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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
24 February 2005 (24.02.2005)

PCT

(10) International Publication Number
WO 2005/016381 A2

(51) International Patent Classification[7]: A61K 39/395

(21) International Application Number:
PCT/US2004/023097

(22) International Filing Date: 16 July 2004 (16.07.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/489,036 21 July 2003 (21.07.2003) US

(71) Applicant *(for all designated States except US)*: **MEDIMMUNE, INC.** [US/US]; One MedImmune Way, Gaithersburg, MD 20878 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **KINCH, Michael, S.** [US/US]; 19627 Hoover Farm Drive, Laytonsville, MD 20882 (US). **CARLES-KINCH, Kelly** [US/US]; 19627 Hoover Farm Drive, Laytonsville, MD 20882 (US). **KIENER, Peter** [US/US]; 1017 Gorky Drive, Potomac, MD 20854 (US). **LANGERMANN, Solomon** [US/US]; 6606 Cross Country Blvd., Baltimore, MD 20878 (US). **MCCARTHY, Michael, P.** [US/US]; 16920 Hoskinson Road, Poolesville, MD 20837 (US). **TICE, David** [US/US]; 15316 Bunchberry Court, Gaithersburg, MD 20878 (US). **WOESSNER, Richard** [US/US]; 2039 Buchanan Point, Lafayette, CO 80026 (US).

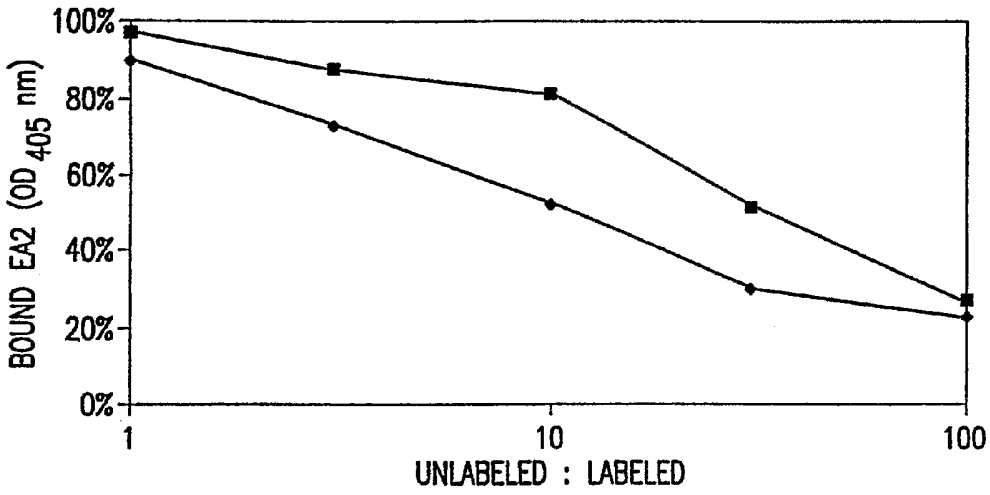
(74) Agent: **ALBAN, Patrick, Scott**; Medimmune, Inc., One Medimmune Way, Gaithersburg, MD 20878 (US).

(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, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH,

[Continued on next page]

(54) Title: COMBINATION THERAPY FOR THE TREATMENT AND PREVENTION OF CANCER USING EPHA2, PCDGF, AND HAAH



(57) Abstract: The present invention relates to methods and compositions designed for the treatment, management, or prevention of a hyperproliferative disorder, particularly cancer, more particularly metastatic cancer. The methods of the invention comprise the administration of an effective amount of one or more agents that decrease/inhibit EphA2 expression or activity in combination with one or more agents that decrease/inhibit PCDGF or HAAH expression or activity. In another embodiment, the methods of the invention comprise the administration of an effective amount of one or more EphA2, PCDGF, and/or HAAH agents of the invention that inhibit cancer cell colony formation in soft agar or tubular network formation in three-dimensional basement membrane or extracellular matrix preparation. The invention also provides pharmaceutical compositions comprising one or more EphA2 agents of the invention in combination with one or more PCDGF agents of the invention and/or one or more HAAH agents of the invention. In some embodiments, the agents of the invention can be administered with other cancer therapeutic agents that are not EphA2-, PCDGF-, or HAAH-based. Diagnostic methods and methods for screening for therapeutically useful agents of the invention are also provided.

WO 2005/016381 A2

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, IT, LU, 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:

— *without international search report and to be republished upon receipt of that report*

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.

COMBINATION THERAPY FOR THE TREATMENT AND PREVENTION OF CANCER USING EPHA2, PCDGF, AND HAAH

FIELD OF THE INVENTION

[0001] The present invention relates to therapeutic and prophylactic protocols and pharmaceutical compositions designed for the treatment, management or prevention of a hyperproliferative disease, particularly, cancer. Such protocols involve the administration of an effective amount of one or more EphA2-based therapies in combination with the administration of an effective amount of one or more PCDGF- or HAAH-based therapies useful in cancer therapy. The invention also provides pharmaceutical compositions comprising one or more EphA2 agents of the invention in combination with one or more PCDGF agents of the invention and/or HAAH agents of the invention useful for cancer therapy. Diagnostic methods and methods for screening for therapeutically useful agents of the invention are also provided.

BACKGROUND OF THE INVENTION

Cancer

[0002] A neoplasm, or tumor, is a neoplastic mass resulting from abnormal uncontrolled cell growth which can be benign or malignant. Benign tumors generally remain localized. Malignant tumors are collectively termed cancers. The term "malignant" generally means that the tumor can invade and destroy neighboring body structures and spread to distant sites to cause death (for review, see Robbins and Angell, 1976, *Basic Pathology*, 2d Ed., W.B. Saunders Co., Philadelphia, pp. 68-122). Cancer can arise in many sites of the body and behave differently depending upon its origin. Cancerous cells destroy the part of the body in which they originate and then spread to other part(s) of the body where they start new growth and cause more destruction.

[0003] More than 1.2 million Americans develop cancer each year. Cancer is the second leading case of death in the United States and, if current trends continue, cancer is expected to be the leading cause of the death by the year 2010. Lung and prostate cancer are the top cancer killers for men in the United States. Lung and breast cancer are the top cancer killers for women in the United States. One in two men in the United States will be

diagnosed with cancer at some time during his lifetime. One in three women in the United States will be diagnosed with cancer at some time during her lifetime.

[0004] A cure for cancer has yet to be found. Current treatment options, such as surgery, chemotherapy and radiation treatment, are oftentimes either ineffective or present serious side effects.

Metastasis

[0005] The most life-threatening forms of cancer often arise when a population of tumor cells gains the ability to colonize distant and foreign sites in the body. These metastatic cells survive by overriding restrictions that normally constrain cell colonization into dissimilar tissues. For example, typical mammary epithelial cells will generally not grow or survive if transplanted to the lung, yet lung metastases are a major cause of breast cancer morbidity and mortality. Recent evidence suggests that dissemination of metastatic cells through the body can occur long before clinical presentation of the primary tumor. These micrometastatic cells may remain dormant for many months or years following the detection and removal of the primary tumor. Thus, a better understanding of the mechanisms that allow for the growth and survival of metastatic cells in a foreign microenvironment is critical for the improvement of therapeutics designed to fight metastatic cancer and diagnostics for the early detection and localization of metastases.

Cancer Cell Signaling

[0006] Cancer is a disease of aberrant signal transduction. Aberrant cell signaling overrides anchorage-dependent constraints on cell growth and survival (Rhim, et al., *Critical Reviews in Oncogenesis* 8:305, 1997; Patarca, *Critical Reviews in Oncogenesis* 7:343, 1996; Malik, et al., *Biochimica et Biophysica Acta* 1287:73, 1996; Cance, et al., *Breast Cancer Res Treat* 35:105, 1995). Tyrosine kinase activity is induced by ECM anchorage and indeed, the expression or function of tyrosine kinases is usually increased in malignant cells (Rhim, et al., *Critical Reviews in Oncogenesis* 8:305,1997; Cance, et al., *Breast Cancer Res Treat* 35:105, 1995; Hunter, *Cell* 88:333, 1997). Based on evidence that tyrosine kinase activity is necessary for malignant cell growth, tyrosine kinases have been targeted with new therapeutics (Levitzki, et al., *Science* 267:1782, 1995; Kondapaka, et al., *Molecular & Cellular Endocrinology* 117:53, 1996; Fry, et al., *Current Opinion in BioTechnology* 6: 662, 1995). Unfortunately, obstacles associated with specific targeting to tumor cells often limit the application of these drugs. In particular, tyrosine kinase activity

is often vital for the function and survival of benign tissues (Levitzki, et al., *Science* 267:1782, 1995). To minimize collateral toxicity, it is critical to identify and then target tyrosine kinases that are selectively overexpressed in tumor cells.

EphA2

[0007] EphA2 is a 130 kDa receptor tyrosine kinase that is expressed in adult epithelia, where it is found at low levels and is enriched within sites of cell-cell adhesion (Zantek, et al, *Cell Growth & Differentiation* 10:629, 1999; Lindberg, et al., *Molecular & Cellular Biology* 10: 6316, 1990). This subcellular localization is important because EphA2 binds ligands (known as EphrinsA1 to A5) that are anchored to the cell membrane (Eph Nomenclature Committee, 1997, *Cell* 90:403; Gale, et al., 1997, *Cell & Tissue Research* 290: 227). The primary consequence of ligand binding is EphA2 autophosphorylation (Lindberg, et al., 1990, *supra*). However, unlike other receptor tyrosine kinases, EphA2 retains enzymatic activity in the absence of ligand binding or phosphotyrosine content (Zantek, et al., 1999, *supra*). EphA2 is upregulated on a large number of aggressive carcinoma cells.

PCDGF

[0008] PC Cell Derived Growth Factor (PCDGF) was first discovered as a secreted N-linked glycoprotein in the culture medium of highly tumorigenic PC cells, an insulin-independent variant isolated from the teratoma-derived adipogenic cell line 1246 (Zhou et al., 1993, *J. Biol. Chem.* 268, 10863-9). Determination of the amino acid sequence of PCDGF indicated similarities with the mouse granulin/epithelin precursor protein. Granulins/epithelins are 6 kDa polypeptides that belong to a family of double cysteine rich polypeptides (see *e.g.*, Plowman et al., 1992, *J. Biol. Chem.* 267: 13073-8; Bateman et al., 1990, *Biochem. Biophys. Res. Commun.* 173, 1161-8; U.S. Patent No. 5,416,192). Granulin/epithelin precursor polypeptide was initially thought to be processed into the small biologically active granulins/epithelins immediately after its synthesis. Additionally, the precursor polypeptide was assigned no biological activity prior to processing. However, Serrero (International Patent Publication WO 98/52607) demonstrated that the precursor polypeptide was not always processed immediately after synthesis and that it did have biological activity. Granulin/epithelin precursor polypeptide (or PCDGF) has growth promoting activity, particularly as an autocrine growth factor for the producer cells, and is implicated in tumorigenicity.

HAAH

[0009] Human aspartyl (asparaginyl) beta-hydroxylase (HAAH) belongs to the alpha-ketoglutarate dependent dioxygenase family of prolyl and lysyl hydroxylases. HAAH has been shown to hydroxylate aspartic acid or asparagine residues in EGF-like domains of several proteins in the presence of ferrous iron. These EGF-like domains contain conserved motifs that form repetitive sequences in proteins such as clotting factors, extracellular matrix proteins, LDL receptor, Notch homologues, or Notch ligand homologues. The abundant expression of HAAH in several malignant neoplasms and low levels of HAAH in many non-cancer cells indicate a role of this enzyme in malignancy. In fact, Wands et al. (International Patent Publication No. WO 01/35102) demonstrated that overexpression of HAAH resulted in high levels of beta hydroxylase activity which lead to invasive growth of transformed neoplastic cells.

Cancer Therapy

[0010] Currently, cancer therapy may involve surgery, chemotherapy, hormonal therapy and/or radiation treatment to eradicate neoplastic cells in a patient (see, for example, Stockdale, 1998, "Principles of Cancer Patient Management", in Scientific American: Medicine, vol. 3, Rubenstein and Federman, eds., Chapter 12, Section IV). Recently, cancer therapy may also involve biological therapy or immunotherapy. All of these approaches can pose significant drawbacks for the patient. Surgery, for example, may be contraindicated due to the health of the patient or may be unacceptable to the patient. Additionally, surgery may not completely remove the neoplastic tissue. Radiation therapy is only effective when the neoplastic tissue exhibits a higher sensitivity to radiation than normal tissue, and radiation therapy can also often elicit serious side effects. Hormonal therapy is rarely given as a single agent and, although it can be effective, is often used to prevent or delay recurrence of cancer after other treatments have removed the majority of the cancer cells. Biological therapies/immunotherapies are limited in number and each therapy is generally effective for a very specific type of cancer.

[0011] With respect to chemotherapy, there are a variety of chemotherapeutic agents available for treatment of cancer. A significant majority of cancer chemotherapeutics act by inhibiting DNA synthesis, either directly, or indirectly by inhibiting the biosynthesis of the deoxyribonucleotide triphosphate precursors, to prevent DNA replication and concomitant cell division (see, for example, Gilman et al., Goodman and Gilman's: The

Pharmacological Basis of Therapeutics, Eighth Ed. (Pergamom Press, New York, 1990)). These agents, which include alkylating agents, such as nitrosourea, anti-metabolites, such as methotrexate and hydroxyurea, and other agents, such as etoposides, campathecins, bleomycin, doxorubicin, daunorubicin, etc., although not necessarily cell cycle specific, kill cells during S phase because of their effect on DNA replication. Other agents, specifically colchicine and the vinca alkaloids, such as vinblastine and vincristine, interfere with microtubule assembly resulting in mitotic arrest. Chemotherapy protocols generally involve administration of a combination of chemotherapeutic agents to increase the efficacy of treatment.

[0012] Despite the availability of a variety of chemotherapeutic agents, chemotherapy has many drawbacks (see, for example, Stockdale, 1998, "Principles Of Cancer Patient Management" in Scientific American Medicine, vol. 3, Rubenstein and Federman, eds., ch. 12, sect. 10). Almost all chemotherapeutic agents are toxic, and chemotherapy causes significant, and often dangerous, side effects, including severe nausea, bone marrow depression, immunosuppression, etc. Additionally, even with administration of combinations of chemotherapeutic agents, many tumor cells are resistant or develop resistance to the chemotherapeutic agents. In fact, those cells resistant to the particular chemotherapeutic agents used in the treatment protocol often prove to be resistant to other drugs, even those agents that act by mechanisms different from the mechanisms of action of the drugs used in the specific treatment; this phenomenon is termed pleiotropic drug or multidrug resistance. Thus, because of drug resistance, many cancers prove refractory to standard chemotherapeutic treatment protocols.

[0013] There is a significant need for alternative cancer treatments, particularly for treatment of cancer that has proved refractory to standard cancer treatments, such as surgery, radiation therapy, chemotherapy, and hormonal therapy. Further, it is uncommon for cancer to be treated by only one method. Thus, there is a need for development of new therapeutic agents for the treatment of cancer and new, more effective, therapy combinations for the treatment of cancer.

1. SUMMARY OF THE INVENTION

[0014] The present invention is also based, in part, on the recognition that EphA2-based cancer therapies can potentiate, synergize with, and/or enhance the effectiveness of PCDGF- and/or HAAH-based cancer therapies. Thus, the invention encompasses therapeutic or prophylactic protocols comprising treatment with EphA2 agents in

combination with PCDGF agents and/or HAAH agents. Encompassed in the invention are combination therapies that have additive potency or an additive therapeutic or prophylactic effect. The invention also encompasses synergistic combinations where the therapeutic or prophylactic efficacy is greater than additive. Preferably, such combinations also reduce or avoid unwanted or adverse effects. In certain embodiments, the combination therapies encompassed by the invention provide an improved overall therapy relative to administration of any component alone. Given the invention, in certain embodiments, doses of cancer therapies can be reduced or administered less frequently which increases patient compliance, improves therapy and reduces unwanted or adverse effects.

