens

<400>  213

Arg Ala Ser Gln Ser Val Ser Ser Ser Ser Leu Ala
1               5                   10
```

<210> 214
<211> 7
<212> PRT
<213> Homo sapiens

<400> 214

Ala Ala Ser Ser Arg Ala Thr
1 5

<210> 215
<211> 9
<212> PRT
<213> Homo sapiens

<400> 215

Gln His Tyr Gly Ser Ser Pro Arg Thr
1 5

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2005/037911

A. CLASSIFICATION OF SUBJECT MATTER
C07K16/22 C12N15/13 C07K16/46 C12P21/08 C12N15/20
A61K39/385 A61K39/395 A61P35/00 A61P9/00 G01N33/68
G01N33/577

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 03/030883 A (KOSMOS PHARMA; FUISZ, RICHARD, C; YANG, ROBERT, K; MYERS, GARY, L) 17 April 2003 (2003-04-17) page 88, line 7 - line 15; claims 16,26-29,35; examples 4,5 page 7, line 21 - line 23 page 8, line 7 - line 9 page 11, line 24 - line 27	1-47
X	WO 00/57901 A (REGENERON PHARMACEUTICALS, INC; SURI, CHITRA; YANCOPOULOS, GEORGE, D;) 5 October 2000 (2000-10-05) page 11, line 26 - page 12, line 7; claims 6-10 page 12, paragraph 2 - paragraph 3	22-27

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 March 2006	06/04/2006

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Siaterli, M-Z

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2005/037911

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	OLINER J ET AL: "Suppression of angiogenesis and tumor growth by selective inhibition of angiopoietin-2" CANCER CELL, XX, US, vol. 6, no. 5, November 2004 (2004-11), pages 507-516, XP002348738 ISSN: 1535-6108 the whole document	1-47
A	US 2003/236193 A1 (OLINER JONATHAN DANIEL ET AL) 25 December 2003 (2003-12-25)	
A	CAI M ET AL: "Single chain Fv antibody against angiopoietin-2 inhibits VEGF-induced endothelial cell proliferation and migration in vitro" BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, ACADEMIC PRESS INC. ORLANDO, FL, US, vol. 309, no. 4, 3 October 2003 (2003-10-03), pages 946-951, XP004455833 ISSN: 0006-291X	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2005/037911

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 16-19, 22-31 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
[redacted]/US2005/037911

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 03030883 A	17-04-2003	CA 2473970 A1	17-04-2003
		EP 1458367 A1	22-09-2004
		JP 2005536443 T	02-12-2005
WO 0057901 A	05-10-2000	AT 241380 T	15-06-2003
		AU 769198 B2	22-01-2004
		AU 3763400 A	16-10-2000
		CA 2368670 A1	05-10-2000
		DE 60003011 D1	03-07-2003
		DE 60003011 T2	08-04-2004
		DK 1165115 T3	22-09-2003
		EP 1165115 A1	02-01-2002
		ES 2199804 T3	01-03-2004
		JP 2002540165 T	26-11-2002
		NO 20014673 A	22-11-2001
		NZ 514434 A	28-11-2003
		PT 1165115 T	31-10-2003
US 2003236193 A1	25-12-2003	AU 2002365179 A1	24-07-2003
		BR 0213223 A	26-04-2005
		CA 2462610 A1	17-07-2003
		CN 1596266 A	16-03-2005
		EP 1434791 A2	07-07-2004
		HU 0402162 A2	28-01-2005
		JP 2005514024 T	19-05-2005
		MX PA04003342 A	08-07-2004
		WO 03057134 A2	17-07-2003
		US 2003229023 A1	11-12-2003

[19] 中华人民共和国国家知识产权局

[12] 发明专利申请公布说明书

[21] 申请号 200580043569.5

[51] Int. Cl.
C07K 16/22 (2006.01)
C12N 15/13 (2006.01)
C07K 16/46 (2006.01)
C12P 21/08 (2006.01)
C12N 15/20 (2006.01)
A61K 39/385 (2006.01)

[43] 公开日 2009 年 7 月 29 日

[11] 公开号 CN 101495513A

[51] Int. Cl. (续)
A61K 39/395 (2006.01)
A61P 35/00 (2006.01)
A61P 9/00 (2006.01)
G01N 33/68 (2006.01)
G01N 33/577 (2006.01)

[22] 申请日 2005.10.19

[21] 申请号 200580043569.5

[30] 优先权

[32] 2004.10.19 [33] US [31] 60/620,161

[32] 2004.11. 4 [33] US [31] 10/982,440

[86] 国际申请 PCT/US2005/037911 2005.10.19

[87] 国际公布 WO2006/045049 英 2006.4.27

[85] 进入国家阶段日期 2007.6.18

[71] 申请人 安姆根有限公司

地址 美国加利福尼亚州

[72] 发明人 J·D·奥利纳 K·格雷汉

[74] 专利代理机构 中国专利代理(香港)有限公司

代理人 刘 冬 梁 谋

权利要求书 7 页 说明书 111 页 序列表 76 页
附图 4 页

[54] 发明名称

促血管生成素 -2 特异性结合剂

[57] 摘要

本发明公开了可结合于促血管生成素 -2 的特异性结合剂，例如完全人抗体。 也公开了这种抗体的重链片段、轻链片段和 CDR，以及这种抗体的制备和使用方法。

1. 一种特异性结合促血管生成素-2 的分离的多肽，其中所述多肽至少包含一种互补决定区（CDR），其中所述 CDR 为：

a) 含下式的氨基酸序列的 CDR1 区域：

$X^1X^2X^3X^4X^5X^6X^7X^8X^9X^{10}X^{11}X^{12}X^{13}X^{14}X^{15}X^{16}X^{17}$ (SEQ ID NO: 199)，其中

X^1 为 R、S、T、G、E、D;

X^2 为 S、G、A、R、N、T、Y、H;

X^3 为 S、D、N、Q、T、Y、A、G、E;

X^4 为 Q、K、S、N、A、G、I、M、W、L;

X^5 为 S、L、G、A、M、H、N;

X^6 为 L、G、N、P、V、D、W、S、T、I，或不存在;

X^7 为 L、Y、I、K、S、N、V，或不存在;

X^8 为 H、T、G、Q、S、A、D，或不存在;

X^9 为 S、Y、N、A、T、E、G、F，或不存在;

X^{10} 为 N、T、A、G、S、Y、K、D、F、L，或不存在;

X^{11} 为 G、S、Y、F、L、P、A、F、D、N，或不存在;

X^{12} 为 Y、V、D、A、F、G、N，或不存在;

X^{13} 为 N、S、V、H、Q、K、T，或不存在;

X^{14} 为 Y、H、S、T，或不存在;

X^{15} 为 L、Y，或不存在;

X^{16} 为 D、N、L，或不存在;

X^{17} 为 D，或不存在;

b) 含下式的氨基酸序列的 CDR2 区域：

$X^1X^2X^3X^4X^5X^6X^7X^8X^9X^{10}X^{11}X^{12}X^{13}X^{14}X^{15}X^{16}X^{17}X^{18}$ (SEQ ID NO: 200)，其中，

X^1 为 L、Q、D、N、K、G、H、A、Y、E、T、R、V、

W;

X^2 为 G、D、N、A、V、T、I、F、M;

X^3 为 S、F、N、H、G、D、K、T、I、W、Y、R;

X^4 为 N、K、E、L、S、Q、H、T、G、P、Y、A;

X^5 为 R、V、L、S、I、D、G、E、Y、T、N;

X^6 为 A、P、T、F、G、L、N、H、S;

X^7 为 S、T、G、K;

X^8 为 S、T、I、N、E、W，或不存在;

X^9 为 T、A、I、K、N、Y、D，或不存在;

X^{10} 为 Y、N、K、F、I、S、G，或不存在;

X^{11} 为 Y、N、D、A，或不存在;

X^{12} 为 A、S、P、Y、D，或不存在;

X^{13} 为 D、Q、S、A、F，或不存在;

X^{14} 为 S、K、L、V，或不存在;

X^{15} 为 V、F、K、S、L，或不存在;

X^{16} 为 K、Q、S、L、F、G，或不存在;

X^{17} 为 G、R，或不存在;

X^{18} 为 G，或不存在；或者

c) 含下式的氨基酸序列的 CDR3 区域:

$X^1X^2X^3X^4X^5X^6X^7X^8X^9X^{10}X^{11}X^{12}X^{13}X^{14}X^{15}X^{16}X^{17}X^{18}X^{19}$

(SEQ ID NO: 201)，其中，

X^1 为 D、M、G、Q、F、A、P、E、S;

X^2 为 L、Q、V、A、R、T、S、Y、E、P、H、G、I;

X^3 为 L、A、V、W、E、P、G、S、Y、I、D、T、Q、F、R;

X^4 为 D、L、G、F、T、S、W、V、Y、P、N、H、I、E;

X^5 为 Y、Q、D、S、T、H、A、F、W、G、M、R、N;

X^6 为 D、T、F、A、S、E、W、R、G、Y、I、M、N、L;

X^7 为 I、P、D、T、M、L、S、V、G、H、Y、W、A、N、R;

X^8 为 L、P、W、A、V、S、D、G、F、N、Y、I、R、E、T;

X^9 为 T、L、V、F、A、G、P、D、S、H、N、M、Y，或不存在;

X^{10} 为 G、S、V、N、F、A、M、D、Y、L、W、I、T，或不存在;

X^{11} 为 P、F、Y、W、D、E、I、G、A、V、L、S、M，或不存在;

X^{12} 为 Y、F、V、G、I、V、M、A、D，或不存在;

X^{13} 为 A、D、Y、V、F、H、I、G，或不存在;

X^{14} 为 Y、V、D、I、S、F、M，或不存在;

X^{15} 为 I、D，或不存在;

X^{16} 为 A、I、V，或不存在;

X^{17} 为 F，或不存在;

X^{18} 为 D，或不存在；和

X^{19} 为 I，或不存在。

2. 权利要求 1 的多肽，其中所述多肽至少含有一个选自 SEQ ID NO: 69-104 和 SEQ ID NO: 213 的氨基酸序列。

3. 权利要求 1 的多肽，其中所述多肽至少含有一个选自 SEQ ID NO: 105-143 和 SEQ ID NO: 214 的氨基酸序列。

4. 权利要求 1 的多肽，其中所述多肽至少含有一个选自 SEQ ID NO: 144-198、SEQ ID NO: 212 和 SEQ ID NO: 215 的氨基酸序列。

5. 权利要求 1 的分离多肽，其中所述多肽为抗体。

6. 权利要求 5 的抗体，其中所述抗体是多抗、单抗、嵌合抗体、

人源化抗体或完全人抗体。

7. 权利要求 6 的抗体，其中所述抗体是单链抗体。

8. 一种杂交瘤，所述杂交瘤产生权利要求 6 所述的单抗。

9. 一种核酸分子，所述核酸分子编码权利要求 1 的多肽。

10. 一种载体，所述载体含有权利要求 9 的核酸分子。

11. 一种宿主细胞，所述细胞含有权利要求 10 的载体。

12. 一种核酸分子，所述核酸分子编码权利要求 5、6 或 7 的抗体。

13. 一种载体，所述载体含有权利要求 12 的核酸分子。

14. 一种宿主细胞，所述细胞含有权利要求 13 的载体。

15. 一种制备抗体的方法，其中所述方法包括:

(a)使用至少一个编码权利要求 5、6 或 7 抗体的核酸分子来转化宿主细胞;

(b)在所述宿主细胞中表达核酸分子; 和

(c)分离所述特异性结合剂。

16. 一种抑制哺乳动物不希望有的血管生成的方法，所述方法包括给予治疗有效量的权利要求 1 的分离多肽。

17. 一种治疗哺乳动物癌症的方法，所述方法包括给予治疗有效量的权利要求 1 的分离多肽。

18. 一种抑制哺乳动物不希望有的血管生成的方法，所述方法包括给予治疗有效量的权利要求 5、6 或 7 的抗体。

19. 一种治疗哺乳动物癌症的方法，所述方法包括给予治疗有效量的权利要求 5、6 或 7 的抗体。

20. 一种药用组合物，所述组合物含有权利要求 1 的分离多肽和药学上可接受的赋形剂。

21. 一种药用组合物，所述组合物含有权利要求 5、6 或 7 的抗体和药学上可接受的赋形剂。

22. 一种调节或抑制促血管生成素-2 活性的方法，所述方法包

括给予患者权利要求 1 的分离多肽。

23. 一种调节或抑制促血管生成素-2 活性的方法，所述方法包括给予患者权利要求 5、6 或 7 的抗体。

24. 一种调节哺乳动物血管渗透性和/或血浆渗漏的方法，所述方法包括给予哺乳动物治疗有效量的权利要求 1 的分离多肽。

25. 一种治疗以下哺乳动物疾病中至少一种的方法：眼新生血管性疾病、肥胖、成血管细胞瘤、血管瘤、动脉硬化、炎性疾病、炎症、动脉粥样硬化、子宫内膜异位、肿瘤性疾病、骨相关疾病或牛皮癣，所述方法包括给予治疗有效量的权利要求 1 的分离多肽。

26. 一种调节哺乳动物血管渗透性和/或血浆渗漏的方法，所述方法包括给予治疗有效量的权利要求 5、6 或 7 的抗体。

27. 一种治疗以下哺乳动物疾病中至少一种的方法：眼新生血管性疾病、肥胖、成血管细胞瘤、血管瘤、动脉硬化、炎性疾病、炎症、动脉粥样硬化、子宫内膜异位、肿瘤性疾病、骨相关疾病或牛皮癣，所述方法包括给予治疗有效量的权利要求 5、6 或 7 的抗体。

28. 一种治疗哺乳动物癌症的方法，所述方法包括给予治疗有效量的权利要求 1 的分离多肽和化疗药物。

29. 权利要求 28 的方法，其中分离多肽和化疗药物被同时给予。

30. 权利要求 28 的方法，其中分离多肽和化疗药物并非被同时给予。

31. 一种治疗哺乳动物癌症的方法，所述方法包括给予治疗有效量的权利要求 5、6 或 7 的抗体和化疗药物。

32. 一种可结合促血管生成素-2 的分离抗体，所述抗体含有 CDR1，其中 CDR1 包含 SEQ ID NO: 69-104 或 SEQ ID NO: 213 中任一项所示的氨基酸序列。

33. 一种可结合促血管生成素-2 的分离抗体，所述抗体含有 CDR2，其中 CDR2 包含 SEQ ID NO: 105-143 或 SEQ ID NO: 214 中任一项所示的氨基酸序列。

34. 一种可结合促血管生成素-2 的分离抗体，所述抗体含有CDR3，其中 CDR3 包含 SEQ ID NO: 144-198、SEQ ID NO: 212 或 SEQ ID NO: 215 中任一项所示的氨基酸序列。

35. 一种核酸分子，所述核酸分子编码权利要求 32、33 或 34 的抗体。

36. 一种载体，所述载体含有权利要求 35 的核酸分子。

37. 一种宿主细胞，所述细胞含有权利要求 36 的载体。

38. 一种检测生物样品中促血管生成素-2 水平的方法，所述方法包括:

(a) 用所述样品接触权利要求 1 的分离多肽；和

(b) 确定特异性结合剂与所述样品的结合程度。

39. 一种检测生物样品中促血管生成素-2 水平的方法，所述方法包括:

(a) 用所述样品接触权利要求 32、33 或 34 的抗体；和

(b) 确定抗体与所述样品的结合程度。

40. 一种含重链和轻链的抗体，其中所述重链中含有的重链可变区选自以下序列:

SEQ ID NO: 1、SEQ ID NO: 3、SEQ ID NO: 5、SEQ ID NO: 7、SEQ ID NO: 9、SEQ ID NO: 11、SEQ ID NO: 13、SEQ ID NO: 15、SEQ ID NO: 17、SEQ ID NO: 19、SEQ ID NO: 21、SEQ ID NO: 23、SEQ ID NO: 25、SEQ ID NO: 27、SEQ ID NO: 29、SEQ ID NO: 31、SEQ ID NO: 33、SEQ ID NO: 35、SEQ ID NO: 37、SEQ ID NO: 39、SEQ ID NO: 41、SEQ ID NO: 43、SEQ ID NO: 45、SEQ ID NO: 47、SEQ ID NO: 49、SEQ ID NO: 51、SEQ ID NO: 53、SEQ ID NO: 55、SEQ ID NO: 57、SEQ ID NO: 59、SEQ ID NO: 61；及其抗原结合片段;

且所述轻链含有的轻链可变区含有 SEQ ID NO: 12、SEQ ID NO: 210 或 SEQ ID NO: 211 所示的氨基酸序列，或其抗原结合片段。

41. 一种核酸分子，所述核酸分子编码权利要求 40 的抗体。

42. 一种载体，所述载体含有权利要求 41 的核酸分子。

43. 一种宿主细胞，所述细胞含有权利要求 42 的载体。

44. 一种可特异性结合促血管生成素-2 的抗体，所述抗体含重链和轻链，其中所述轻链含有的轻链可变区包含 SEQ ID NO: 12、SEQ ID NO: 210 或 SEQ ID NO: 211 所示的氨基酸序列，或其抗原结合片段。

45. 一种核酸分子，所述核酸分子编码权利要求 44 的抗体。

46. 一种载体，所述载体含有权利要求 45 的核酸分子。

47. 一种宿主细胞，所述细胞含有权利要求 46 的载体。

促血管生成素-2 特异性结合剂

本文是美国专利申请 10/269,805(申请于 2002 年 10 月 10 日)的部分继续申请，也是 PCT 申请 PCT/US02/32613(申请于 2002 年 10 月 11 日)的部分继续申请，该 PCT 申请要求对美国临时专利申请 60/328,604(申请于 2001 年 10 月 11 日)享有优先权，全部专利均通过引用并入本文。本文也要求对美国临时专利申请 60/620,161(申请于 2004 年 10 月 19 日)享有优先权，该文通过引用并入本文。

发明领域

本发明涉及能识别并结合于促血管生成素-2(Ang-2)的特异性结合剂(binding agent)。更具体来说，本发明涉及能特异性结合于 Ang-2 的多抗、单抗及其片段的生产及诊断应用和治疗应用。

发明背景

血管生成，即从已有血管形成新血管，是许多生理学过程和病理学过程所必需的。通常，血管生成被促血管生成因子和抗血管生成因子所严格调控，但是在疾病状态下，例如癌症、眼内皮生长因子疾病、关节炎和牛皮癣，这个过程会发生偏差。(Folkman, *J., Nat. Med.*, 1:27-31 (1995))

已知许多疾病与血管生成失控和不希望有的血管生成有关。这些疾病包括但不限于眼新生血管疾病，如视网膜病 (包括糖尿病视网膜病)、老年黄斑变性、牛皮癣、成血管细胞瘤、血管瘤、动脉硬化，炎症如类风湿或风湿性炎症，尤其是关节炎(包括类风湿性关节炎)，

或其它慢性炎症如慢性哮喘，动脉硬化或移植后动脉硬化、子宫内膜异位，肿瘤如所谓的实体瘤和液体(或血液)瘤(如白血病和淋巴瘤)。其它与血管生成失控和不希望有的血管生成有关的疾病对本领域技术人员是显而易见的。

虽然血管生成调控涉及到许多信号转导系统，但其中鉴定最多最好的系统之一是内皮细胞选择系统，其包括 Tie-2 受体酪氨酸激酶(即 Tie-2 或 Tie-2R，或者 ORK；鼠 Tie-2 也被称为 tek)及其配体----促血管生成素(Gale, N. W. and Yancopoulos, G. D., *Genes Dev.* 13:1055-1066 (1999))。现在已知有 4 种促血管生成素：促血管生成素-1(Ang-1)到促血管生成素-4(Ang-4)。这些促血管生成素也被称为“Tie-2 配体”。(Davis, S., 等, *Cell*, *87*:1161-1169 (1996)；Grosios, K., 等, *Cytogenet Cell Genet*, *84*:118-120 (1999)；Holash, J., 等, *Investigative Ophthalmology & Visual Science*, *42*:1617-1625 (1999)；Koblizek, T. I., 等, *Current Biology*, *8*:529-532 (1998)；Lin, P., 等, *Proc Natl Acad Sci USA*, *95*:8829-8834 (1998)；Maisonpierre, P. C., 等, *Science*, *277*:55-60 (1997)；Papapetropoulos, A., 等, *Lab Invest*, *79*:213-223 (1999)；Sato, T. N., 等, *Nature*, *375*:70-74 (1998)；Shyu, K. G., 等, *Circulation*, *98*:2081-2087 (1998)；Suri, C., 等, *Cell*, *87*:1171-1180 (1996)；Suri, C., 等, *Science*, *282*:468-471 (1998)；Valenzuela, D. M., 等, *Proceedings of the National Academy of Sciences of the USA*, *96*:1904-1909 (1999)；Witzenbichler, B., 等, *J Biol Chem*, *273*:18514-18521 (1998))。尽管在培养的内皮细胞中 Ang-1 结合于 Tie-上会激发受体磷酸化作用，但已经观察到 Ang-2 既会激活和也会拮抗 Tie-2 受体磷酸化作用(Davis, S., 等, (1996), *supra*; Maisonpierre, P.C., 等, (1997), *supra*; Kim, I., J.H. Kim, 等, Oncogene **19**(39): 4549-4552 (2000); Teichert-Kuliszewska, K., P.C. Maisonpierre, 等, Cardiovascular Research **49**(3): 659-70 (2001))。

敲除掉 Tie-2 的小鼠和敲除掉 Ang-1 的小鼠表型相似，这提示由 Ang-1 激发的 Tie-2 磷酸化作用通过维持内皮细胞-支撑细胞粘着，

介导了胚胎血管的重塑和稳化。(Dumont, D. J., 等, *Genes & Development*, *8*:1897-1909 (1994); Sato, T. N., 等, *Nature in utero*, *376*:70-74 (1995); Suri, C., 等, (1996), *supra*)。Ang-1 在血管稳化中的作用被认为可以保持到成年，它被广泛并持续的表达(Hanahan, D., *Science*, *277*:48-50 (1997); Zagzag, D., 等, *Experimental Neurology*, *159*:391-400 (1999))。与之相比，Ang-2 的表达主要被限制在血管重塑的位点上，在这里 Ang-2 被认为阻遏了 Ang-1 的功能，从而诱导血管的可塑状态，有利于血管生成(Hanahan, D., (1997), *supra*; Holash, J., 等, *Science*, *284*:1994-1998 (1999); Maisonpierre, P. C., 等, (1997), *supra*)。

许多公开的研究证实了在与血管生成相关的疾病状态下的血管选择性的 Ang-2 表达。这些病理状况包括如牛皮癣、黄斑变性和癌症(Bunone, G., 等, *American Journal of Pathology*, *155*:1967-1976 (1999); Etoh, T., 等, *Cancer Research*, *61*:2145-2153 (2001); Hangai, M., 等, *Investigative Ophthalmology & Visual Science*, *42*:1617-1625 (2001); Holash, J., 等, (1999) *supra*; Kuroda, K., 等, *Journal of Investigative Dermatology*, *116*:713-720 (2001); Otani, A., 等, *Investigative Ophthalmology & Visual Science*, *40*:1912-1920 (1999); Stratmann, A., 等, *American Journal of Pathology*, *153*:1459-1466 (1998); Tanaka, S., 等, *J Clin Invest*, *103*:34-345 (1999); Yoshida, Y., 等, *International Journal of Oncology*, *15*:1221-1225 (1999); Yuan, K., 等, *Journal of Periodontal Research*, *35*:165-171 (2000); Zagzag, D., 等, (1999) *supra*)。这些研究中的大多数都把注意力集中在癌症上，许多种肿瘤都显示有血管 Ang-2 的表达。与其病理性血管生成中的表达相比，正常组织中 Ang-2 的表达受到极大的限制(Maisonpierre, P. C., 等, (1997), *supra*; Mezquita, J., 等, *Biochemical and Biophysical Research Communications*, *260*:492-498 (1999))。正常成人中的三个主要血管生成位置是卵巢、胎盘和子宫；这些位置都是可检测到 Ang-

2 mRNA 的正常(即非癌症组织)组织中的主要组织。

某些功能研究提示 Ang-2 可能涉及到肿瘤的血管生成。Ahmad 等人(*Cancer Res.*, 61:1255-1259 (2001))描述了异种移植小鼠模型中的 Ang-2 过量表达，并称它与肿瘤生长提高有关。也参见上文 Etoh 等人和 Tanaka 的报道，称 Ang-2 的过度表达与肿瘤血管增生相关。但是与之相反，Yu 等人(*Am. J. Path.*, 158:563-570 (2001))报道称在小鼠转染子中，Lewis 肺癌和乳腺癌细胞中 Ang-2 的过度表达延长了小鼠的寿命。

在过去的几年里，多种公开报道已经指出 Ang-1、Ang-2 和/或 Tie-2 可能是抗癌治疗的靶点。例如美国专利 No. 6,166,185, 5,650,490, 和 5,814,464 都公开了抗 Tie-2 配体抗体和受体抗体的概念。Lin 等人(*Proc. Natl. Acad. Sci USA*, 95:8829-8834 (1998))将表达可溶性 Tie-2 的腺病毒注射到小鼠体内；称可溶性 Tie-2 降低了小鼠肿瘤的数目和大小。在一项相关研究中，Lin 等人 (*J. Clin. Invest.*, 100:2072-2078 (1997))将可溶性 Tie-2 注射到大鼠体内；据称该化合物减小了大鼠体内的肿瘤大小。Siemeister 等人(*Cancer Res.*, 59:3185-3189 (1999))制得了表达 Tie-2 胞外结构域的人黑素瘤细胞系，将这种细胞注射到裸鼠体内，并称可溶性 Tie-2 引起了对肿瘤生长和肿瘤血管生成的“显著抑制”。考虑到这些信息，并且因为 Ang-1、Ang-2 都结合于 Tie-2，从这些研究中并不能清楚了解 Ang-1、Ang-2 或 Tie-2 是否可能是具有吸引力的抗癌治疗靶点。

将特定肽融合于稳定的胞质蛋白(如 Ig 恒定区)上以提高该分子的半衰期，这方面已经有多种方法，见如美国专利 5,480,981; Zheng 等, *J. Immunol.*, 154:5590-5600, (1995); Fisher 等, *N. Engl. J. Med.*, 334:1697-1702, (1996); Van Zee, K. 等, *J. Immunol.*, 156:2221-2230, (1996); 美国专利 5,808,029, 授权日：1998 年 9 月 15 日; Capon 等, *Nature*, 337:525-531, (1989); Harvill 等, *Immunotech.*, 1:95-105, (1995); WO 97/23614, 公开日： 1997 年 7 月 3 日; PCT/US 97/23183,

提交日： 1997 年 12 月 11 日； Linsley, *J. Exp. Med.*, 174:561-569, (1991); WO 95/21258, 公开日： 1995 年 8 月 10 日)。

有效的抗 Ang-2 治疗可能对大量的癌症患者都有利，因为大多数实体瘤都需要新血管生成以生长到大于 1-2mm 的直径。这种疗法可能在其它血管生成相关疾病也有广泛应用，例如视网膜病、关节炎和牛皮癣。

现在需要有可特异性识别并结合于 Ang-2 的新型药物来填补空白。这些药物可能在 Ang-2 活性相关疾病的诊断筛选和治疗中发挥作用。

因此本发明的目的是提供可特异性结合于 Ang-2 并调节 Ang-2 活性的 Ang-2 结合剂。

发明概述

本发明提供了包含重链和轻链的抗体，其中所述重链包含的重链可变区序列选自 526 HC (SEQ ID NO: 1); 528 HC (SEQ ID NO: 3); 531 HC (SEQ ID NO: 5); 533 HC (SEQ ID NO: 7); 535 HC (SEQ ID NO: 9); 536 HC (SEQ ID NO: 11); 537 HC (SEQ ID NO: 13); 540 HC (SEQ ID NO: 15); 543 HC (SEQ ID NO: 17); 544 HC (SEQ ID NO: 19); 545 HC (SEQ ID NO: 21); 546 HC (SEQ ID NO: 23); 551 HC (SEQ ID NO: 25); 553 HC (SEQ ID NO: 27); 555 HC (SEQ ID NO: 29); 558 HC (SEQ ID NO: 31); 559 HC (SEQ ID NO: 33); 565 HC (SEQ ID NO: 35); F1-C6 HC (SEQ ID NO: 37); FB1-A7 HC (SEQ ID NO: 39); FD-B2 HC (SEQ ID NO: 41); FE-B7 HC (SEQ ID NO: 43); FJ-G11 HC (SEQ ID NO: 45); FK-E3 HC (SEQ ID NO: 47); G1D4 HC (SEQ ID NO: 49); GC1E8 HC (SEQ ID NO: 51); H1C12 HC (SEQ ID NO: 53); IA1-1E7 HC (SEQ ID NO: 55); IF-1C10 HC (SEQ ID NO: 57); IK-2E2 HC (SEQ ID NO: 59); IP-2C11 HC (SEQ ID NO: 61)，以及这些序列的抗原结合片段。

所述轻链包含的轻链可变区选自 526 κ (SEQ ID NO: 2); 536 (THW) κ (SEQ ID NO: 12); 536 (LQT) κ (SEQ ID NO: 210); 543 κ (SEQ ID NO: 18); 544 κ (SEQ ID NO: 20); 551 κ (SEQ ID NO: 26); 553 κ (SEQ ID NO: 28); 555 κ (SEQ ID NO: 30); 558 κ (SEQ ID NO: 32); 565 κ (SEQ ID NO: 36); FE-B7 κ (SEQ ID NO: 44); FJ-G11 κ (SEQ ID NO: 46); FK-E3 κ (SEQ ID NO: 48); IA1-1E7 κ (SEQ ID NO: 56); IP-2C11 κ (SEQ ID NO: 62); 528 λ (SEQ ID NO: 4); 531 λ (SEQ ID NO: 6); 533 λ (SEQ ID NO: 8); 535 λ (SEQ ID NO: 10); 537 λ (SEQ ID NO: 14); 540 λ (SEQ ID NO: 16); 545 λ (SEQ ID NO: 22); 546 λ (SEQ ID NO: 24); 559 λ (SEQ ID NO: 34); F1-C6 λ (SEQ ID NO: 38); FB1-A7 λ (SEQ ID NO: 40); FD-B2 λ (SEQ ID NO: 42); G1D4 λ (SEQ ID NO: 50); GC1E8 λ (SEQ ID NO: 52); H1C12 λ (SEQ ID NO: 54); IF-1C10 λ (SEQ ID NO: 58); IK-2E2 λ (SEQ ID NO: 60)，以及这些序列的抗原结合片段。

本发明也提供了特异性结合剂，至少包含下列肽的其中之一: SEQ ID NO: 1; SEQ ID NO: 3; SEQ ID NO: 5; SEQ ID NO: 7; SEQ ID NO: 9; SEQ ID NO: 11; SEQ ID NO: 13; SEQ ID NO: 15; SEQ ID NO: 17; SEQ ID NO: 19; SEQ ID NO: 21; SEQ ID NO: 23; SEQ ID NO: 25; SEQ ID NO: 27; SEQ ID NO: 29; SEQ ID NO: 31; SEQ ID NO: 33; SEQ ID NO: 35; SEQ ID NO: 37; SEQ ID NO: 39; SEQ ID NO: 41; SEQ ID NO: 43; SEQ ID NO: 45; SEQ ID NO: 47; SEQ ID NO: 49; SEQ ID NO: 51; SEQ ID NO: 53; SEQ ID NO: 55; SEQ ID NO: 57; SEQ ID NO: 59; SEQ ID NO: 61; SEQ ID NO: 2; SEQ ID NO: 12; SEQ ID NO: 18; SEQ ID NO: 20; SEQ ID NO: 26; SEQ ID NO: 28; SEQ ID NO: 30; SEQ ID NO: 32; SEQ ID NO: 36; SEQ ID NO: 44; SEQ ID NO: 46; SEQ ID NO: 48; SEQ ID NO: 56; SEQ ID NO: 62; SEQ ID NO: 4; SEQ ID NO: 6; SEQ ID NO: 8; SEQ ID NO: 10; SEQ ID NO: 14; SEQ ID NO: 16; SEQ ID NO: 22; SEQ ID NO: 24; SEQ ID

NO: 34; SEQ ID NO: 38; SEQ ID NO: 40; SEQ ID NO: 42; SEQ ID NO: 50; SEQ ID NO: 52; SEQ ID NO: 54; SEQ ID NO: 58; 和 SEQ ID NO: 60，以及这些序列的片段。

应当认识到所述特异性结合剂可以是如抗体，如多抗、单抗、嵌合抗体、人源化抗体或者完全人类抗体。这种抗体也可以是单链抗体。本发明进一步涉及到产生本发明单抗的杂交瘤。

本发明还涉及到编码特异性结合剂(如抗体)的核酸分子，也涉及到含有这些核酸分子的载体和含有这些载体的宿主细胞。

另外，本发明也提供了制备特异性结合剂的方法，步骤包括(a)用至少一种编码权利要求 1 特异性结合剂的核酸分子转化宿主细胞; (b)在所述宿主细胞中表达该核酸分子; 和(c)分离所述特异性结合剂。本发明还提供了制备抗体的方法，步骤包括: (a)用至少一种编码本发明抗体的核酸分子转化宿主细胞; (b)在所述宿主细胞中表达该核酸分子; 和(c)分离所述特异性结合剂。

另外本发明也涉及到通过给予治疗有效剂量的本发明特异性结合剂，来抑制哺乳动物的不希望有的血管生成的方法。本发明也提供了通过给予治疗剂量的本发明特异性结合剂，来治疗哺乳动物癌症的方法。

本发明还涉及到通过给予治疗有效剂量的本发明抗体，来抑制哺乳动物的不希望有的血管生成的方法。本发明也提供了通过给予治疗剂量的本发明抗体，来治疗哺乳动物癌症的方法。

应当理解本发明也涉及到包含有本发明特异性结合剂和药学上可接受的赋形剂(formulation agent)的药用组合物。该药用组合物可包含本发明的抗体和药学上可接受的赋形剂。

本发明提供了通过给予一种或多种本发明所述特异性结合剂来调节或抑制促血管生成素-2 活性的方法。本发明也提供了通过给予本发明所述抗体来调节或抑制促血管生成素-2 的方法。

本发明也涉及在哺乳动物体内至少调节血管渗透性和血浆渗漏二

者之一的方法，步骤包括给予治疗有效量的本发明所述特异性结合剂。本发明也涉及到对以下哺乳动物疾病的至少一种进行治疗的方法：眼新生血管疾病、肥胖症、成血管细胞瘤、血管瘤、动脉硬化、炎性疾病、炎症、动脉粥样硬化、子宫内膜异位、肿瘤、骨相关病或牛皮癣，步骤包括给予治疗有效量的本发明所述特异性结合剂。

本发明也涉及到在哺乳动物体内至少调节血管渗透性和血浆渗漏二者之一的方法，步骤包括给予治疗有效量的本发明所述抗体。本发明也涉及到对以下哺乳动物疾病的至少一种进行治疗的方法：眼新生血管疾病、肥胖症、成血管细胞瘤、血管瘤、动脉硬化、炎性疾病、炎症、动脉粥样硬化、子宫内膜异位、肿瘤、骨相关病或牛皮癣，步骤包括给予治疗有效量的本发明所述抗体。

另外本发明也涉及到治疗哺乳动物癌症的方法，步骤包括给予治疗有效量的本发明所述特异性结合剂和化疗药物。本领域技术人员应当理解所述特异性结合剂和化疗药物并不需要同时给予。

本发明也涉及到治疗哺乳动物癌症的方法，步骤包括给予治疗有效量的本发明所述抗体和化疗药物。所述抗体和化疗药物并不需要同时给予。

本发明也提供包含以下序列中任一项的互补决定区 1(CDR1)的特异性结合剂：526 HC (SEQ ID NO: 1); 528 HC (SEQ ID NO: 3); 531 HC (SEQ ID NO: 5); 533 HC (SEQ ID NO: 7); 535 HC (SEQ ID NO: 9); 536 HC (SEQ ID NO: 11); 537 HC (SEQ ID NO: 13); 540 HC (SEQ ID NO: 15); 543 HC (SEQ ID NO: 17); 544 HC (SEQ ID NO: 19); 545 HC (SEQ ID NO: 21); 546 HC (SEQ ID NO: 23); 551 HC (SEQ ID NO: 25); 553 HC (SEQ ID NO: 27); 555 HC (SEQ ID NO: 29); 558 HC (SEQ ID NO: 31); 559 HC (SEQ ID NO: 33); 565 HC (SEQ ID NO: 35); F1-C6 HC (SEQ ID NO: 37); FB1-A7 HC (SEQ ID NO: 39); FD-B2 HC (SEQ ID NO: 41); FE-B7 HC (SEQ ID NO: 43); FJ-G11 HC (SEQ ID NO: 45); FK-E3 HC (SEQ ID NO: 47); G1D4 HC (SEQ ID NO: 49); GC1E8

HC (SEQ ID NO: 51); H1C12 HC (SEQ ID NO: 53); IA1-1E7 HC (SEQ ID NO: 55); IF-1C10 HC (SEQ ID NO: 57); IK-2E2 HC (SEQ ID NO: 59); IP-2C11 HC (SEQ ID NO: 61); 526 κ (SEQ ID NO: 2); 536 (THW) κ (SEQ ID NO: 12); 536 (LQT) κ (SEQ ID NO: 210); 543 κ (SEQ ID NO: 18); 544 κ (SEQ ID NO: 20); 551 κ (SEQ ID NO: 26); 553 κ (SEQ ID NO: 28); 555 κ (SEQ ID NO: 30); 558 κ (SEQ ID NO: 32); 565 κ (SEQ ID NO: 36); FE-B7 κ (SEQ ID NO: 44); FJ-G11 κ (SEQ ID NO: 46); FK-E3 κ (SEQ ID NO: 48); IA1-1E7 κ (SEQ ID NO: 56); IP-2C11 κ (SEQ ID NO: 62); 528 λ (SEQ ID NO: 4); 531 λ (SEQ ID NO: 6); 533 λ (SEQ ID NO: 8); 535 λ (SEQ ID NO: 10); 537 λ (SEQ ID NO: 14); 540 λ (SEQ ID NO: 16); 545 λ (SEQ ID NO: 22); 546 λ (SEQ ID NO: 24); 559 λ (SEQ ID NO: 34); F1-C6 λ (SEQ ID NO: 38); FB1-A7 λ (SEQ ID NO: 40); FD-B2 λ (SEQ ID NO: 42); G1D4 λ (SEQ ID NO: 50); GC1E8 λ (SEQ ID NO: 52); H1C12 λ (SEQ ID NO: 54); IF-1C10 λ (SEQ ID NO: 58); 和 IK-2E2 λ (SEQ ID NO: 60)。

本发明也提供包含以下序列中任一项的互补决定区 2(CDR2)的特异性结合剂: 526 HC (SEQ ID NO: 1); 528 HC (SEQ ID NO: 3); 531 HC (SEQ ID NO: 5); 533 HC (SEQ ID NO: 7); 535 HC (SEQ ID NO: 9); 536 HC (SEQ ID NO: 11); 537 HC (SEQ ID NO: 13); 540 HC (SEQ ID NO: 15); 543 HC (SEQ ID NO: 17); 544 HC (SEQ ID NO: 19); 545 HC (SEQ ID NO: 21); 546 HC (SEQ ID NO: 23); 551 HC (SEQ ID NO: 25); 553 HC (SEQ ID NO: 27); 555 HC (SEQ ID NO: 29); 558 HC (SEQ ID NO: 31); 559 HC (SEQ ID NO: 33); 565 HC (SEQ ID NO: 35); F1-C6 HC (SEQ ID NO: 37); FB1-A7 HC (SEQ ID NO: 39); FD-B2 HC (SEQ ID NO: 41); FE-B7 HC (SEQ ID NO: 43); FJ-G11 HC (SEQ ID NO: 45); FK-E3 HC (SEQ ID NO: 47); G1D4 HC (SEQ ID NO: 49); GC1E8 HC (SEQ ID NO: 51); H1C12 HC (SEQ ID NO: 53); IA1-1E7 HC (SEQ ID NO: 55); IF-1C10 HC (SEQ ID NO: 57); IK-2E2 HC (SEQ ID NO:

59); IP-2C11 HC (SEQ ID NO: 61); 526 κ (SEQ ID NO: 2); 536 (THW) κ (SEQ ID NO: 12); 536 (LQT) κ (SEQ ID NO: 210); 543 κ (SEQ ID NO: 18); 544 κ (SEQ ID NO: 20); 551 κ (SEQ ID NO: 26); 553 κ (SEQ ID NO: 28); 555 κ (SEQ ID NO: 30); 558 κ (SEQ ID NO: 32); 565 κ (SEQ ID NO: 36); FE-B7 κ (SEQ ID NO: 44); FJ-G11 κ (SEQ ID NO: 46); FK-E3 κ (SEQ ID NO: 48); IA1-1E7 κ (SEQ ID NO: 56); IP-2C11 κ (SEQ ID NO: 62); 528 λ (SEQ ID NO: 4); 531 λ (SEQ ID NO: 6); 533 λ (SEQ ID NO: 8); 535 λ (SEQ ID NO: 10); 537 λ (SEQ ID NO: 14); 540 λ (SEQ ID NO: 16); 545 λ (SEQ ID NO: 22); 546 λ (SEQ ID NO: 24); 559 λ (SEQ ID NO: 34); F1-C6 λ (SEQ ID NO: 38); FB1-A7 λ (SEQ ID NO: 40); FD-B2 λ (SEQ ID NO: 42); G1D4 λ (SEQ ID NO: 50); GC1E8 λ (SEQ ID NO: 52); H1C12 λ (SEQ ID NO: 54); IF-1C10 λ (SEQ ID NO: 58); 和 IK-2E2 λ (SEQ ID NO: 60)。

本发明也提供包含以下序列中任一项的互补决定区 3(CDR3)的特异性结合剂: 526 HC (SEQ ID NO: 1); 528 HC (SEQ ID NO: 3); 531 HC (SEQ ID NO: 5); 533 HC (SEQ ID NO: 7); 535 HC (SEQ ID NO: 9); 536 HC (SEQ ID NO: 11); 537 HC (SEQ ID NO: 13); 540 HC (SEQ ID NO: 15); 543 HC (SEQ ID NO: 17); 544 HC (SEQ ID NO: 19); 545 HC (SEQ ID NO: 21); 546 HC (SEQ ID NO: 23); 551 HC (SEQ ID NO: 25); 553 HC (SEQ ID NO: 27); 555 HC (SEQ ID NO: 29); 558 HC (SEQ ID NO: 31); 559 HC (SEQ ID NO: 33); 565 HC (SEQ ID NO: 35); F1-C6 HC (SEQ ID NO: 37); FB1-A7 HC (SEQ ID NO: 39); FD-B2 HC (SEQ ID NO: 41); FE-B7 HC (SEQ ID NO: 43); FJ-G11 HC (SEQ ID NO: 45); FK-E3 HC (SEQ ID NO: 47); G1D4 HC (SEQ ID NO: 49); GC1E8 HC (SEQ ID NO: 51); H1C12 HC (SEQ ID NO: 53); IA1-1E7 HC (SEQ ID NO: 55); IF-1C10 HC (SEQ ID NO: 57); IK-2E2 HC (SEQ ID NO: 59); IP-2C11 HC (SEQ ID NO: 61); 526 κ (SEQ ID NO: 2); 536 (THW) κ (SEQ ID NO: 12); 536 (LQT) κ (SEQ ID NO: 210) 543 κ (SEQ ID

NO: 18); 544 κ (SEQ ID NO: 20); 551 κ (SEQ ID NO: 26); 553 κ (SEQ ID NO: 28); 555 κ (SEQ ID NO: 30); 558 κ (SEQ ID NO: 32); 565 κ (SEQ ID NO: 36); FE-B7 κ (SEQ ID NO: 44); FJ-G11 κ (SEQ ID NO: 46); FK-E3 κ (SEQ ID NO: 48); IA1-1E7 κ (SEQ ID NO: 56); IP-2C11 κ (SEQ ID NO: 62); 528 λ (SEQ ID NO: 4); 531 λ (SEQ ID NO: 6); 533 λ (SEQ ID NO: 8); 535 λ (SEQ ID NO: 10); 537 λ (SEQ ID NO: 14); 540 λ (SEQ ID NO: 16); 545 λ (SEQ ID NO: 22); 546 λ (SEQ ID NO: 24); 559 λ (SEQ ID NO: 34); F1-C6 λ (SEQ ID NO: 38); FB1-A7 λ (SEQ ID NO: 40); FD-B2 λ (SEQ ID NO: 42); G1D4 λ (SEQ ID NO: 50); GC1E8 λ (SEQ ID NO: 52); H1C12 λ (SEQ ID NO: 54); IF-1C10 λ (SEQ ID NO: 58); 和 IK-2E2 λ (SEQ ID NO: 60)。

本发明也提供了编码本发明所述特异性结合剂的核酸分子。

而且，本发明也涉及到检测生物样本中促血管生成素-2 的方法，步骤包括(a)使本发明的特异性结合剂与生物样本相接触；和(b)确定该特异性结合剂与该样本的结合程度。本发明也涉及到检测生物样本中促血管生成素-2 的方法，步骤包括(a)用本发明的抗体与生物样本相接触；和(b)确定该抗体与该样本的结合程度。

本发明也涉及到抑制哺乳动物体内不希望有的血管生成的方法，步骤包括给予治疗有效量的本文所述多肽或组合物。本发明也涉及到调节哺乳动物体内血管生成的方法，步骤包括给予治疗有效量的本文所述多肽或组合物。本发明也涉及到抑制哺乳动物体内特征为不希望有的血管生成的肿瘤的方法，步骤包括给予治疗有效量的本文所述多肽或组合物。另外，本发明也涉及到治疗哺乳动物癌症的方法，步骤包括给予治疗有效量的本文所述多肽或组合物，以及化疗药物。在一个优选的实施方案中，化疗药物至少包括 5-Fu、CPT-11 和泰索帝(Taxotere)。当然也可使用其它合适的化疗药物和其它癌症疗法。

当然本发明的特异性结合剂可用来治疗多种与血管生成失调或与

不希望有的血管生成相关的疾病。这些疾病包括(但不仅限于)眼新生血管如视网膜病 (包括糖尿病视网膜病)，牛皮癣，成血管细胞瘤，血管瘤，动脉硬化，炎症如风湿症或风湿性炎症，尤其是关节炎(包括风湿性关节炎)，或其它慢性炎症如慢性哮喘，动脉硬化或移植后动脉硬化，子宫内膜异位，肿瘤如所谓的实体瘤和液体瘤(如白血病)。本领域技术人员将明了其它可通过给予本发明的特异性结合剂来进行治疗的疾病。这些疾病包括(但不仅限于)肥胖症、血管通透性疾病、血浆渗漏、骨相关病包括骨质疏松。因此本发明也涉及到治疗这些与血管生成失调或与不希望有的血管生成相关的疾病的方法。

通过本文的阐述将会很容易理解本发明的其它实施方案。

附图简述

图 1 为肿瘤大小(Y 轴)对时间(X 轴)作图，其中小鼠体内的肿瘤用本发明的抗 Ang-2 抗体(533、537 或 544 克隆)、对照抗体或磷酸盐缓冲液(PBS)进行处理。细节描述见实施例部分。

图 2A、2B 和 2C 为本发明肽体(peptibody)TN8-Con4-C、L1-7-N 和 12-9-3-C，以及对照肽体、Tie2-Fc、C2B8 或 5B12 分别与全长人 Ang-2 (hAng-2)、hAng-2 的 N-末端、hAng-2 的 C-末端结合的表位作图数据(O.D. 370)。详见实施例。

发明详述

本节中所用标题目的仅为组织行文，而不是对相关内容进行任何限定。

可使用标准方法来进行重组 DNA、重组蛋白、制备抗体、组织培养和细胞转化的操作。酶促反应和纯化方法主要根据产品使用说明书进行，或者根据本领域常规方法进行，例如文献 Sambrook 等 (分子克隆：实验指南，Molecular Cloning: A Laboratory Manual. Cold

Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1989))中所列的方法，或者根据本文中所列方法进行。如果没有特别指明，本文中的相关术语、分析化学实验方法和技术、有机合成和医药化学相关内容均为相关领域中熟知和常用内容。可使用标准方法来进行化学合成、化学分析、药物的制备、制剂和给予，以及治疗患者。

定义

如果没有特别指明，当以下概念在本文中使用时，含义均遵照下文所述:

“Ang-2” 指美国专利 No. 6,166,185 图 6 中所列多肽(“Tie-2 配体-2”)或者其片段，也指相关多肽包括等位基因变异体、接合变异体、衍生物、取代/删除和/或插入变异体、融合肽和多肽，以及中间同源体。根据制备方法不同，所述 Ang-2 多肽可以包括或不包括附加末端残基，如前导序列、导向序列、靶向序列、氨基末端蛋氨酸、氨基末端蛋氨酸和赖氨酸残基，和/或标签或融合蛋白序列。

当“生物活性”用来指 Ang-2 或 Ang-2 特异性结合剂时，意为具有至少一种 Ang-2 或 Ang-2 特异性结合剂特征活性的肽或多肽。当提到 Ang-2 的至少一种生物活性时，Ang-2 特异性结合剂可具有激动剂、拮抗剂、中和或阻遏活性。

“特异性结合剂”指一个分子，优选是蛋白分子，它结合于 Ang-2(以及本文定义的其变异体和衍生物)并且亲和力远高于对其它促血管生成素的亲和力。特异性结合剂可以是蛋白、肽、核酸、糖、脂类，或者是可优先结合于 Ang-2 的小分子化合物。在一个优选的实施方案中，本发明的特异性结合剂为抗体，例如单抗、多抗(mAb)、嵌合抗体、CDR-移植抗体、多特异性抗体、双特异性抗体、催化抗体、人源化抗体、人抗体、抗独特型抗体 (anti-Id)，和可以可溶形式或固定形式被标记的抗体，以及上述分子的片段、变异体或衍生

物，它们可以单独使用也可以与其它氨基酸序列联合使用，只要技术已知。这些技术包括(但不仅限于)酶裂解、化学裂解、肽合成或重组技术。本发明的抗 Ang-2 特异性结合剂能够结合于 Ang-2 上可调节(如抑制或促进)Ang-2 生物活性和/或其它 Ang-2 相关活性的部分。

“多抗”指异种抗体的混合物，它能识别并结合于同一抗原的不同表位上。可从天然血清制备物得到多抗，或者使用如抗原亲和层析或 A 蛋白/G 蛋白亲和层析的方法来纯化得到多抗。

“单抗”指由同一核酸分子编码的抗体的集合，该核酸任选由单杂交瘤或其它细胞系或转基因动物产生，这样每种单抗将典型识别抗原上的同一表位。这里“单抗”可得自多种不同方法和多种不同动物，如小鼠、大鼠等。

“嵌合抗体”指一个抗体中重链和/或轻链的一部分，与某一种动物来源抗体或某一种类(或亚种)抗体的相应序列相同或同源，而链的其余部分与另一种动物来源抗体或另一种类(或亚种)抗体的相应序列相同或同源。同时也包括这种抗体的片段，它具有所需的生物活性(即特异性结合 Ang-2 的能力)。参见美国专利 No. 4,816,567 和 Morrison 等, *Proc Natl Acad Sci (USA)*, 81:6851-6855 (1985)。

“CDR 抑制抗体”指某一种类或同型抗体的 CDR 被重组插入到另一相同或不同种类(或同型抗体)抗体的架构区中。

“多特异性抗体”指抗体的可变区可识别一种或多种抗原上的多个表位。这种抗体的一个亚种是“双特异性抗体”，它识别同一抗原或不同抗原上的两种不同表位。

“催化抗体”指一种具毒性或催化活性的抗体，其中在靶结合剂上连接一个或多个细胞毒素基团或者(更常见)一个或多个生物活性基团。

“人源化抗体”指一类特殊的 CDR 移植抗体，其中的架构区来源于人，但每个 CDR 都被其它来源 CDR 所替换，如鼠 CDR。“CDR”在下文定义。

“完全人类”抗体指一个抗体的 CDR 和架构区都来自一种或多种人 DNA 分子。

“抗独特型抗体”指任何能够结合于其它可识别抗原的抗体的抗体。可使用本文所述的任何 Ang-2 特异性结合抗体制备方法来制备抗独特型抗体，以下情况除外：通过使用 Ang-2 特异性抗体或其 Ang-2 结合片段免疫动物(而不是使用 Ang-2 多肽本身或其片段)来得到的这些抗体。

本文所用的“变异体”包括在天然存在(或至少在已知)氨基酸序列上发生残基插入、删除和/或替换的结合剂多肽。本发明的变异体包括下文描述的融合蛋白。

“衍生物”包括那些进行了化学修饰的结合剂，这些修饰方法与插入、删除或替换的变异体不同。

“特异性结合于 Ang-2”指本发明的特异性结合剂(例如抗体或其片段)识别并结合于成熟全长(或部分长度)的人 Ang-2 多肽(或其直系同源物)的能力，其亲和力(通过如本文所述的 Affinity ELISA or BIAcore 检测所确定)或其中和能力(通过如本文所述的中和 ELISA 或类似方法所确定)是对其它血管生成蛋白或其它肽(多肽)相应作用的至少 10 倍，或任选为 50 倍、100 倍、250 倍、500 倍，或至少为 1000 倍。

“抗原结合结构域”或“抗原结合区”指特异性结合剂的一部分：它包含了与抗原相互作用的氨基酸残基(或其它基团)并赋予结合剂以对抗原的特异性和亲和力。在抗体中抗原结合剂结构域通常指“互补决定区”或“CDR”。

“表位”指任意分子中能够被特异性结合剂(如抗体)在一个或多个结合剂抗原结合区上所识别并结合的部分。表位通常由分子的化学活性表面基团所组成，例如氨基酸或糖基侧链，表位具有特异的三维结构特征和特异的电荷特征。本文所指的表位可以为连续的或不连续的。而且由于具有与用来产生抗体的表位相同的三维结构，

可以模拟表位，模拟表位中不含有(或仅含一些)用来引发抗体免疫反应的 Ang -2 的氨基酸残基。

“抑制和/或中和表位”的特征为：在体内、体外或原位被特异性结合剂(如抗体)结合上时，能引起包含该表位的分子、细胞或生物体的生物活性发生丧失(或至少降低)。本文中，中和表位定位在 Ang-2 的生物活性区内，或者与该区相连。或者，“活化表位”指这样一种表位：被本发明的特异性结合剂(如抗体)结合上时，能活化 Ang-2，或至少维持 Ang-2 的生物活性构象。

“抗体片段”指包含完整抗体部分序列的肽或多肽。完整抗体包含两个功能独立的部分或片段：抗原结合片段“Fab”和 C-末端可结晶片段--- "Fc"片段。Fab 片段包括重链和轻链的第一恒定区(CH1 和 CL1)，以及重链和轻链的可变区(与特异性抗原结合)。重链和轻链的可变区都包含三个互补决定区(CDR)和分开单个 CDR 的架构区氨基酸残基。Fc 区包括重链的第二和第三恒定区(CH2 和 CH3)，它涉及到效应器功能，如互补活化和噬菌细胞的攻击。一些抗体中 Fc 和 Fab 区被抗体“铰链区”所隔开，根据全长抗体被如何酶解分裂，铰链区可连接到 Fab 或 Fc 片段上。例如用木瓜蛋白酶裂解抗体会使铰链区连接在所得 Fc 片段上，而用胃蛋白酶裂解则所得片段中铰链同时连接在两个 Fab 上。因为在胃蛋白酶裂解后两个 Fab 片段实际上共价连接在一起，所得片段被命名为 $F(ab')_2$ 片段。

Fc 结构域具有相对长的血清半衰期，而 Fab 寿命较短。(Capon 等, Nature, 337: 525-31 (1989))。当作为融合蛋白的一部分被表达时，Fc 结构域可赋予所融合蛋白更长的寿命，或者使融合蛋白具有如 Fc 受体结合、A 蛋白结合、补体结合（complement fixation）、甚至可能是胎盘转移（placental transfer）的功能。所述 Fc 区域可以是天然 Fc 区域，也可以是某些特性被修饰或改善后的 Fc，例如治疗特性或循环时间。

“可变区”或“可变结构域”指抗体轻链和/或重链的一部分，

典型包括重链氨基末端的约 120-130 个氨基酸残基，和轻链氨基末端约 100-110 个氨基酸残基。即使同类抗体的可变区在氨基酸序列上也有很大不同。抗体的可变区决定了每个特定抗体对其特定抗原的结合特性和特异性。序列的可变性集中在互补决定区(CDR)内，而可变结构域中的高度保守区域则被称作架构区(FR)。轻链和重链的 CDR 区内包含了对抗体与抗原直接相互作用起主要作用的氨基酸，不过 FR 区内的氨基酸也可对抗原结合/识别发生显著影响，如下文所述。

抗体的“轻链”是两个不同类型轻链的统称，根据恒定区氨基酸序列分别称作κ (k) 或λ (l)。

抗体的“重链”是 5 个不同类型重链的统称，根据重链恒定区氨基酸序列分别称作α、δ、ε、γ和 μ。已知重链和轻链的组合形成 5 种抗体: IgA、IgD、IgE、IgG 和 IgM，IgG 包含 4 个亚种，称作 IgG_1、IgG_2、IgG_3 和 IgG_4。

当用“天然存在”来形容生物材料(例如核酸分子、多肽、宿主细胞等)时，指这些材料都是天然形式而未经人工修饰。

当用“分离的”修饰 Ang-2 或其特异性结合剂时，指一种物质至少不含有一种在其天然环境中所发现的污染多肽或污染化合物，优选实质上不含有任何其它可能干扰该物质治疗或诊断用途的污染哺乳动物多肽。

当用“成熟”形容 Ang-2、抗 Ang-2 抗体或任何其它 Ang-2 的特异性结合蛋白分子时，指肽或多肽没有前导或信号序列。当本发明的结合剂被表达时(例如在原核宿主细胞中)，“成熟”肽或多肽也可包含附加氨基酸残基(但仍没有前导序列)，例如氨基末端蛋氨酸，或一个或多个蛋氨酸和赖氨酸残疾。以这种方式制备的肽或多肽在使用时，可包含或不含这些已被去掉的氨基酸残基。

当用“有效剂量”或“治疗有效量”形容 Ang-2 的特异性结合剂时，指特异性结合剂的量可有效或足以支持使 Ang-2 的一种或多种

生物活性水平发生可测性改变。这种 Ang-2 的生物活性水平改变可以是增强也可以是减弱。优选的，这种改变是 Ang-2 活性的减弱。

特异性结合剂和抗体

本文所用的“特异性结合剂”指可特异识别并结合所述 Ang-2 的分子。合适的特异性结合剂包括(但不仅限于)抗体及其衍生物、多肽和小分子。合适的特异性结合剂可使用本领域已知方法进行制备。本发明的多肽 Ang-2 特异性结合剂能够结合于 Ang-2 多肽的特定部分上，并优选可调节 Ang-2 多肽的活性或功能。

特异性结合剂例如可特异性结合于 Ang-2 多肽的抗体和抗体片段包含在本发明的范围之内。所述抗体可以是多抗包括单特异性多抗，单抗(mAb)、重组体、嵌合抗体、人源化抗体例如 CDR 移植抗体、人抗体、单链抗体、催化抗体、多特异性抗体和/或双特异性抗体以及它们的片段，还包括它们的变异体和/或衍生物。

通常多次皮下或腹膜内向动物(如兔、仓鼠、山羊、绵羊、马、猪、大鼠、沙鼠、豚鼠、小鼠，或其它任何合适的哺乳动物，和其它非哺乳动物)注射 Ang-2 多肽或其片段(使用或不使用佐剂)，来制备针对 Ang-2 的多抗。所述佐剂包括(但不仅限于)弗氏完全/不完全佐剂、矿物胶如氢氧化铝、表面活性剂如溶血卵磷脂、普卢兰尼克多元醇、聚阴离子、肽、油乳液(oil emulsions)、匙孔蟅血蓝蛋白和二硝基酚。BCG (卡介苗) 和厌氧棒状杆菌特别适用于人体佐剂。在抗原上偶联一个载体蛋白，而该载体蛋白对待免疫动物具有免疫原性，这可能是有效方法，这种载体蛋白例如匙孔蟅血蓝蛋白、血清白蛋白、牛甲状腺蛋白、大豆胰岛素抑制剂。也可使用絮凝剂(aggregating agents)如明矾来提高免疫反应。免疫后取动物血液，并对血清进行抗 Ang-2 多肽抗体滴度测试，可使用“实施例”一节中所描述的方法。可将多抗与在其中检测到该多抗的血清联用，或者

可使用抗原亲和层析或蛋白 A 或 G 亲和层析等方法从血清中纯化多抗。

可使用如(但不仅限于)传统的杂交瘤方法或更新的噬菌体展示技术来制备针对 Ang-2 多肽的单抗。例如可参照文献 Kohler 等., *Nature* 256:495 (1975); Kosbor 等., *Immunol Today* 4:72 (1983)(人 B 细胞杂交瘤技术); Cote 等, *Proc Natl Acad Sci (USA)* 80: 2026-2030 (1983); Brodeur 等, *Monoclonal Antibody Production Techniques and Applications*, pp. 51-63, Marcel Dekker, Inc., New York, (1987))和 Cole 等, *Monoclonal Antibodies and Cancer Therapy*, Alan R Liss Inc, New York N.Y., pp 77-96, (1985)(EBV 杂交瘤技术)。本发明也提供了可产生具有 Ang-2 多肽反应活性的单抗的杂交瘤细胞系。

可在杂交瘤技术中使用骨髓瘤细胞系。这种细胞系不产生抗体，具有高融合效率，并且缺乏一些酶，使得它不能在某些选择性培养基中生长(只有所需的融合细胞(杂交瘤)才能够生长)，因而适合用在杂交瘤融合方法中。例如在小鼠融合操作中使用的细胞系为 Sp-20、P3-X63/Ag8、P3-X63-Ag8.653、NS1/1.Ag 4 1、Sp210-Ag14、FO、NSO/u、MPC-11、MPC11-X45-GTG 1.7 和 S194/5XX0 Bul; 在大鼠融合操作中使用的细胞系为 R210.RCY3、Y3-Ag 1.2.3、IR983F 和 4B210。其它在细胞融合中使用的细胞系为 U-266、GM1500-GRG2、LICR-LON-HMy2 和 UC729-6。杂交瘤和其它能够产生单抗的细胞系就是本发明所考虑的新型组合物。

也可使用噬菌体展示技术来制备任意种类的单抗。优选使用该技术来制备完全人类单抗，其中在噬菌体颗粒表面上使编码单 Fab 或 Fv 抗体片段的多核苷酸进行表达。(Hoogenboom 等, *J Mol Biol* 227: 381 (1991); Marks 等, *J Mol Biol* 222: 581 (1991); 也参见美国专利 No. 5,885,793))。可使用本文描述的结合实验来对每个噬菌体进行“筛选”，以鉴别出具有 Ang-2 亲和作用的抗体片段。因此，通过将抗体片段库展示在噬菌体丝状(filamentous)表面上，并随后根据对 Ang-2

的结合作用对噬菌体加以选择，这些方法模拟了免疫选择作用。在PCT 申请 No. PCT/US98/17364(申请人 Adams 等)中即描述了一种这样的方法，文中通过这样的途径为 MPL 和 msk 受体纯化了具有高亲和力和拮抗功能的抗体片段。在这种方法中，可按上文所述从外周血淋巴细胞中克隆出天然重排的人类 V 基因，以此制备人类抗体基因的完整文库(Mullinax 等, *Proc Natl Acad Sci (USA) 87*: 8095-8099 (1990))。

一旦鉴别出某多核苷酸序列编码了本发明全长单抗的每条链(或编码了单抗的 Fab 或 Fv 片段)，即可按照本领域熟知并常规进行的重组技术，使用真核或原核宿主细胞来表达单抗多核苷酸。或者可将编码特异性结合剂的多核苷酸，以允许编码单抗或其它特异性结合剂的多核苷酸得以表达的方式，导入受体动物的基因组中来构建转基因动物，例如可使用小鼠、大鼠、山羊、或母牛。一方面可将编码单抗或其它特异性结合剂的多核苷酸连接到乳腺特异性调控序列上，并可将嵌合多核苷酸给予目标动物的种系。所得转基因动物即可在其奶中产生所需抗体(Pollock 等, *J Immunol Meth* 231:147-157 (1999); Little 等, *Immunol Today* 8:364-370 (2000))。另外，也可通过使用合适的编码单抗或其它特异性结合剂的多核苷酸来转染植物，使植物表达并产生 Ang-2 特异性结合剂(例如多抗)。

在本发明的另一实施方案中，非人类来源的单抗或多抗(或它们的片段)可以被“人源化”或“嵌合”。对非人类来源抗体进行人源化的操作方法在本领域中是熟知的。(见美国专利 No. 5,859,205, 5,585,089, 和 5,693,762)。可使用如本领域文献(Jones 等, *Nature* 321: 522-525 (1986); Riechmann 等, *Nature*, 332: 323-327 (1988); Verhoeyen 等, *Science* 239:1534-1536 (1988))所述的方法，通过至少将部分互补决定区(如啮齿类动物)用人类抗体的相应区域加以替换。本文也提供了这些人类抗体的变异体和衍生物，如文中讨论(并且在本领域中所熟知)。

本发明也涉及到可结合 Ang-2 多肽的完全人类抗体，及其片段、变异体和/或衍生物。可使用上文所述的噬菌体展示技术来制备这些抗体。或者可使用转基因动物(如小鼠)来产生这些抗体，前提是这种转基因动物在无内生性免疫球蛋白产生时能够生成人类抗体库。这可通过使用 Ang-2 抗原或其片段(当 Ang-2 片段含有 Ang-2 的独特氨基酸序列时)来免疫动物而实现。这些免疫原可任选与载体偶联。参见如 Jakobovits 等, *Proc Natl Acad Sci (USA)*, 90: 2551-2555 (1993); Jakobovits 等, *Nature* 362: 255-258 (1993); Bruggermann 等, *Year in Immuno*, 7: 33 (1993)。在一种制备转基因动物的方法中，使该动物的内生性免疫原重链和轻链编码基因座失活，然后在该动物的基因组中插入人类重链和轻链蛋白的编码基因座。部分修饰动物是那些没有进行完全这些修饰的动物，对其进行杂交以获得包含全部所需免疫系统修饰的动物。当给予免疫原时，这些转基因动物能够产生含有人类可变区域的抗体，这些抗体包含人类(而非如鼠类)氨基酸序列，具有对所需抗原的免疫特异性。参见 PCT 申请 PCT/US96/05928 和 PCT/US93/06926。其它方法见美国专利 5,545,807、PCT 申请 No. PCT/US91/245、PCT/GB89/01207、EP 546073B1 和 EP 546073A1。也可通过在宿主细胞或杂交瘤中表达重组 DNA 来产生人类抗体，如文中所述。

可通过多种不同方法完成转基因操作。参见如 Bruggeman 等, *Immunol Today 17*:391-7 (1996)。在一种方法中，构建微小基因座以通过人工方式使得种系构造中的基因片段互相靠近。由于长度有限(即通常小于 30kb)，所得微基因座所含的不同基因片段数目有限，但仍能够产生一个大抗体库。微基因座仅含有人类 DNA 序列(包括启动子和增强子)，在转基因动物体内具有完整功能。

当需要在转基因动物体内含有大量基因片段时，可使用酵母人工染色体(YAC)。YAC 长度从几百 kb 到 1Mb 不等，通过直接微注射入卵子导入小鼠体内(或其它合适的动物)，或者将 YAC 转移到胚胎

干细胞系内。通常使用纯化 DNA 进行脂转染将 YAC 给予 ES 细胞，或者通过酵母原生质体融合进行，这时纯化 DNA 被小鼠细胞所携带，融合方法类似于杂交瘤融合法。DNA 转移之后的所需 ES 细胞选择可通过 YAC 上本领域已知的任何选择性标记来进行。

另一可选方法是，在大肠杆菌宿主中扩增 P1 噬菌体载体。由于这些载体所携带的 DNA 通常小于 YAC 内插入的 DNA，因此可更容易的得到足够量的克隆，以对小鼠卵子进行直接微注射。已表明，混合使用多种 P1 载体将得到更高水平的同源重组。

使用本领域已知的任何方法(如 ELISA)检测循环抗体的血清水平，一旦鉴别出合适的转基因小鼠(或其它合适的动物)，使该转基因动物与内生性 Ig 基因座被破坏的小鼠进行杂交。所得后代的所有 B 细胞基本上都表达人类抗体。

另一可选方法是，使用人类 Ig 基因座替换动物的整个基因座，所得动物仅表达人类抗体。在另一方法中，所述动物的部分基因座被相应的特异性人类基因座所取代。在一些情况下，根据小鼠 Ig 基因座的替换特征不同，这种方法所得动物可以表达嵌合抗体(与完全人类抗体相对)。

也可在体外将人脾细胞(B 或 T 细胞)暴露于抗原下，然后在免疫减弱小鼠(如 SCID 或 nod/SCID)体内重建暴露细胞来制备人类抗体。见 Brams 等, *J Immunol*, 160: 2051-2058 (1998); Carballido 等, *Nat Med*, 6: 103-106 (2000)。在一种方法中，将人类胚胎细胞给予 SCID 小鼠(SCID-hu)中，会引起长效造血作用和人类 T 细胞发展(McCune 等, *Science 241*:1532-1639 (1988); Ifversen 等, *Sem Immunol* 8:243-248 (1996))。这些嵌合动物体内的任何体液免疫反应都完全依赖于 T 细胞的协同发展(Martensson 等, *Immunol 83*:1271-179 (1994))。在另一可选方法中，将人类外周血淋巴细胞腹(膜)腔内(或其它方式)给予 SCID 小鼠体内 (Mosier 等, *Nature* 335:256-259 (1988))。当导入的细胞被中性球致活物 (例如葡萄球菌肠毒素 A (SEA))处理(Martensson

等, *Immunol 84*: 224-230 (1995)), 或被抗人 CD40 单抗处理时 (Murphy 等, *Blood* 86:1946-1953 (1995)), 可以检测到更高水平的 B 细胞。

另一可选方法是, 通过将每个人 VH 片段与随机核苷酸的 D 片段以及人 J 片段组合起来, 从未重排 V 基因中制备完全合成的人类重链库(Hoogenboom 等, *J Mol Biol 227*:381-388 (1992))。同样将每个人 V 片段与 J 片段组合起来制备轻链库(Griffiths 等, *EMBO J 13*:3245-3260 (1994))。将编码完整抗体(即重链和轻链)的核苷酸与单链 Fv 片段连接, 再将此多核苷酸与编码丝状噬菌体微包被蛋白的核苷酸相连接。当此融合蛋白在该噬菌体表面被表达时, 即使用固定抗原进行选择以鉴别编码同样小抗体的多核苷酸。

在另一可选方法中, 通过将一条链融合于噬菌体蛋白, 而另一条被分泌到细菌周质中, 抗体片段被组装成两个 Fab 片段(Hoogenboom 等, *Nucl Acids Res* 19:4133-4137 (1991); Barbas 等, *Proc Natl Acad Sci (USA)* 88:7978-7982 (1991))。

大量生产嵌合抗体、人源化抗体、CDR 移植抗体和完全人类抗体(或它们的片段)的典型方法是重组技术。可使用本文所述的材料和方法, 将编码每种抗体重链和轻链(或它们的片段)的多核苷酸分子导入到宿主细胞中并进行表达。在一个优选的实施方案中, 在哺乳动物宿主细胞中生产抗体, 例如 CHO 细胞。此法细节将在下文描述。

特异性结合剂的融合对象

在本发明的另一实施方案中, 可将含有 Ang-2 抗体可变区(例如具有本文所述氨基酸序列的重链可变区或具有本文所述氨基酸序列的轻链可变区)氨基酸序列的多肽融合于人 IgG Fc 区域的一个或多个结构域中(N 端或 C 端)。当与治疗蛋白(例如 Ang-2 特异性抗体的 Fab)共同形成构建体时, Fc 结构域可使融合蛋白具有更长的寿命, 或者向融合蛋白提供诸如 Fc 受体结合、蛋白 A 结合、补体结合, 甚至可能是胎盘转运的功能。(Capon 等, Nature, 337: 525-531 (1989))。

在一个实例中，可使用本领域已知方法将抗体铰链区、CH2 和 CH3 区域融合于特异性结合剂多肽(如从噬菌体展示文库所获得的抗 Ang-2 Fab 或 Fv 片段)的 N 末端或 C 末端。可使用 A 蛋白 或 G 蛋白亲和层析柱来纯化所得的融合蛋白。已经发现融合于 Fc 区域的肽和蛋白在体内比未融合的相同分子具有更足够长的寿命。融合于 Fc 区域也允许融合多肽发生二聚化/多聚化作用。所述 Fc 区域可以是天然 Fc 区域，或者可被修饰以改善某些性质，例如治疗特性、循环时间、减少聚集问题等。本领域已知的其它例子包括，将 Fc 区域(可以为人类或其它种类来源，或者为合成)融合于 CD30L 的 N 末端以治疗 Hodgkin's 症、退行发育性淋巴瘤和 T-cell 白血病 (美国专利 No. 5,480,981)，将 Fc 区域融合于 TNF 受体，以治疗感染性休克 (Fisher 等, N Engl J Med, 334: 1697-1702 (1996))，将 Fc 区域融合于 Cd4 受体，以治疗 AIDS (Capon 等, Nature, 337: 525-31 (1989))。

催化抗体是另一种融合分子，在特异性结合剂(抗体)上连接一个或多个细胞毒素基团或者(更常见)一个或多个生物活性基团。参见如 (Rader 等, *Chem Eur J* 12:2091-2095 (2000))。这种类型的细胞毒性剂可提高抗体介导的细胞毒性，并包含这类直接或间接引起细胞死亡的细胞因子、放射性同位素、化疗药物(包括前药)、细菌毒素(如假单胞菌外毒素、白喉毒素等)、植物毒素(如蓖麻毒素、多花白树毒蛋白等)、化学缀合物(conjugates) (如 maytansinoid 毒素、calechaemicin 等)、放射缀合物(conjugates)、酶耦合物(enzyme conjugates)(RNase 耦合物、抗体导向酶-前体药物疗法(antibody-directed enzyme/prodrug therapy) (ADEPT))等的部分。一种方法是，可通过将细胞毒性剂结合于抗体可选抗原识别位点中的一个上，来将细胞毒性剂连接到双特异性或多特异性抗体的一个部分上。另一方法是，可将编码毒素的多核苷酸连接到编码结合剂的多核苷酸上并进行表达，蛋白细胞毒素即作为与特异性结合剂的融合蛋白而被表达。在另一可选方法中，可对特异性结合剂进行共价修饰以包含进所需细胞毒素。

这种融合蛋白的例子包括免疫多肽、具有长循环半衰期的蛋白(例如免疫球蛋白恒定区)、标记蛋白、有利于所需特异性结合剂多肽纯化的蛋白或多肽，以及促进多聚体蛋白(例如有利于聚合物形成和稳定的亮氨酸拉链基序)形成的多肽。

通常在这种插入性变异体中，全部天然分子或实质性部分(substantial portion) 的 N-末端或 C-末端连接在第二种多肽或其部分上。例如，融合蛋白通常使用其它种类的前导序列，以允许在异种宿主中表达重组体。其它有用的融合蛋白包括添加免疫球蛋白活性结构域(例如抗体表位)，以促进该纯化蛋白的纯化。在融合连接位点或其附近包含进一个裂解位点，可有利于在纯化后除去外来多肽。其它有用的融合包括连接上功能性结构域，例如酶的活性位点、糖基化结构域、细胞靶点信号或跨膜区域。

本发明中可使用多种商品化的融合蛋白表达系统。特别有效的系统包括(但不仅限于)谷胱甘肽-S-转移酶(GST)系统(Pharmacia)，麦芽糖结合蛋白系统(NEB, Beverley, MA)，FLAG 系统 (IBI, New Haven, CT)和 6xHis 系统(Qiagen, Chatsworth, CA)。这些系统能够产生仅携带小数量额外氨基酸的重组多肽，这些额外氨基酸不可能影响重组多肽的抗原活性。例如 FLAG 系统和 6xHis 系统仅添加短序列，这两种系统的添加序列已知都几乎无抗原性，并且不影响多肽折叠成其天然构象。另一种被考虑的有效融合是在蛋白或肽的 N 末端融合 Met-Lys 二肽。这种融合可产生蛋白表达或活性的有利提高。

将特异性结合剂融合于半抗原上以提高特异性结合剂融合构建体(它有利于例如本发明的抗独特型抗体的产生)的免疫原性，这可能是一种特别有效的融合构建体。本领域已知这种提高免疫原性的融合方式，例如将特异性结合剂与辅抗原(例如 hsp70)融合，或与如白喉毒素或细胞因子(如 Il-2)融合，将有利于引发免疫反应。在另一实施方案中，可进行融合以提高抗原结合组合物对特异位点或细胞的靶定位活性。

其它被考虑的融合构建体包括融合具有所需特性的异种多肽(如 Ig 恒定区)以延长血清半衰期，或融合抗体(或其片段)以改善靶定位活性。还有其它融合系统产生杂种多肽，它需要从所需多肽上切除融合伴侣。在一个实施方案中，这种融合伴侣连接在重组特异性结合剂多肽上，其中连接肽序列含有蛋白酶的特异性识别序列。合适的蛋白酶例如烟草蚀纹病毒蛋白酶(Life Technologies, Gaithersburg, MD) 或 Xa 因子(New England Biolabs, Beverley, MA)。

本发明也提供了包含 Ang-2 抗体全部或部分可变区的融合多肽，例如具有本文所述氨基酸序列的重链(或轻链)可变区连接在截短组织因子 (tTF)上，它是一种血管靶分子，由切除了部分序列的人诱血凝蛋白(coagulation-inducing protein)(肿瘤血管凝结剂)组成。将 tTF 融合于抗 Ang-2 抗体(或其片段)上，可能会促进抗 Ang-2 到靶细胞的运输。

特异性结合剂的变异体

本发明特异性结合剂的变异体包括插入、删除和/或替换变异体。本发明一方面提供了插入变异体，其中特异性结合剂添加了一个或多个氨基酸残基。插入可发生在特异性结合剂的末端(同时发生或只发生在一端)或内部序列上。在两个末端或一个末端具有额外残基的插入变异体可包含如融合蛋白和具有氨基酸标签或标记的蛋白。插入变异体包括添加有一个或多个残基的特异性结合剂序列(或其片段)。

本发明的变异体产物也包括成熟特异性结合剂产物。这种特异性结合剂产物的前导序列或信号序列被除去，但所得蛋白相比野生型 Ang-2 多肽仍然具有额外的末端残基。这种额外末端残基可能来自其它蛋白，或者可能含有一个或多个不能被鉴别出具体来自哪个蛋白的残基。也考虑了在位置-1 具有额外蛋氨酸残基的特异性结合剂产物(Met^{-1}-特异性结合剂)，和在位置-2 和-1 具有额外蛋氨酸和赖氨

酸残基的特异性结合剂产物(Met^{-2}-Lys^{-1}-特异性结合剂)。具有额外Met、Met-Lys、Lys(或者通常是一个或多个碱性残基)残基的特异性结合剂变异体特别有利于在细菌宿主细胞中提高重组蛋白生成。

本发明也涉及到来自特殊表达系统的具有额外氨基酸残基的特异下结合剂变异体。例如使用将所需多肽作为谷胱甘肽-S 转移酶(GST)融合产物的一部分进行表达的商品化载体系统，所得产物在从所需蛋白上除去 GST 成分后，氨基酸位置-1 即具有一个额外的甘氨酸残基。也考虑了来自其它载体系统表达的变异体，包括在氨基酸序列上包含了聚-his 标签(通常位于序列的羧基末端或氨基末端)的序列。

插入变异体也包括上文所述的融合蛋白，其中特异性结合剂多肽的氨基和/或羧基末端融合了其它多肽(或其片段)，或者融合了通常不被识别为任何特定蛋白序列部分的氨基酸序列。

另一方面，本发明提供了删除变异体，其中特异性结合剂多肽的一个或多个氨基酸残基被删除。删除可以发生在特异性结合剂的一个或两个末端，或者发生在氨基酸序列内部。删除变异体必须包含特异性结合剂多肽的所有片段。

抗体片段包括那些结合在抗原多肽表位上的部分。这些片段包括如完整抗体在酶切或化学裂解后的 Fab 和 $F(ab')_2$ 片段。其它结合片段包括重组 DNA 技术的产物，例如包含有抗体可变区编码序列的重组质粒的表达产物。本发明也涉及到保持了特异性结合 Ang-2 多肽能力的 Ang-2 结合剂的多肽片段。这里理解为至少包含 5、10、15、20、25、30、35、40、45、50 或更多本发明的肽或多肽的保守性氨基酸残基的片段。优选的多肽片段具有对本发明抗原结合剂的特殊或特异性免疫学特性。可使用任何本领域已知和实践的方法来制备具有所学免疫学特性的本发明所述多肽。

另一方面本发明也提供了特异下结合剂的替换变异体。通常认为替换变异体与原多肽相似或具有一定的序列同一性，替换变异体的一个或多个残基被除去并用可选残基加以替换。本发明不仅考虑了

保守性替换，也考虑了非保守性替换。

可通过已知方法方便的对相关多肽的同一性和相似性进行计算。这些方法包括(但不仅限于)以下文献所述的方法：Computational Molecular Biology, Lesk, A.M., ed., Oxford university Press, New York (1988)； Biocomputing: Informatics and Genome Projects, Smith, D.W., ed., Academic Press, New York (1993)； Computer Analysis of Sequence Data, Part 1, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey (1994)； Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press (1987)； Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M. Stockton Press, New York (1991)； and Carillo 等, *SIAM J. Applied Math.*, 48:1073 (1988)。

用以确定两个多肽间的相关性或同一性百分比的优选方法，被设计成给出待测序列间的最大程度的匹配度。在公开的电脑软件中描述了确定同一性的方法。确定两个序列间同一性的优选电脑软件包括(但不仅限于)GCG软件包，包括GAP (Devereux 等, *Nucl. Acid. Res.*, 12:387 (1984)； Genetics Computer Group, university of Wisconsin, Madison, WI, BLASTP, BLASTN, 和FASTA (Altschul 等, *J. Mol. Biol.*, 215:403-410 (1990))。The BLASTX program 国家生物技术中心 (National Center for Biotechnology Information (NCBI))，以及其它来源 (*BLAST Manual*, Altschul 等 NCB/NLM/NIH Bethesda, MD 20894； Altschul 等, *supra* (1990))都提供了公开的 BLASTX 软件。也可使用广为人知的 Smith Waterman 算法来确定同一性。

两个氨基酸序列间的某一比对可能仅会得到两个序列间一个短区域的匹配，即使在这两个全长序列间没有显著的相关性，这个短比对区域也可能具有高度序列同一性。因此在某些实施方案中，选择的比对方法(GAP 程序)将得到至少跨越目的待测多肽全长 10%的比对结果，即待测多肽至少长 400 氨基酸时至少比对 40 个连续氨基酸，待测多肽至少长 300-400 氨基酸时至少比对 30 个连续氨基酸，待测

多肽至少长 200-300 氨基酸时至少比对 20 个连续氨基酸，待测多肽至少长 100-200 氨基酸时至少比对 10 个连续氨基酸。

例如使用 GAP 算法(Genetics Computer Group, university of Wisconsin, Madison, WI)，对两个待确定序列同一百分比的多肽进行比对以得到两者各自氨基酸的最优匹配(匹配范围，如该算法所确定的)。在某些实施方案中，将缺口开放扣分值(它典型被计算为 3X 平均对角线；“平均对角线”是所用对比矩阵的平均对角线；“对角线”是特定对比矩阵分配给每个最优氨基酸匹配的分数或数值)、缺口延伸扣分值(通常是缺口开放扣分值得 1/10)和对比矩阵例如 PAM 250 或 BLOSUM 62 与该算法联合使用。在一些实施方案中也通过算法使用标准对比矩阵(Dayhoff 等, *Atlas of Protein Sequence and Structure*, 5(3)(1978), PAM 250 对比矩阵; Henikoff 等, *Proc. Natl. Acad. Sci* USA, 89:10915-10919 (1992)，BLOSUM 62 对比矩阵)。

在某些实施方案中，多肽序列对比使用的参数包括:

算法: Needleman 等, J. Mol. Biol., 48:443-453 (1970);

对比矩阵: BLOSUM 62 from Henikoff 等, supra (1992);

缺口扣分值: 12

缺口长度扣分值: 4

相似度阈值: 0

上述参数在 GAP 程序可能是有效的。在某些实施方案中，使用 GAP 算法进行多肽对比(末端缺口无扣分值)的前述参数为默认参数。

在某些实施方案中，多肽分子序列对比使用的参数包括:

算法: Needleman 等, *supra* (1970)

对比矩阵: matches = +10, mismatch = 0

缺口扣分值: 50

缺口长度扣分值: 3

上述参数在 GAP 程序也可能是有效的。在某些实施方案中，进

行多肽对比的前述参数为默认参数。

也可以使用其它范例性算法、缺口开放扣分值、缺口延伸扣分值、对比矩阵、相似度阈值等，包括 Program Manual, Wisconsin Package, Version 9, September, 1997 中所列出的。根据待进行的具体对比(例如 DNA-DNA、蛋白-蛋白、蛋白-DNA；另外根据是在序列间的给定分子对间进行比较(这种情况下通常优选 GAP 或 BedtFit)，还是在序列和大序列数据库间进行比较(这种情况下优选 FASTA 或 BLASTA))进行详细选择，这对本领域技术人员将是显而易见的。

这里按照常规用法使用 20 个常规氨基酸的缩写。见 Immunology--A Synthesis (2nd Edition, E. S. Golub and D. R. Gren, Eds., Sinauer Associates, Sunderland, Mass. (1991))，通过引用并入本文。

氨基酸可具有 L 构型或 D 构型(甘氨酸除外，它没有 L 构型和 D 构型之分)，本发明的多肽和组合物可能包含不同的立体异构体。不过优选 L 构型。本发明也提供了反转分子，其羧基到氨基的氨基酸序列是反转的。例如正常序列 X_1-X_2-X_3 的反转分子为 X_3-X_2-X_1。本发明也提供了转型(retro)-反转分子，如上文所述，一个氨基酸序列从羧基到氨基的氨基酸序列发生反转，并且正常为 L 对映异构体的残基变为 D 构型。

20 个常规氨基酸的立体异构体(如 D-氨基酸)、非天然氨基酸如 α-,α-二取代氨基酸、N-烷基氨基酸、乳酸和其它非常规氨基酸也可能适合作本发明的多肽成分。非常规氨基酸包括如(但不仅限于)氨基己二酸、β-丙氨酸、β-氨基丙酸、氨基丁酸、哌啶酸、氨基己酸(aminocaprioic acid)、氨基庚酸、氨基异丁酸、氨基庚二酸、二氨基丁酸、锁链素、二氨基庚二酸、二氨基丙酸、N-乙基甘氨酸、N-乙基天冬氨酸、羟赖氨酸、别-羟赖氨酸、羟脯氨酸、异锁链素、别-异亮氨酸、N-甲基甘氨酸、肌氨酸、N-甲基异亮氨酸、N-甲基缬氨酸、正缬氨酸、正亮氨酸、鸟氨酸、4-羟基脯氨酸、γ-羧基谷氨酸、ε-N,N,N-三甲基赖氨酸、ε-N-乙酰基赖氨酸、O-磷酸丝氨酸、N-乙酰基丝氨

酸、N-甲酰基蛋氨酸、3-甲基组氨酸、5-羟基赖氨酸、σ-N-甲基精氨酸和其它类似的氨基酸等 (如 4-羟基脯氨酸)。

类似的，如果没有特别指出，单链多核苷酸序列的左手末端为 5'末端；双链多核苷酸序列的左手方向指 5'方向。新生 RNA 转录物的 5'到 3' 添加方向即为转录方向；具有与 RNA 相同序列的 DNA 链上的并位于 RNA 转录物 5'末端的 5'方向的序列区域为“上游序列”；具有与 RNA 相同序列的 DNA 链上的并位于 RNA 转录物 3'末端的 3'方向的序列区域为“下游序列”。

保守性氨基酸替换可能包括非天然氨基酸残基，这典型发生在肽的化学合成而不是生物系统合成中。这包括肽模拟和氨基酸部分的其它反转或转型形式。

根据侧链共性可将天然氨基酸分为以下种类:

1) 疏水氨基酸: Met, Ala, Val, Leu, Ile
2) 中性亲水氨基酸: Cys, Ser, Thr, Asn, Gln
3) 酸性氨基酸: Asp, Glu
4) 碱性氨基酸: His, Lys, Arg
5) 影响链方向的残基: Gly, Pro 和
6) 芳香氨基酸: Trp, Tyr, Phe

例如，非保守性替换可以为一个种类的氨基酸被另一种类的氨基酸所替换。这种替换残基可被导入到人类抗体中与非人类抗体同源的区域内，或者导入到该分子的非同源性区域内。

某些实施方案中在进行这种改变时，可考虑氨基酸的亲水值。根据其疏水性和电荷特性，每个氨基酸都被分配了一个亲水值: 异亮氨酸(+4.5); 缬氨酸(+4.2); 亮氨酸(+3.8); 苯丙氨酸 (+2.8); 半胱氨酸/胱氨酸(+2.5); 甲硫氨酸(+1.9); 丙氨酸(+1.8); 甘氨酸(-0.4); 苏氨酸(-0.7); 丝氨酸(-0.8); 酪氨酸(-0.9); 色氨酸(-1.3); 脯氨酸(-1.6); 组氨酸(-3.2); 谷氨酸(-3.5); 谷氨酰胺(-3.5); 天冬氨酸(-3.5); 天冬酰胺(-3.5); 赖氨酸 (-3.9); 精氨酸(-4.5)。

本领域已知氨基酸亲水值在赋予蛋白交互生物功能中的重要性。Kyte 等, *J. Mol. Biol.*, 157:105-131 (1982)。已知某些氨基酸可以被其它具有相似亲水值的氨基酸所替换，并仍然保持相似的生物功能。某些实施方案中这种改变包括将亲水值差异在±2 内的氨基酸进行替换。某些实施方案中这种改变包括将亲水值差异在±1 内的氨基酸进行替换，某些实施方案中这种改变包括将亲水值差异在±0.5 内的氨基酸进行替换。

本领域也理解根据疏水性可有效进行类似氨基酸的替换，特别是当蛋白或肽的生物功能是被制备以进行免疫学应用时，如本发明。在某些实施方案中，蛋白最大局部平均疏水性(由其邻近氨基酸疏水性决定)，与其免疫原性和抗原性相关，即与该蛋白的生物特性相关。

氨基酸残基的亲水值分配如下：精氨酸(+3.0)；赖氨酸(+3.0)；天冬氨酸(+3.0 ± 1)；谷氨酸(+3.0 ± 1)；丝氨酸(+0.3)；天冬酰胺(+0.2)；谷氨酰胺(+0.2)；甘氨酸(0)；苏氨酸(-0.4)；脯氨酸(-0.5 ± 1)；丙氨酸(-0.5)；组氨酸(-0.5)；半胱氨酸(-1.0)；甲硫氨酸(-1.3)；缬氨酸(-1.5)；亮氨酸(-1.8)；异亮氨酸(-1.8)；酪氨酸(-2.3)；苯丙氨酸(-2.5)和色氨酸(-3.4)。某些实施方案中根据相似亲水值进行改变时，包括将亲水值差异在±2 内的氨基酸进行替换，某些实施方案中包括包括将亲水值差异在±1 内的氨基酸进行替换，某些实施方案中包括将亲水值差异在±0.5 内的氨基酸进行替换。根据亲水性从初级氨基酸序列可以鉴别表位。这些区域也称作“表位核心区域”。

范例性氨基酸替换列于表 1。

表 1

氨基酸替换

原始残基	替换范例	优选替换
Ala	Val, Leu, Ile	Val
Arg	Lys, Gln, Asn	Lys
Asn	Gln, Glu, Asp	Gln

Asp	Glu, Gln, Asn	Glu
Cys	Ser, Ala	Ser
Gln	Asn, Glu, Asp	Asn
Glu	Asp, Asn, Gln	Asp
Gly	Pro, Ala	Ala
His	Asn, Gln, Lys, Arg	Arg
Ile	Leu, Val, Met, Ala, Phe, 正亮氨酸	Leu
Leu	正亮氨酸, Ile, Val, Met, Ala, Phe	Ile
Lys	Arg, 1,4 二氨基-丁酸, Gln, Asn	Arg
Met	Leu, Phe, Ile	Leu
Phe	Leu, Val, Ile, Ala, Tyr	Leu
Pro	Ala	Gly
Ser	Thr, Ala, Cys	Thr
Thr	Ser	Ser
Trp	Tyr, Phe	Tyr
Tyr	Trp, Phe, Thr, Ser	Phe
Val	Ile, Met, Leu, Phe, Ala, 正亮氨酸	Leu

使用熟知方法技术人员能够从列出的多肽中确定合适的变异体。在某些实施方案中，技术人员可鉴别出分子的合适区域，通过靶定位这些区域中相信对活性不重要的部分，这些区域可被替换而不破坏活性。在某些实施方案中，甚至也可对可能对生物活性或结构重要的区域进行保守性氨基酸替换而不破坏生物活性，或者对多肽结构没有负面影响。

另外，本领域技术人员可回顾结构-功能研究，其中鉴别了相似多肽中对活性或结构重要的残基。在进行此类比较时，可以预测一个蛋白中与类似蛋白中对活性或结构起重要作用的残基所相应的氨基酸残基的重要性。

本领域技术人员可分析相似多肽的三维结构和氨基酸序列。考虑这些信息，本领域技术人员可预测抗体的三维结构与其氨基酸残基间的比对。在某些实施方案中，由于这些残基可能涉及到该蛋白与

其它分子间的重要相互作用，本领域技术人员可进行选择，以不在预测位于蛋白表面的氨基酸残基进行剧烈改变。而且，本领域技术人员可以制备试验变异体，其中的每个所需氨基酸残基上都进行替换。然后可使用本领域已知的活性检测方法来筛选这些变异体。例如，如果发现一个特殊氨基酸残基会引起活性丧失、活性的非目的性下降或者不合适的活性，那么具有这种变化的变异体就不被选用。换言之，从这些常规实验收集的信息出发，本领域技术人员可方便的确定不能进行替换(不管是单独进行替换还是与其它突变结合使用)的氨基酸。

已有大量涉及到二级结构预测的科技文献。见 Moult J., *Curr. Op. in Biotech.*, 7(4):422-427 (1996), Chou 等, *Biochemistry*, 13(2):222-245 (1974); Chou 等, *Biochemistry*, 113(2):211-222 (1974); Chou 等, *Adv. Enzymol. Relat. Areas Mol. Biol.*, 47:45-148 (1978); Chou 等, *Ann. Rev. Biochem.*, 47:251-276 and Chou 等, *Biophys. J.*, 26:367-384 (1979)。另外现在有可辅助预测二级结构的商品化电脑程序。一种预测二级结构的方法是依据同源性建模(homology modeling)。两个多肽或蛋白的序列同一性高于 30%或相似性高于 40%，那么这两个分子的拓扑结构通常就相似。蛋白结构数据库(PDB)最近的发展提高了二级结构的可预测性，这包括多肽或蛋白结构内潜在折叠的数目。见 Holm 等, *Nucl. Acid. Res.*, 27(1):244-247 (1999)。有文献(Brenner 等, *Curr. Op. Struct. Biol.*, 7(3):369-376 (1997))提出一个给定多肽或蛋白内折叠数目是有限的，并且一旦结构的临界值被解决，那么就会极大提高结构预测精度。

其它二级结构预测方法包括: "threading"法 (Jones, D., *Curr. Opin. Struct. Biol.*, 7(3):377-87 (1997); Sippl 等, *Structure*, 4(1):15-19 (1996)); "轮廓分析法" ("profile analysis")(Bowie 等, *Science*, 253:164-170 (1991); Gribskov 等, *Meth. Enzym.*, 183:146-159 (1990); Gribskov 等, *Proc. Nat. Acad. Sci.*, 84(13):4355-4358 (1987)); "进化关

系法”("evolutionary linkage")(Holm, *supra* (1999), and Brenner, *supra* (1997))。