[0015] EphA2 is over expressed and has altered activity in a large number of malignant carcinomas and is sufficient to confer metastatic potential to cancer cells. EphA2 that is over expressed on malignant cells exhibits kinase activity independent from ligand binding. EphA2 is also associated with other hyperproliferating cells and thus is implicated in diseases caused by cell hyperproliferation. A decrease in EphA2 levels can decrease proliferation and/or metastatic behavior of a cell. In particular, antibodies that agonize EphA2, *i.e.*, elicit EphA2 signaling, actually decrease EphA2 levels and inhibit cancer, particularly tumor, cell growth and/or metastasis. Although not intending to be bound by any mechanism of action, agonistic EphA2 antibodies may repress hyperproliferation or malignant cell behavior by inducing EphA2 autophosphorylation, thereby causing subsequent EphA2 degradation to down-regulate EphA2 expression.

[0016] In one embodiment, the EphA2 agents of the invention are antibodies, preferably monoclonal antibodies. In a specific embodiment, EphA2 antibodies of the invention are Eph099B-102.147, Eph099B-208.261, Eph099B-210.248, Eph099B-233.152, EA2, and EA5. In a preferred embodiment, the EphA2 antibodies of the invention are human or humanized, including humanized versions of Eph099B-102.147, Eph099B-208.261, Eph099B-210.248, Eph099B-233.152, EA2, and EA5.

[0017] PCDGF is a secreted growth factor that is expressed in a tightly regulated manner in non-cancer cells but is overexpressed and unregulated in highly tumorigenic cells. The mitogenic properties of PCDGF are seen in cells expressing a PCDGF receptor (*i.e.*, Rse), particularly in those cells which produce PCDGF as an autocrine growth factor. A decrease in PCDGF or PCDGF receptor levels or activity can decrease proliferation and/or metastatic behavior of a cell. In particular, i) decrease PCDGF and/or PCDGF receptor expression levels (*e.g.*, antibodies, antisense, RNAi, etc.), ii) decrease PCDGF secretion and/or PCDGF receptor presentation (*e.g.*, intrabodies), or iii) decrease PCDGF

and/or PCDGF receptor activity (*e.g.*, antibodies, PCDGF fragment which binds but does not activate its receptor, soluble ligand binding domain fragment of PCDGF receptor, etc.) inhibit cancer, particularly tumor, cell growth and/or metastasis.

[0018] In one embodiment, the PCDGF agents of the invention are antibodies that bind PCDGF or its receptor, preferably monoclonal antibodies. In a preferred embodiment, the PCDGF antibodies of the invention are human or humanized.

[0019] HAAH is overexpressed in a large number of malignant neoplasms as compared to the low levels of HAAH expression in many non-cancer cells. Overexpression of HAAH results in high levels of beta hydroxylase activity which can lead to invasive growth of transformed neoplastic cells. When HAAH is overexpressed (as in malignant cancer cells), the polypeptide is expressed on the cell surface in addition to intracellularly. A decrease in HAAH levels or HAAH activity can decrease proliferation and/or metastatic behavior of a cell. In particular, HAAH agents that decrease HAAH levels (*e.g.*, antibodies, antisense, RNAi, etc.) or HAAH activity (*e.g.*, intrabodies, substrate fragments, etc.) inhibit cancer, particularly tumor, cell growth and/or metastasis.

[0020] In one embodiment, the HAAH agents of the invention are anti-HAAH antibodies, preferably monoclonal antibodies or intrabodies. In a specific embodiment, HAAH antibodies of the invention are FB50, 8AC, 5C7, and 19B. In a preferred embodiment, the HAAH antibodies of the invention are human or humanized, including humanized versions of FB50, 8AC, 5C7, and 19B.

[0021] Accordingly, the present invention relates to pharmaceutical compositions and prophylactic and therapeutic regimens designed to prevent, treat, or manage a disease associated with a hyperproliferative disorder, particularly cancer, more particularly metastatic cancer, in a subject comprising administering one or more EphA2 agents in combination with one or more PCDGF agents and/or one or more HAAH agents. In preferred embodiments, the EphA2 agent is an antibody, preferably an agonistic monoclonal antibody. In one embodiment, the cancer is of an epithelial cell origin. In another embodiment, the cancer is a cancer of the skin, lung, colon, prostate, breast, bladder, or pancreas or is a renal cell carcinoma or a melanoma. In a preferred embodiment, the tumor cells overexpress an EphA2, PCDGF, PCDGF receptor and/or HAAH polypeptide. In a preferred embodiment, EphA2, PCDGF, PCDGF receptor, and/or HAAH is mislocalized in a cancer cell or in a tissue or organ (*e.g.*, EphA2 is not localized to cell-cell contacts, HAAH is on the cell membrane in addition to being intracellular, or PCDGF or its receptor are in areas of the body where they are not normally found because

cells are inappropriately expressing PCDGF or its receptor). In a preferred embodiment, the methods of the invention are used to prevent, treat, or manage metastasis of tumors.

[0022] The agents for use in the methods of the invention can be administered in further combination with one or more cancer therapies that are not EphA2-, PCDGF-, or HAAH-based. In particular, the present invention provides methods of preventing, treating, or managing cancer in a subject comprising administering to said subject a therapeutically or prophylactically effective amount of one or more EphA2 agents in combination with one or more PCDGF and/or HAAH agents in addition to the administration of a therapeutically or prophylactically effective amount of one or more chemotherapies, hormonal therapies, biological therapies/immunotherapies, radiation therapies, and/or surgery.

[0023] In other embodiments, EphA2, PCDGF, and/or HAAH agents are used to treat, prevent and/or manage a non-cancer hyperproliferative disease or disorder, such as but not limited to asthma, chronic obstructive pulmonary disease (COPD), lung fibrosis, bronchial hyper responsiveness, psoriasis, seborrheic dermatitis, cystic fibrosis, restenosis, hyperproliferative vascular disease, Behcet's Syndrome, atherosclerosis, and macular degeneration. In preferred embodiments, the hyperproliferative cells are epithelial. In preferred embodiments, the hyperproliferative cells overexpress an EphA2, PCDGF, PCDGF receptor and/or HAAH polypeptide. In a preferred embodiment, the hyperproliferative cells mislocalize an EphA2, PCDGF, PCDGF receptor, or HAAH polypeptide (*e.g.*, EphA2 is not localized to cell-cell contacts, HAAH is on the cell membrane in addition to being intracellular, or PCDGF or its receptor are in areas of the body where they are not normally found because cells are inappropriately expressing PCDGF or its receptor).

[0024] In other embodiments, EphA2, PCDGF, and/or HAAH agents are used to treat, prevent and/or manage a pre-cancerous condition, especially in order to prevent, delay, or decrease the likelihood that the pre-cancerous condition will progress to malignant cancer, such as, but not limited to, ductal carcinoma in situ (DCIS) of the breast, fibroadenoma of the breast, fibrocystic disease, cervix dysplasia, squamous intraepithelial lesions (SIL), adenomatous polyps, Barrett's esophageal dysplasia, hepatocellular carcinoma, adenomatous hyperplasia, atypical adenomatous hyperplasia (AAH) of the lung, lymphomatoid granulomatosis, pancreatic ductal lesions, pancreatic hyperplasias, pancreatic dysplasias, prostatic intraepithelial neoplasia (PIN), xeroderma pigmentosum, carcinoma in situ of the skin, squamous cell carcinoma, solar keratosis, compound nevi, actinic cheilitis, leukoplakia, or Bowen's disease. In preferred embodiments, the pre-

cancerous cells are epithelial. In preferred embodiments, the pre-cancerous cells overexpress an EphA2, PCDGF, PCDGF receptor and/or HAAH polypeptide. In a preferred embodiment, the pre-cancerous cells mislocalize an EphA2, PCDGF, or HAAH polypeptide (*e.g.*, EphA2 is not localized to cell-cell contacts, HAAH is on the cell membrane in addition to being intracellular, or PCDGF or its receptor are in areas of the body where they are not normally found because cells are inappropriately expressing PCDGF or its receptor).

[0025] The methods and compositions of the invention are useful not only in untreated patients but are also useful in the treatment of patients partially or completely refractory to current standard and experimental cancer therapies, including but not limited to chemotherapies, hormonal therapies, biological therapies, radiation therapies, and/or surgery as well as to improve the efficacy of such treatments. In particular, EphA2 expression has been implicated in increasing levels of the cytokine IL-6, which has been associated with the development of cancer cell resistance to different treatment regimens, such as chemotherapy and hormonal therapy. In addition, EphA2 may suppress estrogen receptor expression, contributing to tamoxifen resistance in breast cancer cells. Accordingly, in a preferred embodiment, the invention provides therapeutic and prophylactic methods for the treatment or prevention of cancer that has been shown to be or may be refractory or non-responsive to therapies other than those comprising administration of the combination of EphA2, PCDGF, and/or HAAH agents.

[0026] In addition, the present invention provides methods of screening for agents that potentiate (*e.g.*, that synergize with) EphA2 agents, PCDGF agents, and/or HAAH agents particularly for synergistic combinations of EphA2, PCDGF and/or HAAH agents. In particular, combinations of agents may be screened for the increased ability to decrease/inhibit a cancer cell phenotype (such as the ability to prevent or reduce cancer cell colony formation in soft agar or reduce or inhibit tubular network formation in a three-dimensional basement membrane or extracellular matrix preparation or reduce cellular hyperproliferation) when given in combination than when administered alone.

[0027] In other embodiments, the invention provides methods of treating, preventing, or managing cancer by administering nucleic acid therapeutic agents that reduce the expression level of the polypeptides of the invention, for example but not by way of limitation, anti-sense nucleic acids, double stranded RNA that mediates RNA interference, ribozymes, etc.

[0028] The invention further provides diagnostic methods using the EphA2 antibodies, PCDGF antibodies, PCDGF receptor antibodies, and/or HAAH antibodies of the invention to evaluate the efficacy of cancer treatment. Treatment efficacy monitored can be either therapies that are or are not based on EphA2, PCDGF, or HAAH therapeutic agents. In general, increased expression of the EphA2, PCDGF, PCDGF receptor, and/or HAAH polypeptides is associated with increasingly invasive and metastatic cancers. Accordingly, a reduction in expression of EphA2, PCDGF, PCDGF receptor, and/or HAAH polypeptides with a particular treatment indicates that the treatment is reducing the invasiveness and/or metastatic potential of cancer. The diagnostic methods of the invention may also be used to prognose or predict cancer. In particular embodiments, the diagnostic methods of the invention provide methods of imaging and localizing tumors, cancer cells, metastases and methods of diagnosis and prognosis using tissues and fluids distal to the primary tumor site (as well as methods using tissues and fluids of the primary tumor), for example, whole blood, sputum, urine, serum, fine needle aspirates (*i.e.*, biopsies). EphA2, PCDGF, PCDGF receptor, and/or HAAH antibodies may also be used for immunohistochemical analyses of frozen or fixed cells or tissue assays. In addition, the diagnostic methods of the invention may be used to diagnose, prognose or monitor therapy of non-cancer hyperproliferative diseases (particularly associated with overexpression of an EphA2, PCDGF, PCDGF receptor and/or HAAH polypeptide), for example, but not limited to, asthma, COPD, lung fibrosis, bronchial hyper responsiveness, psoriasis, seborrheic dermatitis, cystic fibrosis, restenosis, hyperproliferative vascular disease, Behcet's Syndrome, atherosclerosis, and macular degeneration. In addition, the antibodies and diagnostic methods of the invention may be used to diagnose, prognose or monitor therapy of non-cancer hyperproliferative diseases (particularly associated with overexpression of an EphA2, PCDGF, PCDGF receptor and/or HAAH polypeptide), for example, but not limited to, ductal carcinoma in situ (DCIS) of the breast, fibroadenoma of the breast, fibrocystic disease, cervix dysplasia, squamous intraepithelial lesions (SIL), adenomatous polyps, Barrett's esophageal dysplasia, hepatocellular carcinoma, adenomatous hyperplasia, atypical adenomatous hyperplasia (AAH) of the lung, lymphomatoid granulomatosis, pancreatic ductal lesions, pancreatic hyperplasias, pancreatic dysplasias, prostatic intraepithelial neoplasia (PIN), xeroderma pigmentosum, carcinoma in situ of the skin, squamous cell carcinoma, solar keratosis, compound nevi, actinic cheilitis, leukoplakia, and Bowen's disease.

[0029] In another embodiment, kits comprising the pharmaceutical compositions or diagnostic reagents of the invention are provided.

1.1. DEFINITIONS

[0030] The term "agent" as used herein refers to a molecule that has a desired biological effect. Agents include, but are not limited to, proteinaceous molecules, including, but not limited to, peptides, polypeptides, proteins, post-translationally modified proteins, antibodies etc.; or a small molecule (less than 1000 daltons), an inorganic, or an organic compound; or nucleic acid molecules including, but not limited to, double-stranded or single-stranded DNA, or double-stranded or single-stranded RNA, as well as triple helix nucleic acid molecules. Agents can be derived from any known organism (including, but not limited to, animals, plants, bacteria, fungi, and protista, or viruses) or from a library of synthetic molecules. Agents used in the methods and compositions of the invention can be EphA2 agents, PCDGF agents, or HAAH agents.

[0031] The term "agonist" as used herein refers to any compound, including a protein, polypeptide, peptide, antibody, antibody fragment, large molecule, or small molecule (less than 10 kD), that increases the activity, activation or function of another molecule. EphA2 agonists cause increased phosphorylation (particularly autophosphorylation) and degradation of EphA2 protein. EphA2 antibodies that agonize EphA2 may or may not also inhibit cancer cell phenotype (*e.g.*, colony formation in soft agar or tubular network formation in a three-dimensional basement membrane or extracellular matrix preparation) and may or may not preferentially bind an EphA2 epitope that is exposed in a cancer cell relative to a non-cancer cell and may or may not have a low K_{off} rate. In specific embodiments, EphA2 agonist antibodies are Eph099B-102.147, Eph099B-208.261, Eph099B-210.248, Eph099B-233.152, EA2, and EA5.

[0032] The term "antagonist" or "antagonize" as used herein refers to any compound that either inhibits/decreases a molecule from binding to a natural (or endogenous) binding partner or inhibits/decreases a cellular effect that results from a molecule binding to a natural (or endogenous) binding. In one embodiment, an antagonist inhibits/decreases a molecule (*e.g.*, PCDGF, PCDGF receptor, or HAAH) from binding to its natural (or endogenous) binding partner (*e.g.*, receptor, ligand, or substrate). For example, antagonists can do one or more of the following: 1) decrease/disrupt receptor-ligand binding (*e.g.*, PCDGF antagonists or PCDGF receptor antagonists) or enzyme-substrate binding (*e.g.*, HAAH antagonists); or 2) decrease expression such that amount of

the molecule (*e.g.*, PCDGF, PCDGF receptor, or HAAH) available to bind its natural (or endogenous) binding partner is decreased. In another embodiment, an antagonist inhibits/decreases a cellular effect that results from a molecule (*e.g.*, PCDGF, PCDGF receptor, or HAAH) binding to its natural (or endogenous) binding partner (*e.g.*, receptor, ligand, or substrate) and thus inhibits/decreases a biological effect normally observed when such binding occurs. PCDGF antagonists, PCDGF receptor antagonists, and HAAH antagonists include, but are not limited to, biological or chemical compounds, proteins, polypeptides, peptides, antibodies, antibody fragments, nucleic acids, large or small (less than 1000 daltons) organic or inorganic molecules.