在某些实施方案中，抗体变异体包括糖基化变异体，其中相比原有多肽氨基酸序列，其糖基化位点的数目和/或类型发生改变。在某些实施方案中，相比天然蛋白，蛋白变异体包含的 N-连接糖基化位点数目增多或减少。N-连接糖基化位点的特征序列为：Asn-X-Ser 或 Asn-X-Thr，其中的 X 可以为除脯氨酸外的任意氨基酸。替换氨基酸构造出这个序列，提供了一个新的添加 N-连接糖基侧链的潜在位点。或者，替换以除去这个序列将除去已有的 N-连接糖基侧链。也提供了 N-连接糖基侧链的重排，其中除去或创建了一个或多个 N-连接糖基化位点(通常是天然位点)。其它优选的抗体变异体包括半胱氨酸变异体，相比原有氨基酸序列，变异体中的一个或多个半胱氨酸残基被删除或替换为其它氨基酸(如丝氨酸)。当抗体需要重折叠成生物活性构象时(如从可溶性内涵体分离后)，半胱氨酸变异体可能是有效的。半胱氨酸变异体含有的半胱氨酸残基通常比天然蛋白少，并且典型含有偶数个半胱氨酸以将未配对半胱氨酸引起的相互作用降到最低。

某些实施方案中，氨基酸残基替换目的为：(1)降低对蛋白水解的敏感性，(2)降低对氧化作用的敏感性，(3)改变形成蛋白复合体的结合亲和力，(4)改变结合亲和力，和/或(5)赋予这些多肽的其它功能特性或修饰其功能特性。某些实施方案中，可在天然序列中(某些实施方案中，在形成分子内接触以外的序列部分上)进行单个或多个氨基酸替换(某些实施方案中为保守性替换)。某些实施方案中，保守性替换典型可能不引起原有序列结构特性的实质性变化(如氨基酸替换不倾向于破坏原有序列中的螺旋(或者破坏原有序列的其它特征二级结构))。识别多肽二级和三级结构的方法例如见“蛋白，结构和分子规则” Proteins, Structures and Molecular Principles (Creighton, Ed., W. H. Freeman and Company, New York (1984))；“蛋白结构信息”

Introduction to Protein Structure (C. Branden and J. Tooze, eds., Garland Publishing, New York, N.Y. (1991)); 和 Thornton et at. Nature 354:105 (1991)。

本发明特异性结合剂为多肽或肽替换变异体，可含有的氨基酸替换比例可高至 12%。对抗体变异体来说，重链中可有 50、49、48、47、46、45、44、43、42、41、40、39、38、37、36、35、34、33、32、31、30、29、28、27、26、25、24、23、22、21、20、19、18、17、16、15、14、13、12、11、10、9、8、7、6、5、4、3、2 或 1 个氨基酸被替换，轻链中可有 25、24、23、22、21、20、19、18、17、16、15、14、13、12、11、10、9、8、7、6、5、4、3、2 或 1 个氨基酸被替换。

特异性结合剂的衍生物

本发明也提供了特异性结合剂的衍生物。衍生物包括被修饰(除氨基酸残基的插入、删除、替换)的特异性结合剂。这些修饰优选为共价性质，包括如与聚合物、脂质、其它有机和无机基团的化学连接。本发明衍生物的制备目的可以为提高特异性结合剂多肽的循环半衰期，或者设计成能改善多肽对目的细胞、组织或器官的靶定位能力。

本发明也涉及到的结合剂衍生物可共价修饰成包含一个或多个水溶性聚合物连接物，例如聚乙烯乙二醇、聚氧乙二醇或者聚丙二醇，见美国专利 4,640,835、4,496,689、4,301,144、4,670,417、4,791,192、4,179,337。其它本领域已知可用的聚合物包括单甲氧基-聚乙二醇、右旋糖苷、纤维素或其它碳水化合物聚合物、聚-(N-乙烯基吡咯烷酮)-聚乙二醇、丙二醇均聚物、聚丙烯氧化物/乙烯氧化物共聚物、聚氧乙基化多羟基化合物 (如甘油)和聚乙烯醇，以及它们的混合物。特别优选用聚乙烯(PEG)亚单位对特异性结合剂进行共价修饰。水溶性聚合物可连接到特异位置，例如特异性结合剂产物的氨基末端，

或者随机连接到多肽的一个或多个侧链上。使用 PEG 以改善特异性结合剂的治疗能力(尤其用于人源化抗体)，见美国专利 6,133,426 和 Gonzales 等, 授权日： 2000 年 10 月 17 日。

抗体诱变的靶位点

可采取某些措施来操纵 Ang-2 特异性抗体的内在特性，例如抗体对其靶点的亲和力。这些措施包括对编码抗体的多核苷酸进行定点突变或随机突变已得到抗体变异体，然后进行筛选以回收展现所需变化(如提高或降低的亲和力)的抗体变异体。

最常用的氨基酸残基突变位点是 CDR 内的残基。如上文所述，CDR 内含有与 Ang-2 发生实际作用的残基，以及影响这些作用残基的空间重排的残基。不过，CDR 区域外的可变区架构区内的残基也被发现对抗体的抗原结合特性做出了实质性贡献，这些残基也可作为突变靶点以对这些特性进行操纵。见 Hudson, *Curr Opin Biotech*, 9:395-402 (1999)及其参考文献。

可以在体细胞（somatic）亲和力成熟过程中，对 CDR 内相应于易于“超突变”区域的位点进行限制性随机突变或定点突变，以得到更小并且更有效的抗体筛选库。见 Chowdhury and Pastan, *Nature Biotech*, 17: 568-572 (1999)及其参考文献。已知这种方法中限定超突变位点的 DNA 元件类型包括正向和反向重复(direct and inverted repeats), 某些共有序列、二级结构和回文结构。其中共有 DNA 序列包括四碱基序列嘌呤-G-嘧啶-A/T(即 A 或 G - G- C 或 T- A 或 T)和丝氨酸密码 AGY(其中 Y 可以为 C 或 T)。

因此，本发明的一个实施方案中使用突变策略的目的是提高抗体对其靶点的亲和力。这些策略包括在整个可变重链和轻链内的突变、仅在 CDR 区域内进行突变、在 CDR 内的共有突变热点位点进行突变、在架构区内进行突变，或者联合使用这些突变方法(这里的突变可以是随机突变或定点突变)。可使用本领域已知方法如 X 射线结晶

学技术来分析待测抗体和抗体-配体复合体的结构，从而准确描绘 CDR 区域并鉴别残基(包括结合位点)。根据这种抗体晶体结构的分析和鉴定，本领域已知有多种方法可用来近似描绘(尽管不准确)CDR。常用的方法如 Kabat、Chothia、AbM 和接触-描绘(contact definition)。

Kabat 描绘(Kabat definition)是最常用的 CDR 区域预测描绘方法，它基于序列可变性。(Johnson and Wu, *Nucleic Acids Res*, 28: 214-8 (2000)) Chothia 描绘(Chothia definition)基于 loop 结构的位置。(Chothia 等, *J Mol Biol*, 196: 901-17 (1986); Chothia 等, *Nature*, 342: 877-83 (1989))。 AbM 描绘(AbM definition)介于上述两者之间，它是一整套用于抗体结构模型的方法，来自 Oxford Molecular Group (Martin 等, *Proc Natl Acad Sci (USA)* 86:9268-9272 (1989); Rees, 等, "ABM™, 一种抗体可变区模型软件" ABM™, a computer program for modeling variable regions of antibodies, Oxford, UK; Oxford Molecular, Ltd.)。它联合使用数据库和从头算法，模拟了抗体的一级结构到三级结构。另一种方法接触-描绘，基于对商品化晶体结构复合体的分析。

按照惯例，重链中的 CDR 区域典型称为 H1、H2 和 H3，从氨基末端到羧基末端计数。轻链中的 CDR 区域典型称为 L1、L2 和 L3 从氨基末端到羧基末端计数。

CDR-H1 大约长 10-12 个残基，根据 Chothia 和 AbM 描绘，通常从 Cys 后的 4 个残基处开始，或者根据 Kabat 描绘，从其后 5 个残基开始。典型的 H1 后为 Trp，通常为 Trp-Val，但也有 Trp-Ile 或 Trp-Ala。根据 AbM 描绘 H1 长约 10-12 个残基，但是 Chothia 描绘除去了最后 4 个残基。

根据 Kabat 和 AbM 描绘，CDR-H2 通常从 H1 末端后的 15 个残基开始。H2 前面的典型残基为 Leu-Glu-Trp-Ile-Gly，但是也有很多变化。H2 后的典型序列为 Lys/Arg-Leu/Ile/Val/Phe/Thr/Ala-Thr/Ser/Ile/Ala。根据 Kabat 描绘，H2 长约 16-19 个残基，而 AbM

描绘预测它的典型长度为 9-12 个残基。

CDR-H3 典型在 H2 末端后的 33 个残基开始，它前面的典型序列为 Cys-Ala-Arg。H3 后通常为 Gly。H3 的长度在 3-25 残基之间。

CDR-L1 通常从约 24 号残基开始，前面通常为 Cys。通常 CDR-L1 后的残基是 Trp，其后面的典型序列为 Trp-Tyr-Gln、Trp-Leu-Gln、Trp-Phe-Gln 或 Trp-Tyr-Leu。CDR-L1 长约 10-17 残基。本发明抗体的推定性（punitive）CDR-L1 严格遵守这个模式，位于 Cys 残基之后，其后面跟随 15 个氨基酸，然后才是 Trp-Tyr-Gln。

CDR-L2 约开始于 L1 末端后的约 16 个残基处。它前面的残基通常为 Ile-Tyr、Val-Tyr、Ile-Lys 或 Ile-Phe，其长度约为 7 个残基。

CDR-L3 通常开始于 L2 末端后 33 个残基处，它前面的典型残基为 Cys。L3 后面通常为 Phe-Gly-XXX-Gly 序列，其长度约为 7-11 个残基。

本领域中已有多种抗体修饰方法。例如美国专利 5,530,101 (Queen 等, June 25, 1996)描述了产生人源化抗体的方法，其中人源化免疫球蛋白重链可变区架构区的序列与来源免疫球蛋白相应序列有 65-95% 的同一性。每个人源化免疫球蛋白链除了 CDR 外，通常还包含了来源免疫球蛋白架构区中(例如)能够与 CDR 相互作用从而影响结合亲和力的残基，例如来源免疫球蛋白中与 CDR 直接相邻的残基，或者是那些(与 CDR)相距 3 埃以内的残基(如分子模型技术所预测的)。当组合成一个完整抗体后，本发明的人源化免疫球蛋白将对人体基本没有免疫原性，但保持了与来源免疫球蛋白基本相同的对抗原(例如含有表位的蛋白或其它化合物)的亲和力。相关方法见美国专利 5,693,761(Queen, 等, 授权于(issued)12 月 2 日，1997 ，“编码具有改善人源化性能的免疫球蛋白的多核苷酸” “Polynucleotides encoding improved humanized immunoglobulins”); 美国专利 5,693,762(Queen, 等, 授权于 12 月 2 日，1997 ，“人源化免疫球蛋白” “Humanized Immunoglobulins”); 美国专利 5,585,089(Queen, 等, 授权于 12 月 17

日，1996，“人源化免疫球蛋白” “Humanized Immunoglobulins”)。

例如美国专利 5,565,332(Hoogenboom 等，授权于 10 月 15 日，1996，“嵌合抗体的制备-一种组合方法” “Production of chimeric antibodies - a combinatorial approach”)中描述了制备具有与亲代抗体相似的结合活性但人类抗体特征减少的抗体(和抗体片段)的方法。使用如噬菌体展示技术、链重排(shuffling)来得到人源化抗体，包含有非人类抗体的重链(或轻链)可变区，并特异于目的抗原的多肽与人类互补(轻或重)链可变区库进行组合。鉴别出特异于目的抗原的杂交对，来自所选杂交对的人类链与人类互补(轻或重)链可变区库进行组合。在另一实施方案中，来自非人类抗体的 CDR 成分与来自人类的 CDR 成分库进行组合。从所得抗体多肽二聚物库中选择杂种并用以进行第二轮人源化洗牌程序。或者不进行这个步骤，前提是杂种已的人类特征已经足够进行治疗。也有文献描述了进行修饰以提高人类特征的方法，也见 Winter, *FEBS Letts* 430:92-92 (1998)。

又例如美国专利 5,766,886(Studnicka 等, 授权于 6 月 16 日，1998，“修饰的抗体可变区” “Modified antibody variable domains”)描述的方法可鉴别抗体可变区中的这样一种残基，它们可被修饰而不减少抗原结合结构域的天然亲和力，同时减少了抗体对异类动物的免疫原性，该文同时也描述了制备这种修饰的抗体可变区的方法，修饰后可的可变区有助于将抗体导入异类动物。也见美国专利 5,869,619(Studnicka ，授权于 2 月 9 日, 1999)。

如本文所述，使用本领域任何已知方法来修饰抗体的目的通常为提高抗体对抗原的结合亲和力和/或降低抗体对受者的免疫原性。在一种方法中，可修饰人源化抗体以除去糖基化位点，从而提高抗体对其同类抗原的亲和力(Co 等, *Mol Immunol* 30:1361-1367 (1993))。用来制备具有更高治疗能力的人源化抗体的方法例如重塑法(”reshaping”)、超嵌合法(“hyperchimerization”)和镶面/重塑表面法(“veneering/resurfacing”)。(Vaswami 等, *Annals of Allergy, Asthma, &*

Immunol 81:105 (1998)； Roguska 等, *Prot Engineer* 9:895-904 (1996))。也见美国专利6,072,035(Hardman 等,授权于6月6日, 2000)其中描述了重塑抗体的方法。虽然这些技术通过减少外源残基的数目降低了抗体的免疫原性，但它们不能防止在重复给予这些抗体后发生的抗独特型和抗同种异型抗原反应。除这些方法外，其它可选降低免疫原性的方法见 Gilliland 等, *J Immunol* 62(6): 3663-71 (1999)。

在许多例子中，人源化抗体引起了抗原结合能力的丢失。因此优选对人源化抗体进行“恢复突变”(“back mutate”)，以在人源化抗体中包含进一个或多个原有抗体(绝大多数情况下为啮齿类)中的氨基酸残基，从而试图恢复抗体的结合亲和力。见如 Saldanha 等, *Mol Immunol* 36:709-19 (1999)。

非肽类特异性结合剂的类似物/拟蛋白(Protein Mimetic)

另外本发明也考虑了肽类特异性结合剂的非肽类似物，它提供了稳定化的结构或者减弱的生物降解作用。从选出的抑制肽出发，将其中一个或多个残基替换为非肽成分，从而制备特异性结合剂的拟肽类似物。非肽成分优选使得肽保持其天然构象，或者可稳定一个优选的(例如生物活性)构象，而该构象保持了识别并结合 Ang-2 的能力。一方面所得类似物/模拟物对 Ang-2 具有提高的结合亲和力。文献 Nachman 等, *Regul Pept* 57:359-370 (1995)描述了从肽类特异性结合剂出发制备非肽模拟类似物的方法。如果需要可对本发明的肽特异性结合剂进行修饰，例如糖基化、酰胺化、羧化或者磷酸化，或者形成本发明肽的酸加成盐、酰胺、酯，特别是 C-末端酯以及 N-酰基衍生物。也可使肽类特异性结合剂与其他成分一起形成共价或非共价复合体来进行修饰。可将化学成分连接到肽类特异性结合剂侧链的功能基团上，或者连接到 N-末端或 C-末端上来形成共价结合复合体。

特别的，预测肽类特异性结合剂可以与受体基团进行偶联，受体基团包括(但不仅限于)放射性标记、荧光标记、酶(如催化比色反应或荧光反应的酶)、底物、固体基质或者载体(例如生物素或抗生物素)。因此本发明提供了包含抗体分子的分子，该分子优选还包含受体选自以下物质的受体基团：放射性标记、荧光标记、酶、底物、固体基质或者载体。这些标记为本领域技术人员所熟知，例如尤其可考虑使用生物素标记。本领域技术人员也熟知对这些标记的使用，相关描述见美国专利3,817,837；美国专利 No. 3,850,752；美国专利 No. 3,996,345；美国专利 No. 4,277,437。其它可使用的标记包括(但不仅限于)放射性标记、荧光标记和化学发光标记。涉及到这些标记使用的美国专利包括美国专利 No. 3,817,837；美国专利 No. 3,850,752；美国专利 No. 3,939,350；美国专利 No. 3,996,345。本发明的任何肽都可包含这些标记的任意种类，标记数目可为一个、两个或更多。

特异性结合剂制备方法

本发明的蛋白类特异性结合剂可根据常规方法在溶液或固体支持物上通过化学合成而制备。目前固相合成蛋白的长度上限约为 85-100 个氨基酸残基。但是经常使用化学合成技术将一系列较小的肽组合成全长多肽。有多种商品化自动合成装置，并可按照已知方法来使用。例如见 Stewart and Young, “固相肽合成”，Solid Phase Peptide Synthesis, 2d. ed., Pierce Chemical Co., (1984); Tam 等, *J Am Chem Soc*, 105:6442, (1983); Merrifield, Science, 232:341-347, (1986); Barany and Merrifield, The Peptides, Gross and Meienhofer, eds, Academic Press, New York, 1-284; Barany 等, Int. J. Peptide Protein Res., 30, 705-739 (1987)；美国专利 No. 5,424,398，这些文献均通过引用并入本文。

固相肽合成方法使用共聚(苯乙烯-二乙烯基苯)，其中每克聚合物含有 0.1-1.0 mM 胺。这些合成方法使用叔丁氧羰基(t-BOC) 或 9-芴

甲氧羰基(FMOC))保护α-氨基。两种保护方法都为逐步合成，从肽的 C-末端开始每一步加上一个氨基酸(见 Coligan 等, “当前免疫学方法” Current Protocols in Immunology, Wiley Interscience, 1991, unit 9)。在化学合成完成后，可除去 t-BOC 或 FMOC 氨基酸封阻基团以对合成肽去保护，并在低温下用酸处理(例如液体 HF-10%苯甲醚在0℃处理约 1h)以将肽从聚合物上切下。蒸发掉试剂后，使用 1%醋酸溶液从聚合物中提取肽类特异性结合剂，得到粗材料。通常可使用 Sephadex G-15，以 5%醋酸为溶剂进行凝胶过滤等方法来对粗材料进行纯化。对层析柱的合适部分进行冻干，将产生均一的肽类特异性结合剂或肽衍生物，然后可使用标准方法如氨基酸分析、薄层色谱、高效液相色谱、紫外吸收光谱、摩尔旋光度、溶解性检测来对产物进行鉴定，并使用固相 Edman 降解来进行定量。

在抗 Ang-2 抗体、衍生物、变异体(以及这些分子的片段)和其它基于蛋白的 Ang-2 结合剂的化学合成中，允许在结合剂中包含进非天然氨基酸。

重组 DNA 技术是一种便利的方法，可用来制备本发明的抗体和其它蛋白类特异性结合剂(以及它们的片段)。可将编码抗体或其片段的 cDNA 分子插入到表达载体内，然后再将此载体插入到宿主细胞中以产生该抗体或片段。当然可对编码这些抗体的 cDNA 加以修饰以使它们与“原始” cDNA(从 mRNA 翻译而来)不同，从而在不同宿主细胞中提供密码子简并性或允许密码子偏好使用。

一般情况下，可使用本文实施例部分所述方法来得到编码抗体的 DNA 分子。当需要获得原始抗体分子相关的 Fab 片段或 CDR 时，技术人员可以使用标准方法从合适的库中(噬菌体展示文库; 淋巴细胞文库)进行筛选以鉴别并克隆到相关 Fab/CDR。筛选所用的探针可以是编码原始抗体 Fab 部分的全长或部分 Fab 探针，针对原始抗体 Fab 部分中一个或多个 CDR 的探针，或者是其它合适的探针。但使用 DNA 片段作为探针时，典型的杂交条件见 Ausubel et. al. (“精编

分子生物学试验指南”，Current Protocols in Molecular Biology, Current Protocols Press (1994))。根据诸如探针大小、预测的探针与克隆间的同源性、所用文库的类型以及筛选的克隆数目等因素，在合适的严紧性条件下洗涤探针印记。高严紧性筛选条件如 50-65°C ，0.1 X SSC 和 0.1%SDS。

可使用多种表达载体/宿主系统来包含并表达编码本发明特异性结合剂的多核苷酸。这些系统包括(但不仅限于)微生物如用重组噬菌体、质粒或粘粒 DNA 表达载体转化了的细菌；用酵母表达载体转化了的酵母；用病毒表达载体(如杆状病毒)感染了的昆虫细胞；用病毒表达载体(如花椰菜镶嵌病毒 CaMV 和烟草镶嵌病毒 TMV)转染了的或用细菌表达载体(如 Ti 或 pBR322 质粒)转化了的植物细胞系统 ；或者是动物细胞系统。

可有效用在重组特异性结合剂蛋白生产中的哺乳动物细胞包括(但不仅限于)VERO 细胞、HeLa 细胞、中国仓鼠卵巢细胞系（Chinese hamster ovary (CHO) cell lines）、COS 细胞(如 COS-7)、W138、BHK、HepG2、3T3、RIN、MDCK、A549、PC12、K562 和 293，以及本文所述的杂交瘤细胞。在制备如被典型糖基化并需要正确重折叠的抗体和抗体片段这样的特异性结合剂时，优选使用哺乳动物细胞。优选的哺乳动物细胞包括 CHO 细胞、杂交瘤细胞和骨髓细胞。

一些关于特异性结合剂重组表达的例子见于下文。

“表达载体”指用来表达 DNA(RNA)序列的多核苷酸的质粒、病毒或载体。表达载体可包含转录单位，其中包含以下成分的组合，(1)基因元件或在基因表达中具有调节作用的元件，例如启动字或增强子，(2)结构单位或编码被转录到 mRNA 并被翻译为蛋白的特异性结合剂的序列，(3)合适的转录起始和终止序列。用在酵母或真核表达系统的结构单位优选包含前导序列，从而使宿主细胞可将翻译的蛋白分泌到胞外。或者，当重组特异性结合剂蛋白表达没有前导序列或转运(transport)序列时，结构单位可包含氨基酸终止子蛋氨酸残

基。随后可从表达的重组蛋白上切下或保留该残基，以提供特异性结合剂的最终产物。

例如可使用商品化表达系统，按照使用说明书在酵母中重组表达特异性结合剂，如毕赤酵母表达系统（Pichia Expression System）(Invitrogen, San Diego, CA)。这个系统依赖前-α（pre- pro-α）序列进行直接分泌，而插入序列的转录在甲醇的诱导下由乙醇氧化酶(AOX1)进行驱动。

使用如从细菌和哺乳动物细胞培养上清液中纯化肽的方法，从酵母生长培养基中纯化分泌的肽类特异性结合剂。

另一可选方法是，将编码肽类特异性结合剂的 cDNA 克隆到杆状病毒表达系统 pVL1393 中，(PharMingen, San Diego, CA)。可根据使用说明书，在 sF9 无蛋白培养基中使用该载体感染草地贪夜蛾(Spodoptera frugiperda)细胞，以生成重组蛋白。可使用肝磷脂-琼脂糖柱(Pharmacia)从培养基中纯化并浓缩特异性结合剂蛋白。

另一可选方法是，使用昆虫系统表达肽。本领域技术人员熟知用于蛋白表达的昆虫系统。在一个昆虫表达系统中，可使用苜蓿尺蠖核多角体病毒(Autographa californica nuclear polyhedrosis virus (AcNPV))作为载体，在草地贪夜蛾或粉纹夜蛾(Trichoplusia larvae)中表达外源基因。可将肽类特异性结合剂的编码序列克隆到该病毒的非必需区域中，如克隆到如多角体蛋白基因中并置于如多角体蛋白启动子的控制之下。肽类特异性结合剂的成功插入会导致如多角体蛋白基因失活，从而生产缺乏衣壳蛋白(coat protein coat)的重组病毒。可用该重组病毒感染草地贪夜蛾或粉纹夜蛾，外源肽即被表达。(Smith 等, *J Virol* 46: 584 (1983); Engelhard 等, *Proc Nat Acad Sci (USA)* 91: 3224-7 (1994))

在另一实例中，可使用 PCR 扩增肽类特异性结合剂的编码 DNA 序列，并克隆到合适的载体中，如 pGEX-3X (Pharmacia)。pGEX-3X 载体编码了谷胱甘肽-S-转移酶(GST)，它产生包含此酶和特异性结

合剂蛋白(由插入到该载体克隆位点的 DNA 插入片段所编码)的融合蛋白。PCR 引物可包含如合适的裂解位点。当与特异性结合剂融合的部分是仅用以促进表达时，或者不是它插入蛋白所需的连接物时，可从融合蛋白中 GST 部分上切下重组特异性结合剂。使用 pGEX-3X/特异性结合剂肽转化大肠杆菌 XL-1 Blue 细胞 (Stratagene, La Jolla CA)，分离单转化子病进行培养。可从单转化子中纯化质粒 DNA 并使用自动测序仪进行部分测序，以确认特异性结合剂编码核酸是否以正确方向被插入。

使用上文所述重组系统表达编码抗 Ang-2 抗体及其片段，可能会得到需要进行“重折叠”(以建立多种正确的二硫键)以发挥生物活性的抗体或其片段。这些抗体典型的重折叠方法见下节的实施例中所述。

在细菌细胞中制备的特异性结合剂可以细菌不溶性包涵体形式而生成，可采用如下方法进行纯化。可使用离心法处理宿主细胞；用 0.15 M NaCl, 10 mM Tris, pH 8, 1 mM EDTA 进行洗涤; 并用 0.1 mg/ml 溶菌酶(Sigma, St. Louis, MO) 在室温下处理 15 min。可用超声裂解使溶菌液澄清，然后 12,000 Xg 离心 10min 沉淀细胞碎片。用 50 mM Tris，pH 8，和 10 mM EDTA 重悬含有特异性结合剂的沉淀，用 50% 甘油分层，并 6000 Xg 离心 30min。用没有 Mg^{++}和 Ca^{++}的标准磷酸盐溶液(PBS)重悬沉淀。然后可用变性 SDS 聚丙烯酰胺凝胶分离进一步纯化特异性结合剂 (Sambrook 等, *supra*)。 可用 0.4 M KCl 浸泡凝胶以显现蛋白，可切下凝胶并在没有 SDS 的凝胶电泳缓冲液中进行电泳。如果在细菌中产生 GST 融合蛋白(可溶性蛋白)，则可使用 GST Purification Module (Pharmacia)进行纯化。

本领域技术人员熟知使用哺乳动物细胞来表达重组蛋白。可根据加工所表达蛋白或产生某种翻译后修饰(它将有利于产生蛋白活性)的特殊能力来选择宿主细胞系。这种多肽修饰包括(但不仅限于)乙酰化、羧基化、糖基化、磷酸化、脂化和酰化。多种宿主细胞如 CHO、

HeLa、MDCK、293 和杂交瘤细胞系都具有与这种翻译后修饰活性相关的特殊细胞机制和特征机理，可选用它们来确保对所给予外源蛋白的正确修饰和加工。

可使用多种选择系统来回收被转化了的细胞(用于重组蛋白生产)。这些选择系统包括(但不仅限于)分别在 tk-、hgprt-或 aprt-细胞中的 HSV 胸腺嘧啶激酶、次黄嘌呤-鸟嘌呤磷酸核糖转移酶、腺嘌呤磷酸核糖转移酶基因。也可利用抗代谢物抗性来进行选择，DHFR 赋予细胞对氨甲喋呤的抗性；gpt 赋予细胞对霉酚酸的抗性；neo 赋予细胞对氨基糖苷(aminoglycoside) G418 和氯磺隆的抗性；hygro 赋予细胞对潮霉素的抗性。其它可用的选择基因包括 trpB(它允许细胞利用吲哚代替色氨酸)或 hisD(它允许细胞利用组胺醇(histinol)代替组氨酸)。可目测鉴别转化子的标记物包括花青素、β-葡萄糖苷酶及其底物 GUS、荧光素酶及其底物荧光素。

特异性结合剂的纯化和重折叠

在一些情况下，使用上文所述方法产生的特异性结合剂可能需要“重折叠”和氧化，以形成正确的四级结构并建立二硫键以发挥生物活性。可使用本领域已知的多种方法来进行重折叠。这些方法包括如在离液剂存在时使溶解的多肽暴露在 pH7 以上(一般情况下)。离液剂的选择类似于包涵体溶解方面的选择，但是离液剂典型在低浓度下使用。离液剂例如胍。在大多数情况下，重折叠/氧化溶液中也含有还原性试剂及其氧化形式，二者浓度保持在特定比例以产生特定的氧化还原电位，从而使二硫键改组(dusykfide shuffling)得以发生以形成二硫键。通常使用的一些氧化还原分子对包括半胱氨酸/胱胺、谷胱甘肽/二硫 GSH、氯化亚铜、二硫苏糖醇 DTT/ 二噻烷 DTT 和 2-巯基乙醇(bME)/二硫-bME。很多情况下可使用助溶剂以提高重折叠效率。通常使用的助溶剂包括甘油、各种分子量的聚乙二醇和精氨酸。

需要纯化本发明的特异性结合剂蛋白或其变异体。本领域技术人员熟知蛋白纯化方法。这些方法在一种水平上包括多肽和非多肽片段的粗分离。从其它蛋白中分离出特异性结合剂多肽后，可使用色谱和电泳方法纯化目的多肽，以达到部分或完整纯化(或纯化到同质(homogeneity))。特别适合于纯特异性结合剂肽的分析方法是离子交换色谱、排阻色谱法、聚丙烯酰胺电泳、等电点聚焦。一种特别有效的肽纯化方法是快速蛋白液相色谱或 HPLC。

本发明的某些方面涉及到纯化，在特定实施方案中涉及到特异性结合剂蛋白或肽的基本提纯。这里所用的“纯化的特异性结合剂蛋白或肽”是指从其它成分分离的组合物，其中特异性结合剂蛋白或肽被纯化到相对于其天然可得状态的任何程度。因此纯化的特异相结合剂蛋白或肽，也指脱离其可能天然存在环境的特异性结合剂蛋白或肽。

通常“纯化的”指已被分离除去了多种其它成分的特异性结合剂组合物，并且该组合物保持其被表达的充分生物活性。所用的“基本提纯的”指特异性结合剂蛋白或肽为主要组合物成分的特异性结合剂组合物，例如特异性结合剂蛋白或肽占组合物蛋白的比例为约50%、60%、70%、80%、90%、95%或更高。

根据本发明本领域技术人员将了解多种用于定量特异性结合剂纯化程度的方法。这些方法包括如确定部分纯化物的特异结合活性，或者通过 SDS/PAGE 在一部分纯化物中检测特异性结合剂多肽的量。评价特异性结合剂的一部分纯化物纯化程度的优选方法是计算该部分的结合剂活性，并于起始提取物进行比较，从而计算出纯化程度，这里记为“纯化倍数”。当然用以代表结合活性的实际单位依赖于纯化后所选用的具体检测方法，以及表达的纯化结合剂蛋白或肽是否展示出可检测性结合活性。

本领域技术人员熟知多种可用于特异性结合剂蛋白纯化的方法。这些方法包括如硫酸铵沉淀、PEG、抗体法(免疫沉淀)、或者着热变

性后离心、色谱法如亲和层析(如 A 蛋白-Sepharose)、离子交换层析、反相色谱、凝胶过滤、羟基磷灰石和亲和层析、等电点聚焦、凝胶电泳以及这些方法的联用。如本领域公知的，不同纯化步骤次序可发生变化，或者某些步骤可被省略，仍可得到合适的纯化方法以得到基本纯化特异性结合剂。

通常并不要求总是提供最纯状态的特异性结合剂。事实上在特定实施方案中可考虑使用特异性不是特别高的结合剂产品。可在联合方法中使用较少的纯化步骤或使用相同通过纯化方法中的不同形式，来得到部分纯化物。例如，使用 HPLC 装置进行阳离子交换柱层析所得纯化倍数一般高于使用低压色谱系统进行同样操作所得倍数。具有相对较低纯化程度的方法可能对总回收特异性结合剂蛋白产物有利，或者有利于保持表达的特异性结合剂蛋白的结合活性。

已知多肽在不同 SDS/PAGE 条件下的迁移可能不同，有时显著不同 (Capaldi 等, *Biochem Biophys \ Res Comm*, 76: 425 (1977))。因此在不同电泳条件下，纯化或部分纯化的特异性结合剂表达产物的表观分子量当然可能不同。

结合实验

免疫结合实验通常使用捕获试剂来特异性结合于并经常固定住被分析物的靶抗原。捕获试剂是特异性结合于被分析物的基团。在本发明的一个实施方案中，捕获试剂是特异性结合于 Ang-2 的抗体或其片段。本领域熟知这些免疫结合实验(Asai, ed., “细胞生物学方法” Methods in Cell Biology, Vol. 37, “细胞生物学中的抗体” Antibodies in Cell Biology, Academic Press, Inc., New York (1993))。

免疫结合实验经常使用标记物以标出捕获试剂和抗原形成的固定复合体的存在。标记物可以是包括固定复合体的分子中的一个，即标记物可以是被标记的特异性结合剂或被标记的抗特异性结合剂的抗体。或者标记物可以是第三种分子，通常是第三种抗体，它结合

于固定复合体。标记物可以是如携带标记的抗特异性结合剂的抗体。第二种抗体特异于固定复合体，可没有标记，但是它可被第四种分子所固定，而这种分子特异于第二种抗体所属种类的抗体。例如，第二种抗体可以修饰加上一个可检测的基团如生物素，该基团可被第四种分子所固定，例如标记了的链霉抗生物素。其它能够特异结合免疫球蛋白恒定区的蛋白，如A蛋白或G蛋白也可被用作标记物。这些标记蛋白通常是链球菌细胞壁的成分，展示出与多种免疫球蛋白恒定区的强烈非免疫反应活力(通常见 Akerstrom, *J Immunol*, 135:2589-2542 (1985); Chaubert, *Mod Pathol*, 10:585-591 (1997))

贯穿这些实验，可能在每一次物质反应后都需要温育和/或洗涤步骤。温育时间可能从5s到数小时不等，优选从约5min到约24h。但是温育时间依赖于检测形式、被分析物、溶液体积、浓度等因素。通常这些检测在环境温度下进行，不过也可在一个温度范围内进行。

A.非竞争性结合实验:

免疫结合实验可以使非竞争性结合实验。这些实验中有许多被捕获的分析物，对它们进行直接测量。例如在一种优选的“三明治”实验中，捕获试剂(抗体)可被直接固定在固体基质上。这些固定化抗体然后被待测样品中的抗原所捕获(结合)。这样固定的蛋白然后被固定到标记物上，例如携带标记的第二种抗体。在另一优选的“三明治”实验中，第二种抗体没有携带标记，但是可被携带标记的另一抗体所固定，这种抗体特异于第二种抗体所属种类的抗体。第二种抗体也可被修饰添加上可检测的基团例如生物素，第三种被标记的分子可特异性结合于该基团上(见 Harlow and Lane, “抗体实验手册” Antibodies, A Laboratory Manual, Ch 14, Cold Spring Harbor Laboratory, NY (1988),通过引用并入本文)。

B.竞争性结合实验

免疫结合实验可以是竞争性结合实验。通过测量额外添加分析物中被样品中分析物所取代或竞争掉的量，来间接测量样品中存在的分析物的量。在一个优选的竞争性结合实验中，向样品中添加已知量的分析物(通常被标记)，然后使样品与一种抗体(捕获试剂)接触。被该抗体所固定的标记量与样品中分析物的量成反比(见 See, Harlow and Lane, "抗体实验指南"Antibodies, A Laboratory Manual, Ch 14, pp. 579-583, *supra*)。

在另一优选的竞争性结合实验中，抗体被固定在固体基质上。可通过测量蛋白/抗体复合体中蛋白的量或者测量未与抗体结合的剩余蛋白的量，来确定固定在抗体上的蛋白量。可通过提供被标记蛋白来检测蛋白量(见 See, Harlow and Lane, "抗体实验指南" Antibodies, A Laboratory Manual, Ch 14, pp. 579-583, *supra*)。

在另一优选的竞争性结合实验中利用半抗原抑制。这里将已知分析物固定在固体基质上。向样品中加入已知量的抗体，然后使样品与固定的分析物相接触。被固定分析物所捕获的抗体量与样品中分析物的量成反比。可通过检测被固定的抗体量或者检测溶液中剩余的抗体量来检测固定的抗体量。可进行直接检测，这时抗体被标记，或者随后加入被标记了的基团(它特异于该抗体，如上文所述)来进行间接检测。

C.竞争性结合实验的使用

技术人员可使用竞争性结合实验确定交叉反应活性，以确定被本发明特异性结合剂所识别的蛋白或酶复合体是否为所需蛋白，并且不是交叉反应分子，或者确定抗体是否特异于抗原并且不结合于非目的抗原上。在这种类型的实验中，抗原可被固定到固体支持物上，并向实验液中添加未知的蛋白混合物，该混合物将与特异性结合剂对固定的蛋白发生竞争性结合。竞争分子也结合于与目的抗原无关的一种或多种抗原上。将蛋白与特异性结合剂抗体间的对固定抗原

的结合竞争能力，与固体支持物上所固定的相同蛋白的结合进行比较，以确定蛋白混合物的交叉反应活性。

D.其它结合实验

本发明也提供了 Western 印迹方法来检测或量化样品中的 Ang-2。该方法步骤通常包括根据分子量使用凝胶电泳分离样品蛋白，将蛋白转移到合适的固体支持物上，如硝化纤维膜、尼龙膜或衍生尼龙膜。然后样品与特异结合 Ang-2 的抗体或其片段共温育，并检测所得复合体。这些抗体可被直接标记，或者随后使用特异结合第一种抗体的被标记抗体进行检测。

检测那些破坏 Ang-2 与其受体结合作用的 Ang-2 特异性结合剂的结合实验，见本文实施实例部分。

诊断实验

本发明的抗体或其片段有利于对特征为 Ang-2(或其亚基)表达的症状或疾病的诊断，或者有利于在实验中对被 Ang-2 诱导物及其片段、Ang-2 活性拮抗剂或抑制剂所处理患者的监测。Ang-2 诊断实验方法包括使用特异性结合剂和标记物以检测人体液或细胞(或组织)提取物中的 Ang-2。使用本发明的特异性结合剂时可加以修饰或者不修饰。在优选的诊断实验中，通过连接上如标记物或受体分子来对特异性结合剂进行标记。已知有多种标记物和受体分子，其中一些已在本文中被描述。本发明对人类疾病尤其有效。

本领域已知多种使用特异于各自受体蛋白的多抗或单抗来测量 Ang-2 蛋白的方法。包括如酶联免疫反应(ELISA)，放射性免疫测定(RIA)和荧光激活细胞分选术(fluorescence activated cell sorting，FACS)。 优选使用一种基于单抗的双位点免疫测定方法，其中使用了可与 Ang-2 上两个互不干扰的表位发生反应的单抗，不过可使用竞争性结合实验。这些实验的描述见如 Maddox 等, *J Exp Med*,

158:1211 (1983)。

为了向诊断提供依据，通常建立人体正常或标准 Ang-2 表达值。可在本领域熟知的适于复合体形成的条件下，联合使用正常受试者(优选为人类)的体液或细胞提取物及 Ang-2 的特异性结合剂(如抗体)来建立该值。可通过将特异性结合剂与已知量的 Ang-2 蛋白的结合作用，与对照和疾病样本的相应作用进行对比并量化对比结果，来量化标准复合体的形成作用。然后可将从正常样本得到的标准值，与从可能被感染的受试者得到的样本值加以对比。标准值和受试者值之间的偏差提示了 Ang-2 在疾病状态中的作用。

在某些实施方案中，为了诊断目的典型将特异性结合剂用可检测性基团加以标记。这种可检测性基团可以是任何可直接或间接产生可检测性信号的分子/基团。例如为放射性同位素如 ^{3}H、^{14}C、^{32}P、^{35}S、^{125}I；荧光或化学发光化合物如荧光异硫氰酸盐、若丹明或虫萤光素；或酶如碱性磷酸酶、β-半乳糖苷酶或辣根过氧化物酶 (Bayer 等, *Meth Enz*, 184: 138-163, (1990))。

疾病

本发明提供了有效于治疗人类疾病和改善症状的结合于 Ang-2 的特异性结合剂。可联合使用可调节 Ang-2 结合活性或其它细胞活性的物质与其它治疗药物，以增强它们的治疗作用或降低可能的副作用。

一方面，本发明提供了有效于治疗特征为细胞中非必需或异常水平 Ang-2 活性的疾病或症状的药物和方法。这些疾病包括癌症和其它过度增生症状，如增生、牛皮癣、接触性皮炎、免疫失调和不育不孕症。

本发明也提供了治疗动物(包括人类)癌症的方法，步骤包括向动物给予有效剂量的可抑制或降低 Ang-2 活性的特异性结合剂。本发明也涉及到抑制癌细胞生长(过程包括细胞增殖、入侵和生物系统中

转移)的方法。这些方法包括将本发明的化合物用作癌细胞生长抑制剂。优选使用这些方法来抑制或减少活体动物如哺乳动物体内的癌细胞生长、入侵、转移或肿瘤范围。本发明的方法也可在检测系统中方便的加以调节，如在癌细胞生长及其特性的检测中，和对可影响癌细胞生长的化合物的鉴别中。

可使用本发明的方法治疗的癌症优选为哺乳动物癌症。哺乳动物包括如人和其它灵长类，宠物或伴侣动物如狗和猫，实验室动物如大鼠、小鼠和兔子，以及农场动物如马、猪、绵羊和牛。

肿瘤或赘生物包括细胞增殖不受控制并具有入侵性的组织细胞生长。这种生长的其中一些是良性的，可是另一些预期是恶性的，并可能引起有机体的死亡。恶性肿瘤或癌症与良性生长的区别在于，癌细胞除了表现出入侵性细胞增殖外，它们可能侵入周围组织病发生转移。另外恶性肿瘤的特征还在于它们的分化能力丧失更多(退行发育程度更大)，以及相对彼此和它们周围的组织，它们的组织程度下降更多。这个特性被称为“退行性发育”。

可使用本发明内容进行治疗的肿瘤包括实体肿瘤(solid tumor)，即癌(carcinomas)和肉瘤(sarcomas)。癌包括那些起源于渗透(入侵)到周围组织并引起转移的上皮细胞的恶性肿瘤。腺癌起源于腺体组织，或者起源于形成可识别腺体结构的组织。另一大类癌包括肉瘤，这种肿瘤的细胞埋入纤维质或均质体中，例如胚胎结缔组织。本发明也可治疗骨髓系统或淋巴系统癌症，包括白血病、淋巴瘤和其它典型不以肿瘤块形式出现而在血管或淋巴管系统中分布存在的癌症。

本发明可有效治疗的癌症或肿瘤类型包括如产 ACTH 肿瘤、急性淋巴细胞性白血病、急性非淋巴细胞性白血病、肾上腺皮质癌、膀胱癌、脑癌、乳癌、宫颈癌、慢性淋巴细胞性白血病、慢性髓细胞白血病、结肠癌、皮肤 T 细胞淋巴瘤、子宫内膜癌、食道癌、尤文氏肉瘤、胆囊癌、毛细胞白血病、头颈癌、何杰金瘤、Kaposi 肉瘤、肾癌、肝癌、肺癌 (小和非小细胞)、恶性腹腔积液、恶性胸腔

积液、黑素瘤、间皮瘤、多发性骨髓瘤、成神经细胞瘤、神经胶质瘤、非何杰金瘤、骨肉瘤、卵巢癌、生殖细胞卵巢癌、胰腺癌、阴茎癌、前列腺癌、眼癌、皮肤癌、软组织瘤、鳞状细胞癌、胃癌、睾丸癌、甲状腺癌、滋养细胞肿瘤、子宫癌、阴道癌、阴户癌和肾母细胞瘤。

本文中特别举例阐明了某些本发明可治疗的癌症。在这些说明性的实例中，使用当前科技水平上标准的体外和体内模型。这些方法可被用来鉴别那些被预测对体内治疗有效的试剂。但是本发明的方法当然不仅限于只可治疗这些类型的肿瘤，本发明的方法可用来治疗起源于任何器官系统的任何实体肿瘤。入侵或转移与 Ang-2 表达(或活性)相关的癌症，尤其容易被本方法的方法所抑制，或者甚至被诱导而恢复。

应用本发明的方式也可以是使用本发明的特异性结合剂如抗体。将本发明的特异性结合剂与其它抗癌化疗药物联合使用，例如任何常规的化疗药物。联合使用特异性结合剂和化疗药物从而可以增强化疗方法的效果。本领域技术人员将发现许多能够被包含进本发明方法的化疗方法。可使用任何化疗药物，包括烷基化药物、抗代谢物、激素和拮抗剂、放射性同位素和天然产物。例如本发明的化合物可以以下药物联合给予，抗生素如阿霉素和其它蒽环类似物，氮芥例如环磷酰胺，嘧啶类似物例如 5-氟尿嘧啶，顺铂，羟基脲，紫杉醇及其天然或合成衍生物等。又例如在治疗混合物瘤如乳腺癌时(肿瘤细胞包括促性腺激素依赖和不依赖促性腺激素两种细胞)，本发明化合物可以与亮丙瑞林(leuprolide)或戈舍瑞林(goserelin)(LH-RH 的合成类似物)一起给药。其它抗肿瘤方法包括联合使用四环素化合物和其它治疗形式(如手术、放疗等，在本文中也称作“辅助抗肿瘤方法”)。因此可将本发明的方法与这些常规治疗方法联合使用，以减少副作用改善治疗效果。

因此，本发明提供了可有效于许多种癌症(包括实体瘤和白血病)

治疗的组合物和方法。可治疗的癌症类型包括(但不仅限于): 乳腺癌、前列腺癌和结肠癌; 所有形式的肺支气管癌; 骨髓瘤; 黑素瘤; 肝细胞瘤; 成神经细胞瘤; 乳突淋瘤; APUD 肿瘤; 迷芽瘤; 鳃原性瘤; 恶性综合征(malignant carcinoid syndrome); 类癌性心脏病; 癌症(如 Walker 癌、基底细胞癌、基层鳞状细胞癌、Brown-Pearce 癌、导管癌, Ehrlich 瘤, Krebs 2, merkel 细胞癌, 粘液性癌, 非细胞细胞肺癌, 燕麦细胞肺癌, 乳头癌, 硬癌, 支气管癌, 支气管肺癌, 鳞状细胞癌, 移行细胞癌); 组织细胞失调; 白血病; 恶性组织细胞增多; 何杰金瘤; 非小细胞免疫增生性肺癌; 非何杰金淋巴瘤; 浆细胞瘤; 网状内皮增生症; 黑素瘤; 脂肪瘤; 软骨瘤; 软骨骨肉瘤 ; 纤维瘤; 纤维肉瘤; 巨细胞瘤 ; 组织细胞瘤; 脂肪瘤; 脂肪肉瘤; 间皮瘤; 粘液瘤; 粘液肉瘤; 骨瘤; 骨肉瘤; 脊索瘤; 颅咽管瘤; 无性细胞瘤; 错构瘤; 间质瘤; 中肾瘤; 肌肉瘤; 造釉细胞瘤; 牙骨质瘤; 牙瘤; 畸胎瘤; 胸腺瘤; 滋养细胞肿瘤（tophoblastic tumor）。其它可治疗的癌症种类为 : 腺瘤; 胆管瘤; 珠光瘤; cyclindroma; 囊腺癌; 囊腺瘤; 粒层细胞瘤; 两性胚细胞瘤; 肝细胞瘤; 汗腺腺瘤; 胰岛细胞瘤; 间质细胞瘤; 乳突淋瘤; 塞尔托利氏细胞瘤 ; 卵泡膜细胞瘤; 平滑肌瘤; 平滑肌瘤; 成肌细胞瘤; 神经胶质瘤; 肌肉瘤; 横纹肌瘤; 横纹肌肉瘤; 室鼓膜瘤; 神经节细胞瘤; 神经胶质瘤; 成神经管细胞瘤; 脑膜瘤; 神经鞘瘤; 成神经细胞瘤; 上皮瘤; 纤维神经瘤; 神经瘤; 副神经节瘤; 非嗜铬副神经节瘤; 血管胶质瘤; 血管淋巴样增生伴嗜酸性白细胞增多; 硬化型血管瘤; 血管瘤; 血管球瘤; 血管内皮瘤; 血管瘤; 血管外皮细胞瘤; 血管肉瘤; 淋巴管瘤; 淋巴管肌瘤; 淋巴管肉瘤; 松果体瘤; 癌肉瘤; 软骨肉瘤; 叶状囊肉瘤; 纤维肉瘤; 血管肉瘤; 平滑肌肉瘤; 白色肉瘤; 脂肪肉瘤; 淋巴管肉瘤; 肌肉瘤; 粘液肉瘤; 卵巢癌; 横纹肌肉瘤; 肉瘤; 瘤; 神经纤维瘤病; 和宫颈异常。

另外本发明的材料和方法也可用于预防和/或治疗任何皮肤过度

增生，包括牛皮癣和接触性皮炎以及其它过度增生性疾病。已经证实牛皮癣和接触性皮炎患者的 Ang-2 活性在这些疾病状态中提高(Ogoshi 等, *J. Inv. Dermatol.*, 110:818-23 (1998))。优选联合使用特异于 Ang-2 的特异性结合剂和其它药物来治疗表现出这些临床症状的患者。可使用多种载体通过本文所属的途径和其它本领域技术人员熟知的途径将特异性结合剂给予患者体内。

另外本发明包括治疗涉及到血管生成的视网膜病(包括糖尿病视网膜病和年龄相关的黄斑变性)以及女性生殖道紊乱/疾病，例如子宫内膜异位、子宫纤维瘤和其它与女性生殖循环中机能不良血管增生增生(包括子宫内膜微脉管生长)相关的此类症状。

另外本发明也涉及到治疗非正常血管生长，包括脑动静脉畸形(AVM)，胃肠黏膜损害与修复，具有消化器官溃疡病史(包括)的患者的胃十二指肠黏膜溃疡，包括由心脏病发作引起的局部缺血，肝病中的一大类肺部血管失调和患非肝性门静脉高压症患者的门静脉高压症。

本发明也涉及到使用本发明的组合物和方法来预防癌症。这些药物将包括 Ang-2 的特异性结合剂。

药用组合物

本发明也包括 Ang-2 特异性结合剂的药用组合物。药用组合物包含了如美国专利 6,171,586(Lam 等,授权于 1 月 9 日, 2001)中详述的抗体。这些组合物中包含了有效治疗或预防剂量的特异性结合剂(例如本文所述的抗体或抗体的片段、变异体、衍生物和融合蛋白)，它与药学上可接受的试剂形成混合物。在一个优选的实施方案中，药用组合物包含了起拮抗作用的特异性结合剂，它部分或完全调节至少一种 Ang-2 活性，并与药学上可接受的试剂形成混合物。典型的，该特异性结合剂被充分纯化以允许导入动物体内。

药用组合物可包含制剂材料以修饰、维持或保持组合物的性质，

如 pH、摩尔渗透压浓度、粘性、透明度、颜色、等渗性、气味、无菌性、稳定性、分散或释放速率、吸附或渗透特性。合适的制剂材料包括(但不仅限于)氨基酸(如甘氨酸、谷氨酸、天冬氨酸、精氨酸或赖氨酸); 抗菌剂、抗氧化剂(如抗坏血酸、亚硫酸钠或亚硫酸氢钠)、缓冲液(如硼酸盐缓冲液、种碳酸盐缓冲液、Tris-HCl、柠檬酸盐缓冲液、磷酸盐缓冲液、其它有机酸缓冲液)、湿胀剂(如甘露醇或甘氨酸)、螯合剂(如 EDTA)、复合剂 (如咖啡因、聚乙烯吡咯烷酮、β-环式糊精或羟基丙基-β-环式糊精)、填充剂、单糖、二糖和其它碳水化合物(如葡萄糖、甘露糖、或糊精)、蛋白(如血清白蛋白、白明胶或免疫球蛋白)、着色剂、调味剂或稀释剂、乳化剂、亲水聚合物(如聚乙烯吡咯烷酮)、低分子量多肽、成盐反离子（salt-forming counter ions）（如钠)、防腐剂(如氯苯甲烷铵、安息香酸、水杨酸、硫汞撒、苯乙基乙醇、羟苯甲酯、羟苯丙酯、双氯苯双胍己烷、山梨酸或双氧水)、溶剂(如甘油、丙二醇或聚乙二醇)、糖醇 (如甘露醇或山梨醇)、悬浮剂、表面活性剂或润湿剂(如普朗尼克类(pluronics)、PEG、山梨聚糖酯、聚山梨醇酯如聚山梨醇酯 20、聚山梨醇酯 80、triton、氨基丁酸、卵磷脂、胆固醇、泰洛沙泊)、增稳剂(蔗糖或山梨醇)、张度增强剂(tonicity enhancing agents)(如碱金属卤化物 (优选氯化钾或钾、甘露醇、山梨醇))、运输载体、稀释剂、赋形剂和/或药用佐剂 (Remington's Pharmaceutical Sciences,18th Edition,A.R. Gennaro,ed.,Mack Publishing Company,1990)。

本领域技术人员可根据如目的给药途径、运输形式和所需剂量来确定最优的药用组合物。如见上文的 Remington's Pharmaceutical Sciences。这样的组合物可以影响特异性结合剂的物理状体、稳定性、体内释放速率、体内清除率。

药用组合物中的初级媒介或载体可以为天然水成或者非水成的。例如合适的媒介物或载体可以为用于注射的水、生理盐水或人工脑脊髓液，并可以补充如其它在组合物中常用的注射给药材料。载体

又例如为中性盐缓冲液或血清白蛋白-盐混合液。其它药用组合物的例子包含 Tirs 缓冲液(pH 约 7.0-8.5)或醋酸缓冲液(pH 约 4.0-5.5)，还可进一步包含山梨醇或其合适的替代物。在本发明的一个实施方案中，制备储存用的结合剂组合物时，可将选出的组合物(具有所需纯化度)与任选冻干块或水溶液形式的赋形剂(Remington's Pharmaceutical Sciences, *supra*)相混合。然后可以使用合适的赋形剂如蔗糖，将此结合剂产品制成冷冻干产物。

可选择通过注射方式给予药用组合物。或者通过呼吸道或肠道给药，例如口服给药，耳部给药，眼部给药(opthlmically)，直肠给药，或者阴道内给药。本领域技术人员了解这种药学上可接受的组合物的制备方法。

制剂成分的浓度应可被导入位置所接受。例如，用缓冲液将组合物的维持在生理 pH 或稍低的 pH 初，典型在约 5-8 之间。

当考虑通过注射给药时，本发明中所用的治疗组合物可以为无热源且可注射的水溶液形式，其中包含了所需的特异性结合剂以及容纳它的药学上可接受的载体。特别合适的注射用载体为无菌蒸馏水，特异性结合剂在其中被制成无菌无离子溶液，并妥善保存。可采用的另一种方式是将联合使用所需分子与一种试剂，例如可注射的微球体，可生物降解的颗粒，聚合物(聚乳酸，聚羟基乙酸)，珠子或脂质体，这些试剂使得所需产物发生控制的或持续释放，制成后将制剂通过储库型注射给药。也可使用透明质酸，它具有延长(药物)循环的持续时间的功能。所需分子的其它合适给药方式包括移植药物输送装置。

另一方面，适合注射给药的药用组合物也可制成水溶液剂型，优选在生理条件可容的缓冲液中，例如 Hanks 溶液，ringer 溶液，或生理盐水缓冲液。注射用水悬液可包含提高悬液粘性的物质，例如羧甲基纤维素钠、山梨醇和右旋糖苷。另外，活性混合物悬液也可制成合适的油性悬液形式。合适的亲脂溶剂或载体包括脂肪例如芝麻

油，合成脂肪酸酯例如乙基油酸、甘油三酸酯，脂质体。输送也可使用非脂质聚阳离子氨基聚合物(Non-lipid polycationic amino polymers)。悬液可任选包含合适的稳定剂或能够提高化合物溶解性的物质，以允许进行高浓度溶液制备。

在另一实施方案中可将药用组合物制成呼吸给药的形式。例如将特异性结合剂制成可吸入的干粉。也可将多肽或核酸分子吸入溶液与抛射剂制成气雾剂形式。在另一实施方案中，可将溶液制成喷雾。此外，肺部给药内容见 PCT 申请 No. PCT/US94/001875，其中描述了通过肺部给予化学修饰后的蛋白。

也考虑某些可通过口服给药的剂型。在本发明的一个实施方案中，通过这种方式给药的特异性结合剂分子可与那些固体制剂形式中(例如片剂或胶囊)常用的载体一起制剂，也可不与这些载体一起制备。例如，可将胶囊设计成当生物药效率最大并且系统预降解最低时，组合物的活性部分在胃肠道发生释放。还可采用其它试剂来促进结合剂分子的吸收。也可使用稀释剂、调味剂、低熔点蜡、植物油、润滑剂、悬浮剂、片剂崩解剂、和粘合剂。

也可使用本领域熟知的适合口服给药剂型的药学上可接受的载体，将药用组合物制成口服给药形式。这些载体能够使药用组合物形成被患者所吸收的形式，如片剂、丸剂、糖衣丸、胶囊、溶液、凝胶、糖浆、浆(slurries)、混悬液。

可通过使活性化合物与固体赋形剂结合，并将所得混合物制成细颗粒状(可任选在粉碎后)以得到片剂或糖衣丸核心，从而得到口服给药的药用制备物。如果需要可添加合适的辅助物。合适的赋形剂包括碳水化合物或蛋白填充物，例如糖，包括乳糖、蔗糖、甘露醇和山梨醇；玉米淀粉、小麦淀粉、水稻淀粉、马铃薯淀粉或其它植物淀粉；纤维素如甲基纤维素、羟丙基甲基纤维素、羧甲基纤维素钠；树脂包括阿拉伯胶和黄芪胶；蛋白包括白明胶和胶原质。如果需要可添加崩解剂或增溶剂，如交联聚乙烯吡咯烷酮、琼脂和褐藻酸或

它们的盐，例如藻酸钠。

糖衣丸核心可与合适的包被联合使用，如浓缩糖溶液，其中也可含有阿拉伯胶、滑石粉、聚乙烯吡咯烷酮卡波姆凝胶（carbopol gel）、聚乙二醇和/或二氧化钛、漆溶液，以及合适的有机溶剂或溶剂混合物。可在片剂或包被中添加染料或色素，以鉴别产品或标出活性化合物的特征，即剂量。

口服使用的药用制备物也可包含白明胶制成的推入配合（push-fit）胶囊，或者白明胶和包被(如甘油和山梨醇)制成的柔软密封胶囊。推入配合胶囊可含有活性成分和与其混合的填充物或结合剂，如蔗糖或淀粉，润滑剂如滑石粉或硬脂酸镁，以及(任选含或不含)稳定剂。在柔软胶囊中，活性化合物可分散或悬浮在合适的液体中，如脂肪、液体(liquid)或聚乙二醇液体(liquid)，其中可含有或不含有稳定剂。

药用制备物种也可将有效剂量的结合剂与无毒并适合丸剂制造的赋形剂相混合。通过将丸剂溶剂在无菌水或其它载体中，溶液可被制成单位剂量形式。合适的赋形剂包括(但不仅限于)稀释剂如碳酸钙、碳酸钠或重碳酸钠、乳糖、磷酸钙；或粘合剂如淀粉、白明胶或阿拉伯树胶；润滑剂如硬脂酸镁、硬脂酸或滑石粉。

本领域技术人员也明了其它形式的药用组合物，包括使结合剂分子持续或受控释放的剂型。本领域技术人员熟知多种涉及其它持续或受控释放输送方式的制剂方法，例如脂质体载体、生物可降解微粒或多孔珠。如见 PCT/US93/00829，其中描述了通过多孔聚合物微粒输送药用组合物进行受控释放。其它持续释放的例子包括包括成型的半透性聚合物基质，如膜或微胶囊。持续释放机制可包括聚酯，水凝胶，聚交酯(U.S. 3,773,919, EP 58,481)、L-谷氨酸和γ乙基-L-谷氨酸盐的共聚物 (Sidman 等, *Biopolymers*, 22:547-556 (1983))，聚(2-羟乙基-甲基丙烯酸酯) (Langer 等, *J Biomed Mater Res*, 15:167-277, (1981))和(Langer 等, *Chem Tech*, 12:98-105(1982))，乙烯-醋酸乙烯共聚物(Langer 等, *supra*)或 poly-D(-)-3-羟丁酸 (EP 133,988)。持续释

放组合物也包括脂质体，可通过若干种本领域已知方法进行制备。如见 Eppstein 等, *Proc Natl Acad Sci (USA)*, 82:3688-3692 (1985); EP 36,676; EP 88,046; EP 143,949。

用于体内给药的药用组合物典型必须无菌。这可通过使用无军旅膜过滤来实现。当组合物为冻干形式时，这种除菌方法可在冻干之前或之后(重新溶解后)进行。用于注射的组合物可以在冻干形式下保存或在溶液中保存。另外盛装注射用组合物的容器通常具有无菌阀门，例如使用静脉注射溶液包或者经由一个可被皮下注射针头所刺穿的塞子。

一旦药用组合物制剂完成，可以将其以溶液、悬液、凝胶、乳状液、固体、脱水或冻干粉的形式装在无菌小瓶中。这些制剂的储存方式可以为即用的形式，或者为使用前需要重溶的形式(如冻干)。

在本发明的一个特定实施方案中，使用成套包装盒以得到单剂量给药单位。成套包装盒中可含有两种容器，第一种含有干燥蛋白，第二种含有水状制剂。本发明也考虑使用这类成套包装盒，即含有单腔或多腔预填充注射器(如液体注射器和液体溶胶注射器(lyosyringes))。

药用组合物的使用有效剂量依赖于如治疗环境和目标。本领域技术人员将理解合适的治疗剂量水平因此将高低不同，部分依赖于输送分子、待使用结合剂分子的使用指导、给药途径、以及患者的体格(体种、体表面积或器官体积)和条件(年龄和总体健康状况)。因此临床医生可改变剂量并调整给药途径已达到最优治疗效果。根据上述因素，典型剂量范围可为约 0.1 mg/kg-100 mg/kg。在其它实施方案中，剂量范围为约 0.1 mg/kg-100 mg/kg，或者约 1 mg/kg-100 mg/kg，或者约 5 mg/kg-100 mg/kg。

任何化合物的治疗有效剂量开始都可通过细胞培养试验或动物模型进行估计，如使用小鼠、大鼠、兔、狗或猪。可使用动物模型来确定合适的浓度范围和给药途径。然后可使用这些信息来确定人体

的有效剂量和给药途径。

精确剂量要根据需要治疗的受试者的相关因素来确定。调整剂量和给药方式以提供足够水平的活性化合物，或保持所需效果。被纳入考虑范围的因素可包括疾病状态的严重程度、受试者的总体健康状况、年龄、体重、性别、给药的时机和频率、药物化合物、反应敏感性和治疗反应。根据特定组合物的半衰期和清除速率，长效药用组合物的给药频率可为每 3d 或 4d 一次，每周一次，或两周一次。

给药频率要依赖于所用剂型中结合剂的药代动力学参数。典型的，给予组合物直至药物达到所需效果为止。因此可单次给予组合物，或者在一个时期内多次给药(以相同或不同浓度/剂量)，或者持续灌输。精确确定合适的剂量将是例行工作。可通过使用合适的给药反应数据来估计合适的剂量。

药用组合物的给药途径与已知方法一致，如口服、静脉注射、腹膜内、脑内(脑实质内)、脑室内、肌内、眼内、动脉内、门静脉、病灶内途径、髓内、脑脊髓膜内、心室内、穿皮、皮下、腹膜内、鼻内、肠内、局部给药、舌下、尿道、阴道、或直肠途径、通过持续释放系统或移植设备。如果需要，可使用静脉推注或持续灌注。

或者可选择局部给药，通过移植膜、海绵或其它吸收了所需分子或将其包装进胶囊的合适材料来实现。使用移植装置时，可将移植物给予任何合适的组合或器官，而所需分子可通过扩散、定时释放推注或持续给予来进行输送。

在一些情况下，可能需要以先体外后体内(*ex vivo*)方式使用药用组合物。在这种方式下，从患者取出细胞、组织或器官然后暴露在药用组合物下，再移植回患者体内。

另外一些情况下，当结合剂为多肽分子时，可使用如本文所述方法来对某些细胞进行基因操作，然后将这些细胞移植入受试者体内，以表达并分泌该多肽。这种细胞可以是动物细胞或者人类细胞，也可以是自体同源细胞、异源细胞或异种细胞。任选可使用无限增殖

化细胞。为减少免疫反应的机会，这些细胞可被装入胶囊以避免渗透到周围组织。胶囊材料通常为生物相容、半透性聚合物外壳或包膜，它们必须允许蛋白释放并且可防止细胞被患者免疫系统或其它环境有害因子所破坏。

联合疗法

可联合使用本发明的特异性结合剂和 Ang-2 相关疾病治疗中的其它治疗方法。这些治疗方法包括入放疗、化疗和靶疗法，如下文所述。本发明也考虑了其它没有在本文中特别列出的连用疗法。

因此，本发明包括联合使用本发明的一种或多种特异性结合剂和一种或多种其它合适药物，来给予同一患者体内，给药均按照各自药物合适的方法进行。这包括同时给予本发明的特异性结合剂和一种或多种合适药物。这里所用的“同时给药”指本发明的一种或多种特异性结合剂和其它一种或多种合适药物基本上同时给药。

这里所用的“非同时给药”指本发明的一种或多种选择性结合剂与另一种或多种合适试剂的在不同时间给药，不管给药重叠与否，而二者给药的前后顺序随意。这包括(但不仅限于)联用成分顺序给药(如预处理、后处理或重叠处理)，和轮流给予药物，以及一种成分长期给药而其它则间歇给药。可以在同一制剂中给予各种成分或者在分开的制剂中给予，给予途径也可相同或不同。

在某些实施方案中，联合治疗包括使用能够结合 Ang-2 的特异性结合剂，以及至少一种其它抗促血管生成素剂。这种药物包括(但不仅限于)体外合成的化学组合物、抗体、抗原结合区域、放射性核以及这些物质的组合。在某些实施方案中，使用的药物可抑制或激发其靶子(如受体、酶激活或抑制)，从而起始细胞死亡或破坏细胞生长。

化疗可使用抗肿瘤药物，如烷基化合物，包括：氮芥如二氯甲基二乙胺、环磷酰胺、异磷酰胺、美法仑和苯丁酸氮芥；亚硝基脲如亚硝脲氮芥(BCNU)、洛莫司汀(CCNU)、司莫司汀(甲基-CCNU)；乙

烯亚胺/甲基三聚氰胺如三亚乙基三聚氰胺(TEM)、三乙烯硫代磷酰胺(噻替派)、六甲基三聚氰胺(HMM，六甲蜜胺)；磺酸烷基酯如白消安；三嗪如达卡巴嗪(DTIC)；抗代谢物包括叶酸类似物如甲氨喋呤和三甲曲沙；嘧啶类似物如 5-氟尿嘧啶、氟脱氧尿苷、吉西他滨、阿糖胞苷 (AraC，阿糖胞苷)、5-氮杂胞嘧啶核苷、2,2′-双氟去氧胞苷；嘌啉类似物如 6-巯基嘌啉、6-硫鸟嘌啉、咪唑巯嘌啉、2'-脱氧助间型霉素(喷司他丁)、红-9-(2-羟基-3-壬基)腺嘌呤(EHNA)、磷酸氟达拉滨、2-氯去氧腺苷(克拉屈滨(cladribine)、2-CdA)；天然产物包括抗有丝分裂药物如紫杉醇(paclitaxel)、长春花生物碱包括长春碱(VLB)、长春新碱和长春瑞宾(vinorelbine)、多烯紫杉醇、雌莫司汀(Estramustine) 和磷雌氮芥；表鬼臼毒素(ppipodophylotoxins)如依托泊甙和替尼泊苷；抗生素如纺线菌素 D、道诺霉素(红比霉素)、阿霉素、米托蒽醌、去甲氧基柔红霉素、博来霉素、普卡霉素(光神霉素)、丝裂霉素 C、放射菌素；酶如 L-天冬酰胺酶；生物反应修饰物如α干扰素、IL-2、G-CSF 和 GM-CSF；各种药物包括铂配合物如顺铂和顺铂、蒽醌类(anthracenediones)如米托蒽醌、取代的脲如羟基脲、甲苄肼衍生物包括 N-甲苄肼(MIH) 和甲基苄肼；肾上腺皮质抑制剂如米托坦(o,p′-DDD)和氨鲁米特；激素和拮抗剂包括肾上腺皮质类固醇如强的松和等效物、地塞米松和氨鲁米特；孕酮如己酸孕酮单酯、醋酸甲羟孕酮和醋酸甲地孕酮；雌激素如己烯雌酚和炔雌醇等效物；抗雌激素如三苯氧胺；男性激素包括丙酸睾酮和氟羟甲基睾丸素/等效物；抗雄激素如如氟他胺、促性腺激素-释放激素类似物和亮丙瑞林；和非类固醇抗雄激素如氟他胺。

癌症治疗(可通过给予 Ang-2 的特异性结合剂)也包括(但不仅限于)本文所述的靶向疗法。靶向疗法例如(但不仅限于)使用治疗性抗体。治疗性抗体包括如(但不仅限于)鼠源抗体、鼠-人嵌合抗体、CDR-移植抗体、人源化抗体或完全人类抗体，以及合成抗体，包括(但不仅限于)从抗体文库中筛选所得的抗体。抗体包括如(但不仅限于)可结

合于肿瘤细胞上的细胞表面蛋白 Her2、CDC20、CDC33、粘蛋白样糖蛋白(mucin-like glycoprotein)，以及表皮生长因子受体(EGFR)，并可对展示这些蛋白的肿瘤细胞任选产生抑细胞生长和/或细胞毒性作用的抗体。抗体也包括如 HERCEPTIN™ (trastuzumab)(它可被用来治疗乳腺癌和其它癌症)、ITUXAN™ (rituximab)、ZEVALIN™ (替伊莫单抗)、GLEEVEC™，和 LYMPHOCIDE™ (依帕珠单抗)(它可被用来治疗非何杰金瘤和其它癌症)。某些抗体例子也包括 ERBITUX™ (IMC-C225)、IRESSA™ (ertinolib)、BEXXAR™ (碘-131 托西莫单抗)、KDR (激酶结构域受体) 抑制剂、抗 VEGF 抗体和拮抗剂(如 AVASTIN™ 和 VEGAF-TRAP)、抗 VEGF 受体抗体和抗原结合区、抗 Ang-1 抗体和抗原结合区、针对 Tie-2 和其它 Ang-1/Ang-2 受体的抗体、Tie-2 配基、针对 Tie-2 激酶抑制剂的抗体，和 Campath® (阿仑单抗)。在某些实施方案中，抗癌药物为能够选择性引起肿瘤细胞凋亡的多肽，包括(但不仅限于) TNF-相关多肽，如 TRAIL (TNF 受体凋亡诱导配体)。

在某些实施方案中，已知合适的抗癌药物具有抗血管生成作用。这种药物中的某些包括(但不仅限于)IL-8，Campath™，B-FGF，FGF 拮抗剂，Tek 拮抗剂(Cerretti 等, U.S. 公开 No. 2003/0162712，Cerretti 等，美国专利 No. 6,413,932; Cerretti 等，美国专利 No. 6,521,424，均通过引用并入本文)，抗 TWEAK 药物(包括(但不仅限于)抗体和抗原结合区)，可溶性 TWEAK 受体拮抗剂 (Wiley, 美国专利 No. 6,727,225)，ADAM 去整合素(distintegrins) (或其片段以抑制整合素与其配体的结合，Fanslow 等, U.S. 公开 No. 2002/0042368)，抗 eph 受体和抗 ephrin 抗体(和抗原结合区)或拮抗剂 (美国专利 No. 5,981,245、5,728,813、5,969,110、6,596,852、6,232,447、6,057,124 和它们的专利家族成员)，本文所述的抗 VEGF 药物(如特异性结合 VEGF 的抗体和抗原结合区，或可溶性 VEGF 受体或其配基结合区)如 AVASTIN™或 VEGF-TRAP™，抗 VEGF 受体药物(如特异性结

合它的抗体和抗原结合区)，EGFR 抑制剂(如特异性结合它的抗体和抗原结合区)如 panitumumab, IRESSA™ (gefitinib), TARCEVA™ (erlotinib), 抗 Ang-1 和 抗 Ang-2 药物 (如特异性结合它的抗体和抗原结合区或特异性结合它们的受体(如 Tie-2/TEK)的抗体和抗原结合区), 和抗 Tie-2 激酶抑制剂 (特异性结合生长因子并抑制其活性的抗体，如干细胞生长因子抑制剂(HGF, 也称分散因子(Scatter Factor))，和特异性结合其受体“c-met”的抗体和抗原结合区，抗 PDGF-BB 拮抗剂，针对 PDGF-BB 配体的抗体和抗原结合区，PDGFR 激酶抑制剂。

在某些实施方案中，癌症治疗药物为促血管生成素抑制剂。其中一些包括(但不仅限于)SD-7784 (Pfizer, USA)；西仑吉肽(cilengitide)(Merck KGaA, Germany, EPO 770622)；pegaptanib octasodium，(Gilead Sciences, USA)；肿瘤血管抑制肽(Alphastatin)，(BioActa, UK)；M-PGA，(Celgene, USA, US 5712291)；伊洛马司他(ilomastat)(Arriva, USA, US 5892112)；semaxanib，(Pfizer, USA, US 5792783)；vatalanib，(Novartis, Switzerland)；2-甲氧雌二醇，(EntreMed, USA)；TLC ELL-12，(Elan, Ireland)；醋酸阿奈可他，(Alcon, USA)；α-D148 Mab，(Amgen, USA)；CEP-7055，(Cephalon, USA)；抗 Vn Mab，(Crucell, Netherlands)；DAC：抗血管生成，(ConjuChem, Canada)；Angiocidin，(InKine Pharmaceutical, USA)；KM-2550，(Kyowa Hakko, Japan)；SU-0879，(Pfizer, USA)；CGP-79787，(Novartis, Switzerland, EP 970070)；ARGENT technology, (Ariad, USA)；YIGSR-Stealth, (Johnson & Johnson, USA)；纤维蛋白原-E 片段，(BioActa, UK)；血管生成抑制剂，(Trigen, UK)；TBC-1635, (Encysive Pharmaceuticals, USA)；SC-236, (Pfizer, USA)；ABT-567, (Abbott, USA)；Metastatin, (EntreMed, USA)；血管生成抑制剂(Tripep, Sweden)；maspin，(Sosei, Japan)；2-甲氧雌二醇，(Oncology Sciences Corporation, USA)；ER-68203-00, (IVAX, USA)；氟草胺(Benefin),

(Lane Labs, USA); Tz-93, (Tsumura, Japan); TAN-1120, (Takeda, Japan); FR-111142, (Fujisawa, Japan, JP 02233610); 血小板第四因子, (RepliGen, USA, EP 407122); 血管上皮生长因子抑制剂, (Borean, Denmark); 癌症治疗(cancer therapy), (university of South Carolina, USA); bevacizumab (pINN), (Genentech, USA); 血管生成抑制剂, (SUGEN, USA); XL 784, (Exelixis, USA); XL 647, (Exelixis, USA); MAb, α5β3 整合素,第二代, (Applied Molecular Evolution, USA and MedImmune, USA); 基因治疗, 视网膜病(gene therapy, retinopathy), (Oxford BioMedica, UK); enzastaurin hydrochloride (USAN), (Lilly, USA); CEP 7055, (Cephalon, USA and Sanofi-Synthelabo, France); BC 1, (Genoa Institute of Cancer Research, Italy); 血管生成抑制剂, (Alchemia, Australia); VEGF 拮抗剂, (Regeneron, USA); rBPI 21 和 BPI-起源的抗血管生成剂, (XOMA, USA); PI 88, (Progen, Australia); 西仑吉肽(cilengitide) (pINN), (Merck KGaA; Munich Technical university, Germany, Scripps Clinic and Research Foundation, USA); cetuximab (INN), (Aventis, France); AVE 8062, (Ajinomoto, Japan); AS 1404, (Cancer Research Laboratory, New Zealand); SG 292 (Telios, USA); Endostatin, (Boston Childrens Hospital, USA); ATN 161, (Attenuon, USA); 人血管抑素(angiostatin), (Boston Childrens Hospital, USA); 2-甲氧雌二醇, (Boston Childrens Hospital, USA); ZD 6474, (AstraZeneca, UK); ZD 6126, (Angiogene Pharmaceuticals, UK); PPI 2458, (Praecis, USA); AZD 9935, (AstraZeneca, UK); AZD 2171, (AstraZeneca, UK); vatalanib (pINN), (Novartis, Switzerland and Schering AG, Germany); 组织因子突击抑制剂 (EntreMed, USA); pegaptanib (Pinn), (Gilead Sciences, USA); 黄姜根醇(xanthorrhizol), (Yonsei university, South Korea); 基因疫苗 VEGF-2, (Scripps Clinic and Research Foundation, USA); SPV5.2, (Supratek, Canada); SDX 103, (university of California at San Diego, USA); PX 478, (ProlX, USA);

METASTATIN, (EntreMed, USA); 肌钙蛋白 I, (Harvard university, USA); SU 6668, (SUGEN, USA); OXI 4503, (OXiGENE, USA); o-胍, (Dimensional Pharmaceuticals, USA); motuporamine C, (British Columbia university, Canada); CDP 791, (Celltech Group, UK); atiprimod (pINN), (GlaxoSmithKline, UK); E 7820, (Eisai, Japan); CYC 381, (Harvard university, USA); AE 941, (Aeterna, Canada); 疫苗，血管生成(vaccine, angiogenesis)(EntreMed, USA); 尿激酶纤维蛋白溶酶原激活质抑制剂 (Dendreon, USA); oglufanide (pINN), (Melmotte, USA); HIF-1alfa inhibitors, (Xenova, UK); CEP 5214, (Cephalon, USA); BAY RES 2622, (Bayer, Germany); Angiocidin, (InKine, USA); A6, (Angstrom, USA); KR 31372, (Korea Research Institute of Chemical Technology, South Korea); GW 2286, (GlaxoSmithKline, UK); EHT 0101, (ExonHit, France); CP 868596, (Pfizer, USA); CP 564959, (OSI, USA); CP 547632, (Pfizer, USA); 786034, (GlaxoSmithKline, UK); KRN 633, (Kirin Brewery, Japan); 药物给予系统，眼内，2-甲氧雌二醇(drug delivery system, intraocular, 2-methoxyestradiol)(EntreMed, USA); anginex, (Maastricht university, Netherlands, and Minnesota university, USA); ABT 510, (Abbott, USA); AAL 993, (Novartis, Switzerland); VEGI, (ProteomTech, USA); 肿瘤坏死因子α抑制剂, (National Institute on Aging, USA); SU 11248, (Pfizer, USA and SUGEN USA); ABT 518, (Abbott, USA); YH16, (Yantai Rongchang, China); S-3APG , (Boston Childrens Hospital, USA and EntreMed, USA); MAb, KDR, (ImClone Systems, USA); MAb, α5 β1, (Protein Design, USA); KDR 激酶抑制剂(Celltech Group, UK, and Johnson & Johnson, USA); GFB 116, (South Florida university, USA and Yale university, USA); CS 706, (Sankyo, Japan); combretastatin A4 前体药物, (Arizona State university, USA); 软骨素 AC, (IBEX, Canada); BAY RES 2690, (Bayer, Germany); AGM 1470, (Harvard university, USA, Takeda, Japan, and

TAP, USA); AG 13925, (Agouron, USA); 四硫钼酸铵, (university of Michigan, USA); GCS 100, (Wayne State university, USA) CV 247, (Ivy Medical, UK); CKD 732, (Chong Kun Dang, South Korea); MAb, 血管上皮生长因子(Xenova, UK); irsogladine (INN), (Nippon Shinyaku, Japan); RG 13577, (Aventis, France); WX 360, (Wilex, Germany); squalamine (pINN), (Genaera, USA); RPI 4610, (Sirna, USA); cancer therapy, (Marinova, Australia); heparanase 抑制剂, (InSight, Israel); KL 3106, (Kolon, South Korea); Honokiol, (Emory university, USA); ZK CDK, (Schering AG, Germany); ZK Angio, (Schering AG, Germany); ZK 229561, (Novartis, Switzerland, and Schering AG, Germany); XMP 300, (XOMA, USA); VGA 1102, (Taisho, Japan); VEGF 受体调节物, (Pharmacopeia, USA); VE-cadherin-2 拮抗剂, (ImClone Systems, USA); Vasostatin, (National Institutes of Health, USA); vaccine, Flk-1, (ImClone Systems, USA); TZ 93, (Tsumura, Japan); TumStatin, (Beth Israel Hospital, USA); 部分长度可溶性 FLT 1 (vascular endothelial growth factor receptor 1), (Merck & Co, USA); Tie-2 配体, (Regeneron, USA); 血小板反应蛋白 1 抑制剂, (Allegheny Health, Education and Research Foundation, USA);; 2- 苯磺酰胺,4-(5-(4-氯苯基)-3-(三氟甲基)-1H-吡唑-1-基)-; Arriva; 和 C-Met. AVE 8062 ((2S)-2-氨基-3-羟基-N-[2-甲氧基-5-[(1Z)-2-(3,4,5-三甲氧基苯基)乙烯基] 苯基丙酰胺单氢氯); metelimumab (pINN)(免疫球蛋白 G4, 抗-(人转化生长因子β.1 (人单克隆 CAT 192 . γ.4-链)), 二硫化人单抗 CAT 192 .κ.-链二聚物); Flt3 配体; CD40 配体; 白介素-2; 白介素-12; 4-1BB 配体; 抗 4-1BB 抗体; TNF 拮抗剂和 TNF 受体拮抗剂, 包括 TNFR/Fc, TWEAK 拮抗剂和 TWEAK-R 拮抗剂包括 TWEAK-R/Fc; TRAIL; VEGF 拮抗剂包括抗 VEGF 抗体; VEGF 受体(包括 VEGF-R1 和 VEGF-R2, 也称为 Flt1 和 Flk1 或 KDR) 拮抗剂; CD148 (也称为 DEP-1, ECRTP, 和 PTPRJ, 见 Takahashi 等, J. Am.

Soc. Nephrol. 10: 2135-45 (1999), 通过引用并入本文) 拮抗剂；血小板反应蛋白 1 抑制剂，和 Tie-2 或 Tie-2 配体(如 Ang-2)之一的抑制剂，或二者同时的抑制剂。 本领域已知许多 Ang-2 抑制剂，包括某些抗 Ang-2 抗体(美国专利申请 No. 20030124129 (相应于 PCT 申请 No. WO03/030833)和美国专利 No. 6,166,185，其内容通过引用并入本文)。另外本领域也已知 Ang-2 肽体（peptibody），可见如美国专利申请 No. 20030229023 (相应于 PCT 申请 No. WO03/057134)和美国专利申请 No. 20030236193，其内容通过引用并入本文。

某些癌症治疗药物包括(但不仅限于)萨立多胺(thalidomide)和类似物(N-(2,6-二氧代-3-哌啶基)苯邻二甲酰亚胺)；硫化多糖钠（tecogalan sodium） (硫酸多糖肽聚糖(sulfated polysaccharide peptidoglycan))；TAN 1120 (8-乙酰基-7,8,9,10-四氢-6,8,11-三羟基-1-甲氧基-10-[[八氢-5-羟基-2-(2-羟丙基)-4,10-二甲基吡喃[3,4-d]-1,3,6-二氧杂氮杂环辛因(dioxazocin)-8-基]氧基]-5,12-萘二酮)；suradista (7,7'-[羰基双[亚氨基(1-甲基-1H-吡咯-4,2-二基)羰基亚氨基(1-甲基-1H-吡咯-4,2-二基)羰基亚氨基]]双-1,3-萘二磺酸四钠盐)；SU 302；SU 301；SU 1498 ((E)-2-氰基-3-[4-羟基-3,5-双(1-甲基乙基)苯基]-N-(3-苯基丙基)-2-丙酰胺)；SU 1433 (4-(6,7-二甲基-2-喹喔啉基)-1,2-苯二醇)；ST 1514；SR 25989；可溶性 Tie-2；SERM 衍生物，Pharmos；semaxanib (pINN)(3-[(3,5-二甲基-1H-吡咯-2-基)亚甲基]-1,3-二氢-2H-吲哚-2-酮)；S 836；RG 8803；RESTIN；R 440 (3-(1-甲基-1H-吲哚-3-基)-4-(1-甲基-6-硝基-1H-吲哚-3-基)-1H-吡咯-2,5-二酮)；R 123942 (1-[6-(1,2,4-噻二唑-5-基)-3-哒嗪基]-N-[3-(三氟甲基)苯基]-4-哌啶胺)；脯氨酰羟化酶抑制剂；进展加速基因(progression elevated genes)；普啉司他(INN) ((S)-2,2-二甲基-4-[[对-(4-吡啶氧基)苯基]磺酰基]-3-硫吗啉异甲羟肟酸)；NV 1030；NM 3 (8-羟基-6-甲氧基-α-甲基-1-氧代-1H-2-苯并吡喃基-3 醋酸)；NF 681；NF 050；MIG；METH 2；METH 1；manassantin B (α-[1-[4-[5-[4-[2-(3,4-二甲氧基苯基)-2-羟基-1-甲基乙氧基]-3-甲氧

基苯基]四氢-3,4-二甲基-2-呋喃]-2-甲氧基苯氧基]乙基]-1,3-苯并二氧杂环戊烯-5-甲醇); KDR 单抗; α5β3 整合素单抗; LY 290293 (2-氨基-4-(3-吡啶基)-4H-萘并[1,2-b]吡喃-3-腈); KP 0201448; KM 2550; 整合素特异性肽; INGN 401; GYKI 66475; GYKI 66462; greenstatin (101-354-血纤维蛋白溶酶原(人)); 风湿性关节炎、前列腺癌、卵巢癌、神经胶质瘤、内皮抑素、肠癌的基因治疗剂, ATF BTPI、抗血管生成基因、血管生成抑制剂或拮抗剂; 白明胶酶抑制剂、FR 111142 (4,5-二羟基-2-己烯酸 5-甲氧基-4-[2-甲基-3-(3-甲基-2-丁烯基)环氧乙烷基]-1-氧杂螺[2.5]辛-6-基酯); forfenimex (pINN) (S)-α-氨基-3-羟基-4-(羟甲基)苯乙酸); 纤维连接蛋白拮抗剂(1-乙酰基-L-脯氨酰-L-组氨酰-L-丝氨酰-L-半胱氨酰-L-天冬酰胺); 纤维组织母细胞生长因子受体抑制剂; 组织母细胞生长因子拮抗剂; FCE 27164 (7,7'-[羰基双[亚氨基(1-甲基-1H-吡咯-4,2-二基)羰基亚氨基(1-甲基-1H-吡咯-4,2-二基)羰基亚氨基]]双-1,3,5-萘三磺酸六钠盐); FCE 26752 (8,8'-[羰基双[亚氨基(1-甲基-1H-吡咯-4,2-二基)羰基亚氨基(1-甲基-1H-吡咯-4,2-二基)羰基亚氨基]]双-1,3,6-萘三磺酸); 内皮单核细胞活性多肽 II; VEGFR 反义寡核苷酸; 抗血管生成和肝营养因子; ANCHOR 拮抗剂; 内皮抑素; Del-1 血管生成蛋白; CT 3577; contortrostatin; CM 101; 软骨素酶 AC; CDP 845; CanStatin; BST 2002; BST 2001; BLS 0597; BIBF 1000; ARRESTIN; apomigren (1304-1388- XV 类胶原质(人基因 COL15A1α 链前体)); 血管抑制素; aaATIII; A 36; 醋酸 9α-氟甲氧基孕酮(fluoromedroxyprogesterone)((6-α)-17-(乙酰氧基)-9-氟-6-甲基-孕甾-4-烯-3,20-二酮); 2-甲基-2-酞酰亚氨基-戊二酸(2-(1,3-二氢-1-氧代-2H-异吲哚-2-基)-2-甲基戊二酸); Yttrium 90 标记的单抗 BC-1; Semaxanib (3-(4,5-二甲基吡咯-2-基亚甲基)吲哚-2-酮)(C15 H14 N2 O); PI 88 (硫酸化磷酸甘露戊糖); Alvocidib (4H-1-苯并吡喃-4-酮, 2-(2-氯苯基)-5,7-二羟基-8-(3-羟基-1-甲基-4-哌嗪基)-顺-(-)-) (C21 H20 Cl N O5); E 7820; SU 11248 (5-[3-氟-2-氧代-1,2-二氢吲哚-(3Z)-

亚基甲基]-2,4-二甲基-1H-吡咯-3-羧酸 (2-二乙基氨基乙基)酰胺) (C22 H27 F N4 O2); 角鲨胺(胆甾烷-7,24-二醇, 3-[[3-[(4-氨基丁基)氨基丙基]氨基]-, 24-(硫酸氢盐), (3.β.,5.a.,7.a.)-) (C34 H65 N3 O5 S); Eriochrome Black T; AGM 1470 (氨基甲酸, (氯乙酰)-, 5-甲氧基-4-[2-甲基-3-(3-甲基-2-丁烯基)环氧乙烷基]-1-氧杂螺[2,5]辛-6-基酯 [3R-[3α, α(2R, 3R), 5β, 6β]]) (C19 H28 Cl N O6); AZD 9935; BIBF 1000; AZD 2171; ABT 828; KS-白细胞介素-2; uteroglobin; A 6; NSC 639366 延胡索酸(1-[3-(二乙基氨基)-2-羟基丙基氨基]-4-(氧杂丙烷(oxyran)-2-基甲基氨基)蒽醌) (C24 H29 N3 O4 . C4 H4 O4); ISV 616; 抗-ED-B 融合蛋白; HUI 77; Troponin I; BC-1 单抗; SPV 5.2; ER 68203; CKD 731 (3-(3,4,5-三甲氧基苯基)-2(E)-丙烯酸(3R,4S,5S,6R)-4-[2(R)-甲基-3(R)-3(R)-(3-甲基-2-丁烯基)氧杂丙烷-2-基]-5-甲氧基-1-氧杂螺[[2.5] 辛-6-基酯) (C28 H38 O8); IMC-1C11; aaATIII; SC 7; CM 101; Angiocol; Kringle 5; CKD 732 (3-[4-[2-(二甲基氨基)乙氧基]苯基]-2(E)-丙烯酸)(C29 H41 N O6); U 995; Canstatin; SQ 885; CT 2584 (1-[11-(十二氨基)-10-羟基十一烷基]-3,7-二甲基黄嘌呤)(C30 H55 N5 O3); Salmosin; EMAP II; TX 1920 (1-(4-甲基哌嗪基)-2-(2-硝基-1H-1-咪唑酰基)-1-乙酮) (C10 H15 N5 O3); α-v β-x 抑制剂; CHIR 11509 (N-(1-丙炔基)甘氨酰-[N-(2-萘基)]甘氨酰-[N-(氨基甲酰甲基)]甘氨酸双(4-甲氧基苯基)甲基酰胺)(C36 H37 N5 O6); BST 2002; BST 2001; B 0829; FR 111142; 4,5-二羟基-2(E)-己烯酸(3R,4S,5S,6R)-4-[1(R),2(R)-环氧-1,5-二甲基-4-己烯基]-5-甲氧基-1-氧杂螺[2.5]辛烷-6-基酯 (C22 H34 O7); 和激酶抑制剂包括(但不仅限于)N-(4-氯苯基)-4-(4-吡啶基甲基)-1-酞嗪胺; 4-[4-[[[[4-氯-3-(三氟甲基)苯基]氨基]羰基]氨基]苯氧基]-N-甲基-2-吡啶羧酰胺; N-[2-(二乙基氨基)乙基]-5-[(5-氟-1,2-二氢-2-氧代-3H-吲哚-3-亚基)甲基]-2,4-二甲基-1H-吡咯-3-羧酰胺; 3-[(4-溴-2,6-二氟苯基)甲氧基]-5-[[[[4-(1-吡咯烷基)丁基]氨基]羰基]氨基]-4-异噻唑羧酰胺; N-(4-溴-2-氟苯基)-6-甲氧基-7-[(1-甲基

-4-哌啶基)甲氧基]-4-喹唑啉胺；3-[5,6,7,13-四氢-9-[(1-甲基乙氧基)甲基]-5-氧代-12H-茚并[2,1-a]吡咯并[3,4-c] 咔唑-12-基]丙基酯 N,N-二甲基-甘氨酸；N-[5-[[[5-(1,1-二甲基乙基)-2-噁唑基]甲基]硫基]-2-噻唑基]-4-哌啶羧酰胺；*N*-[3-氯-4-[(3-氟苯基)甲氧基]苯基]-6-[5-[[[2-(甲基磺酰基)乙基]氨基]甲基]-2-呋喃基]-4-喹唑啉胺；4-[(4-甲基-1-哌嗪)甲基]-N-[4-甲基-3-[[4-(3-吡啶基)-2-哌嗪]氨基]-苯基]苯甲酰胺；*N*-(3-氯-4-氟苯基)-7-甲氧基-6-[3-(4-吗啉基)丙氧基]-4-喹唑啉胺；*N*-(3-乙基苯基)-6,7-双(2-甲氧基乙氧基)-4-喹唑啉胺；N-(3-((((2R)-1-甲基-2-吡咯烷基)甲基)氧基)-5-(三氟甲基)苯基)-2-((3-(1,3-氧氮杂茂-5-基)苯基)氨基)-3-吡啶羧酰胺；2-(((4-氟甲基)甲基)氨基)-N-(3-((((2R)-1-甲基-2-吡咯烷基)甲基)氧基)-5-(三氟甲基)苯基)-3-吡啶羧酰胺；N-[3-(吖丁啶-3-基甲氧基)-5-三氟甲基)苯基]-2-(4-氟-苄氨基)-烟酰胺；6-氟-N-(4-(1-甲基乙基)苯基)-2-((4-吡啶甲基)氨基)-3-吡啶羧酰胺；2-((4-吡啶甲基)氨基)-N-(3-(((2S)-2-吡咯烷基甲基)氧基)-5-(三氟甲基)苯基)-3-吡啶羧酰胺；N-(3-(1,1-二甲基乙基)-1H-吡唑-5-基)-2-((4-吡啶甲基)氨基)-3-吡啶羧酰胺；N-(3,3-二甲基-2,3-二氢-1-苯并呋喃-6-基)-2-((4-吡啶甲基)氨基)-3-吡啶羧酰胺；N-(3-((((2S)-1-甲基-2-吡咯烷基)甲基)氧基)-5-(三氟甲基)苯基)-2-((4-吡啶甲基)氨基)-3-吡啶羧酰胺；2-((4-吡啶甲基)氨基)-N-(3-((2-(1-吡咯烷基)乙基)氧基)-4-(三氟甲基)苯基)-3-吡啶羧酰胺；N-(3,3-二甲基-2,3-二氢-1H-吲哚-6-基)-2-((4-吡啶甲基)氨基)-3-吡啶羧酰胺；N-(4-(五氟乙基)-3-(((2S)-2-吡咯甲基)氧基)苯基)-2-((4-吡啶甲基)氨基)-3-吡啶羧酰胺；N-(3-((3-吖丁啶基甲基)氧基)-5-(三氟甲基)苯基)-2-((4-吡啶甲基)氨基)-3-吡啶羧酰胺；N-(3-(4-哌啶基氧基)-5-(三氟甲基)苯基)-2-((2-(3-吡啶)乙基)氨基)-3-吡啶羧酰胺；N-(4,4-二甲基-1,2,3,4-四氢-异喹啉-7-基)-2-(1H-吲哚-6-基氨基)-烟酰胺；2-(1H-吲哚-6-基氨基)-N-[3-(1-甲基吡啶-2-基甲氧基)-5-三氟甲基-苯基]-烟酰胺；N-[1-(2-二甲基氨基-乙酰基)-3,3-二甲基-2,3-二氢-1H-吲哚-6-基]-2-(1H-吲唑-6-基氨基)-烟酰胺；2-

(1H-吲唑-6-基氨基)-N-[3-(吡啶-2-基甲氧基)-5-三氟甲基-苯基]-烟酰胺；N-(1-乙酰基-3,3-二甲基-2,3-二氢-1H-吲哚-6-基)-2-(1H-吲唑-6-基氨基)-烟酰胺;N-(4,4-二甲基-1-氧代-1,2,3,4-四氢-异喹啉-7-基)-2-(1H-吲唑-6-基氨基)-烟酰胺；N-[4-(叔丁基)-3-(3-哌啶丙基)苯基][2-(1H-吲唑-6-基氨基)(3-吡啶基)]羧酰胺；N-[5-(叔丁基)异噁唑-3-基][2-(1H-吲唑-6-基氨基)(3-吡啶基)]羧酰胺；和 N-[4-(叔丁基)苯基][2-(1H-吲唑-6-基氨基)(3-吡啶基)]羧酰胺和激酶抑制剂，见美国专利 No. 6,258,812；6,235,764；6,630,500；6,515,004；6,713,485；5,521,184；5,770,599；5,747,498；5,990,141；U.S. 公开 No. US20030105091；和 PCT 公开 No. WO01/37820；WO01/32651；WO02/68406；WO02/66470；WO02/55501；WO04/05279；WO04/07481；WO04/07458；WO04/09784；WO02/59110；WO99/45009；WO98/35958；WO00/59509；WO99/61422；WO00/12089；和 WO00/02871，均通过引用并入本文。

可与生长因子联合使用的治疗药物包括细胞因子、生长因子或其它造血因子如 M-CSF、GM-CSF、TNF、IL-1、IL-2、IL-3、IL-4、IL-5、IL-6、IL-7、IL-8、IL-9、IL-10、IL-11、IL-12、IL-13、IL-14、IL-15、IL-16、IL-17、IL-18、IFN、TNF0、TNF1、TNF2、G-CSF、Meg-CSF、GM-CSF、血小板生成素、干细胞因子和红细胞生产素。其它成分可包括已知的血管生产素如 Ang-1、-2、-4、-Y 和/或人类促血管生成素多肽，和/或血管内皮生长因子(VEGF)。(P50-58)生长因子包括促血管生成素、骨形态发生蛋白-1、骨形态发生蛋白-2、骨形态发生蛋白-3、骨形态发生蛋白-4、骨形态发生蛋白-5、骨形态发生蛋白-6、骨形态发生蛋白-7、骨形态发生蛋白-8、骨形态发生蛋白-9、骨形态发生蛋白-10、骨形态发生蛋白-11、骨形态发生蛋白-12、骨形态发生蛋白-13、骨形态发生蛋白-14、骨形态发生蛋白-15、骨形态发生蛋白受体-IA、骨形态发生蛋白受体 IB、脑元神经营养因子、睫状神经营养因子、睫状神经营养因子受体、细胞因子诱导嗜中性白细胞

趋化因子-1、细胞因子诱导嗜中性白细胞趋化因子、内皮细胞生长因子、内皮素-1、表皮生长因子、上皮细胞来源的嗜中性粒细胞趋化物、纤维原细胞生长因子-4、纤维原细胞生长因子-5、纤维原细胞生长因子-6、f 纤维原细胞生长因子-7、纤维原细胞生长因子-8、纤维原细胞生长因子-8b、纤维原细胞生长因子-8c、纤维原细胞生长因子-9、纤维原细胞生长因子-10、酸性纤维原细胞生长因子、碱性纤维原细胞生长因子、胶质细胞源性神经营养因子-1、胶质细胞源性神经营养因子-2、生长相关蛋白、生长相关蛋白-2、生长相关蛋白-3、肝素结合的表皮生长因子、肝细胞生长因子、肝细胞生长因子受体、胰岛素样生长因子 I、胰岛素样生长因子受体、胰岛素样生长因子 II、胰岛素样生长因子结合蛋白、角化细胞生长因子、白血病抑制因子、白血病抑制因子受体-1、神经生长因子、水晶石中因子受体、神经营养因子-3、神经营养因子-4、胎盘生长因子、胎盘生长因子-2、血小板衍化内皮细胞生长因子、血小板衍化生长因子、血小板衍化生长因子 A 链、血小板衍化生长因子 AA、血小板衍化生长因子 AB、血小板衍化生长因子 B 链、血小板衍化生长因子 BB、血小板衍化生长因子受体-1、血小板衍化生长因子受体-2、促进 B 细胞增殖刺激因子、肝细胞因子、肝细胞因子受体、转化生长因子-1、转化生长因子-2、转化生长因子-3、转化生长因子-1.2、转化生长因子-4、转化生长因子-5、潜在转化生长因子-1、转化生长因子-1 结合蛋白 I、转化生长因子-1 结合蛋白 II、转化生长因子-1 结合蛋白 III、肿瘤坏死因子 I 型受体 (TNF-R1)、肿瘤坏死因子 II 型受体 (TNF-R2)、尿激酶型纤溶酶原激活物受体、血管内皮生长因子、和上述分子的嵌合蛋白以及生物活性或免疫活性片段。

当然本发明的特异性结合剂可与一种或多种抗炎症药物联合给药。这里所用的“抗炎症因子”通常指任何降低患者炎症反应或肿胀的药物。本文提到了许多抗炎症因子实例，不过当然还有未被提及的其它何时抗炎症因子，但这些药物也在本发明的考虑范围之内。

抗炎症因子可以为如抑制了炎症细胞因子与其受体间作用的化合物。可与本发明特异性结合剂联合使用的细胞因子抑制剂包括如TGF-β拮抗剂(如抗体)和直接针对涉及到炎症的白介素的拮抗剂(如抗体)。本文描述了这些白介素，优选的包括(但不仅限于)IL-1、IL-2、IL-3、IL-4、IL-5、IL-6、IL-8、IL-9、IL-11、IL-12、IL-13、IL-17和IL-18。见Feghali,等,*Frontiers in Biosci.*,2:12-26 (1997)。

本发明的特异性结合剂可与第一类蛋白激酶 A 抑制剂联合给药，以增强接受抗逆转录病毒治疗的HIV感染者体内T细胞的增殖。

也可联合使用本发明的特异性结合剂和神经生长因子(NGF)，以治疗某些疾病。这些疾病包括神经退化性疾病、脊索伤害和多发性硬化症，还包括青光眼和糖尿病。

优选一种或多种 IL-1 抑制剂与本发明的特异性结合剂进行联合治疗。IL-1 抑制剂包括(但不仅限于)IL-1 受体肽片段，直接针对 IL-1 或 IL-1 β或第一类 IL-1 受体的抗体，以及包含所有或部分 IL-1 受体的重组蛋白(或其修饰变异体)，这包括基因修饰的突变蛋白、多聚体形式和持续释放制剂。特异拮抗剂包括 IL-1ra 多肽，IL-1 β转换酶(ICE)抑制剂，第一类 IL-1 受体的拮抗抗体，第一类 IL-1 受体的 IL-1 结合形式和第二类 IL-1 受体，针对 IL-1(包括 IL-1 α、IL-1 β 和其它 IL-1 家族成员)的抗体和治疗剂 IL-1 Trap (Regeneron) 。IL-1ra 包括美国专利 No. 5,075,222 中所述的 IL-1ra 形式和修饰和变异体形式，包括美国专利 5,922,573，WO 91/17184，WO 92 16221 和 WO 96 09323 中所述。IL-1 β 转换酶(ICE)抑制剂包括肽酰和小分子 ICE 抑制剂，包括 PCT 专利申请 WO 91/15577，WO 93/05071，WO 93/09135，WO 93/14777 ，WO 93/16710，欧洲专利申请 0 547 699 中所述。非肽化合物包括下列文献中所述的此类化合物，PCT 申请 WO 95/26958、美国专利 No. 5,552,400、美国专利 No. 6,121,266、以及 Dolle 等,*J. Med. Chem.*,39,pp. 2438-2440 (1996)。其它 ICE 抑制剂的描述见美国专利 No. 6,162,790、6,204,261、6,136,787、6,103,711、

6,025,147、6,008,217、5,973,111、5,874,424、5,847,135、5,843,904、5,756,466、5,656,627、5,716,929。I 型 IL-1 受体的 IL-1 结合形式和 II 型 IL-1 受体的描述见美国专利 No. 4,968,607、4,968,607、5,081,228、Re 35,450、5,319,071、和 5,350,683。其他合适的 IL-1 拮抗剂包括(但不仅限于)可竞争性结合于 IL-1 信号受体的 IL-1 起源肽、I 型 IL-1 受体。其它一些 IL-1(和其它细胞因子)的拮抗剂的描述可见美国专利 No. 6,472,179。

此外，TNF 抑制剂也是合适的，这包括(但不仅限于)TNFα的受体结合肽片段、抑制 TNFα 生成的反义寡核苷酸或核酶、直接针对 TNFα抗体、包含 TNFα受体全部或部分的重组蛋白或其修饰变异体，包括基因修饰变异蛋白嵌合形式和持续释放制剂。TACE (肿瘤坏死因子-α 转换酶)抑制剂也是合适的，如 TAPI (Immunex Corp.) 和 GW-3333X (Glaxo Wellcome Inc.)。抑制 IgA-α_1AT 复合体(如 EP 0 614 464 B 中所述)形成的分子或者针对此复合体的抗体也是合适的。其它合适的分子包括(但不仅限于)TNFα-抑制二糖、葡萄糖胺的硫酸盐衍生物，或其它类似的碳水化合物，见美国专利 No. 6,020,323，还包括 TNFα肽抑制剂(美国专利 No. 5,641,751 和 5,519,000)，含有 D-氨基酸的肽(美国专利 No. 5,753,628)。此外，TNFα转换酶的抑制剂也是合适的。WO 01/03719 中进一步描述了可参与本发明联合使用的药物。

其它合适的分子包括(但不仅限于)小分子如萨立多胺或萨立多胺类似物、己酮可可碱(pentoxifylline)、基质金属蛋白酶(MMP) 抑制剂或其它小分子。适于本发明的 MMP 抑制剂包括如美国专利 No. 5,883,131、5,863,949 和 5,861,510 中所述分子，和美国专利 No. 5,872,146 中所述的烷基肽酰化合物。其它能够减少 TNFα生成的小分子包括如美国专利 5,508,300、5,596,013 和 5,563,143 中所述分子。其它合适的小分子包括(但不仅限于)美国专利 5,747,514,和 5,691,382 中所述的 MMP 抑制剂，和美国专利 No. 5,821,262 中所述的氧肟酸