[0033] The term "antibodies" or "antigen binding fragments thereof" as used herein refers to antibodies or antigen binding fragments thereof that specifically bind an antigen, particularly that specifically bind to either 1) an EphA2 polypeptide or a fragment of an EphA2 polypeptide and do not specifically bind to other polypeptides; 2) a PCDGF polypeptide or a fragment of a PCDGF polypeptide and do not specifically bind to other polypeptides; 3) a PCDGF receptor polypeptide or a fragment of a PCDGF receptor polypeptide and do not specifically bind to other polypeptides; or 4) an HAAH polypeptide or a fragment of an HAAH polypeptide and do not specifically bind to other polypeptides. Preferably, antibodies or antigen binding fragments that immunospecifically bind to an EphA2, PCDGF, PCDGF receptor, or HAAH polypeptide or fragment thereof do not cross-react with other antigens. Antibodies or antigen binding fragments that immunospecifically bind to an EphA2, PCDGF, PCDGF receptor, or HAAH polypeptide can be identified, for example, by immunoassays or other techniques known to those of skill in the art. Antibodies for use in the methods of the invention include, but are not limited to, synthetic antibodies, monoclonal antibodies, recombinantly produced antibodies, intrabodies, multispecific antibodies (including bi-specific antibodies), human antibodies, humanized antibodies, chimeric antibodies, single-chain Fvs (scFv) (including bi-specific scFvs), single chain antibodies Fab fragments, F(ab') fragments, disulfide-linked Fvs (sdFv), anti-idiotypic (anti-Id) antibodies, and bispecific T cell engagers, and epitope-binding fragments of any of the above. In particular, antibodies for use in the methods of the present invention include immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, *i.e.*, molecules that contain an antigen binding site that immunospecifically binds to an antigen of an EphA2, PCDGF, PCDGF receptor, or HAAH polypeptide (*e.g.*, one or more complementarity determining regions (CDRs) of an antibody directed to an EphA2, PCDGF, PCDGF receptor, or HAAH polypeptide). Preferably

EphA2 agonistic antibodies or antigen binding fragments that immunospecifically bind to an EphA2 polypeptide or fragment thereof only agonize EphA2 and do not significantly agonize other activities. Preferably, PCDGF or HAAH antagonistic antibodies or antigen binding fragments that immunospecifically bind to a PCDGF or HAAH polypeptide or fragment thereof only antagonize PCDGF or HAAH and do not significantly antagonize other activities.

[0034] The term "cancer" as used herein refers to a disease involving cells that have the potential to metastasize to distal sites and exhibit phenotypic traits that differ from those of non-cancer cells, for example, formation of colonies in a three-dimensional substrate such as soft agar or the formation of tubular networks or weblike matrices in a three-dimensional basement membrane or extracellular matrix preparation, such as MATRIGEL™. Non-cancer cells do not form colonies in soft agar and form distinct sphere-like structures in three-dimensional basement membrane or extracellular matrix preparations. Cancer cells acquire a characteristic set of functional capabilities during their development, albeit through various mechanisms. Such capabilities include evading apoptosis, self-sufficiency in growth signals, insensitivity to anti-growth signals, tissue invasion/metastasis, limitless replicative potential, and sustained angiogenesis. The term "cancer cell" is meant to encompass both pre-malignant and malignant cancer cells.

[0035] The term "cancer cell phenotype inhibiting" as used herein refers to the ability of an agent to prevent or reduce cancer cell colony formation in soft agar or tubular network formation in a three-dimensional basement membrane (*e.g.*, MATRIGEL™) or extracellular matrix preparation or any other method that detects a reduction in a cancer cell phenotype, for example, assays that detect an increase in contact inhibition of cell proliferation (*e.g.*, reduction of colony formation in a monolayer cell culture) or reduce hyperproliferation of cancer cells. Cancer cell phenotype inhibiting compounds may also cause a reduction or elimination of colonies when added to established colonies of cancer cells in soft agar or the extent of tubular network formation in a three-dimensional basement membrane or extracellular matrix preparation. EphA2 agents, PCDGF agents, and/or HAAH agents can have cancer cell phenotype inhibiting properties.

[0036] The term "derivative" as used herein refers to a polypeptide that comprises an amino acid sequence of an EphA2, PCDGF, PCDGF receptor, or HAAH polypeptide, a fragment of an EphA2, PCDGF, PCDGF receptor, or HAAH polypeptide, an antibody that immunospecifically binds to an EphA2, PCDGF, PCDGF receptor, or HAAH polypeptide, or an antibody fragment that immunospecifically binds to an EphA2, PCDGF, PCDGF

receptor, or HAAH polypeptide which has been altered by the introduction of amino acid residue substitutions, deletions or additions. The term "derivative" as used herein also refers to a polypeptide that comprises an amino acid sequence of an EphA2, PCDGF, PCDGF receptor, or HAAH polypeptide, a fragment of an EphA2, PCDGF, PCDGF receptor, or HAAH polypeptide, an antibody that immunospecifically binds to an EphA2, PCDGF, PCDGF receptor, or HAAH polypeptide, or an antibody fragment that immunospecifically binds to an EphA2, PCDGF, PCDGF receptor, or HAAH polypeptide which has been modified, *i.e*, by the covalent attachment of any type of molecule to the polypeptide. For example, a polypeptide may be modified, *e.g.*, by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. A derivative of a polypeptide may be modified by chemical modifications using techniques known to those of skill in the art, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, etc. Further, a derivative of a polypeptide may contain one or more non-classical amino acids. In one embodiment, a polypeptide derivative possesses a similar or identical function as its underivatized counterpart. In another embodiment, a derivative of polypeptide has an altered activity when compared to an underivatized counterpart. For example, a derivative antibody or fragment thereof can bind to its epitope more tightly or be more resistant to proteolysis.

[0037] The term "EphA2 agent" as used herein refers to an agent that binds EphA2 or its mRNA and reduces EphA2 expression and/or activity (other than autophosphorylation). In certain embodiments, the EphA2 agent of the invention is an EphA2 agonist and/or preferentially binds an EphA2 epitope exposed in cancer cells. In preferred embodiments, EphA2 agents are antibodies, preferably monoclonal antibodies, preferably that bind EphA2 with a low K_{off} (*e.g.*, less than 3×10^{-3} s^{-1}). In a most preferred embodiment, EphA2 agents are Eph099B-102.147, Eph099B-208.261, Eph099B-210.248, Eph099B-233.152, EA2, and EA5. In other embodiments, EphA2 agents are antisense molecules, ribozyme molecules, RNAi molecules, or EphA2 ligands (*e.g.*, Ephrin A1) or fragments and fusion proteins (*e.g.*, Ephrin A1-Fc fusions) thereof.

[0038] The term "epitope" as used herein refers to portions of a polypeptide having antigenic or immunogenic activity in an animal, preferably in a mammal, and most preferably in a human. An epitope having immunogenic activity is a portion of a polypeptide that elicits an antibody response in an animal. An epitope having antigenic

activity is a portion of a polypeptide to which an antibody immunospecifically binds as determined by any method well known in the art, for example, by immunoassays. Antigenic epitopes need not necessarily be immunogenic.

[0039] The term "exposed EphA2 epitope" as used herein refers to an epitope of EphA2 which is selectively exposed or accessible to an antibody when it is present on cancer cells but not on non-cancer cells. In non-cancer cells, EphA2 is bound to its ligand, EphrinA1, and localizes at areas of cell-cell contacts. However, malignant cells generally display decreased cell-cell contacts as well as overexpress EphA2 in excess of its ligand. Thus, in malignant cells, there is an increased amount of unbound EphA2 that is not localized to cell-cell contacts. Differences in EphA2 membrane distribution and ligand binding between non-cancer cells and malignant cancer cells expose certain epitopes on malignant cells that are not exposed normally. Accordingly, the invention also provides EphA2 agents that are antibodies that specifically bind EphA2 but preferably bind an EphA2 epitope exposed in cancer cells rather than non-cancer cells ("exposed EphA2 epitope antibodies").

[0040] The term "fragments" as used herein includes a peptide or polypeptide comprising an amino acid sequence of at least 5 contiguous amino acid residues, at least 10 contiguous amino acid residues, at least 15 contiguous amino acid residues, at least 20 contiguous amino acid residues, at least 25 contiguous amino acid residues, at least 40 contiguous amino acid residues, at least 50 contiguous amino acid residues, at least 60 contiguous amino residues, at least 70 contiguous amino acid residues, at least contiguous 80 amino acid residues, at least contiguous 90 amino acid residues, at least contiguous 100 amino acid residues, at least contiguous 125 amino acid residues, at least 150 contiguous amino acid residues, at least contiguous 175 amino acid residues, at least contiguous 200 amino acid residues, or at least contiguous 250 amino acid residues of the amino acid sequence of an EphA2, PCDGF, PCDGF receptor, or HAAH polypeptide, an antibody that immunospecifically binds to an EphA2, PCDGF, PCDGF receptor, or HAAH polypeptide, or a polypeptide that is an endogenous binding partner of an EphA2, PCDGF, PCDGF receptor, or HAAH polypeptide (*e.g.*, ligand, receptor, substrate, etc.). Preferably, EphA2 fragments are the extracellular domain, the ligand binding domain, or a portion thereof. Preferably, PCDGF fragments are the PCDGF receptor binding domain or a portion thereof. Preferably, PCDGF receptor fragments are the extracellular domain, the ligand binding domain, or a portion thereof. Preferably, HAAH fragments are the substrate binding

domain, the catalytic domain, the extracellular domain, or a potion thereof. Preferably, antibody fragments are epitope-binding fragments.

[0041] The term "HAAH" as used herein refers to human aspartyl (asparaginyl) beta-hydroxylase. (see, *e.g.*, International Publication Nos. WO 01/35102 and WO 02/092782, which are incorporated by reference in their entireties herein, and Genbank Accession Nos. S83325, NM032466, NM032468, NM004318, NM032467, and NM020164, nucleic and amino acid sequences of HAAH are incorporated by reference in their entireties herein)

[0042] The term "HAAH agent" as used herein refers to an agent that binds HAAH or its mRNA and reduces expression and/or activity of HAAH or a dominant negative HAAH. In certain embodiments, an HAAH agent is an HAAH antagonist or inhibits/decreases binding of HAAH to its substrate. In one embodiment, an HAAH agent is a competitive, un-competitive, or non-competitive inhibitor of HAAH. An HAAH agent inhibits/decreases a biological effect normally observed when HAAH binds its endogenous binding partner (*e.g.*, substrate) such as HAAH hydroxylase activity (*e.g.*, hydroxylation of aspartic acid or asparagine residues in EGF-like domains), increased bcl-2 or proliferating cell nuclear antigen (PCNA) expression, and/or decreased p21/wafl or p16 expression. In other embodiments, HAAH agents are antibodies, preferably monoclonal antibodies or intrabodies. In a specific embodiment, monoclonal antibodies disclosed in International Publication Nos. WO 01/35102 and WO 02/092782 (which are incorporated by reference in their entireties herein) are used in the methods of the invention. In another specific embodiment, intrabodies comprising at least one of the VH or VL domains of the monoclonal antibodies disclosed in International Publication Nos. WO 01/35102 and WO 02/092782 are used in the methods of the invention.

[0043] The term "humanized antibody" as used herein refers to forms of non-human (*e.g.*, murine) antibodies that are chimeric antibodies which contain minimal sequence derived from a non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which hypervariable region residues of the recipient are replaced by hypervariable region residues from a non-human species (donor antibody) such as mouse, rat, rabbit or non-human primate having the desired specificity, affinity, and capacity. In some instances, Framework Region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues which are not found in the recipient antibody or in the donor antibody. These modifications are made to further refine antibody

performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable regions correspond to those of a non-human immunoglobulin and all or substantially all of the FRs are those of a human immunoglobulin sequence. The humanized antibody optionally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. In some embodiments, a humanized antibody is a derivative that has been altered by the introduction of amino acid residue substitutions, deletions or additions (*i.e.,* mutations) that immunospecifically binds to an EphA2, PCDGF, PCDGF receptor, or HAAH polypeptide. Such a humanized antibody comprises amino acid residue substitutions, deletions or additions in one or more non-human CDRs. The humanized antibody derivative may have substantially the same binding, better binding, or worse binding when compared to a non-derivative humanized antibody. In specific embodiments, one, two, three, four, or five amino acid residues of the CDR have been substituted, deleted or added (*i.e.,* mutated). For further details in humanizing antibodies, see European Patent Nos. EP 239,400, EP 592,106, and EP 519,596; International Publication Nos. WO 91/09967 and WO 93/17105; U.S. Patent Nos. 5,225,539, 5,530,101, 5,565,332, 5,585,089, 5,766,886, and 6,407,213; and Padlan, 1991, *Molecular Immunology* 28(4/5):489-498; Studnicka et al., 1994, *Protein Engineering* 7(6):805-814; Roguska et al., 1994, *PNAS* 91:969-973; Tan et al., 2002, *J. Immunol.* 169:1119-25; Caldas et al., 2000, *Protein Eng.* 13:353-60; Morea et al., 2000, *Methods* 20:267-79; Baca et al., 1997, *J. Biol. Chem.* 272:10678-84; Roguska et al., 1996, *Protein Eng.* 9:895-904; Couto et al., 1995, *Cancer Res.* 55 (23 Supp):5973s-5977s; Couto et al., 1995, *Cancer Res.* 55:1717-22; Sandhu, 1994, *Gene* 150:409-10; Pedersen et al., 1994, *J. Mol. Biol.* 235:959-73; Jones et al., 1986, *Nature* 321:522-525; Reichmann et al., 1988, *Nature* 332:323-329; and Presta, 1992, *Curr. Op. Struct. Biol.* 2:593-596.

[0044] The term "hypervariable region" as used herein refers to the amino acid residues of an antibody that are responsible for antigen binding. The hypervariable region comprises amino acid residues from a "Complementarity Determining Region" or "CDR" (*i.e.*, residues 24-34 (L1), 50-56 (L2) and 89-97 (L3) in the light chain variable domain and 31-35 (H1), 50-65 (H2) and 95-102 (H3) in the heavy chain variable domain; Kabat et al., *Sequences of Proteins of Immunological Interest,* 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)) and/or those residues from a "hypervariable loop" (*i.e.*, residues 26-32 (L1), 50-52 (L2) and 91-96 (L3) in the light chain variable domain and 26-32 (H1), 53-55 (H2) and 96-101 (H3) in the heavy chain variable domain;

Chothia and Lesk, 1987, *J. Mol. Biol.* 196:901-917). CDR residues for Eph099B-208.261 and Eph099B-233.152 are listed in Table 1. "Framework Region" or "FR" residues are those variable domain residues other than the hypervariable region residues as herein defined.

[0045] The term "in combination" as used herein refers to the use of more than one prophylactic and/or therapeutic agents. The use of the term "in combination" does not restrict the order in which prophylactic and/or therapeutic agents are administered to a subject with a hyperproliferative cell disorder, especially cancer. A first prophylactic or therapeutic agent can be administered prior to (*e.g.*, 1 minute, 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks before), concomitantly with, or subsequent to (*e.g.*, 1 minute, 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks after) the administration of a second prophylactic or therapeutic agent to a subject which had, has, or is susceptible to a hyperproliferative cell disorder, especially cancer. The prophylactic or therapeutic agents are administered to a subject in a sequence and within a time interval such that the agent of the invention can act together with the other agent to provide an increased benefit than if they were administered otherwise. In one embodiment, one or more EphA2 agents are administered with one or more PCDGF agents and/or one or more HAAH agents. Any additional prophylactic or therapeutic agent can be administered in any order with the other additional prophylactic or therapeutic agents.

[0046] The term "inhibitor" as used herein refers to an agent that decreases or suppresses the activity of an EphA2, PCDGF, PCDGF receptor, or HAAH polypeptide. Inhibitor agents can be competitive inhibitors wherein the inhibitor agent competes for binding with the endogenous (or natural) binding partner of the molecule to be inhibited. For example, competitive inhibitors can prevent enzyme-substrate binding or receptor-ligand binding. Inhibitor agents can be non-competitive inhibitors wherein the inhibitor agent binds to the molecule to be inhibited at some site other than the endogenous binding partner site but still inhibits the action of the bound molecule. For example, non-competitive inhibitors do not prevent enzyme-substrate binding or receptor-ligand binding but make that binding unproductive. Inhibitor agents can be un-competitive inhibitors wherein the inhibitor agent binds to and inhibits the complex of the molecule to be inhibited bound to its endogenous binding partner. For example, un-competitive inhibitors bind to

the enzyme-substrate complex or receptor-ligand complex and make that binding unproductive.