衍生物。其它合适的分子包括如可抑制磷酸二酯酶 IV 和 TNFα生成的小分子，如取代的肟衍生物(WO 96/00215)、喹啉磺胺(美国专利 No. 5,834,485)、芳基呋喃衍生物 (WO 99/18095)杂双环(heterobicyclic)衍生物 (WO 96/01825; GB 2 291 422 A)。抑制 TNFα 和 IFNγ 的噻唑衍生物(WO 99/15524)和抑制 TNFα和其它炎症细胞因子的的黄嘌呤衍生物(见如美国专利 No. 5,118,500、5,096,906 和 5,196,430)也是合适的。美国专利 No. 5,547,979 中所述分子也适用于本发明。

可在联合治疗中使用的其它药物和药物类型实例包括(但不仅限于)抗病毒药物、抗生素、止痛剂(如醋氨酚、可待因、萘磺酸丙氧芬、盐酸羟考酮、重酒石酸二氢可待因酮、曲马多)、皮质类固醇、炎症细胞因子拮抗剂、疾病调节抗风湿病药物(Disease-Modifying Anti-Rheumatic Drugs ，DMARD)、非甾体类抗炎药(NSAID)、慢作用抗风湿药物(SAARD)。

疾病修饰抗风湿病药物(DMARD)包括如(但不仅限于)Rheumatrex™ (甲氨喋呤); Enbrel® (依那西普(etanercept)); Remicade® (infliximab); Humira™ (阿达木单抗(Adalimumab)); Segard® (阿非莫单抗(afelimomab)); Arava™ (来氟米特(leflunomide)); Kineret™ (阿那白滞素(anakinra)); Arava™ (来氟米特(leflunomide)); D-青霉胺; 金硫基代丁二酸钠; 羟氯喹; Ridaura™ (金诺芬(Auranofin)); 硫代葡萄糖金油剂(Solganal); 来那西普(lenercept)(Hoffman-La Roche); CDP870 (Celltech); CDP571 (Celltech)，以及 EP 0 516 785 B1、美国专利 No. 5,656,272、EP 0 492 448 A1 中所述药物; p55 肿瘤坏死因子连接蛋白(onercept) (Serono; CAS reg. no. 199685-57-9); MRA (Chugai); Imuran™ (咪唑硫嘌啉); NFKB 抑制剂; Cytoxan™ (环磷酰胺); 环孢霉素; 硫酸羟氯喹; 二甲胺四环素; 柳氮磺吡啶(sulfasalazine); 以及金化合物如口服金、硫代苹果酸金钠和金硫葡萄糖。

其它合适的分子包括如起源于 TNFα受体分子胞外区的 TNFR 衍

生物(不同于 p55 和 p75 TNFR)，例如 WO 99/04001 中所述的 TNFR，包括起源于该 TNFR 的 TNFR-Ig。此外合适的 TNFα抑制剂也适合于本文所述用途。这不仅包括用作本文所述的针对 TNFα或 TNFR 的抗体，也包括用作为可发挥 TNFα的竞争性抑制剂作用的 TNFα衍生肽(如美国专利美国专利 No. 5,795,859 或 No. 6,107,273 所述)，用作 TNFR-IgG 融合蛋白(如一个含有 p55 TNFα受体胞外部分的蛋白)，用作可溶性 TNFR(不是 IgG 融合蛋白)，用作其它可减少内生性 TNFα水平的分子，如 TNFα转换酶抑制剂(见如美国专利 5,594,106)，或小分子或 TNFα抑制剂。以上许多在本文中具有描述。

尽管 TNF 抗体的最优剂量需要富有经验的医疗人员根据患者的具体需要而加以确定，针对 TNFα的抗体的优选的一个剂量范围为 0.1-20mg/kg，更优选为 1-10 mg/kg。其它合适的抗 TNFα抗体的剂量为 0.75-7.5 mg/kg 体重。

本发明也可使用特异性结合剂和任意一种或多种非甾体抗炎药(NSAID)。NSAID 的抗炎功能至少部分上依赖于它们对前列腺素合成的抑制。(Goodman and Gilman,*The Pharmacological Basis of Therapeutics*,MacMillan 7th Edition (1985))。NSAID 的特征基团有 9 个：(1) 水杨酸衍生物；(2) 丙酸衍生物；(3) 粗酸衍生物；(4) 芬那酸衍生物；(5)羧酸衍生物；(6) 丁酸衍生物；(7) ,2-苯并噻嗪类；(8) 吡唑和(9) 吡唑啉酮。NSAID 包括如(但不仅限于)Anaprox™、Anaprox DS™ (甲氧萘丙酸钠)； Ansaid™ (氟比洛芬钠(flurbiprofen))； Arthrotec™ (双氯芬酸钠 + 米索前列醇(Misoprostil))； Cataflam™/Voltaren™ (双氯芬酸钾)； Clinoril™ (米索前列醇)； Daypro™ (奥沙普秦(oxaprozin))； Disalcid™ (水杨酸水杨酸酯)； Dolobid™ (二氟尼柳(Diflunisal))； EC Naprosyn™ (甲氧萘丙酸钠)； Feldene™ (吡罗昔康(piroxicam))； Indocin™、Indocin SR™ (吲哚美辛(indomethacin))； Lodine™、Lodine XL™ (依托度酸(etodolac))； Motrin™ (异丁苯丙酸)； Naprelan™ (甲氧萘丙酸)； Naprosyn™ (甲氧

萘丙酸); Orudis™、(酮洛芬(ketoprofen)); Oruvail™ (酮洛芬); Relafen™ (萘丁美酮(nabumetone)); Tolectin™、(托美汀钠(tolmetin sodium)); Trilisate™ (三柳胆镁); Cox-1 抑制剂; Cox-2 抑制剂如 Vioxx™ (rofecoxib); Arcoxia™ (依托考昔(etoricoxib))、Celebrex™ (celecoxib); Mobic™ (美洛昔康(meloxicam)); Bextra™ (valdecoxib)、Dynastat™ (甲氧萘丙酸钠); Prexige™ (lumiracoxib)和 nambumetone。其它合适的 NSAID 包括(但不仅限于): ε-乙酰氨基己酸、S-腺苷甲硫氨酸、3-氨基-4-羟丁酸、阿米西群(amixetrine)、阿尼扎芬(anitrazafen)、安曲非宁 (antrafenine)、bendazac、苄达赖氨酸 (bendazac lysinate)、benzydamine、beprozin、溴哌莫(broperamole)、布可隆(bucolome)、丁苯唑酸(bufezolac)、环丙喹宗(ciproquazone)、cloximate、达齐胺(dazidamine)、地波沙美(deboxamet)、detomidine、吡啶基联苯基乙酰胺、difenpyramide、difisalamine、ditazol、依莫法宗(Emorfazone)、fanetizole mesylate、芬氟咪唑 (fenflumizole)、氟奎氨苯酯 (floctafenine)、flumizole、flunixin、氟丙喹宗 (fluproquazone)、fopirtoline、磷霉素、胍美柳(guaimesal)、guaiazolene、isonixirn、盐酸利非他明(lefetamine HCl)、来氟米特(leflunomide)、洛非咪唑(lofemizole)、氯替法唑(lotifazole)、clonixinate 溶菌酶、美西拉宗(meseclazone)、萘丁美酮(nabumetone)、尼克吲哚(nictindole)、尼美舒利(Nimesulide)、orgotein、奥帕诺辛(orpanoxin)、oxaceprolm、奥沙帕朵(oxapadol)、瑞尼托林(paranyline)、哌立索唑(perisoxal)、柠檬酸哌立酮、哌福肟(pifoxime)、萘普生哌嗪酸(Piproxen)、吡拉唑酸(pirazolac)、pirfenidone、普鲁奎松(proquazone)、普罗沙唑(proxazole)、thielavin B、替氟咪唑(tiflamizole)、替美加定(timegadine)、托来汀(tolectin)、托帕朵(tolpadol)、tryptamid 和分配为如下列公司编码的药物 480156S、AA861、AD1590、AFP802、AFP860、AI77B、AP504、AU8001、BPPC、BW540C、CHINOIN 127、CN100、EB382、EL508、F1044、FK-506、GV3658、ITF182、KCNTEI6090、KME4、LA2851、

MR714、MR897、MY309、ONO3144、PR823、PV102、PV108、R830、RS2131、SCR152、SH440、SIR133、SPAS510、SQ27239、ST281、SY6001、TA60、TAI-901 (4-苯酰-1-吲丹羧酸)、TVX2706、U60257、UR2301 和 WY41770。具有类似止痛和抗炎功能的 NSAID 结构相关物也可包括在其中。

合适的 SAARD 或 DMARDS 包括(但不仅限于)阿洛酮钠(allocupreide sodium)、金诺芬(Auranofin)、金硫葡萄糖(aurothioglucose)、α-硫金基乙酰苯胺(aurothioglycanide)、咪唑硫嘌啉、布喹那(Brequinar Sodium)、布溪那明(bucillamine)、钙 3-硫金(aurothio)-2-丙醇-1-磺酸钠、苯丁酸氮芥、氯喹、氯丁扎利(clobuzarit)、铜克索林(cuproxoline)、环磷酰胺、环胞霉素、氨苯砜、15-脱氧精胍菌素(15-deoxyspergualin)、双醋瑞因(Diacerein)、葡萄糖胺、金盐 (如环喹金盐、硫代苹果酸金钠、硫代硫酸金钠)、羟化氯喹、羟基脲、凯布宗(kebuzone)、左咪唑、氯苯扎利(lobenzarit)、蜂毒肽、6-巯基嘌呤、甲氨蝶呤、咪唑立宾(mizoribine)、霉酚酸吗啉乙酯(mycophenolate mofetil)、金硫乙酸钙、氮芥、D-青霉胺、羟基吡啶吡唑如 SKNF86002 和 SB203580、雷帕霉素(Rapamycin)、硫醇、促胸腺生成素和长春新碱。具有类似止痛和抗炎功能的 SAARD 或 DMARD 结构相关物也可包括在其中。

信号级联中的激酶抑制剂也适合与本发明特异性结合剂联合使用。这包括(但不仅限于)能够抑制 P-38 的药物(又称 “RK” 或 “SAPK-2”、Lee *et al*,*Nature*、372:739 (1994))。P-38 被描述为丝氨酸/苏氨酸激酶(见 Han 等、*Biochimica Biophysica Acta*、1265:224-227 (1995))。已发现 P-38 抑制剂通过抑制依赖于信号途径的激酶，干扰了胞外刺激与 IL-1 和 TNFα与(涉及到阻遏信号传导(blocking signal transduction)的)细胞中分泌之间的联系。

MK-2 抑制剂和 tpl-2 抑制剂也合适。此外合适的还有 T 细胞抑制剂包括如 ctla-4、CsA、Fk-506、OX40、OX40R-Fc、OX40 抗体、

OX40 配体、OX40 配体的抗体、ck 和 ZAP70。类维生素 A 包括口腔类维生素 A 和 TGF-β拮抗剂也是合适的。

其它合适与本发明特异性结合剂联合使用的药物包括如任意一种或多种水杨酸衍生物，及其酯前体药物或药学上可接受的盐。这包括：醋氨沙洛(acetaminosalol)、阿洛普令(aloxiprin)、阿司匹林、扑炎痛(benorylate)、溴水杨醇、乙酰水杨酸钙、三柳胆镁、diflusinal、依特柳酯(etersalate)、芬度柳(fendosal)、2,5-二羟基苯甲酸、水杨酸乙二醇、水杨酸咪唑、赖氨匹林、mesalamine、水杨酸吗啉、1-萘基水杨酸、水杨嗪、帕沙米特(parsalmide)、苯基乙酰水杨酸、苯基水杨酸、醋水杨胺(salacetamide)、水杨酰胺 O-乙酸、水杨酸水杨酸酯和水杨嗪。具有类似止痛和抗炎功能的水杨酸结构相关物也可包括在其中。其它合适的药物包括如丙酸衍生物，及其酯前体药物或药学上可接受的盐，这包括：阿米洛芬(Alminoprofen)、苯恶洛芬(benoxaprofen)、布氯酸(Bucloxic acid)、卡洛芬(Carprofen)、右吲哚洛芬(dexindoprofen)、苯氧布洛芬(fenoprofen)、氟诺洛芬(flunoxaprofen)、氟洛芬(fluprofen)、氟比洛芬钠(flurbiprofen)、呋洛芬(furcloprofen)、异丁苯丙酸、异丁苯丙酸铝、黄原酸异丙酯、吲哚洛芬(indoprofen)、异洛芬(isoprofen)、苯酮苯丙酸(ketoprofen)、洛索洛芬(loxoprofen)、米洛芬(miroprofen)、甲氧萘丙酸、oxaprozin、吡酮洛芬(piketoprofen)、pimeprofen、吡氯布洛芬(pirprofen)、普拉洛芬(Pranoprofen)、丙替嗪酸、pyridoxiprofen、舒洛芬(suprofen)、噻洛芬酸(tiaprofenic acid) 和硫噁洛芬。具有类似止痛和抗炎功能的丙酸结构相关物也可包括在其中。其它合适的药物包括如醋酸衍生物，及其酯前体药物或药学上可接受的盐，这包括：阿西美辛、阿氯芬酸(alclofenac)、氨基苯酰基苯乙酸、丁苯羟酸、吲哚拉新(cinmetacin)、氯吡酸(clopirac)、地美辛(delmetacin)、双氯芬酸钠(diclofenac sodium)、依托度酸(etodolac)、联苯乙酸(FELBINAC)、芬氯酸(fenclofenac)、苯克洛酸(fenclorac)、芬克洛酸(fenclozic acid)、芬替

酸(fentiazac)、乙基二氢苯并呋喃乙酸、葡美辛(glucametacin)、异丁苯乙酸(ibufenac)、茚甲新、三苯唑酸(isofezolac)、伊索克酸(Isoxepac)、氯那唑酸(lonazolac)、甲嗪酸(metiazinic acid)、奥沙美辛(oxametacin)、oxpinac、吡美辛 (pimetacin)、丙谷美辛(proglumetacin)、舒林酸(sulindac)、他美辛(talmetacin)、羟哌苯酮(tiaramide)、二氢氧二苯并硫杂、痛灭定(tolmetin)、齐多美辛(zidometacin)、佐灭酸(zomepirac)。具有类似止痛和抗炎功能的醋酸结构相关物也可包括在其中。此外合适的还有灭酸衍生物，及其酯前体药物或药学上可接受的盐，这包括：苯乙氨茴酸、依托芬那酯(etofenamate)、氟灭酸(flufenamic acid)、异尼辛(isonixin)、甲氯灭酸(meclofenamic acid)、甲氯胺苯酸钠(meclofenamate sodium)、甲氯芬那酸(medofenamic Acid)、甲灭酸(mefanamic acid)、尼氟灭酸(niflumic acid)、他尼氟酯(talniflumate)、特罗氨酯(terofenamate)、托灭酸 (tolfenamic acid)和乌芬那酯(ufenamate)。具有类似止痛和抗炎功能的灭酸结构相关物也可包括在其中。

此外合适的还有羧酸衍生物，及其酯前体药物或药学上可接受的盐，这包括：克力丹酸(clidanac)、二氟尼柳(Diflunisal)、氟苯柳(flufenisal)、inoridine、酮咯酸(ketorolac)和替诺立啶(tinoridine)。具有类似止痛和抗炎功能的羧酸结构相关物也可包括在其中。此外合适的还有丁酸衍生物，及其酯前体药物或药学上可接受的盐，这包括：丁丙二苯肼(bumadizon)、布替布芬(butibufen)、苯布芬(fenbufen)和联苯丁酸(xenbucin)。具有类似止痛和抗炎功能的丁酸结构相关物也可包括在其中。此外合适的还有昔康衍生物，及其酯前体药物或药学上可接受的盐，这包括：德罗喜康(droxicam)、依诺利康(enolicam)、异恶噻酰胺(isoxicam)、吡罗昔康(piroxicam)、湿痛喜康(Sudoxicam)、替诺昔康(tenoxicam)和 4-羟基-1,2-苯并噻嗪 1,1-二氧 4-(N-苯基)-羧酰胺。具有类似止痛和抗炎功能的昔康结构相关物也可包括在其中。此外合适的还有吡唑衍生物，及其酯前体药物或药

学上可接受的盐，这包括：二苯米唑(difenamizole)和依匹唑(epirizole)。具有类似止痛和抗炎功能的吡唑结构相关物也可包括在其中。此外合适的还有吡唑啉酮衍生物，及其酯前体药物或药学上可接受的盐，这包括：阿扎丙宗(apazone)、阿扎丙宗(azapropazone)、苄哌立隆(benzpiperylon)、非普拉宗(feprazone)、莫非布宗(mofebutazone)、吗拉宗(morazone)、羟基保泰松、苯基丁氮酮、哌布宗(pipebuzone)、propylphenazone、雷米那酮(ramifenazone)、琥布宗(suxibuzone)和 thiazolinobutazone。具有类似止痛和抗炎功能的吡唑啉酮结构相关物也可包括在其中。

此外，皮质甾类酯前体药物和药学可接受的盐适于用在对 TNF 介导的疾病的治疗中。皮质甾类酯前体药物和药学可接受的盐包括氢化可的松以及来源于氢化可的松的化合物，如 21-乙酸基- 孕烯醇酮、alclomerasone、阿尔孕酮(algestone)、安西奈德(amcinonide)、氯地米松(beclomethasone)、倍他米松(β-methasone)、戊酸倍他米松(betamethasone valerate)、布地奈德(budesonide)、氯泼尼松(chloroprednisone)、氯倍他索(clobetasol)、丙酸氯倍米松(clobetasol propionate)、氯倍他松(clobetasone)、丁酸氯倍他松(clobetasone butyrate)、氯可托龙 (clocortolone)、氯泼尼醇(cloprednol)、肾上腺酮、可的松、可的伐唑(cortivazol)、deflazacon、地索奈德(desonide)、desoximerasone?、地塞米松、二氟拉松(diflorasone)、二氟可龙(diflucortolone)、二氟泼尼酯(difluprednate)、甘草次酸(enoxolone)、氟扎可特(fluazacort)、氟氯奈德(flucloronide)、flumethasone、氟米松匹酯(flumethasone pivalate)、氟尼缩松(Flunisolide)、氟轻松（flucinolone acetonide）、醋酸氟轻松(fluocinonide)、氟氢松醋酸酯(fluorocinolone acetonide)、氟可丁(fluocortin butyl)、氟可龙(fluocortolone)、己酸氟可龙(fluorocortolone hexanoate)、双氟可龙戊酸酯(diflucortolone valerate)、氟甲松龙(Fluorometholone)、醋酸氟培龙(fluperolone acetate)、醋酸氟泼尼定(fluprednidene acetate)、氟泼尼

龙(fluprednisolone)、氟羟可舒松(flurandenolide)、福莫可他(formocortal)、氯氟松、卤米松、醋酸卤泼尼松(halopredone acetate)、氢可他酯、氢化可的松、醋酸氢化可的松、丁酸氢化可的松、磷酸氢化可的松、21-钠琥珀酸氢化可的松、叔丁乙酸盐、氢化可的松、马泼尼酮(mazipredone)、甲羟松(medrysone)、甲泼尼松(meprednisone)、methylprednicolone、莫美他松糠酸酯(Mometasone Furoate)、帕拉米松(paramethasone)、泼尼卡酯、氢化泼尼松、21-二乙氨基乙酸(diedryaminoacetate)泼尼松龙、氢化泼尼松磷酸钠、氢化泼尼松琥珀酸钠、氢化泼尼松 21-*m*-苯甲酸钠、21-硬脂酰甘油酸钠、强的松龙叔丁乙酯、21-三甲基醋酸氢化泼尼松、强的松、泼尼松龙戊酸酯(prednival)、泼尼立定(prednylidene)、21-二乙基氨基醋酸泼尼立定、巯氢可的松(tixocortol)、氟羟氢化泼尼松、丙酮氟羟氢化泼尼松、苯曲安奈德(triamcinolone benetonide)和己曲安缩松(triamcinolone hexacetonide)。具有类似止痛和抗炎功能的皮质甾类结构相关物也可包括在其中。

抗微生物剂(及其酯前体药物和药学可接受的盐)也可应用在本文所述的联合使用中。合适的抗菌剂包括如氨苄青霉素、羟氨苄青霉素、金霉素(aureomicin)、杆菌肽、头孢他啶、头孢曲松、头孢噻肟、cephachlor、头孢氨苄、头孢拉定、环丙沙星、克拉维酸、氯唑青霉素、双氯青霉素、红霉素、氟氯青霉素、庆大霉素、短杆菌肽、甲氧苯青霉素、新霉素、苯唑西林、青霉素以及万古霉素。具有类似止痛和抗炎功能的抗菌剂结构相关物也可包括在其中。

其它合适的化合物包括(但不仅限于)BN 50730；替尼达普(tenidap)；E 5531；tiapafant PCA 4248；尼美舒利(Nimesulide)；panavir；咯利普兰(Rolipram)；RP 73401；T 肽(peptide T)；MDL 201,449A；(1R,3S)-顺式-1-[9-(2,6-二氨基嘌呤基)]-3-羟基-4-盐酸环戊烯；(1R,3R)-反式-1-[9-(2,6-二氨基)嘌啉]-3-乙酰氧基环戊烷；(1R,3R)- 反式-1-[9-腺嘌啉)-3-叠氮基环戊烷甲酸和(1R,3R)- 反式-1-

[6-羟基-嘌啉-9-基)-3-叠氮基环戊烷。

已经发现 IL-4 在某些情况下可引起炎症反应，如在哮喘中 IL-4 在肺部的过量表达引起上皮细胞肥大，和淋巴细胞、嗜曙红细胞、嗜中性粒细胞的的积累。该反应代表了由其它 Th2 细胞因子所引起的前炎症反应的主要特征。因此如上文所述，IL-4 的抑制剂也可在本发明中发挥作用。另外，某些免疫抑制剂药物当然也可用于治疗关节炎，这些药物包括(但不仅限于)iNOS 抑制剂和 5-脂肪氧合酶抑制剂。

已发现 Ginger 具有一定的抗炎作用，因此也适合作为本发明中的抗炎药物，类似情况还有软骨素。

在某些实施方案中，Ang-2 特异性结合剂的给予可发生在抗癌药物治疗的之前、同时或之后。癌症包括如(但不仅限于乳癌、肠癌、胃癌、神经胶质瘤、头颈鳞状细胞癌、遗传散发性乳头状肾细胞癌、白血病、淋巴瘤、Li-Fraumeni 综合征、胸膜恶性间皮瘤、黑素瘤、多骨髓瘤、非小细胞肺癌、骨肉瘤、卵巢癌、胰腺癌、前列腺癌、小细胞肺癌、滑膜肉瘤、甲状腺癌、膀胱移行癌。

在某些实施方案中，可以单独使用 Ang-2 特异性结合剂或与至少一种其它癌症治疗药物联合使用。在某些实施方案中，Ang-2 特异性结合剂与一种有效剂量的癌症治疗药物联合使用。这种可与 Ang-2 特异性结合剂联合使用的癌症治疗药物包括如(但不仅限于)anisamycin 抗生素中格尔德霉素(geldanamycin)家族的成员；Pro-HGF; NK2; a c-蛋氨酸肽抑制剂; 生长因子受体结合蛋白 2(Grb2) SH2 (Src homology 2)结构域的拮抗剂；Gab1 调节物；显性阴性 Src; von-Hippel-Landau 抑制剂，包括(但不仅限于)渥曼青霉素；P13 激酶抑制剂、其它抗受体(anti-receptor)疗法、抗 EGFR、COX-2 抑制剂、Celebrex™、Vioxx™；血管内皮生长因子(VEGF)、VEGF 调节物、纤维原细胞生长因子 (FGF)、FGF 调节物、内皮生长因子(EGF); EGF 调节物；角化细胞生长因子 (KGF)、KGF 相关分子、KGF 调

节物；基质金属蛋白酶(MMP) 调节物。

在本发明的某些实施方案中，所用药物包括 Ang-2 特异性结合剂和至少一种丝氨酸蛋白酶抑制剂，并使用这些药物进行治疗。在某些实施方案中，所用药物包括 Ang-2 特异性结合剂、丝氨酸蛋白酶抑制剂和至少一种其它本文中所述的分子。

在某些情况下，蛋白酶/蛋白酶(protease/protease)平衡的破坏可引起蛋白酶-介导的组织破坏，包括(但不仅限于)肿瘤入侵正常组织，从而引起肿瘤转移。

在某些实施方案中，Ang-2 特异性结合剂可与至少一种炎症治疗药物联合使用。在某些实施方案中，Ang-2 特异性结合剂可与至少一种免疫失调治疗药物联合使用。炎症和免疫失调治疗药物包括如(但不仅限于)环氧酶 1 (COX-1) 和环氧酶 2 (COX-2) 抑制剂；38 kDa 的有丝分裂原活化的蛋白激酶(mitogen-activated protein kinase (p38-MAPK))的小分子调节物；参与炎症途径的胞内分子的小分子调节物，这种胞内分子包括(但不仅限于)jnk、IKK、NF-κB、ZAP70、和 lck。某些炎症治疗药物例子可见如 C.A. Dinarello and L.L. Moldawer "风湿性关节炎中的前炎症和抗炎细胞因子：治疗依据"，*Proinflammatory and Anti-Inflammatory Cytokines in Rheumatoid Arthritis: A Primer for Clinicians* Third Edition (2001) Amgen Inc. Thousand Oaks, CA。

在某些实施方案中，药用组合物包含一种以上的不同 Ang-2 特异性结合剂。在某些实施方案中，药用组合物包含一种以上的 Ang-2 特异性结合剂，它们结合于一种以上的表位上。

免疫治疗

通常免疫治疗依赖于使用免疫影响物细胞和分子以靶定位并摧毁癌症细胞。免疫影响物例如可以是本发明的抗体，它识别靶细胞表面上的一些标记。这种抗体可单独作为治疗影响物，或者可激发其

它细胞发挥实际杀死细胞的功能。这种抗体也可以与药物或毒素(化疗药物、放疗药物、蓖麻毒素 A 链、霍乱毒素、百日咳毒素等)联合使用，抗体仅扮演定位剂的角色。

根据本发明，免疫治疗剂(本发明的抗体或抗体偶联物)可靶定位突变体形式的 Ang-2。特别需要的支出的是，本发明的抗体组合物可以与 Ang-2 靶定位剂一起用在联合疗法中。

已证明被动免疫疗法对许多癌症尤其有效。见如 WO 98/39027。

下文实例仅用以阐述本发明，而不对本发明做出任何限定。

实施例 1

在病理性组织和正常组织中表达 Ang-2

使用原位杂交检测正常组织和病理性组织中 Ang-2 的表达。通过逆转录 PCR 从人或鼠胎儿肺脏 cDNA 中扩增出人 Ang-2 序列片段(Genbank 检索号: AF004327, 核苷酸 1274-1726)和鼠 Ang-2 片段(Genbank 检索号: AF004326, 核苷酸 1135-1588)，并克隆到 pGEM-T 质粒载体中，通过测序确证。使用 ^{33}P-UTP 和 RNA 聚合酶以线性化质粒为模板转录得到 ^{33}P 标记的反义 RNA 探针。经甲醛封阻并包埋在石蜡中的组织被切片(5 μm)并收集在荷电的（charged）玻片上。原位杂交之前，用 0.2M HCl 浸透（permeabilized）组织，然后用蛋白酶 K 消化，并用三乙醇胺和醋酸酐乙酰化。在 55°C 下使放射性标记探针与切片杂交过夜，然后用 RNase 消化，并用约 0.1X SSC 在 55°C 下进行高严紧性洗涤。将玻片浸没在 Kodak NTB2 液中，并在 4°C 下暴露 2-3 周，显影并复染色。使用暗视野和标准光照检查切片，以便同时评价组织形态和杂交信号。

结果表明，在正常出生后的人体中，Ang-2 表达被限制在少数几个含有新生血管的组织中，如卵巢、胎盘和子宫。正常成人心脏、大脑、肾脏、肝脏、肺、胰脏、脾脏、肌肉、扁桃腺、胸腺、阑尾、淋巴结、胆囊、前列腺和睾丸中都没有 Ang-2 表达。在 5 周龄小鼠

肾脏的直小血管中有显著的 Ang-2 表达，而在成年猴或人中则没有。为确认此表达是否是胚胎发育的残留，使用鼠 Ang-2 探针和上述反应条件对不同大小的小鼠(直至 1 岁)肾脏进行检测。发现随着出生后的生长，Ang-2 表达降低，但在 1 岁小鼠肾脏中仍明显存在。

几乎所有试验的肿瘤类型中均检测到了 Ang-2 表达，这包括人原发性肿瘤如如结肠癌 (5 例)，乳癌(10 例)，肺癌(8 例)，多形性胶质母细胞瘤(1 例)，人转移瘤如乳癌 (2 例)，肺癌(2 例)和卵巢癌 (2 例)(已转移到大脑)，和啮齿类肿瘤模型如 C6 (大鼠神经胶质瘤)，HT29 (人结肠癌)，Colo-205(人结肠癌)，HCT116 (人结肠癌)，A431 (人表皮样癌)，A673 (人横纹肌肉瘤)，HT1080 (人纤维肉瘤)，PC-3 (人前列腺癌)，B16F10 (鼠黑素瘤)，MethA (鼠肉瘤)，和 Lewis 肺癌转移 (Lewis lung carcinoma mets)。另外，对响应 VEGF 而生长进入基质胶塞(Matrigel plug)的新生血管，和小鼠早熟视网膜病缺氧模型也进行了 Ang-2 表达检测。

实例例 2

重组 mAng-2 蛋白和兔多克隆抗 Ang-2 抗血清的制备

使用扩增全长人 Ang-2 所用的 PCR 引物，从 15 日龄鼠胚胎 cDNA 文库 (Marathon-Ready-cDNA, Cat.# 7459-1, Clonetech, Inc.)通过 PCR(Clontech Advantage PCR Kit, Cat. # K1905-01) 得到带有 His 标签的全长鼠 Ang-2 cDNA。将 PCR 产物连接到 CMV 启动子表达载体上，用 FuGENE6 转染试剂 (Roche, Cat. #1814443)将所得质粒转染进 HT1080 人纤维肉瘤细胞(得自 ATCC)。通过 G418 选择分离稳定克隆。使用抗 His 标签 ELASA 和 Western 印迹筛选出表达 mAng-2-his 的克隆。

从这些细胞的条件培养基(C.M.)中纯化重组 mAng-2 多肽。使用两步色谱法纯化含有 mAng-2-His 的条件培养基。简短来说，向条件培养基中添加 Tris 缓冲液(pH 9.5)直至终浓度为约 20mM，调节 pH

到 8.9。另外也添加去污剂 CHAPS 直至终浓度为约 5mM。然后使培养基直接上阴离子交换柱 Q-sepharose ff (Pharmacia)。用浓度约为 10 mM 的 Tris 缓冲液(pH 8.0，含有约 50 mM NaCl)洗涤柱子。使用浓度为 10 mM 的 Tris 缓冲液(pH 8.0，含有约 350 mM NaCl 和 5mM 的 CHAPS)在一个步骤中洗脱重组 mAng-2-His。

Q-sepharose 柱洗出液用浓度约 4mM 的咪唑调节，并通过固定金属亲合柱(Ni-NTA superflow (Qiagen))。用含有浓度约 5mM 的 CHAPS 和约 100mM 的咪唑的 PBS 洗脱结合的蛋白。然后将洗出液浓缩到约 1.0mg/ml，并用 PBS 透析。SDS-PAGE 考马斯蓝染色表明 mAng-2-His 的纯度高于 90%。

通过注射约 0.2mg 的 mAng-2 来免疫兔子，以产生抗体。向兔子体内注射 1mL Hunter's TiterMax® (Sigma) 和 mAng-2 的混合液(比例为 1:1)。4 周后，每只兔子均接受重复注射或加强免疫；两周后，再次接受加强剂；在第七周抽血并评价 mAng-2 滴度。如果血清滴度高，则每周抽血 50mL，连续 6 周。但是如果血清滴度低，就给予兔以附加加强剂，并从第九周开始每周抽血 50mL，连续 6 周。抽血 6 周后，对兔不进行任何处理，时间为 6 周。如果需要更多血清则在最后抽血后的一个月后再次对兔进行加强免疫。

使用中和 ELISA(见下文)，观察从 5245 号和 5255 号兔所得的兔抗 mAng-2 多克隆抗血清对 mAng-2:Tie2 相互作用的中和。

实施例 3

评价 Ang-2 抗体的分子实验

开发了分子实验(亲和 ELISA、中和 ELISA 和 BIAcore)以对结合于 Ang-2 及相关家族成员的抗体进行直接评价，并评价抗体对 mAng-2:Tie2 相互作用的影响。下文描述了这些基于细胞的体外实验。

A.亲和 ELISA

为了对候选抗 Ang-2 抗体进行初始筛选，使用纯化的人 Ang-2(R and D Systems, Inc; 目录号 623-AN; 其中 Ang-2 以两种切短片段混合物形式提供)或鼠 Ang-2 多肽(按上文所述方法制备)。为了进行确认性结合实验，使用全长人 Ang-2 DNA 转染人 293T 细胞，并在无血清 DMEM 中(含有约 50 微克/ml 的牛血清白蛋白(BSA)进行培养，从该条件培养基中得到人 Ang-2。

使用微量滴定板，向每孔中加入约 100 微升 Ang-2，并温育约 2h。然后用磷酸盐缓冲液(PBS，含有约 0.1%的 Tween-20)洗涤微量滴定板 4 次。每孔使用约 250 微升约含 5%BSA 的 PBS 进行封阻，然后在室温下温育约 2h。温育后除去过量的封阻液，向每孔中加入约 100 微升候选抗 Ang-2 抗体，其稀释梯度起始为约 40nmol，然后用含有约 1%BSA 的 PBS 连续稀释四倍。然后在室温下温育过夜。温育后，用含有约 0.1%Tween-20 的 PBS 洗涤微量滴定板 5 次，然后向每孔加入约 100 微升羊抗人 IgG(Fc)-HRP (Pierce Chemical Co., 目录号# 31416，预先用含 1%BSA 的 PBS 稀释为 1:5000)。微量滴定板在室温下温育约 1h 后，用含约 0.1%Tween-20 的 PBS 洗涤 5 次，然后向每孔加入约 100 微升 TMB (3,3',5,5'-四甲基对二氨基联苯 Liquid Substrate System; Sigma chemical Company, St. Louis, MO, 目录号 T8665)底物，并温育 5-15min 直到蓝色出现。然后 370nm 处测量吸光度。

B. 中和 ELISA

如亲和 ELISA 部分所述在微量滴定板上固定人 Ang-2 多肽。用含有约 1%BSA 和约 1nM Tie2(以 Tie2-Fc 分子形式提供，其中 Tie2 部分仅含有该分子的可溶性胞外部分，R 和 D 系统, 目录号 313-TI)的 PBS 溶液如上文亲和 ELISA 部分所述对候选抗 Ang-2 抗体进行梯度稀释。向每孔中加入约 100 微升抗体/Tie2 溶液后，室温下温育过

夜，用含 0.1%Tween-20 的 PBS 洗涤 5 次。然后向每孔加入约 100 微升抗 Tie2 抗体(Pharmingen Inc.，目录号 557039)使终浓度为约 1 微克/ml，室温温育约 1h。然后向每孔加入约 100 微升羊抗鼠 IgG-HRP (Pierce Chemical Co.，目录号 31432，预先用含 1%BSA 的 PBS 稀释为 1:10000)。微量滴定板在室温下温育约 1h 后，用含 0.1%Tween-20 的 PBS 洗涤 5 次，然后向每孔加入约 100 微升 TMB 底物(如上文所述)，并温育直到颜色出现。然后在 370nm 处测量吸光度。

C.亲和 BIAcore

以 PBS 和 0.005% 的 P20 表面活性剂 (BIAcore, Inc.)作为运行缓冲液，在 BIAcore®2000 (Biacore, Inc., Piscataway, NJ) 上对每个候选 Ang-2 抗体均进行亲和分析。使用 Amine Coupling Kit (Biacore, Inc.) 根据使用说明书通过伯胺基团将重组 G 蛋白 (Repligen, Needham, MA)固定在研究级别的 CM5 传感器芯片上 (Biacore, Inc.) 。

结合实验步骤如下。首先将每种候选抗 Ang-2 抗体约 100Ru 连接在固定的 G 蛋白上，然后在固定抗体表面上方注射入不同浓度(0-100nM)的 huAng-2 或 mAng-2，流速约为 50 μl/min，持续约 3min。使用 BIA evaluation 3.1 版计算机程序(BIAcore, Inc.)确定抗体结合动力学，包括 k_a (结合速率常数)，k_d (解离速率常数)和 K_D (解离平衡常数) 。低解离平衡常数说明 Ang-2 抗体的亲和力更高。

实施例 4

噬菌体展示法制备全长人 Ang-2 抗体

根据下文描述，针对人 Ang-2 多肽(R and D Systems Inc.，目录号 623-AN)从靶向查询噬菌体 Fab 文库(Target Quest Phage Display Fab library) (Target Quest, Inc.)中淘选得到全长人 Ang-2 抗体。

通过两种方法将人 Ang-2 固定在聚苯乙烯磁力珠表面上：(1) 直接包被，用 Ang-2 4℃下反应过夜；(2)间接捕获，用 50μg/ml 的羊抗 Ang-2 抗体在 4℃下反应过夜捕获 Ang-2。用含 2%牛奶的 PBS(MPBS)

封阻珠子表面。对人 Fab 噬菌体文库进行预选择以除去与未包被磁力珠反应或与羊抗 Ang-2 抗体反应的噬菌体。然后使被 Ang-2 包被的珠子与噬菌体文库在室温下温育 1.5h。在噬菌体结合步骤后，用含 0.1%Tween-20 的 MPBS 洗涤珠子 6 次，再用含 0.1%Tween-20 的 PBS 洗涤 6 次，再用 PBS 洗涤 2 次。首先用约 10μg/ml 的 Tie2-Fc(R and D Systems, Minneapolis, MN)洗脱固定的噬菌体，然后用约 100mM 的三乙醇胺洗脱。用洗脱所得噬菌体感染大肠杆菌 TG1 细胞，扩增后回收用于进一步筛选。在连续筛选回合中，通过采用更严紧的洗涤条件和减少噬菌体输入量，来增大选择压力。3 轮筛选后鉴别得到 18 个独特 Ang-2 结合 Fab 克隆，通过上文所述的 ELISA 亲和实验检测，它们几乎都识别人 Ang-2、大鼠 Ang-2 和小鼠 Ang-2。这些噬菌体中约有 10%也结合人 Ang-1。按下文所述将这些克隆转化为 IgG1 抗体。

为得到额外独特噬菌体，进行第二轮筛选，使用相同文库只是方法稍有不同。在这个方法中，将人 Ang-2 加到装有 $NaHCO_3$ 缓冲液(pH 9.6)的 Nunc maxisorp 免疫管中，4℃过夜。第 1、2、3 轮淘选的 Ang-2 浓度分别为 1.5、0.74、0.3μg/ml。使用含 2%牛奶的 PBS(MPBS)封阻免疫管表面，然后与上文中所述同一文库(Target Quest)的约 2 万亿噬菌体颗粒(文库中每个独特克隆约 50 个拷贝)在约 4ml 2%MPBS 中温育。噬菌体温育步骤后，用 PBS 加上约 0.1%Tween-20 洗涤表面 20 次，再用 PBS 洗涤 20 次。使用 1μM hAng-2 或 1μM 人 Tie2(R and D Systems，同上文)洗脱固定的噬菌体。用洗脱所得噬菌体感染大肠杆菌 TG1 细胞(带有噬菌体文库)，扩增后回收用于下一轮筛选。通过对所有固定 hAng-2 或 Tie2 的噬菌体进行 PCR 扩增，鉴别到 16 个独特 Ang-2 结合 Fab 克隆，并使用限制性酶切分析了这些克隆。对这 16 个克隆的 DNA 都进行了测序。

每个噬菌体的每条重链可变区的编码序列均使用互补引物扩增。引物设计成在可变区的 5'末端含有 HindIII 和 XbaI 位点、Kozak

序列和信号序列(翻译的肽为 MDMRVPAQLLGLLLLWLRGARC; SEQ ID NO: 202)，而在 PCR 产物的 3'末端含有 BsmBI 位点。例如如下克隆重链，扩增来自 544 号克隆的噬菌体 DNA 模板(SEQ ID NO: 19)，使用 2248-21 引物(GTG GTT GAG AGG TGC CAG ATG TCA GGT CCA GCT GGT GCA G; SEQ ID NO: 203，添加有信号序列的最后 7 个氨基酸)，2502-31 引物(ATT ACG TCT CAC AGT TCG TTT GAT CTC CAC; SEQ ID NO: 204，可变区末端添加有 BsmBI 位点)。所得产物再次扩增，使用 2148-98 引物(CCG CTC AGC TCC TGG GGC TCC TGC TAT TGT GGT TGA GAG GTG CCA GAT; SEQ ID NO: 205，添加 9 个氨基酸到信号肽 (AQLLGLLLL; SEQ ID NO: 206)) 和 2502-31 引物，再使用 2489-36 引物 (CAG CAG AAG CTT CTA GAC CAC CAT GGA CAT GAG GGT CCC CGC TCA GCT CCT GGG; SEQ ID NO: 207) 和 2502-31 引物再次扩增。2498-36 引物从 5'到 3'添加了 HindIII 和 XbaI 位点，Kozak 序列和信号肽的前 6 个氨基酸。使用 XbaI 和 BsmBI 消化 PCR 产物，然后克隆到含有人 IgG1 恒定区的哺乳动物表达载体中。此载体含有 SV40 启动子和 DHFR 选择区。

来自每个噬菌体的轻链均为κ类或λ类。就轻链而言，互补引物设计成从 5'到 3'含有 HindIII 和 XbaI 位点，Kozak 序列和信号肽(见上文)。克隆那些具有无错(error-free)编码区的链作为全长产物。例如扩增来自 536 噬菌体克隆的轻链(SEQ ID NO: 11 和 SEQ ID NO: 210)作为全长编码区，所用引物为 2627-69 引物 (GTG GTT GAG AGG TGC CAG ATG TGA CAT TGT GAT GAC TCA GTC TCC; SEQ ID NO: 208，添加有信号肽的最后 7 个氨基酸)，和 2458-54 引物 (CTT GTC GAC TTA TTA ACA CTC TCC CCT GTT G; SEQ ID NO: 209，在终止密码子之后添加有 SalI 位点)。再如上文所述分别使用另外的 5'引物 2148-98 和 2489-36(与 2458-54 引物配对)扩增 PCR 产物，以完成信号肽序列和克隆位点的添加。如上文所述将全长轻链作为

XbaI-SalI 片段克隆到哺乳动物表达载体中。

某些λ克隆与天然人恒定区序列相比较，在其恒定区中有错误。为纠正这些错误，使用编码无错误λ恒定区的 DNA 和来自这些噬菌体可变区进行重叠 PCR。如上文所述将这些克隆作为 XbaI-SalI 片段克隆到哺乳动物表达载体中。

当从它们的恒定区独立克隆κ可变区时，向 PCR 产物的 3'末端加上一个 BsmBI 位点。在用 XbaI 和 BsmBI 消化 PCR 产物后，将κ链可变区克隆到含有人κ恒定区的表达载体中。

大体上按照使用说明书，通过磷酸钙转染试剂盒(Calcium Phosphate Transfection Kit)(Invitrogen Corp.)使用来自每个转化的噬菌体的轻链/重链配对构建体共转染 CHO 细胞。转染后 14-16h 更换一次培养基，根据使用说明书在约 48h 后在细胞培养皿中传代细胞，以进行选择。使用 HT 选择分离转染细胞约 3 周，此时转染的 CHO 细胞克隆被用胰蛋白酶消化(trypsinzed)并汇合成转染细胞“池”。

48h 后收集小量的条件培养基，并使用抗人 Fc 抗体、抗人κ抗体或抗人λ抗体进行 Western 印迹来检测抗体的生成。然后使用标准组织培养无菌技术在选择压力下对选出的细胞群进行传代，直到得到足够的细胞，以接种到 4 个 850 cm^2 滚瓶中(每瓶个 $2x10^7$ 活细胞)并使用 DMSO 制备细胞系冻干保存物。接种后使用培养基(含 10%血清的 DMEM (Gibco/BRL, Inc)，并添加谷氨酸和非必需氨基酸)在滚瓶中维持细胞。维持细胞 2-3 天，直到细胞融汇度达到约 80%。此时更换滚瓶中的培养基，使用无血清培养基混合物(50%DMEM、50%F12，Gibco，并添加谷氨酸和非必需氨基酸)。7d 后收集条件培养基，并加入新鲜无血清培养基再收集，同法额外收集 1-2 次。

使用标准方法直接从条件培养基中通过 G 蛋白亲和层析纯化抗体。使用低 pH 缓冲液(pH 约为 3)从 G 蛋白柱中洗脱抗体，然后用 1M Tris(pH8.5)中和洗脱的抗体，再用 10 kD 分子量截断离心浓缩器进行浓缩。然后将浓缩所得抗体通过缓冲液交换转移到 PBS 中。

建立了 31 个抗体，每个均由两个重链和两个轻链(κ或λ)构成，如下表 2 所示。

表 2

抗体重链	抗体轻链#
526 HC*	526 κ
528 HC*	528 λ C1
531 HC*	531 λ C3
533 HC*	533 -κ
535 HC*	535 λ C3
536 HC*	536 κ
537 HC*	537 λ C3 (G 107A R)
540 HC*	540 λ C3
543 HC*	543 κ
544 HC*	544 -λ C3
545 HC*	545 λ C2
546 HC*	546 λ C1 (G 107A S, N 112 A, T114 S)
551 HC*	551 κ
553 HC*	553 κ
555 HC*	555 κ
558 HC	558 κ
559 HC	559 λ C1 (N 112 A, T 114 S)
565 HC*	565 κ
F1-C6 HC	F1-C6 λ C2
FB1-A7 HC	FB1-A7 λ C2 (G 107A S)
FD-B2 HC	FD-B2 λ C3 (G 107A S)
FE-B7 HC	FE-B7 κ
FJ-G11 HC	FJ-G11 κ
FK-E3 HC	FK-E3 κ
G1D4 HC*	G1D4 λ C2
GC1E8 HC	GC1E8 λ C3 (K 149 R)
H1C12 HC	H1C12 λ C2
IA1-1E7 HC	IA1-1E7 κ
IF-1C10 HC	IF-1C10 λ C3 (T 212 A)
IK-2E2 HC	IK-2E2 λ C2 (T 212 A)
IP-2C11 HC	IP-2C11 κ

* 如本文所述，检测了与 hAng-2、mAng-2 和 hAng-1 的结合。

一些λ轻链恒定区表现为多于 1 个种系λ恒定区基因的嵌合体。指出了最接近的λ恒定区种系基因以及与该种系基因不同的氨基酸，用 Kabat 系统编号。

31 个 Ang-2 抗体(从噬菌体转化来的全长 IgG1 抗体)的重链和轻

链(κ和λ)序列和 SEQ ID NO 序号，见下面 4 个表格。使用 VBASE 数据库(它使用的方法见 Kabat et al,*Sequences of Proteins of Immunological Interest* (NIH 公开 No. 91-3242; U.S. Dept. Health and Human Services, 5th ed.))预测了多抗的互补决定区(CDR)。使用来自 MRC Centre for Protein Engineering(Cambridge, UK)的工具对 Fab 区域用最近缘种系序列与数据库中的序列进行了比对，然后将这些序列进行了目视比较。每个可变区(重链或轻链)的 CDR 列于表 7。

表 3

重链可变区

抗体重链(HC)	序 列
526 HC (SEQ ID NO: 1)	EVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAP GKGLEWVSAISGSGGSTYYADSVKGRFTISRDNAKNSLYLQM NSLRAEDTAVYYCARDLLDYDILTGPYAYWGQGTLVTVSS
528 HC (SEQ ID NO: 3)	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPG QGLEWMGGIIPIFGTANYAQKFQGRVTITADESTSTAYMELSS LRSEDTAVYYCARGVVGDFDWLSFFDYWGQGTLVTVSS
531 HC (SEQ ID NO: 5)	EVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPG QGLEWMGGIIPILGIANYAQKFQGRVTITADKSTNTAYMELTS LTSDDTAVYYCARDREDTAMVFNYWGQGTLVTVSS
533 HC (SEQ ID NO: 7)	EVQLVQSGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAP GKGLEWVSYISSSGSTIYYADSVKGRFTISRDNAKNSLYLQM NSLRAEDTAVYYCARDLLDYDILTGYGYWGQGTLVTVSS
535 HC (SEQ ID NO: 9)	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAP GQGLEWMGGIIPIFGTANYAQKFQGRVTITADKSTSTAYMEL SSLRSEDTAVYYCAAFSPFTETDAFDIWGQGTMVTVSS
536 HC (SEQ ID NO: 11)	EVQLVQSGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAP GKGLEWVSYISSSGSTIYYADSVKGRFTISRDNAKNSLYLQM NSLRAEDTAVYYCARDLLDYDILTGYGYWGQGTLVTVSS
537 HC (SEQ ID NO: 13)	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPG QGLEWMGGIIPILGIANYAQKFQGRVTITADKSTSTAYMELSG LGSEDTAVYYCARGSSDAAVAGMWGQGTLVTVSS
540 HC (SEQ ID NO: 15)	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPG QGLEWMGGIIPILGIANYAQKFQGRVTITADKFTSTAYMELSS LGSEDTAVYYCARAVPGTEDAFDIWGQGTMVTVSS
543 HC (SEQ ID NO: 17)	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPG QGLEWMGRIIPILGIANYAQKFQGRVTITADKSTSTAYMELSSL RSEDTAVYYCARPYYDFWSGPGGMDVWGQGTTVTVSS
544 HC (SEQ ID NO: 19)	QVQLVQSGAEVKKPGASVKVSCKASGGTFSSYAISWVRQAP GQGLEWMGGIIPIFGTANYAQKFQGRVTITADESTSTAYMELS SLRSEDTAVYYCARFESGYWGDAFDIWGQGTMVTVSS