[0047] The term "low tolerance" as used herein refers to a state in which the patient suffers from side effects from treatment so that the patient does not benefit from and/or will not continue therapy because of the adverse effects and/or the harm from the side effects outweighs the benefit of the treatment..

[0048] The terms "manage", "managing" and "management" as used herein refer to the beneficial effects that a subject derives from a prophylactic or therapeutic agent, which does not result in a cure of the disease. In certain embodiments, a subject is administered one or more prophylactic or therapeutic agents to "manage" a disease so as to prevent the progression or worsening of the disease.

[0049] The terms "non-responsive" or "refractory" are used herein to describe patients treated with one or more currently available therapies (*e.g.*, cancer therapies) such as chemotherapy, radiation therapy, surgery, hormonal therapy and/or biological therapy/immunotherapy, particularly a standard therapeutic regimen for the particular cancer, wherein the therapy is not clinically adequate to treat the patients such that these patients need additional effective therapy, *e.g.*, remain unsusceptible to therapy. The phrase can also describe patients who respond to therapy yet suffer from side effects, relapse, develop resistance, etc. In various embodiments, "non-responsive/refractory" means that at least some significant portion of the cancer cells are not killed or their cell division arrested. The determination of whether the cancer cells are "non-responsive/refractory" can be made either *in vivo* or *in vitro* by any method known in the art for assaying the effectiveness of treatment on cancer cells, using the art-accepted meanings of "refractory" in such a context. In various embodiments, a cancer is "non-responsive/refractory" where the number of cancer cells has not been significantly reduced, or has increased during the treatment.

[0050] The term "PCDGF" as used herein refers to PC cell derived growth factor. (see, *e.g.*, International Publication No. WO 98/52607, which is incorporated herein by reference in its entirety, and Genbank Accession Nos. AY124489, NM002087, and M75161, nucleic and amino acid sequences of PCDGF are incorporated by reference in their entireties herein)

[0051] The term "PCDGF receptor" as used herein refers to receptor that can bind PC cell derived growth factor and have a biological consequence from such binding, wherein the biological consequence is one caused by PCDGF. In one embodiment, Rse is a PCDGF receptor (see, *e.g.*, Genbank Accession Nos. BC051756, BC049368, and

NM006293, nucleic and amino acid sequences of Rse are incorporated by reference in their entireties herein). See also US Provisional Patent Application no. 60/474,493 entitled "PCDGF Receptor, Antibodies and Methods of Use" filed May 30, 2003, US Provisional Patent Application no. 60/478,908 entitled "PCDGF Receptor, Antibodies and Methods of Use" filed June 16, 2003, and US Provisional Patent Application no. 60/487,411 entitled "PCDGF Receptor, Antibodies and Methods of Use" filed July 15, 2003, each of which is incorporated by reference in its entirety herein.

[0052] The term "PCDGF agent" as used herein is an agent of the invention that binds PCDGF, PCDGF mRNA, PCDGF receptor, or PCDGF receptor mRNA and reduces PCDGF or PCDGF receptor expression, secretion, and/or activity. In certain embodiments, the PCDGF agent of the invention is a PCDGF antagonist or inhibits/decreases binding of PCDGF to its receptor or inhibits/decreases signaling from the ligand-bound PCDGF receptor. In one embodiment, a PCDGF agent is a competitive, un-competitive, or non-competitive inhibitor of PCDGF. In another embodiment, a PCDGF agent neutralizers PCDGF such that PCDGF cannot bind its receptor. In another embodiment, a PCDGF agent binds a PCDGF receptor without causing signaling and blocks PCDGF binding. An PCDGF agent inhibits/decreases a biological effect normally observed when PCDGF binds its endogenous binding partner (*e.g.*, receptor) such as increased cell proliferation, mitogen-activated protein (MAP) kinase activation, phosphatidylinositol 3' kinase (PI3K) activation, focal adhesion kinase (FAK) activation, increased cyclin D1 expression, increased phosphorylation of pRB, increased expression of matrix metalloproteinase (MMP) 13 and 17. In preferred embodiments, PCDGF agents are antibodies, preferably monoclonal antibodies. In a specific embodiment, PCDGF monoclonal antibodies disclosed in International Publication No. WO 98/52607 are used in the methods of the invention.

[0053] The term "potentiate" as used herein refers to an improvement in the efficacy of a therapeutic or prophylactic agent, *e.g.*, by combining it with one or more other therapeutic or prophylactic agents. In one embodiment, combination therapies that have additive potency or an additive therapeutic or prophylactic effect are potentiated. In a preferred embodiment, combination therapies that have a synergistic (*i.e.*, the effect of the combination is greater than the additive effect of the components of the combination alone) potency or synergistic therapeutic or prophylactic effect are potentiated. In a specific embodiment, EphA2-based therapies are potentiated by PCDGF-based and/or HAAH-based therapies. In another specific embodiment, PCDGF-based therapies are potentiated by EphA2-based and/or HAAH-based therapies. In another specific embodiment, HAAH-

based therapies are potentiated by EphA2 and/or PCDGF-based therapies. In another specific embodiment, non-EphA2-based, PCDGF-based or HAAH-based therapies are potentiated by EphA2-based, PCDGF-based and/or HAAH-based therapies.

[0054] The terms "prevent", " preventing" and "prevention" as used herein refer to the prevention of the onset, recurrence, or spread of a disease in a subject resulting from the administration of a prophylactic or therapeutic agent.

[0055] The term "prophylactic agent" as used herein refers to any agent that can be used in the prevention of the onset, recurrence or spread of a disease or disorder associated with a hyperproliferative disease, particularly cancer. In certain embodiments, the term "prophylactic agent" refers to an EphA2 agent (*e.g.*, the anti-EphA2 antibodies Eph099B-102.147, Eph099B-208.261, Eph099B-210.248, Eph099B-233.152, EA2, and EA5), a PCDGF agent, or an HAAH agent. In certain other embodiments, the term "prophylactic agent" refers to cancer chemotherapeutics, radiation therapy, hormonal therapy, biological therapy (*e.g.*, immunotherapy).

[0056] The term "prophylactically effective amount" as used herein refers to that amount of the prophylactic agent sufficient to result in the prevention of the onset, recurrence or spread of cell hyperproliferative disease, preferably, cancer. A prophylactically effective amount may refer to the amount of prophylactic agent sufficient to prevent the onset, recurrence or spread of hyperproliferative disease, particularly cancer, including but not limited to those predisposed to hyperproliferative disease, for example, those genetically predisposed to cancer or previously exposed to carcinogens. A prophylactically effective amount may also refer to the amount of the prophylactic agent that provides a prophylactic benefit in the prevention of hyperproliferative disease. Further, a prophylactically effective amount with respect to a prophylactic agent that is an EphA2, PCDGF, or HAAH agent means that amount of prophylactic agent alone, or in combination with other agents, that provides a prophylactic benefit in the prevention of hyperproliferative disease. Used in connection with an amount of an EphA2, PCDGF, or HAAH agent, the term can encompass an amount that improves overall prophylaxis or enhances the prophylactic efficacy of or synergies with another prophylactic agent.

[0057] The term "protocol" as used herein includes dosing schedules and dosing regimens.

[0058] The term "side effects" as used herein encompasses unwanted and adverse effects of a prophylactic or therapeutic agent. Adverse effects are always unwanted, but unwanted effects are not necessarily adverse. An adverse effect from a prophylactic or

therapeutic agent might be harmful or uncomfortable or risky. Side effects from chemotherapy include, but are not limited to, gastrointestinal toxicity such as, but not limited to, early and late-forming diarrhea and flatulence, nausea, vomiting, anorexia, leukopenia, anemia, neutropenia, asthenia, abdominal cramping, fever, pain, loss of body weight, dehydration, alopecia, dyspnea, insomnia, dizziness, mucositis, xerostomia, and kidney failure, as well as constipation, nerve and muscle effects, temporary or permanent damage to kidneys and bladder, flu-like symptoms, fluid retention, and temporary or permanent infertility. Side effects from radiation therapy include but are not limited to fatigue, dry mouth, and loss of appetite. Side effects from biological therapies/immunotherapies include but are not limited to rashes or swellings at the site of administration, flu-like symptoms such as fever, chills and fatigue, digestive tract problems and allergic reactions. Side effects from hormonal therapies include but are not limited to nausea, fertility problems, depression, loss of appetite, eye problems, headache, and weight fluctuation. Additional undesired effects typically experienced by patients are numerous and known in the art. Many are described in the *Physicians' Desk Reference* (56^{th} ed., 2002).

[0059] The terms "single-chain Fv" or "scFv" as used herein refer to antibody fragments that comprise the VH and VL domains of antibody, wherein these domains are present in a single polypeptide chain. Generally, the Fv polypeptide further comprises a polypeptide linker between the VH and VL domains which enables the scFv to form the desired structure for antigen binding. For a review of sFvs see Pluckthun in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenburg and Moore eds. Springer-Verlag, New York, pp. 269-315 (1994). In specific embodiments, scFvs include bi-specific scFvs and humanized scFvs.

[0060] The terms "subject" and "patient" as used herein are used interchangeably. As used herein, a subject is preferably a mammal such as a non-primate (*e.g.*, cows, pigs, horses, cats, dogs, rats etc.) and a primate (*e.g.*, monkey and human), most preferably a human.

[0061] The terms "treat", "treating" and "treatment" as used herein refer to the eradication, reduction or amelioration of symptoms of a disease or disorder, particularly, the eradication, removal, modification, or control of primary, regional, or metastatic cancer tissue that results from the administration of one or more prophylactic or therapeutic agents. In certain embodiments, such terms refer to the minimizing or delay of the spread of cancer

resulting from the administration of one or more prophylactic or therapeutic agents to a subject with such a disease.

[0062] The term "therapeutic agent" as used herein refers to any agent that can be used in the treatment, or management of a disease or disorder associated with an hyperproliferative disease or disorder, particularly, cancer. In certain embodiments, the term "therapeutic agent" refers to an EphA2 agent (*e.g.*, the anti-EphA2 antibodies Eph099B-102.147, Eph099B-208.261, Eph099B-210.248, Eph099B-233.152, EA2, and EA5), a PCDGF agent, or an HAAH agent. In certain other embodiments, the terms "therapeutic agent" refer to cancer chemotherapeutics, radiation therapy, hormonal therapy, biological therapy/immunotherapy.

[0063] As used herein, a "therapeutically effective amount" refers to that amount of the therapeutic agent sufficient to treat, manage, or ameliorate the symptoms of a disease or disorder associated with EphA2, PCDGF, PCDGF receptor and/or HAAH overexpression and/or cell hyperproliferation. Preferably, a therapeutic amount is the amount sufficient to destroy, modify, control or remove primary, regional or metastatic cancer tissue. A therapeutically effective amount may refer to the amount of therapeutic agent sufficient to delay or minimize the onset, recurrence or spread of the hyperproliferative disease, *e.g.*, delay or minimize the spread of cancer. A therapeutically effective amount may also refer to the amount of the therapeutic agent that provides a therapeutic benefit in the treatment or management of cancer. Further, a therapeutically effective amount with respect to a therapeutic agent for use in the methods of the invention means that amount of therapeutic agent that provides a therapeutic benefit in the treatment or management of hyperproliferative disease or cancer. Used in connection with an amount of an EphA2, PCDGF, or HAAH agent, the term can encompass an amount that improves overall therapy, reduces or avoids unwanted effects, or enhances the therapeutic efficacy of or synergies with each other or another (*e.g.*, non-EphA2, PCDGF, or HAAH-based) therapeutic agent.

2. DESCRIPTION OF THE FIGURES

[0064] **FIG. 1**: Eph099B-208.261 can compete with EphA2 antibody EA2 for binding to EphA2 in a competitive ELISA assay. The ability of labeled EA2 monoclonal antibody to bind EphA2-Fc was assayed by competitive ELISA in presence of either unlabeled monoclonal antibodies EA2 or Eph099B-208.261. Ratios of unlabeled to labeled antibody used in the assay are indicated on the x-axis. EA2 is indicated by diamonds and Eph099B-208.261 is indicated by squares.

[0065] **FIGS. 2A-2D:** EphA2 antibodies promote EphA2 tyrosine phosphorylation in MDA-MB-231 cells. Monolayers of MDA-MB-231 cells were incubated in the presence of a single dose of 5μg/ml **(A, C)** Eph099B-208.261 or **(B, D)** EA2 for the indicated time at 37°C. Cell lysates were then immunoprecipitated with an EphA2-specific antibody, resolved by SDS-PAGE and subjected to western blot analysis with a phosphotyrosine-specific antibody **(A, B)**. The membranes were stripped and re-probed with the EphA2-specific antibody used in the immunoprecipitation as a loading control **(C, D)**.

[0066] **FIGS. 3A-3D:** EphA2 antibodies promote EphA2 degradation in MDA-MB-231 cells. Monolayers of MDA-MB-231 cells were incubated in the presence of a single dose of 5μg/ml **(A, C)** Eph099B-208.261 or **(B, D)** EA2 for the indicated time at 37°C. Cell lysates were then resolved by SDS-PAGE and subjected to western blot analysis with an EphA2-specific antibody **(A, B)**. The membranes were stripped and re-probed with a β-catenin-specific antibody as a loading control **(C, D)**.

[0067] **FIG. 4**: EphA2 antibodies inhibit malignant tumor cell growth in soft agar. A single dose of 5μg/ml of Eph099B-208.261 (black bar), EA2 (white bar) purified EphA2 antibodies or a negative control antibody, 1A7 (gray bar) were incubated with malignant MDA-MB-231 tumor cells for the indicated time at 37°C in soft agar. Results are reported as colonies per high-powered field (HPF).

[0068] **FIGS. 5A-5B:** EphA2 Eph099B-233.152 antibody inhibits tumor cell growth *in vivo*. MDA-MB-231 cells were implanted subcutaneously into athymic mice. After the tumors had grown to an average volume of 100mm^3, mice were administered 6mg/ml Eph099B-233.152 or PBS control intraperitoneally twice a week for 3 weeks. **(A)** Tumor Growth. Tumor growth was assessed and expressed as a ratio of the tumor volume divided by initial tumor volume (100 mm^3). Control mice are indicated by circles and Eph099B-233.152-treated mice are indicated by squares. Arrows indicate time of Eph099B-233.152 or PBS administration. **(B)** Survival. Tumor growth was allowed to proceed until tumor volume reached 1000mm^3. Survival of the mice was assessed by scoring the percent of mice living each day post treatment. Control mice are indicated by a dashed line and Eph099B-233.152-treated mice are indicated by a solid line.

[0069] **FIGS. 6A-6D**: The EphA2 antibodies, EA2, Eph099B-208.261, and Eph099B-233.152, inhibit tumor cell growth *in vivo*. MDA-MB-231 breast cancer cells were implanted **(A)** orthotopically or **(B)** subcutaneously into athymic mice. **(C)** A549 lung cancer cells were implanted subcutaneously into athymic mice. After the tumors had grown to an average volume of 100mm^3, mice were administered 6 mg/kg of the indicated

antibody or negative control (PBS or 1A7 antibody) intraperitoneally twice a week for 3 weeks. Tumor growth was assessed and expressed as a ratio of the tumor volume divided by initial tumor volume (100 mm^3). **(D)** MDA-MB-231 breast cancer cells were implanted subcutaneously into athymic mice. After the tumors had grown to an average volume of 100mm^3, mice were administered 6 mg/kg of the indicated antibody or negative control intraperitoneally twice a week for 3 weeks. Total tumor volume was determined after sacrifice. Negative control is black, EA2 is white, Eph099B-208.261 is dark grey, and Eph099B-233.152 is light grey.

[0070] **FIGS. 7A-7B**: EphA2 overexpression selectively increases malignant cell growth. **(A)** $1x10^5$ control (white bar) or MCF-7^{EphA2} cells (black bar) were suspended in soft agar in the presence of 1 mg/ml 17β-estradiol for 14 days prior to microscopic evaluation. EphA2-transfected cells formed more colonies (47 colonies/high powered field (HPF)) than matched controls (1 colony/HPF; $P<0.01$). **(B)** Monolayer growth assays did not distinguish between the growth of control (white circles) and MCF-7^{EphA2} cells (black squares).

[0071] **FIGS. 8A-8B:** EphA2 overexpression increases tumorigenic potential. **(A)** $1x10^6$ control (white circle) or MCF-7^{EphA2} cells (black square) were implanted into the mammary fatpad of athymic mice (n=20 mice per group) in the presence of supplemental estrogen (1 μM 17β-estradiol). The tumors formed by MCF-7^{EphA2} cells were significantly larger than tumors formed by matched controls ($P = 0.027$). **(B)** Equal amounts of protein lysate, isolated from input cells or resected tumors (T) were evaluated by western blot analyses with an EphA2 antibody (D7). The membranes were stripped and re-probed with a β-catenin-specific antibody as a loading control.

[0072] **FIGS. 9A-9C:** EphA2 overexpression decreases estrogen dependence. **(A)** $1x10^5$ control (white bar) or MCF-7^{EphA2} cells (black bar) were suspended in soft agar in the absence of exogenous estrogen and colony formation was evaluated microscopically after 14 days. The monolayer growth **(B)** and tumorigenic potential **(C)** of MCF-7^{EphA2} (black square) cells were increased relative to matched controls (white circle) in the absence of supplemental estrogen ($P<0.01$ and $P<0.004$, respectively).

[0073] **FIGS. 10A-10B:** EphA2 overexpression decreases tamoxifen sensitivity. **(A)** $1x10^5$ MCF-7 or MCF-7^{EphA2} cells were suspended in soft agar in the presence of 1μM tamoxifen (TAM) and or 1μM 17β-estradiol and colony formation was evaluated microscopically after 14 days. **(B)** MCF-7 (circles) or MCF-7^{EphA2} cells (squares) were

implanted into the mammary fatpad (n=15 mice per group) in the presence of supplemental estrogen. Tamoxifen treatment was initiated 17 days post-implantation. Tumor volume of tamoxifen treated (black circles and squares) and saline treated (white circles and squares) animals was measured at the indicated time. Note the lower inhibitory effects of tamoxifen on MCF-7^{EphA2} relative to control cells (P=0.01).

[0074] **FIGS. 11A-11F**: Estrogen receptor is expressed but functionally altered in MCF-7^{EphA2} cells. **(A)** ERα and **(B)** ERβ levels were evaluated in MCF-7^{neo} control cells and MCF-7^{EphA2} cells by western blot analyses with an EphA2-specific antibody (D7). **(C, D)** The membranes were stripped and re-probed with a β-catenin-specific antibody as a loading control. **(E, F)** Estrogen receptor activity was measured using a CAT reporter system, revealing comparable estrogen receptor activity in control and MCF-7^{EphA2} cells. The average results from three experiments are graphed in **(F)**. E2 indicates estrogen treatment; TAM indicates tamoxifen treatment; % conversion indicates the amount of substrate converted from non-acetylated substrate (non-AC) to acetylated substrate (AC) by CAT enzyme.

[0075] **FIGS. 12A-12C:** EphA2 agonistic antibody EA2 decreases malignant growth. MCF-7^{EphA2} cells were incubated in the presence of 3 μg/ml of EA2 for the time indicated prior to sample extraction and western blot analyses with an EphA2-specific antibody (D7). **(B)** The membrane was stripped and re-probed with a β-catenin-specific antibody as a loading control. **(C)** $1x10^5$ control or MCF-7^{EphA2} cells were suspended in soft agar in the presence or absence of tamoxifen (TAM, 1μM) and EphA2 agonistic antibody (EA2, 10μg/ml). Note that EA2 increased the sensitivity of MCF-7^{EphA2} cells to tamoxifen.

[0076] **FIG. 13**: Kinetic analysis of EphA2 monoclonal antibody binding. BIACORE™ assays were used to assay the kinetics of EphA2 monoclonal antibody binding to immobilized EphA2-Fc. Eph099B-208.261 is indicated by a solid line, Eph099B-233.152 is indicated by a dotted line, EA2 is indicated by a dashed line, and the negative control is indicated by squares.

[0077] **FIGS. 14A-14B**: Decreased EphA2 protein levels are sufficient to reduce tumor cell colonization of soft agar. Monolayers of MDA-MB-231 cells were transfected with 2 μg/ml of EphA2 antisense or inverse antisense (IAS) oligonucleotides at 37°C for 24 hours. **(A)** Western blot analysis of whole cell lysates with EphA2-specific D7 antibody confirms that transfection with antisense oligonucleotides decreases EphA2 protein levels.

The membranes were stripped and reprobed with paxillin antibodies as a loading control. The relative mobility of molecular mass standards is shown on the left. **(B)** MDA-MB-231 cell monolayers, treated with antisense oligonucleotides as detailed above, were suspended in soft agar for 7 days before microscopic analysis of colony formation. Note that colony formation by MDA-MB-231 cells was significantly impaired by EphA2 antisense oligonucleotides as compared to the inverted antisense control ($P < 0.002$). Results are reported as colonies per high-powered field (HPF).

[0078] **FIGS. 15A-15D:** Sequences of VL and VH of EphA2 antibodies. Amino acid and nucleic acid sequences of Eph099B-208.261 **(A)** VL (SEQ ID NOs:1 and 9, respectively) and **(B)** VH (SEQ ID NOs:5 and 13, respectively); Eph099B-233.152 **(C)** VL (SEQ ID NOs.:17 and 25, respectively) and **(D)** VH (SEQ ID NOs:21 and 29, respectively); and EA2 **(E)** VL (SEQ ID NOs:33 and 41, respectively) and **(F)** VH (SEQ ID NOs:37 and 45, respectively). Sequences of the CDRs are indicated.

3. DETAILED DESCRIPTION OF THE INVENTION

[0079] The present invention is based, in part, on the inventors' discovery that EphA2 agonistic agents can inhibit cancer cell proliferation and invasiveness by reducing the levels of EphA2 expression in these cancer cells. Decreased EphA2 activity selectively inhibits malignant cancer cell growth. In particular, such decreased levels of EphA2 can be achieved with EphA2 agonistic monoclonal antibodies. Although not intending to be bound by any mechanism of action, this inhibition of cell growth and/or metastasis is achieved by stimulating (*i.e.*, agonizing) EphA2 signaling thereby causing EphA2 phosphorylation which leads to the degradation of EphA2. Cancer cell growth is decreased due to the decreased EphA2 levels and, therefore, the decreased ligand-independent EphA2 signaling. Decreased EphA2 activity may also be achieved with antibodies that preferentially bind an EphA2 epitope exposed in cancer cells or with antibodies that bind EphA2 with a low K_{off} (*e.g.*, less than less than $3 \times 10^{-3}\ s^{-1}$).

[0080] The present invention is also based, in part, on the recognition that EphA2-based cancer therapies can potentiate, synergize with, and/or enhance the effectiveness of PCDGF- and/or HAAH-based cancer therapies. Thus, the invention encompasses therapeutic or prophylactic protocols comprising treatment with EphA2 agents in combination with PCDGF agents and/or HAAH agents. Encompassed in the invention are combination therapies that have additive potency or an additive therapeutic or prophylactic effect. The invention also encompasses synergistic combinations where the therapeutic or

prophylactic efficacy of the combination is greater than the additive effect of the components of the combination alone. Preferably, such combinations also reduce or avoid unwanted or adverse effects.

[0081] Accordingly, the present invention relates to methods and compositions that provide for the treatment, inhibition, and management of diseases and disorders associated with hyperproliferative diseases and disorders, particularly cancer. A particular aspect of the invention relates to methods and compositions containing compounds that inhibit cancer cell proliferation and invasion, particularly those cancer cells that overexpress an EphA2, PCDGF, PCDGF receptor and/or HAAH polypeptide. The present invention further relates to methods and compositions that treat, inhibit, or manage metastases of cancers, especially cancers of epithelial cell origin (*e.g.*, cancers of the breast, lung, skin, prostate, bladder, and pancreas, and renal cell carcinomas and melanomas).

[0082] Further compositions and methods of the invention include other types of active ingredients in combination with the EphA2 agents, PCDGF agents, and/or HAAH agents. In some embodiments, the methods of the invention are used to treat, prevent or manage other diseases or disorders associated with cell hyperproliferation, for example but not limited to, asthma, COPD, lung fibrosis, bronchial hyper responsiveness, psoriasis, seborrheic dermatitis, cystic fibrosis, restenosis, hyperproliferative vascular disease, Behcet's Syndrome, atherosclerosis, and macular degeneration, etc. In some embodiments, the methods of the invention are used to treat, prevent or manage other diseases or disorders associated with pre-cancerous conditions, for example but not limited to, ductal carcinoma in situ (DCIS) of the breast, fibroadenoma of the breast, fibrocystic disease, cervix dysplasia, squamous intraepithelial lesions (SIL), adenomatous polyps, Barrett's esophageal dysplasia, hepatocellular carcinoma, adenomatous hyperplasia, atypical adenomatous hyperplasia (AAH) of the lung, lymphomatoid granulomatosis, pancreatic ductal lesions, pancreatic hyperplasias, pancreatic dysplasias, prostatic intraepithelial neoplasia (PIN), xeroderma pigmentosum, carcinoma in situ of the skin, squamous cell carcinoma, solar keratosis, compound nevi, actinic cheilitis, leukoplakia, and Bowen's disease, etc.

[0083] The present invention also relates to methods for the treatment, inhibition, and management of cancer or other hyperproliferative cell disorder or disease that has become partially or completely refractory to current treatment (*e.g.*, a non-EphA2, PCDGF, and/or HAAH-based therapy), such as chemotherapy, radiation therapy, hormonal therapy, or biological therapy.

[0084] The invention further provides diagnostic methods using a combination of antibodies wherein the combination comprises antibodies that immunospecifically bind to EphA2 polypeptides, antibodies that immunospecifically bind to PCDGF polypeptides, antibodies that immunospecifically bind to PCDGF receptor polypeptides, and/or antibodies that immunospecifically bind to HAAH polypeptides to evaluate the efficacy of a treatment of cancer or other hyperproliferative cell disorder. Treatment efficacy monitored can be either therapies that are or are not based on EphA2, PCDGF, and/or HAAH agents. The diagnostic methods of the invention can also be used to prognose or predict the progression of cancer or other hyperproliferative cell disorder. In particular embodiments, the diagnostic methods of the invention provide methods of imaging and localizing metastases and methods of diagnosis and prognosis using tissues and fluids distal to the primary tumor site (as well as methods using tissues and fluids of the primary tumor).

[0085] In an additional embodiment, the invention provides methods of screening for synergistic combinations of EphA2, PCDGF, and/or HAAH agents by screening combinations of EphA2, PCDGF, and/or HAAH agents for the ability to decrease cell colonization in soft agar and/or tubular network formation in three-dimensional basement membrane and extracellular matrix preparations (such as MATRIGEL™) which is greater than the additive effect of the components of the combination alone. In other embodiments, the invention provides methods of screening for synergistic combinations of EphA2, PCDGF, and/or HAAH by assaying for the ability to reduce the extent of existing cell colonization in soft agar and/or tubular network formation in three-dimensional basement membrane which is greater than the additive effect of the components of the combination alone.

[0086] EphA2, PCDGF, and HAAH agents include, but are not limited to, proteinaceous molecules, including, but not limited to, peptides, polypeptides, proteins, including post-translationally modified proteins, antibodies etc.; or small molecules (less than 1000 daltons), inorganic or organic compounds; or nucleic acid molecules including, but not limited to, double-stranded or single-stranded DNA, or double-stranded or single-stranded RNA, as well as triple helix nucleic acid molecules.

3.1. Polypeptide Agents

[0087] Methods of the present invention encompasses use of EphA2, PCDGF, and/or HAAH agents that are polypeptides. In one embodiment, a polypeptide agent is an antibody or fragment thereof that immunospecifically binds EphA2, PCDGF, PCDGF receptor, or HAAH polypeptides and decreases polypeptide expression and/or function.

[0088] In another embodiment, a polypeptide agent is binding partner of EphA2, PCDGF, PCDGF receptor, or HAAH polypeptides such as a ligand, receptor, or substrate or fragment thereof that decreases polypeptide expression and/or function. In a specific embodiment, a polypeptide agent is an EphA2 ligand or fragment thereof such as Ephrin A1, including Ephrin A1-F_c fusion protein, that is capable of binding EphA2 and agonizing EphA2 (*e.g.*, increases EphA2 cytoplasmic tail phosphorylation, increases EphA2 degradation, decreases survival of EphA2 expressing cells, increases EphA2 autophosphorylation, reduces EphA2 activity other than autophosphorylation). In another specific embodiment, a polypeptide agent is a PCDGF receptor or fragment thereof (*e.g.*, ligand binding domain which may or may not be soluble) that binds PCDGF and decreases polypeptide expression and/or function. In another specific embodiment, a polypeptide agent is a modified or derivatized PCDGF or PCDGF fragment (*e.g.*, receptor binding domain) that binds PCDGF receptor but does not elicit PCDGF receptor activation and/or decreases polypeptide expression and/or function. In another specific embodiment, a polypeptide agent is an HAAH substrate or fragment thereof (*e.g.*, EGF-like cysteine-rich repeat sequences) that binds HAAH and decreases HAAH expression and/or function (*e.g.*, hydroxylation), for example, by competing for HAAH binding with an endogenous HAAH substrate (*e.g.*, notch or EGF-like domain containing protein).

3.1.1. Antibodies as Polypeptide Agents

[0089] The invention encompasses agents of the invention that are antibodies (preferably monoclonal antibodies) or fragments thereof that immunospecifically bind to an EphA2, PCDGF, PCDGF receptor, or HAAH polypeptide and decreases or inhibits polypeptide expression and/or activity. Antibodies for use in methods of the invention include, but are not limited to, monoclonal antibodies, synthetic antibodies, recombinantly produced antibodies, intrabodies, multispecific antibodies (including bi-specific antibodies), human antibodies, humanized antibodies, chimeric antibodies, single-chain Fvs (scFv) (including bi-specific scFvs), single chain antibodies, Fab fragments, F(ab') fragments, disulfide-linked Fvs (sdFv), bispecific T cell engagers, and epitope-binding fragments of

any of the above. In particular, antibodies used in the methods of the present invention include immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, *i.e.*, molecules that contain an antigen binding site that immunospecifically binds to an EphA2, PCDGF, PCDGF receptor, or HAAH polypeptide and inhibits or reduces polypeptide expression and/or activity. The immunoglobulin molecules of the invention can be of any type (*e.g.*, IgG, IgE, IgM, IgD, IgA and IgY), class (*e.g.*, IgG_1, IgG_2, IgG_3, IgG_4, IgA_1 and IgA_2) or subclass of immunoglobulin molecule.

[0090] The antibodies used in the methods of the invention may be from any animal origin including birds and mammals (*e.g.*, human, murine, donkey, sheep, rabbit, goat, guinea pig, camel, horse, or chicken). Preferably, the antibodies are human or humanized monoclonal antibodies. As used herein, "human" antibodies include antibodies having the amino acid sequence of a human immunoglobulin and include antibodies isolated from human immunoglobulin libraries or from mice or other animals that express antibodies from human genes.

[0091] The antibodies used in the methods of the present invention may be monospecific, bispecific, trispecific or of greater multispecificity. Multispecific antibodies may immunospecifically bind to different epitopes of an EphA2, PCDGF, PCDGF receptor, or HAAH polypeptide or may immunospecifically bind to both an EphA2, PCDGF, PCDGF receptor, or HAAH polypeptide as well a heterologous epitope, such as a heterologous polypeptide or solid support material. See, *e.g.*, International Publication Nos. WO 93/17715, WO 92/08802, WO 91/00360, and WO 92/05793; Tutt, et al., 1991, *J. Immunol.* 147:60-69; U.S. Patent Nos. 4,474,893, 4,714,681, 4,925,648, 5,573,920, and 5,601,819; and Kostelny et al., 1992, *J. Immunol.* 148:1547-1553.