545 HC (SEQ ID NO: 21)	QVQLQESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAP GKGLEWVAVISYDGSNKYYADSVKGRFTISRDNSKNTLYLQM NSLRAEDTAVYYCAKGPVDFDYGDYAIDYWGQGTLVTVSS

546 HC (SEQ ID NO: 23)	EVQLVDSGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAP GKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQM NSLRAEDTAVYYCAKETISFSTFSGYFDYWAQGTLVTVSS
551 HC (SEQ ID NO: 25)	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPG QGLEWMGGIIPIFGTANYAQKFQGRVTITADESTSTAYMELSS LRSEDTAVYYCARGYDFWSGYSLDAFDIWGQGTMVTVSS
553 HC (SEQ ID NO: 27)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYAMHWVRQA PGQRLEWMGWINAGNGNTKYSQKFQGRVTITRDTSASTAYM ELSGLRSEDTAVYYCARGVDDYGGNSWAFDIWGQGTMVTVS S
555 HC (SEQ ID NO: 29)	QVQLQESGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAP GKGLEWVAVISYDGSNKYYADSVKGRFTISRDNSKNTLYLQM NSLRAEDTAVYYCARSASDHYYDSSGYYSDAFDIWGQGTMV TVSS
558 HC (SEQ ID NO: 31)	QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWSWIRQSP GKGLEWIGEINHSGSTNFNPSLKSRITISVDTSNNQFSLKLSSV TAADTAAYYCARGHDWGMGIGGAAYDIWGQGTMVTVSS
559 HC (SEQ ID NO: 33)	QVQLVQSGAEVKKPGASVKVSCKVSGYTLTESSMHWVRQAP GKGLEWMGGFDPEHGETIYAQKFQGRLTMTEDTSTDTAYME LSSLRSEDTAVYFCARGVQVTSGYHYFDHWGQGTLVTVSS
565 HC (SEQ ID NO: 35)	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPG QGLEWMGGIIPIFGTANYAQKFQGRVTITADESTSTAYMELSS LRSEDTAVYYCARSPIYYDILTGIDAFDIWGQGTMVTVSS
F1-C6 HC (SEQ ID NO: 37)	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPG QGLEWMGRIIPILGIANYAQKFQGRVTITADKSTSTAYMELSSL RSEDTAVYYCARDPIPSGWYFDLWGRGTLVTVSS
FB1-A7 HC (SEQ ID NO: 39)	QVQLVESGGGLVKPGRSLRLSCAASGFTFSSYGMHWVRQAP GKGLEWVAVIWYDGSNKYYADSVKGRFTISRDNSKNTLYLQ MNSLRAEDTAVYYCAREVGNYYDSSGYGYWGQGTLVTVSS
FD-B2 HC (SEQ ID NO: 41)	QVQLQQSGPGLVKPSQTLSLTCAISGDTVSSNSAAWNWIRQSP SRGLEWLGRTYYRSKWYSDYAVSLRGRITINLDTDTSKNQFS LQLNSVTPEDTAVYYCARDRGGYIDSWGQGTLVTVSS
FE-B7 HC (SEQ ID NO: 43)	EVQLVESGGGLGQPGGSLRLSCAATGFSLDDYEMNWVRQAP GRGLEWVSYIIGSGKTIFYADSVKGRFTISRDNGKNSVYLQM NSLRAEDTAIYYCARGGGSAYYLNTSDIWGQGTMVTVSS
FJ-G11 HC (SEQ ID NO: 45)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYGISWVRQAP GQGLEWMGWISAYNGNTNYAQKLQGRVTMTTDTSTSTAYM ELRSLRSDDTAVYYCARDRGIAARSAYYYGMDVWGQGTTVT VSS
FK-E3 HC (SEQ ID NO: 47)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYDLNWVRQAS GQGLEWMGWMNPTSGNTGYAQKFQGRITMTRNTSISTAYME LRSLRSDDTAVYYCARDPPSGGWEFDYWGQGTLVTVSS
G1D4 HC (SEQ ID NO: 49)	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSHAISWVRQAP GQGLEWMGRIIPILGIANYAQKFQGRVTITADESTSTAYMELSS LRSEDTAVYYCATSRLEWLLYLDYWGQGTLVTVSS
GC1E8 HC (SEQ ID NO: 51)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYGISWVRQAP GQGLEWMGWISAYNGNTNYAQKLQGRVTMTTDTSTSTAYM EVRSLRSDDTAVYYCARGGSPYGGYAYPFDYWGQGTLVTVS S
H1C12 HC (SEQ ID NO: 53)	EVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAP GKGLEWVSYISSSGSTIYYADSVKGRFTISRDNAKNSLYLQM NSLRAEDTAVYYCARDLLDYDILTGYGYWGQGTLVTVSS
IA1-1E7 HC (SEQ ID NO: 55)	QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWSWIRQSP GKGLEWIGEINHSGSTNFNPSLKSRITISVDTSNNQFSLKLSSV TAADTAVYYCARGHDWGMGIGGAAYDIWGQGTMVTVSS

IF-1C10 HC (SEQ ID NO: 57)	QVQLVESGGGLVQPGGSLRLSCAASGFTFFSTYAMTWVRQAP GKGLEWVSVIRSNGGTDYADFVKGRFTISRDNSKNTLYLQM NGLRAEDTAVYYCMTDYYWGQGTLVTVSS
IK-2E2 HC (SEQ ID NO: 59)	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPG KGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQMN SLRAEDTAVYYCAKETISFSTFSGYFDYWGQGTLVTVSS
IP-2C11 HC (SEQ ID NO: 61)	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYDINWVRQAT GQGLEWMGWMNPNSGNTGYAQKFQGRVTMTRNTSISTAYM ELSSLRSEDTAVYYCAKEIAVAGTRYGMDVWGQGTTVTVSS

表 4

κ链可变区

抗体轻链(LC)	序 列
526 κ (SEQ ID NO: 2)	DIVMTQSPLSLPVTPGEPASISCRSSQSLLHSNGYNYLDWYLQ KPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAE DVGVYYCMQALQTPPTFGGGTKVEIK
533 κ (SEQ ID NO: 8)	DIVMTQSPLSLPVTPGEPASISCRSSQSLLHSNGYNYLNWYLQ KPGQSPQILIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAED VGVYYCMQGLQTPPTFGQGTKLEIK
536 κ (THW) (SEQ ID NO: 12)	DIVMTQSPLSLPVTPGEPASISCRSSQSLLHSNGYNYLDWYLQ KPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAE DVGVYYCMQGTHWPPTFGQGTKLEIK
536 κ (LQT) (SEQ ID NO: 210)	DIVMTQSPLSLPVTPGEPASISCRSSQSLLHSNGYNYLDWYLQ KPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDF TLKISRVEAEDVGVYYCMQGLQTPPTFGQGTKLEIK
543 κ (SEQ ID NO: 18)	DIVMTQSPLSLPVTPGEPASISCRSSQSLLHSNGYNYLDWYLQ KPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAE DVGVYYCMQALQTPLTFGGGTKVEIK
551 κ (SEQ ID NO: 26)	DIVMTQSPLSLPVTPGEPASISCRSSQSLLHSNGYNYLDWYLQ KPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAE DVGVYYCMQALQTPLTFGGGTKVEIK
553 κ (SEQ ID NO: 28)	DIVMTQSPLSLPVTPGEPASISCRSSQSLLHSNGYNYLDWYLQ KPGQSPQLLIYLGSNRASGVPDRFTGSGSATDFTLRISRVEAE DVGVYYCMQALQTPLTFGGGTKVEIK
555 κ (SEQ ID NO: 30)	DIVMTQSPLSLPVTPGEPASISCRSSQSLLHSNGYNYLDWYLQ KPGQSPQLLIYLASNRASGVPDRFSGSGSGTDFTLRISRVEAE DVGVYYCMQTLQIPITFGPGTKVDIK
558 κ (SEQ ID NO: 32)	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSSLAWYQQKPGQ APRLLVYAASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVY YCQHYGSSPRTFGQGTKVEIK
565 κ (SEQ ID NO: 36)	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSSLAWYQQKPGQ APRLLVYAASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVY YCQHYGSSPRTFGQGTKVEIK
565 κ (2) (SEQ ID NO: 211)	DIVMTQSPLSLPVTPGEPASISCRSSQSLLHSNGYNYLDWYLQ KPGQSPQLLIFLGSSRASGVPDRFSGSGSGTDFTLKISKVEAD DVGIYYCMQALDTPPTFGPGTKVEIK
FE-B7 κ (SEQ ID NO: 44)	DIVMTQSPLSLPVTPGEPASISCRSSQSLLHSKGDNYLDWYLQ KPGQSPQLLIYLGSHRASGVPDRFSGSGSGTDFTLKISRVEAE DVGVYYCMQALQTPLTFGGGTKVEIK
FJ-G11 κ (SEQ ID NO: 46)	DIVMTQTPLSLPVTPGEPASISCRSSQSLLDSDDGKTYLDWYL QRPGQSPQLLMYTTSSRASGVPDRFSGSGSGTDFTLKISRVEA EDVGVYYCMQATQFPYTFGQGTKLEIK

FK-E3 κ (SEQ ID NO: 48)	DIVMTQTPLSSTVTLGQPASISCRSSQSLVHEDGNTYLNWLHQ RPGQPPRLLIYKISKRFSGVPDRFSGSGAGTDFTLKISRVEPED VGVYYCMQSTRFPRTFGQGTKLEIK
IA1-1E7 κ (SEQ ID NO: 56)	EIVLTQSPATLSLSPGERATLSCRASQSVSSSFLAWYQQKAGQ APRLLIYDTSTRATGIADRFSGSGSGTDFTLTISRLEAEDSAVY YCQQYDFSPLTFGGGTKVEIK
IP-2C11 κ (SEQ ID NO: 62)	EIVLTQSPGTLSLSPGERATLSCRASQSISTFLAWYQQKPGQAP RLLIYDASNRATGIPGRFSGSGSGTDFTLTISNLEPEDFAVYYC QHRINWPLTFGGGTKVEIK

表 5

λ链可变区

抗体轻链(LC)	序 列
528 λ (SEQ ID NO: 4)	SYELTQPPSVSVSPGQTASITCSGDKLGYTYTSWFQQKPGQSP VLVIFQDFKRPSGIPERFSGSNSGNTATLTISGTQAMDEADYYC QAWDSTTAVVFGTGTKVTVL
531 λ (SEQ ID NO: 6)	QSVLTQPPSVSAAPGQKVTVSCSGSSSNIGNNYVSWYQQLPG TAPKLLIYDNNKRPSGIPDRFSGSKSGTSATLGITGLQTGDEAD YYCGTWDSSLSAFWVFGGGTKLTVL
535 λ (SEQ ID NO: 10)	QSVLTQPPSVSAAPGQKVTISCSGSNSNIGNNFVSWYQQLPGT APKLLVYDNNKRPSGIPDRFSGSKSGTSATLGITGLQTGDEAD YYCGTWDSSLSAAEVVFGGGTKLTVL
537 λ (SEQ ID NO: 14)	QSVLTQPPSVSAAPGQDVTISCSGNNSNIGNNYVSWYQQVPG TAPKLLVYDNHKRPSGISDRFSGSKSDTSATLDITGLQPGDEA DYYCGTWDTSLSANWVFGGGTKLTVL
540 λ (SEQ ID NO: 16)	QSVLTQPPSVSAAPGQKVTISCSGSSSNIGANYVSWYQQLPGT APKLLIYNNNKRPSGIPDRFSGSKSDTSATLGITGLQTGDEAD YYCGAWDSSLSASWVFGGGTKLTVL
544 λ (SEQ ID NO: 20)	SYELTQPPSVSVSPGQTARITCSGDALPKQYAYWYQQKPGQA PVLVIYKDSERPSGIPERFSGSSSGTTVTLTISGVQAEDEADYY CQSADSSHVVFGGGTKLTVL
545 λ (SEQ ID NO: 22)	QSVLTQPSSVSGAPGQRVTISCTGQSSNIGAGYDVHWYQQFP GRAPKLLIYGNSNRPSGVPDRFSGSKSGTSASLAITGLQPEDE ADYYCQSYDSRLSGSVFGGGTKLTVL
546 λ (SEQ ID NO: 24)	QSVLTQPSSVSEAPRQRVTISCSGSASNIGANGVSWYHQVPGK APRLLLSHDGLVTSGVPDRLSVSKSGTSASLAISGLHSDDEGD YYCAVWDDSLNAVVFGGGTKLTVL
559 λ (SEQ ID NO: 34)	QSALTQPPSASGSPGQSITISCTGTNSDIGSYPFVSWYQRHPGK APKLLIYDVSNRPSGVSDRFSGSKSGNTASLTISGLQAEDEGD YYCSSFTMNSFVIFGGGTKLTVL
F1-C6 λ (SEQ ID NO: 38)	QSVLTQPPSVSEAPRQRVTISCSGSSSNIGNNAVNWYQQLPGK APKLLIYYDDLLPSGVSDRFSGSKSGTSASLAISGLRSEDEAD YYCATWDDSLSGWVFGGGTKLTVL
FB1-A7 λ (SEQ ID NO: 40)	NFMLTQPHSVSESPGKTVTISCTRSGGGIGSSFVHWFQQRPGS SPTTVIFDDNQRPTGVPDRFSAAIDTSSSSASLTISGLTAEDEAD YYCQSSHSTAVVFGGGTKLTVL
FD-B2 λ (SEQ ID NO: 42)	NFMLTQPHSVSESPGKTVTISCTRSSGSIATNYVQWYQQRPGS SPATVIYEDNQRPSGVPDRFSGSIDTSSNSASLTISGLTTEDEAD YFCQSYGDNNWVFGGGTKLTVL
G1D4 λ (SEQ ID NO: 50)	NFMLTQPHSVSESPGKTVIIPCTRSSGSIASNYVQWYQKRPGS APSIVIYEDKQRPSGVPDRFSGSIDSSSNSASLTISGLKTEDEAD YYCQSYNSRGVMFGGGTKLTVL

GC1E8 λ (SEQ ID NO: 52)	NFMLTQPHSVLESAGKTVTISCTRSSGSIASNYVQWYQQRPG TSPTNVIFEDNQRPSGVPDRFSGSIDSSSNSASLTISGLKTEDEA DYFCQSYDSNIWVFGGGTKLTVL
H1C12 λ (SEQ ID NO: 54)	QSVLTQPPSVSAAPGQKVTISCSGSSSNIGNNYVSWYQHLPGT APKLLIYGNTNRPSGVPDRFSGSKSGTSASLAIAGLQAEDEAD YYCQSYDSSLSGSLVFGGGTKLTVL
IF-1C10 λ (SEQ ID NO: 58)	NFMLTQPHSVSESPGKTVTISCTGSGGSIASNYVQWYQQRPG SAPTTVIYEDNQRPSGVPDRFSGSIDSSSNSASLTISGLKTEDE ADYYCQSYDSSTWVFGGGTKLTVL
IK-2E2 λ (SEQ ID NO: 60)	QSALTQPASVSGSPGQSITISCTGTSSDVGGYNYVSWFQQHPG KAPKLMIYKVNNRPSGLSNRFSGSQSGNTASLTISGLQAEDEA DYYCSSYTSSSTLGFGGGTKLTVL

表 6

人恒定区(CR)

抗体恒定区(CR)	序 列
Human λ 恒定区 1 (C1) (SEQ ID NO: 63)	GQPKANPTVTLFPPSSEELQANKATLVCLISDFYPGAVTVAW KADGSPVKAGVETTKPSKQSNNKYAASSYLSLTPEQWKSH RSYSCQVTHEGSTVEKTVAPTECS
Human λ 恒定区 2 (C2) (SEQ ID NO: 64)	GQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAW KADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSHR SYSCQVTHEGSTVEKTVAPTECS
Human λ 恒定区 3 (C3) (SEQ ID NO: 65)	GQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAW KADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSH KSYSCQVTHEGSTVEKTVAPTECS
Human λ 恒定区 7 (C7) (SEQ ID NO: 66)	GQPKAAPSVTLFPPSSEELQANKATLVCLVSDFYPGAVTVA WKADGSPVKVGVETTKPSKQSNNKYAASSYLSLTPEQWKS HRSYSCRVTHEGSTVEKTVAPAECS
Human κ 恒定区 (SEQ ID NO: 67)	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQW KVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKH KVYACEVTHQGLSSPVTKSFNRGEC
Human IgG1 恒定区 (SEQ ID NO: 68)	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSW NSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYIC NVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVF LFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDG VEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS FFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL SPGK

表 7

Ang-2 抗体重链(HC)和轻链(LC)的互补决定区(CDR)

	CDR 1	CDR 2	CDR 3
抗体	残基	残基	残基
Ab 526 HC	SYGMH (SEQ ID NO: 69)	AISGSGGSTYYADSVKG (SEQ ID NO: 105)	DLLDYDILTGPYAY (SEQ ID NO: 144)
Ab 526			

KC	RSSQSLLHSNGYNYLD (SEQ ID NO: 70)	LGSNRAS - (SEQ ID NO: 106)	MQALQTPPT (SEQ ID NO: 145)
Ab 528 HC	SYAIS (SEQ ID NO: 71)	GIIPIFGTANYAQKFQG (SEQ ID NO: 107)	GVVGDFDWLSFFDY (SEQ ID NO: 146)
Ab 528 LC	SGDKLGYTYTS (SEQ ID NO: 72)	QDFKRPS (SEQ ID NO: 108)	QAWDSTTAVV (SEQ ID NO: 147)
Ab 531 HC	SYAIS (SEQ ID NO: 71)	GIIPILGIANYAQKFQG (SEQ ID NO: 109)	DREDTAMVFNY (SEQ ID NO: 148)
Ab 531 LC	SGSSSNIGNNYVS (SEQ ID NO: 73)	DNNKRPS (SEQ ID NO: 110)	GTWDSSLSAFWV (SEQ ID NO: 149)
Ab 533 HC	SYGMH (SEQ ID NO: 69)	YISSSGSTIYYADSVKG (SEQ ID NO: 111)	DLLDYDILTGYGY (SEQ ID NO: 150)
Ab 533 KC	RSSQSLLHSNGYNYLN (SEQ ID NO: 74)	LGSNRAS (SEQ ID NO: 106)	MQGLQTPPT (SEQ ID NO: 151)
Ab 535 HC	SYAIS (SEQ ID NO: 71)	GIIPIFGTANYAQKFQG (SEQ ID NO: 107)	FSPFTETDAFDI (SEQ ID NO: 152)
Ab 535 LC	SGSNSNIGNNFVS (SEQ ID NO: 75)	DNNKRPS (SEQ ID NO: 110)	GTWDSSLSAAEVV (SEQ ID NO: 153)
Ab 536 HC	SYGMH (SEQ ID NO: 69)	YISSSGSTIYYADSVKG (SEQ ID NO: 111)	DLLDYDILTGYGY (SEQ ID NO: 150)
Ab 536 (THW) KC	RSSQSLLHSNGYNYLD (SEQ ID NO: 70)	LGSNRAS (SEQ ID NO: 106)	MQGTHWPPT (SEQ ID NO: 154)
Ab 536 (LQT) KC	RSSQSLLHSNGYNYLD (SEQ ID NO: 70)	LGSNRAS (SEQ ID NO: 106)	MQGLQTPPT (SEQ ID NO: 212)
Ab 537 HC	SYAIS (SEQ ID NO: 71)	GIIPILGIANYAQKFQG (SEQ ID NO: 109)	GSSDAAVAGM (SEQ ID NO: 155)
Ab 537 LC	SGNNSNIGNNYVS (SEQ ID NO: 76)	DNHKRPS (SEQ ID NO: 112)	GTWDTSLSANWV (SEQ ID NO: 156)
Ab 540 HC	SYAIS (SEQ ID NO: 71)	GIIPILGIANYAQKFQG (SEQ ID NO: 109)	AVPGTEDAFDI (SEQ ID NO: 157)
Ab 540 LC	SGSSSNIGANYVS (SEQ ID NO: 77)	NNNKRPS (SEQ ID NO: 113)	GAWDSSLSASWV (SEQ ID NO: 158)
Ab 543 HC	SYAIS (SEQ ID NO: 71)	RIIPILGIANYAQKFQG (SEQ ID NO: 114)	PYYDFWSGPGGMDV (SEQ ID NO: 159)
Ab 543 KC	RSSQSLLHSNGYNYLD (SEQ ID NO: 70)	LGSNRAS (SEQ ID NO: 106)	MQALQTPLT (SEQ ID NO: 160)
Ab 544 HC	SYAIS (SEQ ID NO: 71)	GIIPIFGTANYAQKFQG (SEQ ID NO: 107)	FESGYWGDAFDI (SEQ ID NO: 161)

Ab 544 LC	SGDALPKQYAY (SEQ ID NO: 78)	KDSERPS (SEQ ID NO: 115)	QSADSSHVV (SEQ ID NO: 162)
Ab 545 HC	SYGMH (SEQ ID NO: 69)	VISYDGSNKYYADSVKG (SEQ ID NO: 116)	GPVDFDYGDYAIDY (SEQ ID NO: 163)
Ab 545 LC	TGQSSNIGAGYDVH (SEQ ID NO: 79)	GNSNRPS (SEQ ID NO: 117)	QSYDSRLSGSV (SEQ ID NO: 164)
Ab 546 HC	SYAMS (SEQ ID NO: 80)	AISGSGGSTYYADSVKG (SEQ ID NO: 105)	ETISFSTFSGYFDY (SEQ ID NO: 165)
Ab 546 LC	SGSASNIGANGVS (SEQ ID NO: 81)	HDGLVTS (SEQ ID NO: 118)	AVWDDSLNAVV (SEQ ID NO: 166)
Ab 551 HC	SYAIS (SEQ ID NO: 71)	GIIPIFGTANYAQKFQG (SEQ ID NO: 107)	GYDFWSGYSLDAFDI (SEQ ID NO: 167)
Ab 551 KC	RSSQSLLHSNGYNYLD (SEQ ID NO: 70)	LGSNRAS (SEQ ID NO: 106)	MQALQTPLT (SEQ ID NO: 160)
Ab 553 HC	SYAMH (SEQ ID NO: 82)	WINAGNGNTKYSQKFQG (SEQ ID NO: 119)	GVDDYGGNSWAFDI (SEQ ID NO: 168)
Ab 553 KC	RSSQSLLHSNGYNYLD (SEQ ID NO: 70)	LGSNRAS (SEQ ID NO: 106)	MQALQTPLT (SEQ ID NO: 160)
Ab 555 HC	SYAMH (SEQ ID NO: 82)	VISYDGSNKYYADSVKG (SEQ ID NO: 116)	SASDHYYDSSGYYSDAFDI (SEQ ID NO: 169)
Ab 555 KC	RSSQSLLHSNGYNYLD (SEQ ID NO: 70)	LASNRAS (SEQ ID NO: 120)	MQTLQIPIT (SEQ ID NO: 170)
Ab 558 HC	GYYWS (SEQ ID NO: 83)	EINHSGSTNFNPSLKS (SEQ ID NO: 121)	GHDWGMGIGGAAYDI (SEQ ID NO: 171)
Ab 558 KC	RASQSVSSSSLA (SEQ ID NO: 84)	AASSRAT (SEQ ID NO: 122)	QHYGSSPRT (SEQ ID NO: 172)
Ab 559 HC	ESSMH (SEQ ID NO: 85)	GFDPEHGETIYAQKFQG (SEQ ID NO: 123)	GVQVTSGYHYFDH (SEQ ID NO: 173)
Ab 559 LC	TGTNSDIGSYPFVS (SEQ ID NO: 86)	DVSNRPS (SEQ ID NO: 124)	SSFTMNSFVI (SEQ ID NO: 174)
Ab 565 HC	SYAIS (SEQ ID NO: 71)	GIIPIFGTANYAQKFQG (SEQ ID NO: 107)	SPIYYDILTGIDAFDI (SEQ ID NO: 175)
Ab 565 KC	RASQSVSSSSLA (SEQ ID NO: 213)	AASSRAT (SEQ ID NO: 214)	QHYGSSPRT (SEQ ID NO: 215)
Ab 565 (2) KC	RSSQSLLHSNGYNYLD (SEQ ID NO: 70)	LGSSRAS (SEQ ID NO: 125)	MQALDTPPT (SEQ ID NO: 176)
Ab F1-C6 HC	SYAIS (SEQ ID NO: 71)	RIIPILGIANYAQKFQG (SEQ ID NO: 114)	DPIPSGWYFDL (SEQ ID NO: 177)

Ab F1-C6 LC	SGSSSNIGNNAVN (SEQ ID NO: 87)	YDDLLPS (SEQ ID NO: 126)	ATWDDSLSGWV (SEQ ID NO: 178)
Ab FB1-A7 HC	SYGMH (SEQ ID NO: 69)	VIWYDGSNKYYADSVKG (SEQ ID NO: 127)	EVGNYYDSSGYGY (SEQ ID NO: 179)
Ab FB1-A7 LC	TRSGGGIGSSFVH (SEQ ID NO: 88)	DDNQRPT (SEQ ID NO: 128)	QSSHSTAVV (SEQ ID NO: 180)
Ab FD-B2 HC	SNSAAWN (SEQ ID NO: 89)	RTYYRSKWYSDYAVSLRG (SEQ ID NO: 129)	DRGGYIDS (SEQ ID NO: 181)
Ab FD-B2 LC	TRSSGSIATNYVQ (SEQ ID NO: 90)	EDNQRPS (SEQ ID NO: 130)	QSYGDNNWV (SEQ ID NO: 182)
Ab FE-B7 HC	DYEMN (SEQ ID NO: 91)	YIIGSGKTIFYADSVKG (SEQ ID NO: 131)	GGGSAYYLNTSDI (SEQ ID NO: 183)
Ab FE-B7 KC	RSSQSLLHSKGDNYLD (SEQ ID NO: 92)	LGSHRAS (SEQ ID NO: 132)	MQALQTPLT (SEQ ID NO: 160)
Ab FJ-G11 HC	SYGIS (SEQ ID NO: 93)	WISAYNGNTNYAQKLQG (SEQ ID NO: 133)	DRGIAARSAYYYGMDV (SEQ ID NO: 184)
Ab FJ-G11 KC	RSSQSLLDSDDGKTYLD (SEQ ID NO: 94)	TTSSRAS (SEQ ID NO: 134)	MQATQFPYT (SEQ ID NO: 185)
Ab FK-E3 HC	SYDLN (SEQ ID NO: 95)	WMNPTSGNTGYAQKFQG (SEQ ID NO: 135)	DPPSGGWEFDY (SEQ ID NO: 186)
Ab FK-E3 KC	RSSQSLVHEDGNTYLN (SEQ ID NO: 96)	KISKRFS (SEQ ID NO: 136)	MQSTRFPRT (SEQ ID NO: 187)
Ab G1D4 HC	SHAIS (SEQ ID NO: 97)	RIIPILGIANYAQKFQG (SEQ ID NO: 114)	SRLEWLLYLDY (SEQ ID NO: 188)
Ab G1D4 LC	TRSSGSIASNYVQ (SEQ ID NO: 98)	EDKQRPS (SEQ ID NO: 137)	QSYNSRGVM (SEQ ID NO: 189)
Ab GC1E8 HC	SYGIS (SEQ ID NO: 93)	WISAYNGNTNYAQKLQG (SEQ ID NO: 133)	GGSPYGGYAYPFDY (SEQ ID NO: 190)
Ab GC1E8 LC	TRSSGSIASNYVQ (SEQ ID NO: 98)	EDNQRPS (SEQ ID NO: 130)	QSYDSNIWV (SEQ ID NO: 191)
Ab H1C12 HC	SYGMH (SEQ ID NO: 69)	YISSSGSTIYYADSVKG (SEQ ID NO: 111)	DLLDYDILTGYGY (SEQ ID NO: 150)
Ab H1C12 LC	SGSSSNIGNNYVS (SEQ ID NO: 73)	GNTNRPS (SEQ ID NO: 138)	QSYDSSLSGSLV (SEQ ID NO: 192)
Ab IA1-1E7 HC	GYYWS (SEQ ID NO: 83)	EINHSGSTNFNPSLKS (SEQ ID NO: 121)	GHDWGMGIGGAAYDI (SEQ ID NO: 171)
Ab IA1-1E7 KC	RASQSVSSSFLA (SEQ	DTSTRAT (SEQ ID NO: 139)	QQYDFSPLT (SEQ ID NO:

	ID NO: 99)		193)
Ab IF-1C10 HC	STYAMT (SEQ ID NO: 100)	VIRSNGGTDYADFVKG (SEQ ID NO: 140)	DYY (SEQ ID NO: 194)
Ab IF-1C10 LC	TGSGGSIASNYVQ (SEQ ID NO: 101)	EDNQRPS (SEQ ID NO: 130)	QSYDSSTWV (SEQ ID NO: 195)
Ab IK-2E2 HC	SYAMS (SEQ ID NO: 80)	AISGSGGSTYYADSVKG (SEQ ID NO: 105)	ETISFSTFSGYFDY (SEQ ID NO: 165)
Ab IK-2E2 LC	TGTSSDVGGYNYVS (SEQ ID NO: 102)	KVNNRPS (SEQ ID NO: 141)	SSYTSSSTLG (SEQ ID NO: 196)
Ab IP-2C11 HC	SYDIN (SEQ ID NO: 103)	WMNPNSGNTGYAQKFQG (SEQ ID NO: 142)	EIAVAGTRYGMDV (SEQ ID NO: 197)
Ab IP-2C11 KC	RASQSISTFLA (SEQ ID NO: 104)	DASNRAT (SEQ ID NO: 143)	QHRINWPLT (SEQ ID NO: 198)

使用亲和ELISA和中和ELISA(如上文实施例3所述)以及BIAcore中和实验确定了这些抗体中的17个和阴性对照IgG1(称为RDB1)的亲和力、中和作用和特异性。所得结果列于下文表8，并使用标准方法进行计算。

表8

Ang-2抗体的EC50和IC50

	hAng-2		**mAng-2**		**hAng-1**	
抗体	**IC50 (nM)**	**EC50 (nM)**	**IC50 (nM)**	**EC50 (nM)**	**IC50 (nM)**	**EC50 (nM)**
Ab 536 (THW/LQT 混合物)	0.08	0.005	0.05	0.01	114.65	30
Ab 565	0.26		0.26		无抑制	
Ab 546	0.37		1.09		无抑制	
Ab 543	0.51		0.24		无抑制	
Ab 533	0.3		0.08		无抑制	
Ab 537	0.56		0.62		无抑制	
Ab 540	0.70		1.53		无抑制	
Ab 544	0.97		1.82		23.32	
Ab 545	1.04	0.02	1.30	0.05	8.31	2
Ab 528	1.37		0.73		无抑制	
Ab G1D4	1.39		0.60		69.48	
Ab 551	1.41		2.88		无抑制	

Ab 553	1.47		1.41		无抑制	
Ab 526	1.83		0.27		243.15	
Ab 531	2.15		1.67		无抑制	
Ab 555	2.21		1.76		无抑制	
Ab 535	2.81		2.45		无抑制	
RDB1	无抑制	无结合	无抑制	无结合	无抑制	无结合

按上文所述使用 BIAcore 分析检测了克隆 536 和克隆 545 的抗体。如上文所述用 BIAcore 确定了抗体的结合作用，K_{Ds} 较低说明亲和力较高，所得结果见表 9。

表 9

抗体对 hAng-2 和 mAng-2 的亲和力

	hAng-2			mAng-2		
Ab	**K_D (nM)**	**k_a(1/Ms)**	**k_d(1/s)**	**K_D (nM)**	**k_a(1/Ms)**	**k_d(1/s)**
Ab 536 (THW/LQT 混合物)	0.12	3.2×10^5	3.8×10^{-5}	0.15	6.2×10^5	9.5×10^{-5}
Ab 545	1.2	3.3×10^5	3.9×10^{-4}	0.9	5.9×10^5	5.3×10^{-4}

上文中分析的克隆 536 所含有的是两个变异抗体的混合物，分别为表 4 中的 SEQ ID NO: 12 (536 κ THW)和 SEQ ID NO: 210 (536 κ LQT)。这两个 536 变异体是独立的并单独进行 ELISA 和 HTRF 实验，以确定其作用能力。

ELISA 使用 96 孔板，37℃下用含有重组促血管生成素的 293T 细胞条件培养基(DMEM/50 μg/ml BSA)进行包被，反应 1h。调节条件培养基中促血管生成素的浓度，使得对 1 nM hTie2-Fc (R&D Systems, 目录号 313-TI)的结合力为最大结合作用的 80%。使用 PBS/0.1% Tween-20 洗板，然后在室温下用 PBS/5%BSA 封阻 2h。向 PBS/1% BSA/1 nM Tie2 溶液中滴加促血管生成素中和剂，浓度从 100nM 到 0.4pM，然后将此溶液加到被促血管生成素包被的多孔板上，室温下温育过夜再用 PBS/0.1%Tween-20 洗板。向每个孔中加入鼠源抗 Tie2 抗体(BD Pharmingen Inc., 目录号 557039)直至终浓度达到 1 μg/ml，室温下温育 1h 后，用 PBS/0.1%Tween-20 洗板。向

PBS/1%BSA 中加入羊抗鼠 IgG-HRP (Pierce, 目录号 31432)，稀释度为 1:10000，室温下温育 1h 后，用 PBS/0.1%Tween-20 洗板若干次。加入 TMB 底物(Sigma, 目录号 T8665)，测量 OD_{370nm}，通过与 Tie2 标准曲线相比较确定促血管生成素:Tie2 的中和程度。

为进行 HTRF 实验，向 96 孔板的每个孔加入 50 μl HTRF 缓冲液 (50 mM Tris-HCl, pH 7.5, 100 mM NaCl, 0.05% Tween 20, 0.1% BSA)，其中还含有 0.8 nM 铕-偶联链霉抗生物素(PERKIN ELMER LIFE SCIENCES INC., 目录号 AD0062)和 4.0 nM 生物素酰化的人促血管生成素 1 (R & D Systems)或促血管生成素 2 (Amgen Inc.)，以制备混合板(mix plate)。在另一块板上，向 HTRF 缓冲液中滴加促血管生成素中和剂(从 400nM 到 20pM)，然后取每种连续稀释的促血管生成素中和剂 50μl 转移到混合板上(含有链霉抗生物素-铕/促血管生成素)并混合。然后将此板置于摇床上室温下温育 1h。接下来在混合板的每个孔中取 20μl 转移到“实验板”(96 孔板，每孔中有 HTFR 缓冲液溶液 20μl，其中含 10nM 的人异藻青蛋白(allophycocyanin)-偶联人 Tie2-Fc)。所得的实验板在室温下摇床上温育 2h。实验板上溶液的终浓度为: 1.0 nM 促血管生成素，5.0 nM 人 Tie2-Fc 和 100 nM 到 5.0 pM 梯度稀释的促血管生成素中和剂。使用 Rubystar 读板器 (BMG Labtechnologies, Offenberg, Germany)分析实验板。使用“无促血管生成素中和剂”对照(代表零抑制)和“无促血管生成素”对照(代表完全抑制)，通过计算每种浓度的促血管生成素中和剂的抑制百分比，来确定促血管生成素:Tie2 的中和程度。使用 GRAFIT 5.0 程序 (Erithacus Software Ltd.)分析抑制百分比来计算 IC50 值。

所有结果均表示为用以下公式从样品计算得到的 IC50 曲线，样品进行一式两份检测。IC50 结果使抑制数据符合一个双参数方程，其中数据下限为 0(即该数据经背景校正)，上限为 100(即该数据经范围校正)。

$$y = \frac{100\%}{1 + \left(\frac{x}{IC_{50}}\right)^{s}}$$

该方程中，s 为斜率(slope factor)。该方程假定 y 随着 x 的增加而减少，它使用了软件 GRAFIT 5.0 (Erithacus Software Limited)。

所得结果见表 10 和 11。

表 10

Ab536 变异体 ELISA IC50 结果

样品	人 Ang1 IC50 (nM)	人 Ang2 IC50 (nM)	鼠 Ang2 IC50 (nM)
Ab536 LQT	>100	0.35	0.10
Ab536 THW	>100	0.31	0.088

表 11

Ab536 变异体 HTRF IC50 结果

样品	人 Ang1 IC50 (nM)	人 Ang2 IC50 (nM)
Ab536 LQT	>100	0.072
Ab536 THW	>100	0.071

实施例 5

使用抗 Ang-2 抗体的疗效研究

使用小鼠检测(使用 G 蛋白纯化的)兔抗 Ang-2 多抗的药代动力学。使用兔抗 Ang-2 多抗处理了 24 只小鼠(每只 1mg)。在注射抗体后的 1h、6h、1d、3d、7d 和 14d 分别处死 4 只处理过的小鼠。

结果表明总兔 IgG 的循环血清半衰期大约为 19d，而总 IgG 中的抗 Ang-2 IgG 成分的半衰期约为 8d。

为了评价疗效，使用异种移植了 A431 肿瘤的小鼠(每组 10 只)。在移植后的 1d、5d、6d、7d、8d、12d、13d、14d、15d、18d 向小鼠腹膜内注射 10 个剂量的(使用 G 蛋白纯化的)兔抗 Ang-2 多抗(每只每个剂量约为 10mg IgG)。在第 7d、12d、15d、19d、21d 测量肿

瘤大小。在第 0d、7d、15d、21d 测量体重，发现体重不受影响。重复测量 ANOVA 结果表明，相比用无免疫活性的纯化多抗抗血清(每只每个剂量约 10mg IgG)和载体(PBS)处理的对照，抗 Ang-2 多抗抑制了 A431 肿瘤异种移植物的生长，抑制程度约 50%，p=0.008。

为了评价全长人抗 Ang-2 单抗的体内疗效，使用异种移植了 A431 肿瘤的小鼠(每组 10 只)。分别向小鼠腹膜内注射抗 Ang-2 抗体克隆 533、537、544，或阴性对照(PBS 或人 IgG1-κ)。注射量为，第 1 次每只约 420μg 蛋白，接下来三次每次每只约 140μg 蛋白，接下来四次每次每只约 55μg 蛋白，每只总共注射 8 次。每周记录肿瘤大小和体重两次。在研究的最后阶段处死动物并收集血清，通过 ELISA 测量血清抗体水平。每组动物均收集肿瘤和一组正常组织。

在用抗 Ang-2 抗体治疗组合对照组的肿瘤生长情况间有明显差别，见图 1。所有用抗 Ang-2 抗体治疗的三组相比对照，肿瘤生长均受到抑制(所有 3 个抗体治疗组与 hIgG1 对照的 p< .005，重复测量 ANOVA)。与之相反，对照组的肿瘤继续以更高的速率进行生长。

实施例 6

表位作图

将人 Ang-2(hAng-2)的全长蛋白(1-495 位氨基酸)、N-末端(1-254 位氨基酸)和 C-末端(255-495 位氨基酸)克隆到 CMV 驱动的哺乳动物表达载体中(其中含有 C-末端 6xHis 标签)。在 293T 细胞中瞬时表达所得的 3 个构建体以及对照载体。然后收集转染细胞的条件培养基，并使用抗 6xHis ELISA 和 Western 印迹来估计培养基中的 Ang-2 表达水平。

通过 ELISA 确定它们对三种人 hAng-2 的结合能力，来确定抗 Ang-2 抗体和肽体的结合表位，方法如下：在高效结合实验 96 孔板的每孔中使用 100μl 条件培养基进行包被，37°C 下温育 1h。吸干(aspirated)条件培养基，每孔用 200μl 含 5%BSA 的 PBS 在室温下封

阻 1h。然后吸干封阻液。向每孔加入含有 1μg/ml 抗体、肽体或 Tie2-Fc 以及 1%BSA 的 PBS 溶液 100μl，室温下温育 1h。用含 0.1%Tween 的 PBS 溶液 200μl 洗板 4 次。向每孔中加入 HRP-偶联的羊抗人 IgG 或羊抗鼠 IgG 100μl，室温温育 45min。然后用含 0.1%Tween 的 PBS 溶液 200μl 洗板 4 次。向每孔中加入 100μl TMB 底物，测量 OD_{370nm}。

所得结果见图 2A、2B 和 2C。

<110> Oliner, John
Graham, Kevin

<120> 促血管生成素-2特异性结合剂

<130> 04-881-A

<140> 10/982,440
<141> 2004-11-04

<150> 60/620,161
<151> 2004-10-19

<160> 215

<170> PatentIn version 3.3

<210> 1
<211> 123
<212> PRT
<213> 人(Homo sapiens)

<400> 1

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asp Leu Leu Asp Tyr Asp Ile Leu Thr Gly Pro Tyr Ala Tyr
100 105 110

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 2
<211> 112
<212> PRT
<213> 人 (Homo sapiens)

<400> 2

Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu His Ser
20 25 30

Asn Gly Tyr Asn Tyr Leu Asp Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Leu Gly Ser Asn Arg Ala Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln Ala
85 90 95

Leu Gln Thr Pro Pro Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110

<210> 3
<211> 123
<212> PRT
<213> 人 (Homo sapiens)

<400> 3

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr
20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Glu Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Gly Val Val Gly Asp Phe Asp Trp Leu Ser Phe Phe Asp Tyr
100 105 110

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 4
<211> 107
<212> PRT
<213> 人 (Homo sapiens)

<400> 4

Ser Tyr Glu Leu Thr Gln Pro Pro Ser Val Ser Val Ser Pro Gly Gln
1 5 10 15

Thr Ala Ser Ile Thr Cys Ser Gly Asp Lys Leu Gly Tyr Thr Tyr Thr
20 25 30

Ser Trp Phe Gln Gln Lys Pro Gly Gln Ser Pro Val Leu Val Ile Phe
35 40 45

Gln Asp Phe Lys Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
50 55 60

Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Gly Thr Gln Ala Met
65 70 75 80

Asp Glu Ala Asp Tyr Tyr Cys Gln Ala Trp Asp Ser Thr Thr Ala Val
85 90 95

Val Phe Gly Thr Gly Thr Lys Val Thr Val Leu
100 105

<210> 5
<211> 120
<212> PRT
<213> 人 (Homo sapiens)

<400> 5

Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr
20 25 30