[0092] In a preferred embodiment, antibodies of the invention are bispecific T cell engagers (BiTEs). Bispecific T cell engagers (BiTE) are bispecific antibodies that can redirect T cells for antigen-specific elimination of targets. A BiTE molecule has an antigen-binding domain that binds to a T cell antigen (*e.g.* CD3) at one end of the molecule and an antigen binding domain that will bind to an antigen on the target cell. A BiTE molecule was recently described in WO 99/54440, which is herein incorporated by reference. This publication describes a novel single-chain multifunctional polypeptide that comprises binding sites for the CD19 and CD3 antigens (CD19xCD3). This molecule was derived from two antibodies, one that binds to CD19 on the B cell and an antibody that binds to CD3 on the T cells. The variable regions of these different antibodies are linked by a polypeptide sequence, thus creating a single molecule. Also described, is the linking of the

heavy chain (VH) and light chain (VL) variable domain of a specific binding domain with a flexible linker to create a single chain, bispecific antibody.

[0093] In an embodiment of this invention, an antibody that immunospecifically binds a polypeptide of interest (*e.g.*, EphA2, PCDGF, PCDGF receptor or HAAH) will comprise a portion of the BiTE molecule. For example, the VH and/or VL (preferably a scFV) of an antibody that binds a polypeptide of interest (*e.g.*, EphA2, PCDGF, PCDGF receptor or HAAH) can be fused to an anti-CD3 binding portion such as that of the molecule described above, thus creating a BiTE molecule that targets the polypeptide of interest (*e.g.*, EphA2, PCDGF, PCDGF receptor or HAAH). In addition to the heavy and/or light chain variable domains of an antibody against a polypeptide of interest (*e.g.*, EphA2, PCDGF, PCDGF receptor or HAAH), other molecules that bind the polypeptide of interest (*e.g.*, EphA2, PCDGF, PCDGF receptor or HAAH) can comprise the BiTE molecule, for example ligands (*e.g.*, Ephrin A1 or PCDGF), receptors (*e.g.*, PCDGF receptor), or substrates (*e.g.*, EGF-like domains). In another embodiment, the BiTE molecule can comprise a molecule that binds to other T cell antigens (other than CD3). For example, ligands and/or antibodies that immunospecifically bind to T-cell antigens like CD2, CD4, CD8, CD11a, TCR, and CD28 are contemplated to be part of this invention. This list is not meant to be exhaustive but only to illustrate that other molecules that can immunospecifically bind to a T cell antigen can be used as part of a BiTE molecule. These molecules can include the VH and/or VL portions of the antibody or natural ligands (for example LFA3 whose natural ligand is CD3). A BiTE molecule can be an agonist or antagonist.

[0094] The "binding domain" as used in accordance with the present invention denotes a domain comprising a three-dimensional structure capable of specifically binding to an epitope like native antibodies, free scFv fragments or one of their corresponding immunoglobulin chains, preferably the VH chain. Thus, said domain can comprise the VH and/or VL domain of an antibody or an immunoglobulin chain, preferably at least the VH domain or more preferably the VH and VL domain linked by a flexible polypeptide linker (scFv). On the other hand, said binding domain contained in the polypeptide of interest may comprise at least one complementarity determining region (CDR) of an antibody or immunoglobulin chain recognizing an antigen on the T cell or a cellular antigen. In this respect, it is noted that the binding domain present in the polypeptide of interest may not only be derived from antibodies but also from other T cell or cellular antigen binding protein, such as naturally occurring surface receptors or ligands. It is further contemplated

that in an embodiment of the invention, said first and or second domain of the above-described polypeptide mimic or correspond to a VH and VL region from a natural antibody. The antibody providing the binding site for the polypeptide of interest can be, *e.g.*, a monoclonal antibody, polyclonal antibody, chimeric antibody, humanized antibody, bispecific antibody, synthetic antibody, antibody fragment, such as Fab, Fv or scFv fragments etc., or a chemically modified derivative of any of these.

[0095] The antibodies used in the methods of the invention include derivatives that are modified, *i.e*, by the covalent attachment of any type of molecule to the antibody. For example, but not by way of limitation, the antibody derivatives include antibodies that have been modified, *e.g.*, by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous chemical modifications may be carried out by known techniques, including, but not limited to, specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, etc. Additionally, the derivative may contain one or more non-classical amino acids.

[0096] The present invention also provides antibodies of the invention or fragments thereof that comprise a framework region known to those of skill in the art. Preferably, the antibody of the invention or fragment thereof is human or humanized.

[0097] The present invention encompasses single domain antibodies, including camelized single domain antibodies (see *e.g.*, Muyldermans et al., 2001, *Trends Biochem. Sci.* 26:230; Nuttall et al., 2000, *Cur. Pharm. Biotech.* 1:253; Reichmann and Muyldermans, 1999, *J. Immunol. Meth.* 231:25; International Publication Nos. WO 94/04678 and WO 94/25591; U.S. Patent No. 6,005,079; which are incorporated herein by reference in their entireties). In one embodiment, the present invention provides single domain antibodies comprising two VH domains having the amino acid sequence of any of the VH domains of an antibody which immunospecifically binds an EphA2, PCDGF, PCDGF receptor, or HAAH polypeptide and decreases polypeptide expression and/or function with modifications such that single domain antibodies are formed. In another embodiment, the present invention also provides single domain antibodies comprising two VH domains comprising one or more of the VH CDRs of an antibody which immunospecifically binds an EphA2, PCDGF, PCDGF receptor, or HAAH polypeptide and decreases polypeptide expression and/or function.

[0098] The methods of the present invention also encompass the use of antibodies or antigen binding fragments thereof that have half-lives (*e.g.*, serum half-lives) in a

mammal, preferably a human, of greater than 15 days, preferably greater than 20 days, greater than 25 days, greater than 30 days, greater than 35 days, greater than 40 days, greater than 45 days, greater than 2 months, greater than 3 months, greater than 4 months, or greater than 5 months. The increased half-lives of the antibodies of the present invention or fragments thereof in a mammal, preferably a human, result in a higher serum titer of said antibodies or antibody fragments in the mammal, and thus, reduce the frequency of the administration of said antibodies or antibody fragments and/or reduces the concentration of said antibodies or antibody fragments to be administered. Antibodies or antigen binding fragments thereof having increased *in vivo* half-lives can be generated by techniques known to those of skill in the art. For example, antibodies or antigen binding fragments thereof with increased *in vivo* half-lives can be generated by modifying (*e.g.*, substituting, deleting or adding) amino acid residues identified as involved in the interaction between the Fc domain and the FcRn receptor (see, *e.g.*, International Publication Nos. WO 97/34631 and WO 02/060919, which are incorporated by reference in their entireties herein). Antibodies or antigen binding fragments thereof with increased *in vivo* half-lives can be generated by attaching to said antibodies or antibody fragments polymer molecules such as high molecular weight polyethyleneglycol (PEG). PEG can be attached to said antibodies or antibody fragments with or without a multifunctional linker either through site-specific conjugation of the PEG to the N- or C- terminus of said antibodies or antibody fragments or via epsilon-amino groups present on lysine residues. Linear or branched polymer derivatization that results in minimal loss of biological activity will be used. The degree of conjugation will be closely monitored by SDS-PAGE and mass spectrometry to ensure proper conjugation of PEG molecules to the antibodies. Unreacted PEG can be separated from antibody-PEG conjugates by, *e.g.*, size exclusion or ion-exchange chromatography.

[0099] The present invention also encompasses the use of antibodies or antibody fragments comprising the amino acid sequence of one or both variable domains of an antibody which immunospecifically binds an EphA2, PCDGF, PCDGF receptor, or HAAH polypeptide and decreases polypeptide expression and/or function with mutations (*e.g.*, one or more amino acid substitutions) in the variable regions. Preferably, mutations in these antibodies maintain or enhance the avidity and/or affinity of the antibodies for the particular antigen(s) to which they immunospecifically bind. Standard techniques known to those skilled in the art (*e.g.*, immunoassays) can be used to assay the affinity of an antibody for a particular antigen.

[00100] Standard techniques known to those skilled in the art can be used to introduce mutations in the nucleotide sequence encoding an antibody, or fragment thereof, including, *e.g.*, site-directed mutagenesis and PCR-mediated mutagenesis, which results in amino acid substitutions. Preferably, the derivatives include less than 15 amino acid substitutions, less than 10 amino acid substitutions, less than 5 amino acid substitutions, less than 4 amino acid substitutions, less than 3 amino acid substitutions, or less than 2 amino acid substitutions relative to the original antibody or fragment thereof. In a preferred embodiment, the derivatives have conservative amino acid substitutions made at one or more predicted non-essential amino acid residues.

3.1.1.1. EphA2 Antibodies

[00101] In one embodiment, antibodies for use in the methods of the invention encompass EphA2 antibodies (preferably monoclonal antibodies) or fragments thereof that immunospecifically bind to and agonize EphA2 signaling ("EphA2 agonistic antibodies"); inhibit a cancer cell phenotype, *e.g.*, inhibit colony formation in soft agar or tubular network formation in a three-dimensional basement membrane or extracellular matrix preparation, such as MATRIGEL™ ("cancer cell phenotype inhibiting antibodies"); preferentially bind epitopes on EphA2 that are selectively exposed or increased on cancer cells but not non-cancer cells ("exposed EphA2 epitope antibodies"); and/or bind EphA2 with a K_{off} of less than $3 \times 10^{-3}\ s^{-1}$. In a specific embodiment, the antibody binds to the extracellular domain of EphA2 and, preferably, also agonizes EphA2, *e.g.*, increases EphA2 phosphorylation and, preferably, causes EphA2 degradation. In another specific embodiment, the antibody binds to the extracellular domain of EphA2 and, preferably, also inhibits and, even more preferably, reduces the extent of colony formation in soft agar or tubular network formation in a three-dimensional basement membrane or extracellular matrix preparation (*e.g.*, by cell killing mechanisms such as necrosis and apoptosis). In another specific embodiment, the antibody binds to the extracellular domain of EphA2 at an epitope that is exposed in a cancer cell but occluded in a non-cancer cell. In another specific embodiment, the antibody binds to the extracellular domain of EphA2, preferably with a K_{off} of less than $3 \times 10^{-3}\ s^{-1}$, more preferably less than $1 \times 10^{-3}\ s^{-1}$. In other specific embodiments, the antibody binds to EphA2 with a K_{off} of less than $5 \times 10^{-3}\ s^{-1}$, less than $10^{-3}\ s^{-1}$, less than $8 \times 10^{-4}\ s^{-1}$, less than $5 \times 10^{-4}\ s^{-1}$, less than $10^{-4}\ s^{-1}$, less than $9 \times 10^{-5}\ s^{-1}$, less than $5 \times 10^{-5}\ s^{-1}$, less than $10^{-5}\ s^{-1}$, less than $5 \times 10^{-6}\ s^{-1}$, less than $10^{-6}\ s^{-1}$, less than $5 \times 10^{-7}\ s^{-1}$, less than $10^{-7}\ s^{-1}$, less than $5 \times 10^{-8}\ s^{-1}$, less than $10^{-8}\ s^{-1}$, less than $5 \times 10^{-9}\ s^{-1}$, less than $10^{-9}\ s^{-1}$, or less than $10^{-10}\ s^{-1}$.

[00102] In a more preferred embodiment, the EphA2 antibody is Eph099B-102.147, Eph099B-208.261, Eph099B-210.248, Eph099B-233.152, EA2, or EA5. In another embodiment, the antibody binds to an epitope bound by Eph099B-102.147, Eph099B-208.261, Eph099B-210.248, Eph099B-233.152, EA2, or EA5 and/or competes for EphA2 binding with Eph099B-102.147, Eph099B-208.261, Eph099B-210.248, Eph099B-233.152, EA2, or EA5 *e.g.* as assayed by ELISA or any other appropriate immunoassay. In other embodiments, the antibody for use in the methods of the invention immunospecifically binds to and agonizes EphA2 signaling, inhibits a cancer cell phenotype, preferentially binds an epitope on EphA2 that is selectively exposed or increased on cancer cells but not non-cancer cells, and/or has a K_{off} of less than 3 X 10^{-3} s^{-1} and may or may not compete for binding with an EphA2 ligand, *e.g.*, Ephrin A1.

[00103] Hybridomas producing Eph099B-102.147, Eph099B-208.261, and Eph099B-210.248 have been deposited with the American Type Culture Collection (ATCC, P.O. Box 1549, Manassas, VA 20108) on August 7, 2002 under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedures, and assigned accession numbers PTA-4572, PTA-4573, and PTA-4574, respectively, and incorporated by reference herein. A hybridoma producing Eph099B-233.152 has been deposited with the ATCC on May 12, 2003 under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedures, and assigned accession number PTA-5194, and incorporated by reference herein. Hybridomas producing antibodies EA2 (strain EA2.31) and EA5 (strain EA5.12) have been deposited with the ATCC on May 22, 2002 under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedures, and assigned accession numbers PTA-4380 and PTA-4381, respectively and incorporated by reference The amino acid and nucleic acid sequences of VL and VH of Eph099B-208.261, Eph099B-233.152, and EA2 are shown in FIGS. 15A-15D and Table 1. The sequences of the Eph099B-208.261, Eph099B-233.152, and EA2 CDRs are indicated. In a most preferred embodiment, the antibody is human or has been humanized.

[00104] The present invention also encompasses antibodies or antigen binding fragments thereof that immunospecifically bind to EphA2 and agonize EphA2, inhibit a cancer cell phenotype, preferentially bind an EphA2 epitope exposed in cancer cells, and/or bind EphA2 with a K_{off} of less than 3 X 10^{-3} s^{-1}, said antibodies comprising a VH CDR having an amino acid sequence of any one of the VH CDRs of Eph099B-102.147,

Eph099B-208.261, Eph099B-210.248, Eph099B-233.152, EA2, or EA5. The present invention also encompasses the use of antibodies that immunospecifically bind to EphA2 and agonize EphA2, inhibit a cancer cell phenotype, preferentially bind an EphA2 epitope exposed in cancer cells, and/or bind EphA2 with a K_{off} of less than 3 X 10^{-3} s^{-1}, said antibodies comprising a VL CDR having an amino acid sequence of any one of the VL CDRs of Eph099B-102.147, Eph099B-208.261, Eph099B-210.248, Eph099B-233.152, EA2, or EA5. The present invention also encompasses the use of antibodies that immunospecifically bind to EphA2 and agonize EphA2, inhibit a cancer cell phenotype, preferentially bind an EphA2 epitope exposed in cancer cells, and/or bind EphA2 with a K_{off} of less than 3 X 10^{-3} s^{-1}, said antibodies comprising one or more VH CDRs and one or more VL CDRs of Eph099B-102.147, Eph099B-208.261, Eph099B-210.248, Eph099B-233.152, EA2, or EA5. In particular, the invention encompasses the use of antibodies that immunospecifically bind to EphA2 and agonize EphA2, inhibit a cancer cell phenotype, preferentially bind an EphA2 epitope exposed in cancer cells, and/or bind EphA2 with a K_{off} of less than 3 X 10^{-3} s^{-1}, said antibodies comprising a VH CDR1 and a VL CDR1; a VH CDR1 and a VL CDR2; a VH CDR1 and a VL CDR3; a VH CDR2 and a VL CDR1; VH CDR2 and VL CDR2; a VH CDR2 and a VL CDR3; a VH CDR3 and a VL CDR1; a VH CDR3 and a VL CDR2; a VH CDR3 and a VL CDR3; a VH1 CDR1, a VH CDR2 and a VL CDR1; a VH CDR1, a VH CDR2 and a VL CDR2; a VH CDR1, a VH CDR2 and a VL CDR3; a VH CDR2, a VH CDR3 and a VL CDR1, a VH CDR2, a VH CDR3 and a VL CDR2; a VH CDR2, a VH CDR3 and a VL CDR3; a VH1 CDR1, a VH CDR3 and a VL CDR1; a VH CDR1, a VH CDR3 and a VL CDR2; a VH CDR1, a VH CDR3 and a VL CDR3; a VH CDR1, a VL CDR1 and a VL CDR2; a VH CDR1, a VL CDR1 and a VL CDR3; a VH CDR1, a VL CDR2 and a VL CDR3; a VH CDR2, a VL CDR1 and a VL CDR2; a VH CDR2, a VL CDR1 and a VL CDR3; a VH CDR2, a VL CDR2 and a VL CDR3; a VH CDR3, a VL CDR1 and a VL CDR2; a VH CDR3, a VL CDR1 and a VL CDR3; a VH CDR3, a VL CDR2 and a VL CDR3; a VH CDR1, a VH CDR2, a VH CDR3 and a VL CDR1; a VH CDR1, a VH CDR2, a VH CDR3 and a VL CDR2; a VH CDR1, a VH CDR2, a VH CDR3 and a VL CDR3; a VH CDR1, a VL CDR1, a VL CDR2 and a VL CDR3; a VH CDR2, a VL CDR1, a VL CDR2 and a VL CDR3; a VH CDR3, a VL CDR1, a VL CDR2 and a VL CDR3; a VH CDR1, a VH CDR2, a VL CDR1 and a VL CDR2; a VH CDR1, a VH CDR2, a VL CDR1 and a VL CDR3; a VH CDR1, a VH CDR2, a VL CDR2 and a VL CDR3; a VH CDR1, a VH CDR3, a VL CDR1 and a VL CDR2; a VH CDR1, a VH CDR3, a VL CDR1 and a VL CDR3; ; a VH CDR1, a VH CDR3, a VL CDR2

and a VL CDR3; a VH CDR2, a VH CDR3, a VL CDR1 and a VL CDR2; a VH CDR2, a VH CDR3, a VL CDR1 and a VL CDR3; a VH CDR2, a VH CDR3, a VL CDR2 and a VL CDR3; a VH CDR1, a VH CDR2, a VH CDR3, a VL CDR1 and a VL CDR2; a VH CDR1, a VH CDR2, a VH CDR3, a VL CDR1 and a VL CDR3; a VH CDR1, a VH CDR2, a VH CDR3, a VL CDR2 and a VL CDR3; a VH CDR1, a VH CDR2, a VL CDR1, a VL CDR2, and a VL CDR3; a VH CDR1, a VH CDR3, a VL CDR1, a VL CDR2, and a VL CDR3; a VH CDR2, a VH CDR3, a VL CDR1, a VL CDR2, and a VL CDR3; a VH CDR1, a VH CDR2, a VH CDR3, a VL CDR1, a VL CDR2, and a VL CDR3 or any combination thereof of the VH CDRs and VL CDRs of Eph099B-102.147, Eph099B-208.261, Eph099B-210.248, Eph099B-233.152, EA2, or EA5. In specific embodiments, the VH CDR1 is SEQ ID NO:6, 22, or 38; the VH CDR2 is SEQ ID NO:7, 23, or 39; the VH CDR3 is SEQ ID NO:8, 24, or 40; the VL CDR1 is SEQ ID NO:2, 18, or 34; the VL CDR2 is SEQ ID NO:3, 19, or 35; and the VL CDR3 is SEQ ID NO:4, 20, or 36 (see, *e.g.*, Table 1). In a more specific embodiment, the VH CDR1 is SEQ ID NO:6; the VH CDR2 is SEQ ID NO:7; the VH CDR3 is SEQ ID NO:8; the VL CDR1 is SEQ ID NO:2; the VL CDR2 is SEQ ID NO:3; and the VL CDR3 is SEQ ID NO:4. In another more specific embodiment, the VH CDR1 is SEQ ID NO:22; the VH CDR2 is SEQ ID NO:23; the VH CDR3 is SEQ ID NO:24; the VL CDR1 is SEQ ID NO:18; the VL CDR2 is SEQ ID NO:19; and the VL CDR3 is SEQ ID NO:20. In another more specific embodiment, the VH CDR1 is SEQ ID NO:38; the VH CDR2 is SEQ ID NO:39; the VH CDR3 is SEQ ID NO:40; the VL CDR1 is SEQ ID NO:34; the VL CDR2 is SEQ ID NO:35; and the VL CDR3 is SEQ ID NO:36. The invention also encompasses any of the foregoing with one, two, three, four, or five amino acid substitutions, additions, or deletions that bind EphA2.

[00105] In one embodiment, an antibody that immunospecifically binds to EphA2 and agonizes EphA2, inhibits a cancer cell phenotype, preferentially binds an EphA2 epitope exposed in cancer cells, and/or binds EphA2 with a K_{off} of less than 3 X 10^{-3} s^{-1} comprises a VH CDR1 having the amino acid sequence of SEQ ID NO:6 and a VL CDR1 having the amino acid sequence of SEQ ID NO:2. In another embodiment, an antibody that immunospecifically binds to EphA2 and agonizes EphA2, inhibits a cancer cell phenotype, preferentially binds an EphA2 epitope exposed in cancer cells, and/or binds EphA2 with a K_{off} of less than 3 X 10^{-3} s^{-1} comprises a VH CDR1 having the amino acid sequence of SEQ ID NO:6 and a VL CDR2 having the amino acid sequence of SEQ ID NO:3. In another embodiment, an antibody that immunospecifically binds to EphA2 and agonizes EphA2, inhibits a cancer cell phenotype, preferentially binds an EphA2 epitope exposed in cancer

cells, and/or binds EphA2 with a K_{off} of less than 3 X 10^{-3} s^{-1} comprises a VH CDR1 having the amino acid sequence of SEQ ID NO:6 and a VL CDR3 having the amino acid sequence of SEQ ID NO:4.

[00106] In another embodiment, an antibody that immunospecifically binds to EphA2 and agonizes EphA2, inhibits a cancer cell phenotype, preferentially binds an EphA2 epitope exposed in cancer cells, and/or binds EphA2 with a K_{off} of less than 3 X 10^{-3} s^{-1} comprises a VH CDR1 having the amino acid sequence of SEQ ID NO:22 and a VL CDR1 having the amino acid sequence of SEQ ID NO:18. In another embodiment, an antibody that immunospecifically binds to EphA2 and agonizes EphA2, inhibits a cancer cell phenotype, preferentially binds an EphA2 epitope exposed in cancer cells, and/or binds EphA2 with a K_{off} of less than 3 X 10^{-3} s^{-1} comprises a VH CDR1 having the amino acid sequence of SEQ ID NO:22 and a VL CDR2 having the amino acid sequence of SEQ ID NO:19. In another embodiment, an antibody that immunospecifically binds to EphA2 and agonizes EphA2, inhibits a cancer cell phenotype, preferentially binds an EphA2 epitope exposed in cancer cells, and/or binds EphA2 with a K_{off} of less than 3 X 10^{-3} s^{-1} comprises a VH CDR1 having the amino acid sequence of SEQ ID NO:22 and a VL CDR3 having the amino acid sequence of SEQ ID NO:20.

[00107] In another embodiment, an antibody that immunospecifically binds to EphA2 and agonizes EphA2, inhibits a cancer cell phenotype, preferentially binds an EphA2 epitope exposed in cancer cells, and/or binds EphA2 with a K_{off} of less than 3 X 10^{-3} s^{-1} comprises a VH CDR1 having the amino acid sequence of SEQ ID NO:38 and a VL CDR1 having the amino acid sequence of SEQ ID NO:34. In another embodiment, an antibody that immunospecifically binds to EphA2 and agonizes EphA2, inhibits a cancer cell phenotype, preferentially binds an EphA2 epitope exposed in cancer cells, and/or binds EphA2 with a K_{off} of less than 3 X 10^{-3} s^{-1} comprises a VH CDR1 having the amino acid sequence of SEQ ID NO:38 and a VL CDR2 having the amino acid sequence of SEQ ID NO:35. In another embodiment, an antibody that immunospecifically binds to EphA2 and agonizes EphA2, inhibits a cancer cell phenotype, preferentially binds an EphA2 epitope exposed in cancer cells, and/or binds EphA2 with a K_{off} of less than 3 X 10^{-3} s^{-1} comprises a VH CDR1 having the amino acid sequence of SEQ ID NO:38 and a VL CDR3 having the amino acid sequence of SEQ ID NO:36.

[00108] In another embodiment, an antibody that immunospecifically binds to EphA2 and agonizes EphA2, inhibits a cancer cell phenotype, preferentially binds an EphA2 epitope exposed in cancer cells, and/or binds EphA2 with a K_{off} of less than 3 X 10^{-3}

s^{-1} comprises a VH CDR2 having the amino acid sequence of SEQ ID NO:7 and a VL CDR1 having the amino acid sequence of SEQ ID NO:2. In another embodiment, an antibody that immunospecifically binds to EphA2 and agonizes EphA2, inhibits a cancer cell phenotype, preferentially binds an EphA2 epitope exposed in cancer cells, and/or binds EphA2 with a K_{off} of less than 3 X 10^{-3} s^{-1} comprises a VH CDR2 having the amino acid sequence of SEQ ID NO:7 and a VL CDR2 having the amino acid sequence of SEQ ID NO:3. In another embodiment, an antibody that immunospecifically binds to EphA2 and agonizes EphA2, inhibits a cancer cell phenotype, preferentially binds an EphA2 epitope exposed in cancer cells, and/or binds EphA2 with a K_{off} of less than 3 X 10^{-3} s^{-1} comprises a VH CDR2 having the amino acid sequence of SEQ ID NO:7 and a VL CDR3 having the amino acid sequence of SEQ ID NO:4.

[00109] In another embodiment, an antibody that immunospecifically binds to EphA2 and agonizes EphA2, inhibits a cancer cell phenotype, preferentially binds an EphA2 epitope exposed in cancer cells, and/or binds EphA2 with a K_{off} of less than 3 X 10^{-3} s^{-1} comprises a VH CDR2 having the amino acid sequence of SEQ ID NO:23 and a VL CDR1 having the amino acid sequence of SEQ ID NO:18. In another embodiment, an antibody that immunospecifically binds to EphA2 and agonizes EphA2, inhibits a cancer cell phenotype, preferentially binds an EphA2 epitope exposed in cancer cells, and/or binds EphA2 with a K_{off} of less than 3 X 10^{-3} s^{-1} comprises a VH CDR2 having the amino acid sequence of SEQ ID NO:23 and a VL CDR2 having the amino acid sequence of SEQ ID NO:19. In another embodiment, an antibody that immunospecifically binds to EphA2 and agonizes EphA2, inhibits a cancer cell phenotype, preferentially binds an EphA2 epitope exposed in cancer cells, and/or binds EphA2 with a K_{off} of less than 3 X 10^{-3} s^{-1} comprises a VH CDR2 having the amino acid sequence of SEQ ID NO:23 and a VL CDR3 having the amino acid sequence of SEQ ID NO:20.

[00110] In another embodiment, an antibody that immunospecifically binds to EphA2 and agonizes EphA2, inhibits a cancer cell phenotype, preferentially binds an EphA2 epitope exposed in cancer cells, and/or binds EphA2 with a K_{off} of less than 3 X 10^{-3} s^{-1} comprises a VH CDR2 having the amino acid sequence of SEQ ID NO:39 and a VL CDR1 having the amino acid sequence of SEQ ID NO:34. In another embodiment, an antibody that immunospecifically binds to EphA2 and agonizes EphA2, inhibits a cancer cell phenotype, preferentially binds an EphA2 epitope exposed in cancer cells, and/or binds EphA2 with a K_{off} of less than 3 X 10^{-3} s^{-1} comprises a VH CDR2 having the amino acid sequence of SEQ ID NO:39 and a VL CDR2 having the amino acid sequence of SEQ ID

NO:35. In another embodiment, an antibody that immunospecifically binds to EphA2 and agonizes EphA2, inhibits a cancer cell phenotype, preferentially binds an EphA2 epitope exposed in cancer cells, and/or binds EphA2 with a K_{off} of less than 3 X 10^{-3} s^{-1} comprises a VH CDR2 having the amino acid sequence of SEQ ID NO:39 and a VL CDR3 having the amino acid sequence of SEQ ID NO:36.

[00111] In another embodiment, an antibody that immunospecifically binds to EphA2 and agonizes EphA2, inhibits a cancer cell phenotype, preferentially binds an EphA2 epitope exposed in cancer cells, and/or binds EphA2 with a K_{off} of less than 3 X 10^{-3} s^{-1} comprises a VH CDR3 having the amino acid sequence of SEQ ID NO:8 and a VL CDR1 having the amino acid sequence of SEQ ID NO:2. In another embodiment, an antibody that immunospecifically binds to EphA2 and agonizes EphA2, inhibits a cancer cell phenotype, preferentially binds an EphA2 epitope exposed in cancer cells, and/or binds EphA2 with a K_{off} of less than 3 X 10^{-3} s^{-1} comprises a VH CDR3 having the amino acid sequence of SEQ ID NO:8 and a VL CDR2 having the amino acid sequence of SEQ ID NO:3. In another embodiment, an antibody that immunospecifically binds to EphA2 and agonizes EphA2, inhibits a cancer cell phenotype, preferentially binds an EphA2 epitope exposed in cancer cells, and/or binds EphA2 with a K_{off} of less than 3 X 10^{-3} s^{-1} comprises a VH CDR3 having the amino acid sequence of SEQ ID NO:8 and a VL CDR3 having the amino acid sequence of SEQ ID NO:4.

[00112] In another embodiment, an antibody that immunospecifically binds to EphA2 and agonizes EphA2, inhibits a cancer cell phenotype, preferentially binds an EphA2 epitope exposed in cancer cells, and/or binds EphA2 with a K_{off} of less than 3 X 10^{-3} s^{-1} comprises a VH CDR3 having the amino acid sequence of SEQ ID NO:24 and a VL CDR1 having the amino acid sequence of SEQ ID NO:18. In another embodiment, an antibody that immunospecifically binds to EphA2 and agonizes EphA2, inhibits a cancer cell phenotype, preferentially binds an EphA2 epitope exposed in cancer cells, and/or binds EphA2 with a K_{off} of less than 3 X 10^{-3} s^{-1} comprises a VH CDR3 having the amino acid sequence of SEQ ID NO:24 and a VL CDR2 having the amino acid sequence of SEQ ID NO:19. In another embodiment, an antibody that immunospecifically binds to EphA2 and agonizes EphA2, inhibits a cancer cell phenotype, preferentially binds an EphA2 epitope exposed in cancer cells, and/or binds EphA2 with a K_{off} of less than 3 X 10^{-3} s^{-1} comprises a VH CDR3 having the amino acid sequence of SEQ ID NO:24 and a VL CDR3 having the amino acid sequence of SEQ ID NO:20.

[00113] In another embodiment, an antibody that immunospecifically binds to EphA2 and agonizes EphA2, inhibits a cancer cell phenotype, preferentially binds an EphA2 epitope exposed in cancer cells, and/or binds EphA2 with a K_{off} of less than 3 X 10^{-3} s^{-1} comprises a VH CDR3 having the amino acid sequence of SEQ ID NO:40 and a VL CDR1 having the amino acid sequence of SEQ ID NO:34. In another embodiment, an antibody that immunospecifically binds to EphA2 and agonizes EphA2, inhibits a cancer cell phenotype, preferentially binds an EphA2 epitope exposed in cancer cells, and/or binds EphA2 with a K_{off} of less than 3 X 10^{-3} s^{-1} comprises a VH CDR3 having the amino acid sequence of SEQ ID NO:40 and a VL CDR2 having the amino acid sequence of SEQ ID NO:35. In another embodiment, an antibody that immunospecifically binds to EphA2 and agonizes EphA2, inhibits a cancer cell phenotype, preferentially binds an EphA2 epitope exposed in cancer cells, and/or binds EphA2 with a K_{off} of less than 3 X 10^{-3} s^{-1} comprises a VH CDR3 having the amino acid sequence of SEQ ID NO:40 and a VL CDR3 having the amino acid sequence of SEQ ID NO:36.