```
Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
        35                  40                  45

Gly Gly Ile Ile Pro Ile Leu Gly Ile Ala Asn Tyr Ala Gln Lys Phe
    50                  55                  60

Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Asn Thr Ala Tyr
65                  70                  75                  80

Met Glu Leu Thr Ser Leu Thr Ser Asp Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Asp Arg Glu Asp Thr Ala Met Val Phe Asn Tyr Trp Gly Gln
            100                 105                 110

Gly Thr Leu Val Thr Val Ser Ser
        115                 120
```

<210> 6
<211> 111
<212> PRT
<213> 人 (Homo sapiens)

<400> 6

```
Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Ala Ala Pro Gly Gln
1               5                   10                  15

Lys Val Thr Val Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Asn Asn
            20                  25                  30

Tyr Val Ser Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
        35                  40                  45

Ile Tyr Asp Asn Asn Lys Arg Pro Ser Gly Ile Pro Asp Arg Phe Ser
    50                  55                  60

Gly Ser Lys Ser Gly Thr Ser Ala Thr Leu Gly Ile Thr Gly Leu Gln
65                  70                  75                  80

Thr Gly Asp Glu Ala Asp Tyr Tyr Cys Gly Thr Trp Asp Ser Ser Leu
                85                  90                  95

Ser Ala Phe Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
            100                 105                 110
```

<210> 7
<211> 122
<212> PRT
<213> 人 (Homo sapiens)

<400> 7

Glu Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Tyr Ile Ser Ser Ser Gly Ser Thr Ile Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asp Leu Leu Asp Tyr Asp Ile Leu Thr Gly Tyr Gly Tyr Trp
100 105 110

Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 8
<211> 112
<212> PRT
<213> 人 (Homo sapiens)

<400> 8

Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu His Ser
20 25 30

Asn Gly Tyr Asn Tyr Leu Asn Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Ile Leu Ile Tyr Leu Gly Ser Asn Arg Ala Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln Gly
85 90 95

Leu Gln Thr Pro Pro Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 9
<211> 121
<212> PRT
<213> 人 (Homo sapiens)

<400> 9

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr
20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Ala Phe Ser Pro Phe Thr Glu Thr Asp Ala Phe Asp Ile Trp Gly
100 105 110

Gln Gly Thr Met Val Thr Val Ser Ser
115 120

<210> 10
<211> 112
<212> PRT
<213> 人 (Homo sapiens)

<400> 10

Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Ala Ala Pro Gly Gln
1 5 10 15

```
Lys Val Thr Ile Ser Cys Ser Gly Ser Asn Ser Asn Ile Gly Asn Asn
            20                  25                  30

Phe Val Ser Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
        35                  40                  45

Val Tyr Asp Asn Asn Lys Arg Pro Ser Gly Ile Pro Asp Arg Phe Ser
    50                  55                  60

Gly Ser Lys Ser Gly Thr Ser Ala Thr Leu Gly Ile Thr Gly Leu Gln
65                  70                  75                  80

Thr Gly Asp Glu Ala Asp Tyr Tyr Cys Gly Thr Trp Asp Ser Ser Leu
                85                  90                  95

Ser Ala Ala Glu Val Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
            100                 105                 110
```

<210> 11
<211> 122
<212> PRT
<213> 人 (Homo sapiens)

<400> 11

```
Glu Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1               5                   10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
            20                  25                  30

Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Ser Tyr Ile Ser Ser Ser Gly Ser Thr Ile Tyr Tyr Ala Asp Ser Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Asp Leu Leu Asp Tyr Asp Ile Leu Thr Gly Tyr Gly Tyr Trp
            100                 105                 110
```

Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 12
<211> 112
<212> PRT
<213> 人 (Homo sapiens)

<400> 12

Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu His Ser
20 25 30

Asn Gly Tyr Asn Tyr Leu Asp Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Leu Gly Ser Asn Arg Ala Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln Gly
85 90 95

Thr His Trp Pro Pro Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 13
<211> 119
<212> PRT
<213> 人 (Homo sapiens)

<400> 13

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr
20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Gly Ile Ile Pro Ile Leu Gly Ile Ala Asn Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Gly Leu Gly Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Gly Ser Ser Asp Ala Ala Val Ala Gly Met Trp Gly Gln Gly
100 105 110

Thr Leu Val Thr Val Ser Ser
115

<210> 14
<211> 111
<212> PRT
<213> 人 (Homo sapiens)

<400> 14

Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Ala Ala Pro Gly Gln
1 5 10 15

Asp Val Thr Ile Ser Cys Ser Gly Asn Asn Ser Asn Ile Gly Asn Asn
20 25 30

Tyr Val Ser Trp Tyr Gln Gln Val Pro Gly Thr Ala Pro Lys Leu Leu
35 40 45

Val Tyr Asp Asn His Lys Arg Pro Ser Gly Ile Ser Asp Arg Phe Ser
50 55 60

Gly Ser Lys Ser Asp Thr Ser Ala Thr Leu Asp Ile Thr Gly Leu Gln
65 70 75 80

Pro Gly Asp Glu Ala Asp Tyr Tyr Cys Gly Thr Trp Asp Thr Ser Leu
85 90 95

Ser Ala Asn Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
100 105 110

<210> 15
<211> 120
<212> PRT
<213> 人 (Homo sapiens)

<400> 15

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr
20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Gly Ile Ile Pro Ile Leu Gly Ile Ala Asn Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Phe Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Gly Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ala Val Pro Gly Thr Glu Asp Ala Phe Asp Ile Trp Gly Gln
100 105 110

Gly Thr Met Val Thr Val Ser Ser
115 120

<210> 16
<211> 111
<212> PRT
<213> 人 (Homo sapiens)

<400> 16

Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Ala Ala Pro Gly Gln
1 5 10 15

Lys Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Ala Asn
20 25 30

Tyr Val Ser Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
35 40 45

Ile Tyr Asn Asn Asn Lys Arg Pro Ser Gly Ile Pro Asp Arg Phe Ser
50 55 60

Gly Ser Lys Ser Asp Thr Ser Ala Thr Leu Gly Ile Thr Gly Leu Gln
65 70 75 80

Thr Gly Asp Glu Ala Asp Tyr Tyr Cys Gly Ala Trp Asp Ser Ser Leu
85 90 95

Ser Ala Ser Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
100 105 110

<210> 17
<211> 123
<212> PRT
<213> 人 (Homo sapiens)

<400> 17

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr
20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Arg Ile Ile Pro Ile Leu Gly Ile Ala Asn Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Pro Tyr Tyr Asp Phe Trp Ser Gly Pro Gly Gly Met Asp Val
100 105 110

Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
115 120

<210> 18
<211> 112
<212> PRT
<213> 人 (Homo sapiens)

<400> 18

Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu His Ser
20 25 30

Asn Gly Tyr Asn Tyr Leu Asp Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Leu Gly Ser Asn Arg Ala Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln Ala
85 90 95

Leu Gln Thr Pro Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110

<210> 19
<211> 121
<212> PRT
<213> 人 (Homo sapiens)

<400> 19

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr
20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Glu Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Phe Glu Ser Gly Tyr Trp Gly Asp Ala Phe Asp Ile Trp Gly
100 105 110

Gln Gly Thr Met Val Thr Val Ser Ser
115 120

<210> 20
<211> 106
<212> PRT
<213> 人 (Homo sapiens)

<400> 20

Ser Tyr Glu Leu Thr Gln Pro Pro Ser Val Ser Val Ser Pro Gly Gln
1 5 10 15

Thr Ala Arg Ile Thr Cys Ser Gly Asp Ala Leu Pro Lys Gln Tyr Ala
20 25 30

Tyr Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Ile Tyr
35 40 45

Lys Asp Ser Glu Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
50 55 60

Ser Ser Gly Thr Thr Val Thr Leu Thr Ile Ser Gly Val Gln Ala Glu
65 70 75 80

Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Ala Asp Ser Ser His Val Val
85 90 95

Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
100 105

<210> 21
<211> 123
<212> PRT
<213> 人 (Homo sapiens)

<400> 21

Gln Val Gln Leu Gln Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Val Ile Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Lys Gly Pro Val Asp Phe Asp Tyr Gly Asp Tyr Ala Ile Asp Tyr
100 105 110

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 22
<211> 111
<212> PRT
<213> 人 (Homo sapiens)

<400> 22

Gln Ser Val Leu Thr Gln Pro Ser Ser Val Ser Gly Ala Pro Gly Gln
1 5 10 15

Arg Val Thr Ile Ser Cys Thr Gly Gln Ser Ser Asn Ile Gly Ala Gly
20 25 30

Tyr Asp Val His Trp Tyr Gln Gln Phe Pro Gly Arg Ala Pro Lys Leu
35 40 45

Leu Ile Tyr Gly Asn Ser Asn Arg Pro Ser Gly Val Pro Asp Arg Phe
50 55 60

Ser Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Leu
65 70 75 80

Gln Pro Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Ser Arg
85 90 95

Leu Ser Gly Ser Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
100 105 110

<210> 23
<211> 123
<212> PRT
<213> 人 (Homo sapiens)

<400> 23

Glu Val Gln Leu Val Asp Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val

35 40 45

Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Lys Glu Thr Ile Ser Phe Ser Thr Phe Ser Gly Tyr Phe Asp Tyr
100 105 110

Trp Ala Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 24
<211> 110
<212> PRT
<213> 人 (Homo sapiens)

<400> 24

Gln Ser Val Leu Thr Gln Pro Ser Ser Val Ser Glu Ala Pro Arg Gln
1 5 10 15

Arg Val Thr Ile Ser Cys Ser Gly Ser Ala Ser Asn Ile Gly Ala Asn
20 25 30

Gly Val Ser Trp Tyr His Gln Val Pro Gly Lys Ala Pro Arg Leu Leu
35 40 45

Leu Ser His Asp Gly Leu Val Thr Ser Gly Val Pro Asp Arg Leu Ser
50 55 60

Val Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu His
65 70 75 80

Ser Asp Asp Glu Gly Asp Tyr Tyr Cys Ala Val Trp Asp Asp Ser Leu
85 90 95

Asn Ala Val Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
100 105 110

<210> 25
<211> 124
<212> PRT

<213> 人 (Homo sapiens)

<400> 25

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr
20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Glu Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Gly Tyr Asp Phe Trp Ser Gly Tyr Ser Leu Asp Ala Phe Asp
100 105 110

Ile Trp Gly Gln Gly Thr Met Val Thr Val Ser Ser
115 120

<210> 26
<211> 112
<212> PRT
<213> 人 (Homo sapiens)

<400> 26

Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu His Ser
20 25 30

Asn Gly Tyr Asn Tyr Leu Asp Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Leu Gly Ser Asn Arg Ala Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln Ala
85 90 95

Leu Gln Thr Pro Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110

<210> 27
<211> 123
<212> PRT
<213> 人 (Homo sapiens)

<400> 27

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
20 25 30

Ala Met His Trp Val Arg Gln Ala Pro Gly Gln Arg Leu Glu Trp Met
35 40 45

Gly Trp Ile Asn Ala Gly Asn Gly Asn Thr Lys Tyr Ser Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Ile Thr Arg Asp Thr Ser Ala Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Gly Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Gly Val Asp Asp Tyr Gly Gly Asn Ser Trp Ala Phe Asp Ile
100 105 110

Trp Gly Gln Gly Thr Met Val Thr Val Ser Ser
115 120

<210> 28
<211> 112
<212> PRT
<213> 人 (Homo sapiens)

<400> 28

Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu His Ser
20 25 30

Asn Gly Tyr Asn Tyr Leu Asp Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Leu Gly Ser Asn Arg Ala Ser Gly Val Pro
50 55 60

Asp Arg Phe Thr Gly Ser Gly Ser Ala Thr Asp Phe Thr Leu Arg Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln Ala
85 90 95

Leu Gln Thr Pro Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110

<210> 29
<211> 128
<212> PRT
<213> 人 (Homo sapiens)

<400> 29

Gln Val Gln Leu Gln Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Val Ile Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Ala Ser Asp His Tyr Tyr Asp Ser Ser Gly Tyr Tyr Ser
100 105 110

Asp Ala Phe Asp Ile Trp Gly Gln Gly Thr Met Val Thr Val Ser Ser
115 120 125

<210> 30
<211> 112
<212> PRT
<213> 人 (Homo sapiens)

<400> 30

Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu His Ser
20 25 30

Asn Gly Tyr Asn Tyr Leu Asp Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Leu Ala Ser Asn Arg Ala Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Arg Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln Thr
85 90 95

Leu Gln Ile Pro Ile Thr Phe Gly Pro Gly Thr Lys Val Asp Ile Lys
100 105 110

<210> 31
<211> 123
<212> PRT
<213> 人 (Homo sapiens)

<400> 31

Gln Val Gln Leu Gln Gln Trp Gly Ala Gly Leu Leu Lys Pro Ser Glu
1 5 10 15

Thr Leu Ser Leu Thr Cys Ala Val Tyr Gly Gly Ser Phe Ser Gly Tyr
20 25 30

Tyr Trp Ser Trp Ile Arg Gln Ser Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45

Gly Glu Ile Asn His Ser Gly Ser Thr Asn Phe Asn Pro Ser Leu Lys
50 55 60

Ser Arg Ile Thr Ile Ser Val Asp Thr Ser Asn Asn Gln Phe Ser Leu

65 70 75 80

Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Ala Tyr Tyr Cys Ala
85 90 95

Arg Gly His Asp Trp Gly Met Gly Ile Gly Gly Ala Ala Tyr Asp Ile
100 105 110

Trp Gly Gln Gly Thr Met Val Thr Val Ser Ser
115 120

<210> 32
<211> 108
<212> PRT
<213> 人 (Homo sapiens)

<400> 32

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Ser
20 25 30

Ser Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
35 40 45

Val Tyr Ala Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
50 55 60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65 70 75 80

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln His Tyr Gly Ser Ser Pro
85 90 95

Arg Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 33
<211> 122
<212> PRT
<213> 人 (Homo sapiens)

<400> 33

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Val Ser Gly Tyr Thr Leu Thr Glu Ser
20 25 30

Ser Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
35 40 45

Gly Gly Phe Asp Pro Glu His Gly Glu Thr Ile Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Arg Leu Thr Met Thr Glu Asp Thr Ser Thr Asp Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Phe Cys
85 90 95

Ala Arg Gly Val Gln Val Thr Ser Gly Tyr His Tyr Phe Asp His Trp
100 105 110

Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 34
<211> 110
<212> PRT
<213> 人(Homo sapiens)

<400> 34

Gln Ser Ala Leu Thr Gln Pro Pro Ser Ala Ser Gly Ser Pro Gly Gln
1 5 10 15

Ser Ile Thr Ile Ser Cys Thr Gly Thr Asn Ser Asp Ile Gly Ser Tyr
20 25 30

Pro Phe Val Ser Trp Tyr Gln Arg His Pro Gly Lys Ala Pro Lys Leu
35 40 45

Leu Ile Tyr Asp Val Ser Asn Arg Pro Ser Gly Val Ser Asp Arg Phe
50 55 60

Ser Gly Ser Lys Ser Gly Asn Thr Ala Ser Leu Thr Ile Ser Gly Leu
65 70 75 80

Gln Ala Glu Asp Glu Gly Asp Tyr Tyr Cys Ser Ser Phe Thr Met Asn
85 90 95

Ser Phe Val Ile Phe Gly Gly Gly Thr Lys Leu Thr Val Leu

100 105 110

<210> 35
<211> 125
<212> PRT
<213> 人 (Homo sapiens)

<400> 35

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr
20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Glu Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Pro Ile Tyr Tyr Asp Ile Leu Thr Gly Ile Asp Ala Phe
100 105 110

Asp Ile Trp Gly Gln Gly Thr Met Val Thr Val Ser Ser
115 120 125

<210> 36
<211> 108
<212> PRT
<213> 人 (Homo sapiens)

<400> 36

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Ser
20 25 30

Ser Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
35 40 45

Val Tyr Ala Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
50 55 60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65 70 75 80

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln His Tyr Gly Ser Ser Pro
85 90 95

Arg Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 37
<211> 120
<212> PRT
<213> 人 (Homo sapiens)

<400> 37

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr
20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Arg Ile Ile Pro Ile Leu Gly Ile Ala Asn Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asp Pro Ile Pro Ser Gly Trp Tyr Phe Asp Leu Trp Gly Arg
100 105 110

Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 38
<211> 110
<212> PRT
<213> 人 (Homo sapiens)

<400> 38

Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Glu Ala Pro Arg Gln
1 5 10 15

Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Asn Asn
20 25 30

Ala Val Asn Trp Tyr Gln Gln Leu Pro Gly Lys Ala Pro Lys Leu Leu
35 40 45

Ile Tyr Tyr Asp Asp Leu Leu Pro Ser Gly Val Ser Asp Arg Phe Ser
50 55 60

Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Arg
65 70 75 80

Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Thr Trp Asp Asp Ser Leu
85 90 95

Ser Gly Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
100 105 110

<210> 39
<211> 122
<212> PRT
<213> 人 (Homo sapiens)

<400> 39

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Val Ile Trp Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Glu Val Gly Asn Tyr Tyr Asp Ser Ser Gly Tyr Gly Tyr Trp
100 105 110

Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 40
<211> 110
<212> PRT
<213> 人 (Homo sapiens)

<400> 40

Asn Phe Met Leu Thr Gln Pro His Ser Val Ser Glu Ser Pro Gly Lys
1 5 10 15

Thr Val Thr Ile Ser Cys Thr Arg Ser Gly Gly Gly Ile Gly Ser Ser
20 25 30

Phe Val His Trp Phe Gln Gln Arg Pro Gly Ser Ser Pro Thr Thr Val
35 40 45

Ile Phe Asp Asp Asn Gln Arg Pro Thr Gly Val Pro Asp Arg Phe Ser
50 55 60

Ala Ala Ile Asp Thr Ser Ser Ser Ser Ala Ser Leu Thr Ile Ser Gly
65 70 75 80

Leu Thr Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Ser His Ser
85 90 95

Thr Ala Val Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
100 105 110

<210> 41
<211> 122
<212> PRT
<213> 人 (Homo sapiens)

<400> 41

Gln Val Gln Leu Gln Gln Ser Gly Pro Gly Leu Val Lys Pro Ser Gln
1 5 10 15

Thr Leu Ser Leu Thr Cys Ala Ile Ser Gly Asp Thr Val Ser Ser Asn
20 25 30

Ser Ala Ala Trp Asn Trp Ile Arg Gln Ser Pro Ser Arg Gly Leu Glu
35 40 45

Trp Leu Gly Arg Thr Tyr Tyr Arg Ser Lys Trp Tyr Ser Asp Tyr Ala
50 55 60

Val Ser Leu Arg Gly Arg Ile Thr Ile Asn Leu Asp Thr Asp Thr Ser
65 70 75 80

Lys Asn Gln Phe Ser Leu Gln Leu Asn Ser Val Thr Pro Glu Asp Thr
85 90 95

Ala Val Tyr Tyr Cys Ala Arg Asp Arg Gly Gly Tyr Ile Asp Ser Trp
100 105 110

Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 42
<211> 110
<212> PRT
<213> 人 (Homo sapiens)

<400> 42

Asn Phe Met Leu Thr Gln Pro His Ser Val Ser Glu Ser Pro Gly Lys
1 5 10 15

Thr Val Thr Ile Ser Cys Thr Arg Ser Ser Gly Ser Ile Ala Thr Asn
20 25 30

Tyr Val Gln Trp Tyr Gln Gln Arg Pro Gly Ser Ser Pro Ala Thr Val
35 40 45

Ile Tyr Glu Asp Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
50 55 60

Gly Ser Ile Asp Thr Ser Ser Asn Ser Ala Ser Leu Thr Ile Ser Gly
65 70 75 80

Leu Thr Thr Glu Asp Glu Ala Asp Tyr Phe Cys Gln Ser Tyr Gly Asp
85 90 95

Asn Asn Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
100 105 110

<210> 43
<211> 122

<212> PRT
<213> 人 (Homo sapiens)

<400> 43

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Gly Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Thr Gly Phe Ser Leu Asp Asp Tyr
20 25 30

Glu Met Asn Trp Val Arg Gln Ala Pro Gly Arg Gly Leu Glu Trp Val
35 40 45

Ser Tyr Ile Ile Gly Ser Gly Lys Thr Ile Phe Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Gly Lys Asn Ser Val Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Ile Tyr Tyr Cys
85 90 95

Ala Arg Gly Gly Gly Ser Ala Tyr Tyr Leu Asn Thr Ser Asp Ile Trp
100 105 110

Gly Gln Gly Thr Met Val Thr Val Ser Ser
115 120

<210> 44
<211> 112
<212> PRT
<213> 人 (Homo sapiens)

<400> 44

Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu His Ser
20 25 30

Lys Gly Asp Asn Tyr Leu Asp Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Leu Gly Ser His Arg Ala Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile

65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln Ala
85 90 95

Leu Gln Thr Pro Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110

<210> 45
<211> 125
<212> PRT
<213> 人 (Homo sapiens)

<400> 45

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
20 25 30

Gly Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Ser Ala Tyr Asn Gly Asn Thr Asn Tyr Ala Gln Lys Leu
50 55 60

Gln Gly Arg Val Thr Met Thr Thr Asp Thr Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Arg Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asp Arg Gly Ile Ala Ala Arg Ser Ala Tyr Tyr Tyr Gly Met
100 105 110

Asp Val Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
115 120 125

<210> 46
<211> 113
<212> PRT
<213> 人 (Homo sapiens)

<400> 46

Asp Ile Val Met Thr Gln Thr Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu Asp Ser
20 25 30

Asp Asp Gly Lys Thr Tyr Leu Asp Trp Tyr Leu Gln Arg Pro Gly Gln
35 40 45

Ser Pro Gln Leu Leu Met Tyr Thr Thr Ser Ser Arg Ala Ser Gly Val
50 55 60

Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys
65 70 75 80

Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln
85 90 95

Ala Thr Gln Phe Pro Tyr Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile
100 105 110

Lys

<210> 47
<211> 120
<212> PRT
<213> 人 (Homo sapiens)

<400> 47

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
20 25 30

Asp Leu Asn Trp Val Arg Gln Ala Ser Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Met Asn Pro Thr Ser Gly Asn Thr Gly Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Arg Ile Thr Met Thr Arg Asn Thr Ser Ile Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Arg Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asp Pro Pro Ser Gly Gly Trp Glu Phe Asp Tyr Trp Gly Gln
100 105 110

Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 48
<211> 112
<212> PRT
<213> 人 (Homo sapiens)

<400> 48

Asp Ile Val Met Thr Gln Thr Pro Leu Ser Ser Thr Val Thr Leu Gly
1 5 10 15

Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Glu
20 25 30

Asp Gly Asn Thr Tyr Leu Asn Trp Leu His Gln Arg Pro Gly Gln Pro
35 40 45

Pro Arg Leu Leu Ile Tyr Lys Ile Ser Lys Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ala Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Pro Glu Asp Val Gly Val Tyr Tyr Cys Met Gln Ser
85 90 95

Thr Arg Phe Pro Arg Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 49
<211> 120
<212> PRT
<213> 人 (Homo sapiens)

<400> 49

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser His
20 25 30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Arg Ile Ile Pro Ile Leu Gly Ile Ala Asn Tyr Ala Gln Lys Phe

50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Glu Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Thr Ser Arg Leu Glu Trp Leu Leu Tyr Leu Asp Tyr Trp Gly Gln
100 105 110

Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 50
<211> 110
<212> PRT
<213> 人 (Homo sapiens)

<400> 50

Asn Phe Met Leu Thr Gln Pro His Ser Val Ser Glu Ser Pro Gly Lys
1 5 10 15

Thr Val Ile Ile Pro Cys Thr Arg Ser Ser Gly Ser Ile Ala Ser Asn
20 25 30

Tyr Val Gln Trp Tyr Gln Lys Arg Pro Gly Ser Ala Pro Ser Ile Val
35 40 45

Ile Tyr Glu Asp Lys Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
50 55 60

Gly Ser Ile Asp Ser Ser Ser Asn Ser Ala Ser Leu Thr Ile Ser Gly
65 70 75 80

Leu Lys Thr Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asn Ser
85 90 95

Arg Gly Val Met Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
100 105 110

<210> 51
<211> 123
<212> PRT
<213> 人 (Homo sapiens)

<400> 51

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
20 25 30

Gly Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Ile Ser Ala Tyr Asn Gly Asn Thr Asn Tyr Ala Gln Lys Leu
50 55 60

Gln Gly Arg Val Thr Met Thr Thr Asp Thr Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Val Arg Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Gly Gly Ser Pro Tyr Gly Gly Tyr Ala Tyr Pro Phe Asp Tyr
100 105 110

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 52
<211> 110
<212> PRT
<213> 人 (Homo sapiens)

<400> 52

Asn Phe Met Leu Thr Gln Pro His Ser Val Leu Glu Ser Ala Gly Lys
1 5 10 15

Thr Val Thr Ile Ser Cys Thr Arg Ser Ser Gly Ser Ile Ala Ser Asn
20 25 30

Tyr Val Gln Trp Tyr Gln Gln Arg Pro Gly Thr Ser Pro Thr Asn Val
35 40 45

Ile Phe Glu Asp Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
50 55 60

Gly Ser Ile Asp Ser Ser Ser Asn Ser Ala Ser Leu Thr Ile Ser Gly
65 70 75 80

Leu Lys Thr Glu Asp Glu Ala Asp Tyr Phe Cys Gln Ser Tyr Asp Ser

85 90 95

Asn Ile Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
100 105 110

<210> 53
<211> 122
<212> PRT
<213> 人 (Homo sapiens)

<400> 53

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Tyr Ile Ser Ser Ser Gly Ser Thr Ile Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asp Leu Leu Asp Tyr Asp Ile Leu Thr Gly Tyr Gly Tyr Trp
100 105 110

Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 54
<211> 111
<212> PRT
<213> 人 (Homo sapiens)

<400> 54

Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Ala Ala Pro Gly Gln
1 5 10 15

Lys Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Asn Asn
20 25 30

Tyr Val Ser Trp Tyr Gln His Leu Pro Gly Thr Ala Pro Lys Leu Leu
35 40 45

Ile Tyr Gly Asn Thr Asn Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
50 55 60

Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ala Gly Leu Gln
65 70 75 80

Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Ser Ser Leu
85 90 95

Ser Gly Ser Leu Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
100 105 110

<210> 55
<211> 123
<212> PRT
<213> 人 (Homo sapiens)

<400> 55

Gln Val Gln Leu Gln Gln Trp Gly Ala Gly Leu Leu Lys Pro Ser Glu
1 5 10 15

Thr Leu Ser Leu Thr Cys Ala Val Tyr Gly Gly Ser Phe Ser Gly Tyr
20 25 30

Tyr Trp Ser Trp Ile Arg Gln Ser Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45

Gly Glu Ile Asn His Ser Gly Ser Thr Asn Phe Asn Pro Ser Leu Lys
50 55 60

Ser Arg Ile Thr Ile Ser Val Asp Thr Ser Asn Asn Gln Phe Ser Leu
65 70 75 80

Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala
85 90 95

Arg Gly His Asp Trp Gly Met Gly Ile Gly Gly Ala Ala Tyr Asp Ile
100 105 110

Trp Gly Gln Gly Thr Met Val Thr Val Ser Ser
115 120

<210> 56
<211> 108

<212> PRT
<213> 人 (Homo sapiens)

<400> 56

Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Ser
20 25 30

Phe Leu Ala Trp Tyr Gln Gln Lys Ala Gly Gln Ala Pro Arg Leu Leu
35 40 45

Ile Tyr Asp Thr Ser Thr Arg Ala Thr Gly Ile Ala Asp Arg Phe Ser
50 55 60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65 70 75 80

Ala Glu Asp Ser Ala Val Tyr Tyr Cys Gln Gln Tyr Asp Phe Ser Pro
85 90 95

Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105

<210> 57
<211> 112
<212> PRT
<213> 人 (Homo sapiens)

<400> 57

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Phe Ser Thr
20 25 30

Tyr Ala Met Thr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp
35 40 45

Val Ser Val Ile Arg Ser Asn Gly Gly Thr Asp Tyr Ala Asp Phe Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Gly Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys

85 90 95

Met Thr Asp Tyr Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
100 105 110

<210> 58
<211> 110
<212> PRT
<213> 人 (Homo sapiens)

<400> 58

Asn Phe Met Leu Thr Gln Pro His Ser Val Ser Glu Ser Pro Gly Lys
1 5 10 15

Thr Val Thr Ile Ser Cys Thr Gly Ser Gly Gly Ser Ile Ala Ser Asn
20 25 30

Tyr Val Gln Trp Tyr Gln Gln Arg Pro Gly Ser Ala Pro Thr Thr Val
35 40 45

Ile Tyr Glu Asp Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
50 55 60

Gly Ser Ile Asp Ser Ser Ser Asn Ser Ala Ser Leu Thr Ile Ser Gly
65 70 75 80

Leu Lys Thr Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Ser
85 90 95

Ser Thr Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
100 105 110

<210> 59
<211> 123
<212> PRT
<213> 人 (Homo sapiens)

<400> 59

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Lys Glu Thr Ile Ser Phe Ser Thr Phe Ser Gly Tyr Phe Asp Tyr
100 105 110

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 60
<211> 110
<212> PRT
<213> 人 (Homo sapiens)

<400> 60

Gln Ser Ala Leu Thr Gln Pro Ala Ser Val Ser Gly Ser Pro Gly Gln
1 5 10 15

Ser Ile Thr Ile Ser Cys Thr Gly Thr Ser Ser Asp Val Gly Gly Tyr
20 25 30

Asn Tyr Val Ser Trp Phe Gln Gln His Pro Gly Lys Ala Pro Lys Leu
35 40 45

Met Ile Tyr Lys Val Asn Asn Arg Pro Ser Gly Leu Ser Asn Arg Phe
50 55 60

Ser Gly Ser Gln Ser Gly Asn Thr Ala Ser Leu Thr Ile Ser Gly Leu
65 70 75 80

Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Ser Ser Tyr Thr Ser Ser
85 90 95

Ser Thr Leu Gly Phe Gly Gly Gly Thr Lys Leu Thr Val Leu
100 105 110

<210> 61
<211> 122
<212> PRT
<213> 人 (Homo sapiens)

<400> 61

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
20 25 30

Asp Ile Asn Trp Val Arg Gln Ala Thr Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Trp Met Asn Pro Asn Ser Gly Asn Thr Gly Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Met Thr Arg Asn Thr Ser Ile Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Lys Glu Ile Ala Val Ala Gly Thr Arg Tyr Gly Met Asp Val Trp
100 105 110

Gly Gln Gly Thr Thr Val Thr Val Ser Ser
115 120

<210> 62
<211> 107
<212> PRT
<213> 人 (Homo sapiens)

<400> 62

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Ile Ser Thr Phe
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
35 40 45

Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Gly Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Asn Leu Glu Pro
65 70 75 80

Glu Asp Phe Ala Val Tyr Tyr Cys Gln His Arg Ile Asn Trp Pro Leu

85 90 95

Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105

<210> 63
<211> 106
<212> PRT
<213> 人 (Homo sapiens)

<400> 63

Gly Gln Pro Lys Ala Asn Pro Thr Val Thr Leu Phe Pro Pro Ser Ser
1 5 10 15

Glu Glu Leu Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp
20 25 30

Phe Tyr Pro Gly Ala Val Thr Val Ala Trp Lys Ala Asp Gly Ser Pro
35 40 45

Val Lys Ala Gly Val Glu Thr Thr Lys Pro Ser Lys Gln Ser Asn Asn
50 55 60

Lys Tyr Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys
65 70 75 80

Ser His Arg Ser Tyr Ser Cys Gln Val Thr His Glu Gly Ser Thr Val
85 90 95

Glu Lys Thr Val Ala Pro Thr Glu Cys Ser
100 105

<210> 64
<211> 106
<212> PRT
<213> 人 (Homo sapiens)

<400> 64

Gly Gln Pro Lys Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser
1 5 10 15

Glu Glu Leu Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp
20 25 30

Phe Tyr Pro Gly Ala Val Thr Val Ala Trp Lys Ala Asp Ser Ser Pro
35 40 45

Val Lys Ala Gly Val Glu Thr Thr Thr Pro Ser Lys Gln Ser Asn Asn
50 55 60

Lys Tyr Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys
65 70 75 80

Ser His Arg Ser Tyr Ser Cys Gln Val Thr His Glu Gly Ser Thr Val
85 90 95

Glu Lys Thr Val Ala Pro Thr Glu Cys Ser
100 105

<210> 65
<211> 106
<212> PRT
<213> 人 (Homo sapiens)

<400> 65

Gly Gln Pro Lys Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser
1 5 10 15

Glu Glu Leu Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp
20 25 30

Phe Tyr Pro Gly Ala Val Thr Val Ala Trp Lys Ala Asp Ser Ser Pro
35 40 45

Val Lys Ala Gly Val Glu Thr Thr Thr Pro Ser Lys Gln Ser Asn Asn
50 55 60

Lys Tyr Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys
65 70 75 80

Ser His Lys Ser Tyr Ser Cys Gln Val Thr His Glu Gly Ser Thr Val
85 90 95

Glu Lys Thr Val Ala Pro Thr Glu Cys Ser
100 105

<210> 66
<211> 106
<212> PRT
<213> 人 (Homo sapiens)

<400> 66

Gly Gln Pro Lys Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser
1 5 10 15

Glu Glu Leu Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Val Ser Asp
20 25 30

Phe Tyr Pro Gly Ala Val Thr Val Ala Trp Lys Ala Asp Gly Ser Pro
35 40 45

Val Lys Val Gly Val Glu Thr Thr Lys Pro Ser Lys Gln Ser Asn Asn
50 55 60

Lys Tyr Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys
65 70 75 80

Ser His Arg Ser Tyr Ser Cys Arg Val Thr His Glu Gly Ser Thr Val
85 90 95

Glu Lys Thr Val Ala Pro Ala Glu Cys Ser
100 105

<210> 67
<211> 107
<212> PRT
<213> 人 (Homo sapiens)

<400> 67

Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
1 5 10 15

Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe
20 25 30

Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
35 40 45

Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
50 55 60

Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
65 70 75 80

Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser
85 90 95

Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
100 105

<210> 68
<211> 330
<212> PRT
<213> 人 (Homo sapiens)

<400> 68

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
1 5 10 15

Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
20 25 30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
35 40 45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
50 55 60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
65 70 75 80

Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
85 90 95

Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys
100 105 110

Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro
115 120 125

Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
130 135 140

Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
145 150 155 160

Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
165 170 175

Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
180 185 190

His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
195 200 205

Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly

210 215 220

Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu
225 230 235 240

Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
245 250 255

Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
260 265 270

Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
275 280 285

Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
290 295 300

Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
305 310 315 320

Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
325 330

<210> 69
<211> 5
<212> PRT
<213> 人 (Homo sapiens)

<400> 69

Ser Tyr Gly Met His
1 5

<210> 70
<211> 16
<212> PRT
<213> 人 (Homo sapiens)

<400> 70

Arg Ser Ser Gln Ser Leu Leu His Ser Asn Gly Tyr Asn Tyr Leu Asp
1 5 10 15

<210> 71
<211> 5
<212> PRT
<213> 人 (Homo sapiens)

<400> 71

Ser Tyr Ala Ile Ser

1 5

<210> 72
<211> 11
<212> PRT
<213> 人 (Homo sapiens)

<400> 72

Ser Gly Asp Lys Leu Gly Tyr Thr Tyr Thr Ser
1 5 10

<210> 73
<211> 13
<212> PRT
<213> 人 (Homo sapiens)

<400> 73

Ser Gly Ser Ser Ser Asn Ile Gly Asn Asn Tyr Val Ser
1 5 10

<210> 74
<211> 16
<212> PRT
<213> 人 (Homo sapiens)

<400> 74

Arg Ser Ser Gln Ser Leu Leu His Ser Asn Gly Tyr Asn Tyr Leu Asn
1 5 10 15

<210> 75
<211> 13
<212> PRT
<213> 人 (Homo sapiens)

<400> 75

Ser Gly Ser Asn Ser Asn Ile Gly Asn Asn Phe Val Ser
1 5 10

<210> 76
<211> 13
<212> PRT
<213> 人 (Homo sapiens)

<400> 76

Ser Gly Asn Asn Ser Asn Ile Gly Asn Asn Tyr Val Ser
1 5 10

<210> 77
<211> 13
<212> PRT

<213> 人 (Homo sapiens)

<400> 77

Ser Gly Ser Ser Ser Asn Ile Gly Ala Asn Tyr Val Ser
1 5 10

<210> 78
<211> 11
<212> PRT
<213> 人 (Homo sapiens)

<400> 78

Ser Gly Asp Ala Leu Pro Lys Gln Tyr Ala Tyr
1 5 10

<210> 79
<211> 14
<212> PRT
<213> 人 (Homo sapiens)

<400> 79

Thr Gly Gln Ser Ser Asn Ile Gly Ala Gly Tyr Asp Val His
1 5 10

<210> 80
<211> 5
<212> PRT
<213> 人 (Homo sapiens)

<400> 80

Ser Tyr Ala Met Ser
1 5

<210> 81
<211> 13
<212> PRT
<213> 人 (Homo sapiens)

<400> 81

Ser Gly Ser Ala Ser Asn Ile Gly Ala Asn Gly Val Ser
1 5 10

<210> 82
<211> 5
<212> PRT
<213> 人 (Homo sapiens)

<400> 82

Ser Tyr Ala Met His
1 5

<210> 83
<211> 5
<212> PRT
<213> 人 (Homo sapiens)

<400> 83

Gly Tyr Tyr Trp Ser
1 5

<210> 84
<211> 12
<212> PRT
<213> 人 (Homo sapiens)

<400> 84

Arg Ala Ser Gln Ser Val Ser Ser Ser Ser Leu Ala
1 5 10

<210> 85
<211> 5
<212> PRT
<213> 人 (Homo sapiens)

<400> 85

Glu Ser Ser Met His
1 5

<210> 86
<211> 14
<212> PRT
<213> 人 (Homo sapiens)

<400> 86

Thr Gly Thr Asn Ser Asp Ile Gly Ser Tyr Pro Phe Val Ser
1 5 10

<210> 87
<211> 13
<212> PRT
<213> 人 (Homo sapiens)

<400> 87

Ser Gly Ser Ser Ser Asn Ile Gly Asn Asn Ala Val Asn
1 5 10

<210> 88
<211> 13
<212> PRT
<213> 人 (Homo sapiens)

<400> 88

Thr Arg Ser Gly Gly Gly Ile Gly Ser Ser Phe Val His
1 5 10

<210> 89
<211> 7
<212> PRT
<213> 人 (Homo sapiens)

<400> 89

Ser Asn Ser Ala Ala Trp Asn
1 5

<210> 90
<211> 13
<212> PRT
<213> 人 (Homo sapiens)

<400> 90

Thr Arg Ser Ser Gly Ser Ile Ala Thr Asn Tyr Val Gln
1 5 10

<210> 91
<211> 5
<212> PRT
<213> 人 (Homo sapiens)

<400> 91

Asp Tyr Glu Met Asn
1 5

<210> 92
<211> 16
<212> PRT
<213> 人 (Homo sapiens)

<400> 92

Arg Ser Ser Gln Ser Leu Leu His Ser Lys Gly Asp Asn Tyr Leu Asp
1 5 10 15

<210> 93
<211> 5
<212> PRT
<213> 人 (Homo sapiens)

<400> 93

Ser Tyr Gly Ile Ser
1 5

<210> 94
<211> 17
<212> PRT
<213> 人 (Homo sapiens)

<400> 94

Arg Ser Ser Gln Ser Leu Leu Asp Ser Asp Asp Gly Lys Thr Tyr Leu
1 5 10 15

Asp

<210> 95
<211> 5
<212> PRT
<213> 人 (Homo sapiens)

<400> 95

Ser Tyr Asp Leu Asn
1 5

<210> 96
<211> 16
<212> PRT
<213> 人 (Homo sapiens)

<400> 96

Arg Ser Ser Gln Ser Leu Val His Glu Asp Gly Asn Thr Tyr Leu Asn
1 5 10 15

<210> 97
<211> 5
<212> PRT
<213> 人 (Homo sapiens)

<400> 97

Ser His Ala Ile Ser
1 5

<210> 98
<211> 13
<212> PRT
<213> 人 (Homo sapiens)

<400> 98

Thr Arg Ser Ser Gly Ser Ile Ala Ser Asn Tyr Val Gln
1 5 10

<210> 99

<211> 12
<212> PRT
<213> 人 (Homo sapiens)

<400> 99

Arg Ala Ser Gln Ser Val Ser Ser Ser Phe Leu Ala
1 5 10

<210> 100
<211> 6
<212> PRT
<213> 人 (Homo sapiens)

<400> 100

Ser Thr Tyr Ala Met Thr
1 5

<210> 101
<211> 13
<212> PRT
<213> 人 (Homo sapiens)

<400> 101

Thr Gly Ser Gly Gly Ser Ile Ala Ser Asn Tyr Val Gln
1 5 10

<210> 102
<211> 14
<212> PRT
<213> 人 (Homo sapiens)

<400> 102

Thr Gly Thr Ser Ser Asp Val Gly Gly Tyr Asn Tyr Val Ser
1 5 10

<210> 103
<211> 5
<212> PRT

<213> 人 (Homo sapiens)

<400> 103

Ser Tyr Asp Ile Asn
1 5

<210> 104
<211> 11
<212> PRT
<213> 人 (Homo sapiens)

<400> 104

Arg Ala Ser Gln Ser Ile Ser Thr Phe Leu Ala
1 5 10

<210> 105
<211> 17
<212> PRT
<213> 人 (Homo sapiens)

<400> 105

Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 106
<211> 7
<212> PRT
<213> 人 (Homo sapiens)

<400> 106

Leu Gly Ser Asn Arg Ala Ser
1 5

<210> 107
<211> 17
<212> PRT
<213> 人 (Homo sapiens)

<400> 107

Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe Gln
1 5 10 15

Gly

<210> 108
<211> 7
<212> PRT
<213> 人 (Homo sapiens)

<400> 108

Gln Asp Phe Lys Arg Pro Ser
1 5

<210> 109
<211> 17
<212> PRT
<213> 人 (Homo sapiens)

<400> 109

Gly Ile Ile Pro Ile Leu Gly Ile Ala Asn Tyr Ala Gln Lys Phe Gln
1 5 10 15

Gly

<210> 110
<211> 7
<212> PRT
<213> 人 (Homo sapiens)

<400> 110

Asp Asn Asn Lys Arg Pro Ser
1 5

<210> 111
<211> 17
<212> PRT
<213> 人 (Homo sapiens)

<400> 111

Tyr Ile Ser Ser Ser Gly Ser Thr Ile Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 112
<211> 7
<212> PRT
<213> 人 (Homo sapiens)

<400> 112

Asp Asn His Lys Arg Pro Ser
1 5

<210> 113
<211> 7
<212> PRT
<213> 人 (Homo sapiens)

<400> 113

Asn Asn Asn Lys Arg Pro Ser
1 5

<210> 114

<211> 17
<212> PRT
<213> 人 (Homo sapiens)

<400> 114

Arg Ile Ile Pro Ile Leu Gly Ile Ala Asn Tyr Ala Gln Lys Phe Gln
1 5 10 15

Gly

<210> 115
<211> 7
<212> PRT
<213> 人 (Homo sapiens)

<400> 115

Lys Asp Ser Glu Arg Pro Ser
1 5

<210> 116
<211> 17
<212> PRT
<213> 人 (Homo sapiens)

<400> 116

Val Ile Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 117
<211> 7
<212> PRT
<213> 人 (Homo sapiens)

<400> 117

Gly Asn Ser Asn Arg Pro Ser
1 5

<210> 118
<211> 7
<212> PRT
<213> 人 (Homo sapiens)

<400> 118

His Asp Gly Leu Val Thr Ser
1 5

<210> 119
<211> 17
<212> PRT
<213> 人 (Homo sapiens)

<400> 119

Trp Ile Asn Ala Gly Asn Gly Asn Thr Lys Tyr Ser Gln Lys Phe Gln
1 5 10 15

Gly

<210> 120
<211> 7
<212> PRT
<213> 人 (Homo sapiens)

<400> 120

Leu Ala Ser Asn Arg Ala Ser
1 5

<210> 121
<211> 16
<212> PRT
<213> 人 (Homo sapiens)

<400> 121

Glu Ile Asn His Ser Gly Ser Thr Asn Phe Asn Pro Ser Leu Lys Ser
1 5 10 15

<210> 122
<211> 7
<212> PRT
<213> 人 (Homo sapiens)

<400> 122

Ala Ala Ser Ser Arg Ala Thr
1 5

<210> 123
<211> 17
<212> PRT
<213> 人 (Homo sapiens)

<400> 123

Gly Phe Asp Pro Glu His Gly Glu Thr Ile Tyr Ala Gln Lys Phe Gln
1 5 10 15

Gly

<210> 124
<211> 7
<212> PRT
<213> 人 (Homo sapiens)

<400> 124

Asp Val Ser Asn Arg Pro Ser
1 5

<210> 125
<211> 7
<212> PRT
<213> 人 (Homo sapiens)

<400> 125

Leu Gly Ser Ser Arg Ala Ser
1 5

<210> 126
<211> 7
<212> PRT
<213> 人 (Homo sapiens)

<400> 126

Tyr Asp Asp Leu Leu Pro Ser
1 5

<210> 127
<211> 17
<212> PRT
<213> 人 (Homo sapiens)

<400> 127

Val Ile Trp Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 128
<211> 7
<212> PRT
<213> 人 (Homo sapiens)

<400> 128

Asp Asp Asn Gln Arg Pro Thr
1 5

<210> 129
<211> 18
<212> PRT
<213> 人 (Homo sapiens)

<400> 129

Arg Thr Tyr Tyr Arg Ser Lys Trp Tyr Ser Asp Tyr Ala Val Ser Leu
1 5 10 15

Arg Gly

<210> 130
<211> 7
<212> PRT
<213> 人 (Homo sapiens)

<400> 130

Glu Asp Asn Gln Arg Pro Ser
1 5

<210> 131
<211> 17
<212> PRT
<213> 人 (Homo sapiens)

<400> 131

Tyr Ile Ile Gly Ser Gly Lys Thr Ile Phe Tyr Ala Asp Ser Val Lys
1 5 10 15

Gly

<210> 132
<211> 7
<212> PRT
<213> 人 (Homo sapiens)

<400> 132

Leu Gly Ser His Arg Ala Ser
1 5

<210> 133
<211> 17
<212> PRT
<213> 人 (Homo sapiens)

<400> 133

Trp Ile Ser Ala Tyr Asn Gly Asn Thr Asn Tyr Ala Gln Lys Leu Gln
1 5 10 15

Gly

<210> 134
<211> 7
<212> PRT
<213> 人 (Homo sapiens)

<400> 134

Thr Thr Ser Ser Arg Ala Ser
1 5

<210> 135
<211> 17
<212> PRT
<213> 人 (Homo sapiens)

<400> 135

Trp Met Asn Pro Thr Ser Gly Asn Thr Gly Tyr Ala Gln Lys Phe Gln
1 5 10 15

Gly

<210> 136
<211> 7
<212> PRT
<213> 人 (Homo sapiens)

<400> 136

Lys Ile Ser Lys Arg Phe Ser
1 5

<210> 137
<211> 7
<212> PRT
<213> 人 (Homo sapiens)

<400> 137

Glu Asp Lys Gln Arg Pro Ser
1 5

<210> 138
<211> 7
<212> PRT
<213> 人 (Homo sapiens)

<400> 138

Gly Asn Thr Asn Arg Pro Ser
1 5

<210> 139
<211> 7
<212> PRT
<213> 人 (Homo sapiens)

<400> 139

Asp Thr Ser Thr Arg Ala Thr
1 5

<210> 140
<211> 16
<212> PRT
<213> 人 (Homo sapiens)

<400> 140

Val Ile Arg Ser Asn Gly Gly Thr Asp Tyr Ala Asp Phe Val Lys Gly
1 5 10 15

<210> 141
<211> 7
<212> PRT
<213> 人 (Homo sapiens)

<400> 141

Lys Val Asn Asn Arg Pro Ser
1 5

<210> 142
<211> 17
<212> PRT
<213> 人 (Homo sapiens)

<400> 142

Trp Met Asn Pro Asn Ser Gly Asn Thr Gly Tyr Ala Gln Lys Phe Gln
1 5 10 15

Gly

<210> 143
<211> 7
<212> PRT
<213> 人 (Homo sapiens)

<400> 143

Asp Ala Ser Asn Arg Ala Thr
1 5

<210> 144
<211> 14
<212> PRT
<213> 人 (Homo sapiens)

<400> 144

Asp Leu Leu Asp Tyr Asp Ile Leu Thr Gly Pro Tyr Ala Tyr
1 5 10

<210> 145
<211> 9
<212> PRT
<213> 人 (Homo sapiens)

<400> 145

Met Gln Ala Leu Gln Thr Pro Pro Thr
1 5

<210> 146
<211> 14
<212> PRT
<213> 人 (Homo sapiens)

<400> 146

Gly Val Val Gly Asp Phe Asp Trp Leu Ser Phe Phe Asp Tyr
1 5 10

<210> 147
<211> 10
<212> PRT
<213> 人 (Homo sapiens)

<400> 147

Gln Ala Trp Asp Ser Thr Thr Ala Val Val
1 5 10

<210> 148
<211> 11
<212> PRT
<213> 人 (Homo sapiens)

<400> 148

Asp Arg Glu Asp Thr Ala Met Val Phe Asn Tyr
1 5 10

<210> 149
<211> 12

<212> PRT
<213> 人 (Homo sapiens)

<400> 149

Gly Thr Trp Asp Ser Ser Leu Ser Ala Phe Trp Val
1 5 10

<210> 150
<211> 13
<212> PRT
<213> 人 (Homo sapiens)

<400> 150

Asp Leu Leu Asp Tyr Asp Ile Leu Thr Gly Tyr Gly Tyr
1 5 10

<210> 151
<211> 9
<212> PRT
<213> 人 (Homo sapiens)

<400> 151

Met Gln Gly Leu Gln Thr Pro Pro Thr
1 5

<210> 152
<211> 12
<212> PRT
<213> 人 (Homo sapiens)

<400> 152

Phe Ser Pro Phe Thr Glu Thr Asp Ala Phe Asp Ile
1 5 10

<210> 153
<211> 13
<212> PRT
<213> 人 (Homo sapiens)

<400> 153

Gly Thr Trp Asp Ser Ser Leu Ser Ala Ala Glu Val Val
1 5 10

<210> 154
<211> 9
<212> PRT
<213> 人 (Homo sapiens)

<400> 154

Met Gln Gly Thr His Trp Pro Pro Thr

1 5

<210> 155
<211> 10
<212> PRT
<213> 人 (Homo sapiens)

<400> 155

Gly Ser Ser Asp Ala Ala Val Ala Gly Met
1 5 10

<210> 156
<211> 12
<212> PRT
<213> 人 (Homo sapiens)

<400> 156

Gly Thr Trp Asp Thr Ser Leu Ser Ala Asn Trp Val
1 5 10

<210> 157
<211> 11
<212> PRT
<213> 人 (Homo sapiens)

<400> 157

Ala Val Pro Gly Thr Glu Asp Ala Phe Asp Ile
1 5 10

<210> 158
<211> 12
<212> PRT
<213> 人 (Homo sapiens)

<400> 158

Gly Ala Trp Asp Ser Ser Leu Ser Ala Ser Trp Val
1 5 10

<210> 159
<211> 14
<212> PRT
<213> 人 (Homo sapiens)

<400> 159

Pro Tyr Tyr Asp Phe Trp Ser Gly Pro Gly Gly Met Asp Val
1 5 10

<210> 160
<211> 9
<212> PRT

<213> 人 (Homo sapiens)

<400> 160

Met Gln Ala Leu Gln Thr Pro Leu Thr
1 5

<210> 161
<211> 12
<212> PRT
<213> 人 (Homo sapiens)

<400> 161

Phe Glu Ser Gly Tyr Trp Gly Asp Ala Phe Asp Ile
1 5 10

<210> 162
<211> 9
<212> PRT
<213> 人 (Homo sapiens)

<400> 162

Gln Ser Ala Asp Ser Ser His Val Val
1 5

<210> 163
<211> 14
<212> PRT
<213> 人 (Homo sapiens)

<400> 163

Gly Pro Val Asp Phe Asp Tyr Gly Asp Tyr Ala Ile Asp Tyr
1 5 10

<210> 164
<211> 11
<212> PRT
<213> 人 (Homo sapiens)

<400> 164

Gln Ser Tyr Asp Ser Arg Leu Ser Gly Ser Val
1 5 10

<210> 165
<211> 14
<212> PRT
<213> 人 (Homo sapiens)

<400> 165

Glu Thr Ile Ser Phe Ser Thr Phe Ser Gly Tyr Phe Asp Tyr
1 5 10

<210> 166
<211> 11
<212> PRT
<213> 人 (Homo sapiens)

<400> 166

Ala Val Trp Asp Asp Ser Leu Asn Ala Val Val
1 5 10

<210> 167
<211> 15
<212> PRT
<213> 人 (Homo sapiens)

<400> 167

Gly Tyr Asp Phe Trp Ser Gly Tyr Ser Leu Asp Ala Phe Asp Ile
1 5 10 15

<210> 168
<211> 14
<212> PRT
<213> 人 (Homo sapiens)

<400> 168

Gly Val Asp Asp Tyr Gly Gly Asn Ser Trp Ala Phe Asp Ile
1 5 10

<210> 169
<211> 19
<212> PRT
<213> 人 (Homo sapiens)

<400> 169

Ser Ala Ser Asp His Tyr Tyr Asp Ser Ser Gly Tyr Tyr Ser Asp Ala
1 5 10 15

Phe Asp Ile

<210> 170
<211> 9
<212> PRT
<213> 人 (Homo sapiens)

<400> 170

Met Gln Thr Leu Gln Ile Pro Ile Thr
1 5

<210> 171
<211> 15
<212> PRT
<213> 人 (Homo sapiens)

<400> 171

Gly His Asp Trp Gly Met Gly Ile Gly Gly Ala Ala Tyr Asp Ile
1 5 10 15

<210> 172
<211> 9
<212> PRT
<213> 人 (Homo sapiens)

<400> 172

Gln His Tyr Gly Ser Ser Pro Arg Thr
1 5

<210> 173
<211> 13
<212> PRT
<213> 人 (Homo sapiens)

<400> 173

Gly Val Gln Val Thr Ser Gly Tyr His Tyr Phe Asp His
1 5 10

<210> 174
<211> 10
<212> PRT
<213> 人 (Homo sapiens)

<400> 174

Ser Ser Phe Thr Met Asn Ser Phe Val Ile
1 5 10

<210> 175
<211> 16
<212> PRT
<213> 人 (Homo sapiens)

<400> 175

Ser Pro Ile Tyr Tyr Asp Ile Leu Thr Gly Ile Asp Ala Phe Asp Ile
1 5 10 15

<210> 176
<211> 9
<212> PRT
<213> 人 (Homo sapiens)

<400> 176

Met Gln Ala Leu Asp Thr Pro Pro Thr
1 5

<210> 177
<211> 11
<212> PRT
<213> 人 (Homo sapiens)

<400> 177

Asp Pro Ile Pro Ser Gly Trp Tyr Phe Asp Leu
1 5 10

<210> 178
<211> 11
<212> PRT
<213> 人 (Homo sapiens)

<400> 178

Ala Thr Trp Asp Asp Ser Leu Ser Gly Trp Val
1 5 10

<210> 179
<211> 13
<212> PRT
<213> 人 (Homo sapiens)

<400> 179

Glu Val Gly Asn Tyr Tyr Asp Ser Ser Gly Tyr Gly Tyr
1 5 10

<210> 180
<211> 9
<212> PRT
<213> 人 (Homo sapiens)

<400> 180

Gln Ser Ser His Ser Thr Ala Val Val
1 5

<210> 181
<211> 8
<212> PRT
<213> 人 (Homo sapiens)

<400> 181

Asp Arg Gly Gly Tyr Ile Asp Ser
1 5

<210> 182

<211> 9
<212> PRT
<213> 人 (Homo sapiens)

<400> 182

Gln Ser Tyr Gly Asp Asn Asn Trp Val
1 5

<210> 183
<211> 13
<212> PRT
<213> 人 (Homo sapiens)

<400> 183

Gly Gly Gly Ser Ala Tyr Tyr Leu Asn Thr Ser Asp Ile
1 5 10

<210> 184
<211> 16
<212> PRT
<213> 人 (Homo sapiens)

<400> 184

Asp Arg Gly Ile Ala Ala Arg Ser Ala Tyr Tyr Tyr Gly Met Asp Val
1 5 10 15

<210> 185
<211> 9
<212> PRT
<213> 人 (Homo sapiens)

<400> 185

Met Gln Ala Thr Gln Phe Pro Tyr Thr
1 5

<210> 186
<211> 11
<212> PRT
<213> 人 (Homo sapiens)

<400> 186

Asp Pro Pro Ser Gly Gly Trp Glu Phe Asp Tyr

1 5 10

<210> 187
<211> 9
<212> PRT
<213> 人 (Homo sapiens)

<400> 187

Met Gln Ser Thr Arg Phe Pro Arg Thr
1 5

<210> 188
<211> 11
<212> PRT
<213> 人 (Homo sapiens)

<400> 188

Ser Arg Leu Glu Trp Leu Leu Tyr Leu Asp Tyr
1 5 10

<210> 189
<211> 9
<212> PRT
<213> 人 (Homo sapiens)

<400> 189

Gln Ser Tyr Asn Ser Arg Gly Val Met
1 5

<210> 190
<211> 14
<212> PRT
<213> 人 (Homo sapiens)

<400> 190

Gly Gly Ser Pro Tyr Gly Gly Tyr Ala Tyr Pro Phe Asp Tyr
1 5 10

<210> 191
<211> 9
<212> PRT
<213> 人 (Homo sapiens)

<400> 191

Gln Ser Tyr Asp Ser Asn Ile Trp Val
1 5

<210> 192
<211> 12
<212> PRT
<213> 人 (Homo sapiens)

<400> 192

Gln Ser Tyr Asp Ser Ser Leu Ser Gly Ser Leu Val
1 5 10

<210> 193

<211> 9
<212> PRT
<213> 人 (Homo sapiens)

<400> 193

Gln Gln Tyr Asp Phe Ser Pro Leu Thr
1 5

<210> 194
<211> 3
<212> PRT
<213> 人 (Homo sapiens)

<400> 194

Asp Tyr Tyr
1

<210> 195
<211> 9
<212> PRT
<213> 人 (Homo sapiens)

<400> 195

Gln Ser Tyr Asp Ser Ser Thr Trp Val
1 5

<210> 196
<211> 10
<212> PRT
<213> 人 (Homo sapiens)

<400> 196

Ser Ser Tyr Thr Ser Ser Ser Thr Leu Gly
1 5 10

<210> 197
<211> 13
<212> PRT
<213> 人 (Homo sapiens)

<400> 197

Glu Ile Ala Val Ala Gly Thr Arg Tyr Gly Met Asp Val
1 5 10

<210> 198
<211> 9
<212> PRT
<213> 人 (Homo sapiens)

<400> 198

Gln His Arg Ile Asn Trp Pro Leu Thr
1 5

<210> 199
<211> 17
<212> PRT
<213> 人工序列

<220>
<223> CDR1区

<220>
<221> misc_feature
<222> (1)..(1)
<223> Xaa为R、S、T、G、E或D

<220>
<221> misc_feature
<222> (2)..(2)
<223> Xaa为S、G、A、R、N、T、Y或H.

<220>
<221> misc_feature
<222> (3)..(3)
<223> Xaa为S、D、N、Q、T、Y、A、G或E

<220>
<221> misc_feature
<222> (4)..(4)
<223> Xaa为Q、K、S、N、A、G、I、M、W或L

<220>
<221> misc_feature
<222> (5)..(5)
<223> Xaa为S、L、G、A、M、H或N

<220>
<221> misc_feature
<222> (6)..(6)
<223> Xaa为L、G、N、P、V、D、W、S、T、I，或不存在

<220>
<221> misc_feature
<222> (7)..(7)
<223> Xaa为L、Y、I、K、S、N、V，或不存在

<220>
<221> misc_feature
<222> (8)..(8)
<223> Xaa为H、T、G、Q、S、A、D，或不存在

<220>
<221> misc_feature
<222> (9)..(9)
<223> Xaa为S、Y、N、A、T、E、G、F，或不存在

<220>
<221> misc_feature
<222> (10)..(10)

<223> Xaa为N、T、A、G、S、Y、K、D、F、L，或不存在

<220>
<221> misc_feature
<222> (11)..(11)
<223> Xaa为G、S、Y、F、L、P、A、F、D、N，或不存在

<220>
<221> misc_feature
<222> (12)..(12)
<223> Xaa为Y、V、D、A、F、G、N，或不存在

<220>
<221> misc_feature
<222> (13)..(13)
<223> Xaa为N、S、V、H、Q、K、T，或不存在

<220>
<221> misc_feature
<222> (14)..(14)
<223> Xaa为Y、H、S、T，或不存在

<220>
<221> misc_feature
<222> (15)..(15)
<223> Xaa为L、Y，或不存在

<220>
<221> misc_feature
<222> (16)..(16)
<223> Xaa为D、N、L，或不存在

<220>
<221> misc_feature
<222> (17)..(17)
<223> Xaa为D，或不存在

<400> 199

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
1 5 10 15

Xaa

<210> 200
<211> 18
<212> PRT
<213> 人工序列

<220>
<223> CDR2区

<220>
<221> misc_feature
<222> (1)..(1)

<223> Xaa为L、Q、D、N、K、G、H、A、Y、E、T、R、V或W

<220>
<221> misc_feature
<222> (2)..(2)
<223> Xaa为G、D、N、A、V、T、I、F或M

<220>
<221> misc_feature
<222> (3)..(3)
<223> Xaa为S、F、N、H、G、D、K、T、I、W、Y或R

<220>
<221> misc_feature
<222> (4)..(4)
<223> Xaa为N、K、E、L、S、Q、H、T、G、P、Y或A

<220>
<221> misc_feature
<222> (5)..(5)
<223> Xaa为R、V、L、S、I、D、G、E、Y、T或N

<220>
<221> misc_feature
<222> (6)..(6)
<223> Xaa为A、P、T、F、G、L、N、H或S

<220>
<221> misc_feature
<222> (7)..(7)
<223> Xaa为S、T、G或K

<220>
<221> misc_feature
<222> (8)..(8)
<223> Xaa为S、T、I、N、E、W，或不存在

<220>
<221> misc_feature
<222> (9)..(9)
<223> Xaa为T、A、I、K、N、Y、D，或不存在

<220>
<221> misc_feature
<222> (10)..(10)
<223> Xaa为Y、N、K、F、I、S、G，或不存在

<220>
<221> misc_feature
<222> (11)..(11)
<223> Xaa为Y、N、D、A，或不存在

<220>
<221> misc_feature
<222> (12)..(12)
<223> Xaa为A、S、P、Y、D，或不存在

<220>
<221> misc_feature
<222> (13)..(13)
<223> Xaa为D、Q、S、A、F，或不存在

<220>
<221> misc_feature
<222> (14)..(14)
<223> Xaa为S、K、L、V，或不存在

<220>
<221> misc_feature
<222> (15)..(15)
<223> Xaa为V、F、K、S、L，或不存在

<220>
<221> misc_feature
<222> (16)..(16)
<223> Xaa为K、Q、S、L、F、G，或不存在

<220>
<221> misc_feature
<222> (17)..(17)
<223> Xaa为G、R，或不存在

<220>
<221> misc_feature
<222> (18)..(18)
<223> Xaa为G，或不存在

<400> 200

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
1 5 10 15

Xaa Xaa

<210> 201
<211> 19
<212> PRT
<213> 人工序列

<220>
<223> CDR3区

<220>
<221> misc_feature
<222> (1)..(1)
<223> Xaa为D、M、G、Q、G、A、P、E或S

<220>
<221> misc_feature
<222> (2)..(2)
<223> Xaa为L、Q、V、A、R、T、S、Y、E、P、H、G或I

<220>
<221> misc_feature
<222> (3)..(3)
<223> Xaa为L、A、V、W、E、P、G、S、Y、I、D、T、Q、F或R

<220>
<221> misc_feature
<222> (4)..(4)
<223> Xaa为D、L、G、F、T、S、W、V、Y、P、N、H、I或E

<220>
<221> misc_feature
<222> (5)..(5)
<223> Xaa为Y、Q、D、S、T、H、A、F、W、G、M、R或N

<220>
<221> misc_feature
<222> (6)..(6)
<223> Xaa为D、T、F、A、S、E、W、R、G、Y、I、M、N或L

<220>
<221> misc_feature
<222> (7)..(7)
<223> Xaa为I、P、D、T、M、L、S、V、G、H、Y、W、A、N或R

<220>
<221> misc_feature
<222> (8)..(8)
<223> Xaa为任何天然氨基酸

<220>
<221> misc_feature
<222> (9)..(9)
<223> Xaa为任何天然氨基酸

<220>
<221> misc_feature
<222> (10)..(10)
<223> Xaa为任何天然氨基酸

<220>
<221> misc_feature
<222> (11)..(11)
<223> Xaa为任何天然氨基酸

<220>
<221> misc_feature
<222> (12)..(12)
<223> Xaa为任何天然氨基酸

<220>
<221> misc_feature
<222> (13)..(13)
<223> Xaa为任何天然氨基酸

<220>
<221> misc_feature
<222> (14)..(14)
<223> Xaa为任何天然氨基酸

<220>
<221> misc_feature
<222> (15)..(15)
<223> Xaa为I、D，或不存在

<220>
<221> misc_feature
<222> (16)..(16)
<223> Xaa为A、I、V，或不存在

<220>
<221> misc_feature
<222> (17)..(17)
<223> Xaa为F，或不存在

<220>
<221> misc_feature
<222> (18)..(18)
<223> Xaa为D，或不存在

<220>
<221> misc_feature
<222> (19)..(19)
<223> Xaa为I，或不存在

<400> 201

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
1 5 10 15

Xaa Xaa Xaa

<210> 202
<211> 22
<212> PRT
<213> 人(Homo sapiens)

<400> 202

Met Asp Met Arg Val Pro Ala Gln Leu Leu Gly Leu Leu Leu Leu Trp
1 5 10 15

Leu Arg Gly Ala Arg Cys
20

<210> 203
<211> 40
<212> DNA
<213> 人(Homo sapiens)

<400> 203
gtggttgaga ggtgccagat gtcaggtcca gctggtgcag 40

<210> 204
<211> 30
<212> DNA
<213> 人(Homo sapiens)

<400> 204
attacgtctc acagttcgtt tgatctccac 30

<210> 205
<211> 48
<212> DNA
<213> 人 (Homo sapiens)

<400> 205
ccgctcagct cctggggctc ctgctattgt ggttgagagg tgccagat 48

<210> 206
<211> 9
<212> PRT
<213> 人 (Homo sapiens)

<400> 206

Ala Gln Leu Leu Gly Leu Leu Leu Leu
1 5

<210> 207
<211> 54
<212> DNA
<213> 人 (Homo sapiens)

<400> 207
cagcagaagc ttctagacca ccatggacat gagggtcccc gctcagctcc tggg 54

<210> 208
<211> 45
<212> DNA
<213> 人 (Homo sapiens)

<400> 208
gtggttgaga ggtgccagat gtgacattgt gatgactcag tctcc 45

<210> 209
<211> 31
<212> DNA
<213> 人 (Homo sapiens)

<400> 209
cttgtcgact tattaacact ctcccctgtt g 31

<210> 210
<211> 112
<212> PRT
<213> 人 (Homo sapiens)

<400> 210

Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu His Ser
20 25 30

Asn Gly Tyr Asn Tyr Leu Asp Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Leu Gly Ser Asn Arg Ala Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln Gly
85 90 95

Leu Gln Thr Pro Pro Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 211
<211> 112
<212> PRT
<213> 人 (Homo sapiens)

<400> 211

Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu His Ser
20 25 30

Asn Gly Tyr Asn Tyr Leu Asp Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Phe Leu Gly Ser Ser Arg Ala Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Lys Val Glu Ala Asp Asp Val Gly Ile Tyr Tyr Cys Met Gln Ala
85 90 95

Leu Asp Thr Pro Pro Thr Phe Gly Pro Gly Thr Lys Val Glu Ile Lys
100 105 110

<210> 212
<211> 9
<212> PRT
<213> 人 (Homo sapiens)

<400> 212

Met Gln Gly Leu Gln Thr Pro Pro Thr
1 5

<210> 213
<211> 12
<212> PRT
<213> 人 (Homo sapiens)

<400> 213

Arg Ala Ser Gln Ser Val Ser Ser Ser Ser Leu Ala
1 5 10

<210> 214
<211> 7
<212> PRT
<213> 人 (Homo sapiens)

<400> 214

Ala Ala Ser Ser Arg Ala Thr
1 5

<210> 215
<211> 9
<212> PRT
<213> 人 (Homo sapiens)

<400> 215

Gln His Tyr Gly Ser Ser Pro Arg Thr
1 5

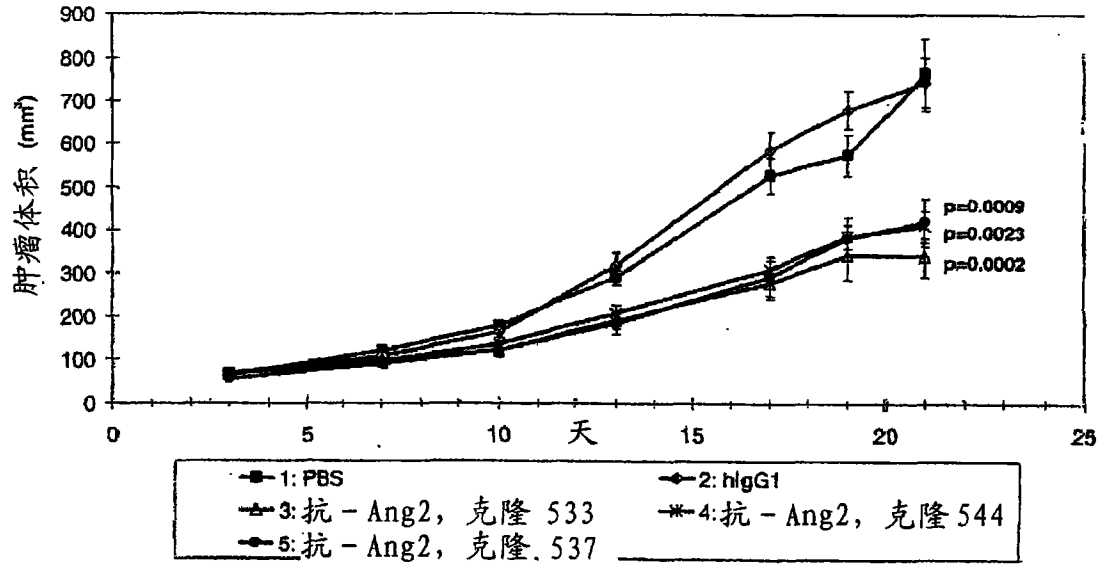

900
800
700
600
500
400
300
200
100
0
肿瘤体积 (mm³)
0
5
10
天
15
20
25
p=0.0009
p=0.0023
p=0.0002
1: PBS
2: hIgG1
3:抗－Ang2，克隆 533
4:抗－Ang2，克隆 544
5:抗－Ang2，克隆 537

图 1

表位作图

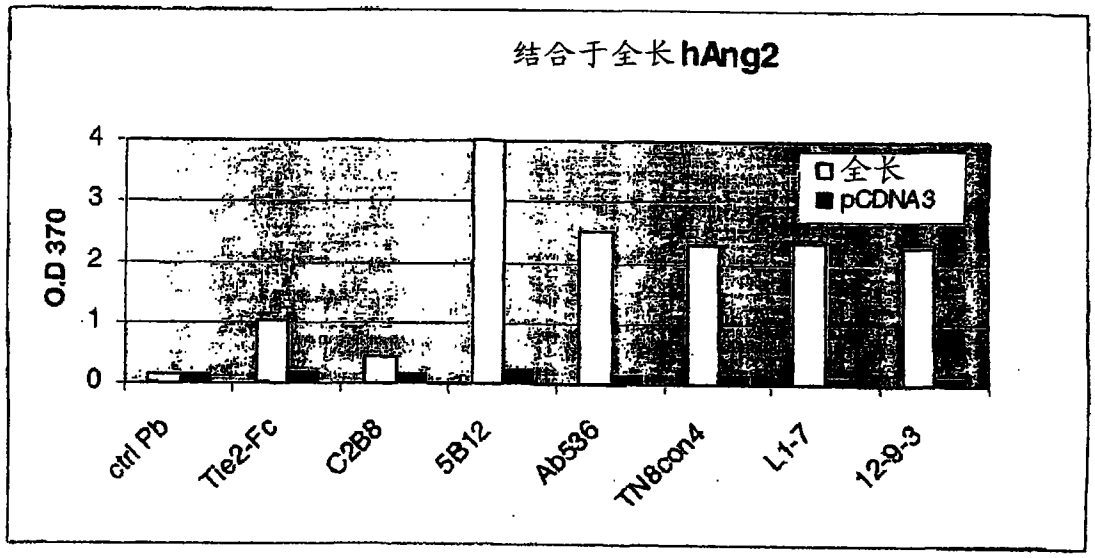

结合于全长hAng2
O.D 370
4
3
2
1
0
全长
pCDNA3
ctrl Pb
Tie2-Fc
C2B8
5B12
Ab536
TN8con4
L1-7
12-9-3

图 2a

表位作图

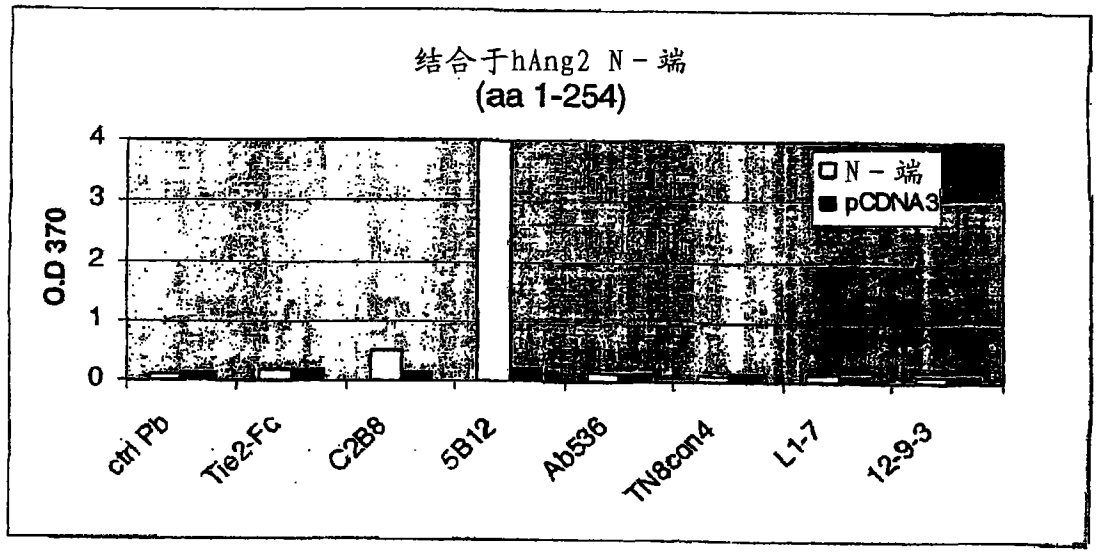

结合于hAng2 N－端
(aa 1-254)
O.D 370
4
3
2
1
0
N－端
pCDNA3
ctrl Pb
Tie2-Fc
C2B8
5B12
Ab536
TN8con4
L1-7
12-9-3

图 2b

表位作图

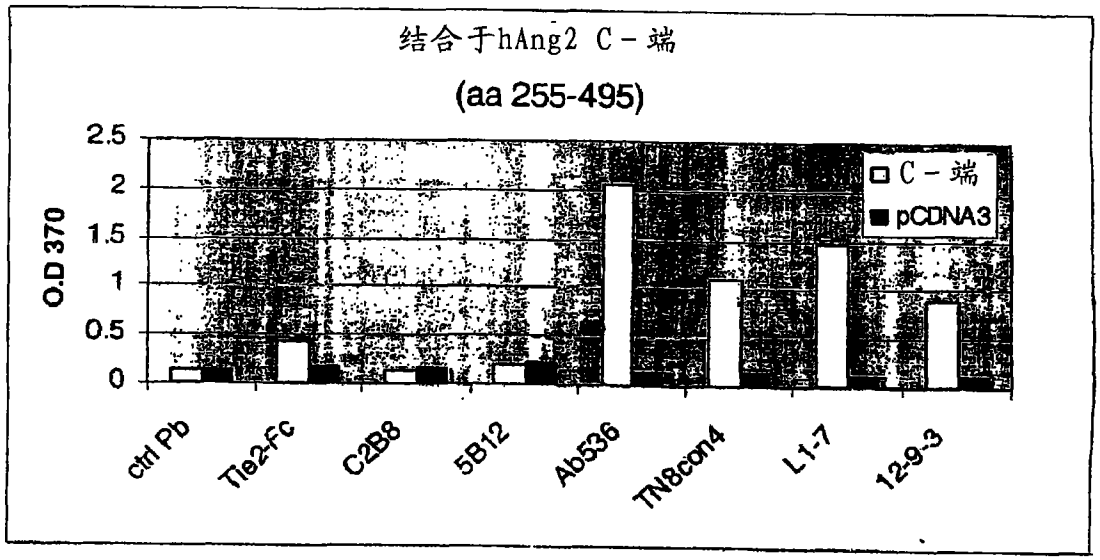

结合于hAng2 C－端
(aa 255-495)
O.D 370
2.5
2
1.5
1
0.5
0
C－端
pCDNA3
ctrl Pb
Tie2-Fc
C2B8
5B12
Ab536
TN8con4
L1-7
12-9-3

图 2c