[00114] The present invention also encompasses methods of using antibodies or antigen binding fragments thereof that immunospecifically bind to EphA2 and agonize EphA2 and/or inhibit a cancer cell phenotype, preferentially bind an EphA2 epitope exposed in cancer cells, and/or bind EphA2 with a K_{off} of less than 3 X 10^{-3} s^{-1}, said antibodies or antibody fragments comprising an amino acid sequence of a variable light chain and/or variable heavy chain that is at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to the amino acid sequence of the variable light chain and/or heavy chain of Eph099B-102.147, Eph099B-208.261, Eph099B-210.248, Eph099B-233.152, or EA2, or EA5. In some embodiments, antibodies or antibody fragments for use in methods of the invention immunospecifically bind to EphA2 and comprise an amino acid sequence of a variable light chain that is at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to SEQ ID NO:1, 17, or 33. In other embodiments, antibodies or antibody fragments for use in the methods of the invention immunospecifically bind to EphA2 and comprise an amino acid sequence of a variable heavy chain that is at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to SEQ ID NO:5, 21, or 37. In other embodiments, antibodies or antibody fragments for use in the methods of the invention immunospecifically bind to EphA2 and

comprise an amino acid sequence of a variable light chain that is at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to SEQ ID NO:1, 17, or 33 and a variable heavy chain that is at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to SEQ ID NO:5, 21, or 37.

[00115] The present invention further encompasses methods of using antibodies or antigen binding fragments thereof that immunospecifically bind to EphA2 and agonize EphA2 and/or inhibit a cancer cell phenotype, preferentially bind an EphA2 epitope exposed in cancer cells, and/or bind EphA2 with a K_{off} of less than 3 X 10^{-3} s^{-1}, said antibodies or antibody fragments comprising an amino acid sequence of one or more CDRs that is at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to the amino acid sequence of one or more CDRs of Eph099B-102.147, Eph099B-208.261, Eph099B-210.248, Eph099B-233.152, EA2, or EA5. In one embodiment, antibodies or antibody fragments for use in methods of the invention immunospecifically bind to EphA2 and comprise an amino acid sequence of a CDR that is at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to SEQ ID NO:2, 3, or 4. In another embodiment, antibodies or antibody fragments for use in methods of the invention immunospecifically bind to EphA2 and comprise an amino acid sequence of a CDR that is at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to SEQ ID NO:18, 19, or 20. In another embodiment, antibodies or antibody fragments for use in methods of the invention immunospecifically bind to EphA2 and comprise an amino acid sequence of a CDR that is at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to SEQ ID NO:34, 35, or 36. In another embodiment, antibodies or antibody fragments for use in methods of the invention immunospecifically bind to EphA2 and comprise an amino acid sequence of a CDR that is at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to SEQ ID NO:6, 7, or 8. In another embodiment, antibodies or antibody fragments for use in methods of the invention immunospecifically bind to EphA2 and comprise an amino acid sequence of a CDR that is

at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to SEQ ID NO:22, 23, or 24. In another embodiment, antibodies or antibody fragments for use in methods of the invention immunospecifically bind to EphA2 and comprise an amino acid sequence of a CDR that is at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to SEQ ID NO:38, 39, or 40.

[00116] The determination of percent identity of two amino acid sequences can be determined by any method known to one skilled in the art, including BLAST protein searches.

[00117] The present invention further encompasses the use of antibodies or antigen binding fragments thereof in the methods of the invention that immunospecifically bind to EphA2 and agonize EphA2 and/or inhibit a cancer cell phenotype, preferentially bind an EphA2 epitope exposed in cancer cells, and/or bind EphA2 with a K_{off} of less than 3 X 10^{-3} s^{-1}, said antibodies or antibody fragments comprising an amino acid sequence of one or more CDRs comprising amino acid residue substitutions, deletions or additions as compared to SEQ ID NO: 2, 3, 4, 6, 7, 8, 18, 19, 20, 22, 23, 24, 34, 35, 36, 38, 39, or 40. The antibody comprising the one or more CDRs comprising amino acid residue substitutions, deletions or additions may have substantially the same binding, better binding, or worse binding when compared to an antibody comprising one or more CDRs without amino acid residue substitutions, deletions or additions. In specific embodiments, one, two, three, four, or five amino acid residues of the CDR have been substituted, deleted or added (*i.e.*, mutated).

[00118] The present invention also encompasses the use of antibodies or antibody fragments in methods of the invention that immunospecifically bind to EphA2 and agonize EphA2 and/or inhibit a cancer cell phenotype, preferentially bind epitopes on EphA2 that are selectively exposed or increased on cancer cells but not non-cancer cells and/or bind EphA2 with a K_{off} less than 3 X 10^{-3} s^{-1}, where said antibodies or antibody fragments are encoded by a nucleotide sequence that hybridizes to the nucleotide sequence of Eph099B-102.147, Eph099B-208.261, Eph099B-210.248, Eph099B-233.152, EA2, or EA5 under stringent conditions. In one embodiment, antibodies or antigen binding fragments thereof for use in the methods of the invention immunospecifically bind to EphA2 and agonize EphA2 and/or inhibit a cancer cell phenotype, preferentially bind an epitope on EphA2 that is selectively exposed or increased on cancer cells but not non-cancer cells and/or bind

EphA2 with a K_{off} less than 3 X 10^{-3} s^{-1}, said antibodies or antibody fragments comprising a variable light chain encoded by a nucleotide sequence that hybridizes under stringent conditions to the nucleotide sequence of the variable light chain of Eph099B-102.147, Eph099B-208.261, Eph099B-210.248, Eph099B-233.152, EA2, or EA5. In a preferred embodiment, antibodies or antigen binding fragments for use in the methods of the invention immunospecifically bind to EphA2 and comprise a variable light chain encoded by a nucleotide sequence that hybridizes under stringent conditions to the nucleotide sequence of SEQ ID NO:9, 25, or 41. In another embodiment, antibodies or antigen binding fragments thereof for use in the methods of the invention immunospecifically bind to EphA2 and agonize EphA2 and/or inhibit a cancer cell phenotype, preferentially bind an epitope on EphA2 that is selectively exposed or increased on cancer cells but not non-cancer cells and/or bind EphA2 with a K_{off} less than 3 X 10^{-3} s^{-1}, said antibodies or antibody fragments comprising a variable heavy chain encoded by a nucleotide sequence that hybridizes under stringent conditions to the nucleotide sequence of the variable heavy chain of Eph099B-102.147, Eph099B-208.261, Eph099B-210.248, Eph099B-233.152, EA2, or EA5. In a preferred embodiment, antibodies or antigen binding fragments thereof for use in the methods of the invention immunospecifically bind to EphA2 and comprise a variable heavy chain encoded by a nucleotide sequence that hybridizes under stringent conditions to the nucleotide sequence of SEQ ID NO:13, 29, or 45. In other embodiments, antibodies or antibody fragments for use in methods of the invention immunospecifically bind to EphA2 and comprise a variable light chain encoded by a nucleotide sequence that hybridizes under stringent conditions to the nucleotide sequence of SEQ ID NO:9, 25, or 41 and a variable heavy chain encoded by a nucleotide sequence that hybridizes under stringent conditions to the nucleotide sequence of SEQ ID NO:13, 29, or 45.

[00119] In another embodiment, antibodies or antigen binding fragments thereof for use in methods of the invention immunospecifically bind to EphA2 and agonize EphA2 and/or inhibit a cancer cell phenotype, preferentially bind an EphA2 epitope exposed on cancer cells but not non-cancer cells and/or bind EphA2 with a K_{off} less than 3 X 10^{-3} s^{-1}, said antibodies or antibody fragments comprising one or more CDRs encoded by a nucleotide sequence that hybridizes under stringent conditions to the nucleotide sequence of one or more CDRs of Eph099B-102.147, Eph099B-208.261, Eph099B-210.248, Eph099B-233.152, EA2, or EA5. In a preferred embodiment, the antibodies or antigen binding fragments for use in the methods of the invention immunospecifically bind to EphA2 and comprise a CDR encoded by a nucleotide sequence that hybridizes under stringent

conditions the nucleotide sequence of SEQ ID NO:10, 11, or 12. In another preferred embodiment, the antibodies or antigen binding fragments for use in the methods of the invention immunospecifically bind to EphA2 and comprise a CDR encoded by a nucleotide sequence that hybridizes under stringent conditions the nucleotide sequence of SEQ ID NO:26, 27, or 28. In another preferred embodiment, the antibodies or antigen binding fragments for use in the methods of the invention immunospecifically bind to EphA2 and comprise a CDR encoded by a nucleotide sequence that hybridizes under stringent conditions the nucleotide sequence of SEQ ID NO:42, 43, or 44. In another preferred embodiment, the antibodies or antigen binding fragments for use in the methods of the invention immunospecifically bind to EphA2 and comprise a CDR encoded by a nucleotide sequence that hybridizes under stringent conditions the nucleotide sequence of SEQ ID NO:14, 15, or 16. In another preferred embodiment, the antibodies or antigen binding fragments for use in the methods of the invention immunospecifically bind to EphA2 and comprise a CDR encoded by a nucleotide sequence that hybridizes under stringent conditions the nucleotide sequence of SEQ ID NO:30, 31, or 32. In another preferred embodiment, the antibodies or antigen binding fragments for use in the methods of the invention immunospecifically bind to EphA2 and comprise a CDR encoded by a nucleotide sequence that hybridizes under stringent conditions the nucleotide sequence of SEQ ID NO:46, 47, or 48.

[00120] Stringent hybridization conditions include, but are not limited to, hybridization to filter-bound DNA in 6X sodium chloride/sodium citrate (SSC) at about 45°C followed by one or more washes in 0.2X SSC/0.1% SDS at about 50-65°C, highly stringent conditions such as hybridization to filter-bound DNA in 6X SSC at about 45°C followed by one or more washes in 0.1X SSC/0.2% SDS at about 60°C, or any other stringent hybridization conditions known to those skilled in the art (see, for example, Ausubel, F.M. et al., eds. 1989 Current Protocols in Molecular Biology, vol. 1, Green Publishing Associates, Inc. and John Wiley and Sons, Inc., NY at pages 6.3.1 to 6.3.6 and 2.10.3).

[00121] The present invention further encompasses antibodies or antigen binding fragments thereof for use in methods of the invention that immunospecifically bind to EphA2 and agonize EphA2 and/or inhibit a cancer cell phenotype, preferentially bind an EphA2 epitope exposed in cancer cells, and/or bind EphA2 with a K_{off} of less than 3 X 10^{-3} s^{-1}, said antibodies or antibody fragments said antibodies or antibody fragments comprising one or more CDRs encoded by a nucleotide sequence of one or more CDRs comprising

nucleic acid residue substitutions, deletions or additions as compared to SEQ ID NO:10, 11, 12, 14, 15, 16, 26, 27, 28, 30, 31, 32, 42, 43, 44, 46, 47, or 48. The antibody comprising the one or more CDRs comprising nucleic acid residue substitutions, deletions or additions may have substantially the same binding, better binding, or worse binding when compared to an antibody comprising one or more CDRs without nucleic acid residue substitutions, deletions or additions. In specific embodiments, one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, or fifteen nucleic acid residues of the CDR have been substituted, deleted or added (*i.e.*, mutated). The nucleic acid substitutions may or may not change the amino acid sequence of the mutated CDR.

Table 1

Antibody	**V chain**	**CDR**	**SEQ ID NO. (amino acid)**	**SEQ ID NO. (nucleic acid)**	**ATCC Deposit No.**
Eph099B-208.261					PTA-4573
	VL		1	9	
		VL1	2	10	
		VL2	3	11	
		VL3	4	12	
	VH		5	13	
		VH1	6	14	
		VH2	7	15	
		VH3	8	16	
Eph099B-233.152					PTA-5194
	VL		17	25	
		VL1	18	26	
		VL2	19	27	
		VL3	20	28	
	VH		21	29	
		VH1	22	30	
		VH2	23	31	
		VH3	24	32	
EA2					PTA-4380
	VL		33	41	
		VL1	34	42	
		VL2	35	43	
		VL3	36	44	
	VH		37	45	
		VH1	38	46	
		VH2	39	47	
		VH3	40	48	

3.1.1.2. PCDGF Antibodies

[00122] In one embodiment, antibodies for use in the methods of the invention encompass PCDGF antibodies (preferably monoclonal antibodies) or fragments thereof that immunospecifically bind to PCDGF and decrease/inhibit PCDGF expression, secretion, and/or activity. In a specific embodiment, the antibody binds to the receptor binding domain of PCDGF and prevents PCDGF from binding to its receptor. In one embodiment, the PCDGF antibody is an antibody disclosed in International Patent Publication No. WO 98/52607. In another embodiment, the antibody immunospecifically binds an epitope in a PCDGF K19T peptide (KKVIAPRRLPDPQILKSDT; SEQ ID NO:49), S14R peptide (SARGTKCLRKKIPR; SEQ ID NO:50), or E19V peptide (EKAPAHLSLPDPQALKRDV; SEQ ID NO:51). In other embodiments, the antibody of the invention immunospecifically binds to PCDGF and decreases/inhibits PCDGF activity (*e.g.*, the ability to stimulate cell proliferation, activate MAP kinase PI3K, and/or FAK, increased expression of cyclin D1 and/or MMP 13 and/or MMP 17 expression, increased phosphorylation of pRB, etc.). In a most preferred embodiment, the antibody is human or has been humanized.

[00123] The present invention further encompasses the use of antibodies or antigen binding fragments thereof that immunospecifically bind to PCDGF and decrease/inhibit PCDGF expression and/or activity, said antibodies or antibody fragments comprising one or more VH, VL, or CDRs comprising amino acid or nucleic acid residue substitutions, deletions or additions as compared to the VH, VL, or CDRs of isolated PCDGF antibodies. The antibody comprising the one or more amino acid or nucleic acid residue substitutions, deletions or additions may have substantially the same binding, better binding, or worse binding when compared to an antibody without the amino acid or nucleic acid residue substitutions, deletions or additions. In a specific embodiment, one, two, three, four, or five amino acid residues have been substituted, deleted or added (*i.e.*, mutated). In another specific embodiments, one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, or fifteen nucleic acid residues have been substituted, deleted or added (*i.e.*, mutated). The nucleic acid substitutions may or may not change the amino acid sequence of the mutated antibody.

3.1.1.3. PCDGF Receptor Antibodies

[00124] In one embodiment, antibodies for use in the methods of the invention encompass PCDGF receptor antibodies (preferably monoclonal antibodies) or fragments thereof that immunospecifically bind to a PCDGF receptor and decrease/inhibit PCDGF

receptor expression, ability to bind PCDGF, and/or activity. In a specific embodiment, the antibody binds to the ligand binding domain of a PCDGF receptor and prevents PCDGF from binding. In one embodiment, the PCDGF receptor is Rse. In other embodiments, the antibody for use in the methods of the invention immunospecifically binds to a PCDGF receptor and decreases/inhibits PCDGF activity (*e.g.* the ability to stimulate cell proliferation, activate MAP kinase PI3K, and/or FAK, increased expression of cyclin D1 and/or MMP 13 and/or MMP 17 expression, increased phosphorylation of pRB, etc.). In a most preferred embodiment, the antibody is human or has been humanized.

[00125] The present invention further encompasses the use of antibodies or antigen binding fragments thereof that immunospecifically bind to a PCDGF receptor and decrease/inhibit PCDGF receptor expression, ability to bind PCDGF, and/or activity, said antibodies or antibody fragments comprising one or more VH, VL, or CDRs comprising amino acid or nucleic acid residue substitutions, deletions or additions as compared to the VH, VL, or CDRs of isolated PCDGF receptor antibodies. The antibody comprising the one or more amino acid or nucleic acid residue substitutions, deletions or additions may have substantially the same binding, better binding, or worse binding when compared to an antibody without the amino acid or nucleic acid residue substitutions, deletions or additions. In a specific embodiment, one, two, three, four, or five amino acid residues have been substituted, deleted or added (*i.e.,* mutated). In another specific embodiments, one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, or fifteen nucleic acid residues have been substituted, deleted or added (*i.e.,* mutated). The nucleic acid substitutions may or may not change the amino acid sequence of the mutated antibody.

3.1.1.4. HAAH Antibodies

[00126] In one embodiment, antibodies for use in the methods of the invention encompass HAAH antibodies (preferably monoclonal antibodies) or fragments thereof that immunospecifically bind to HAAH and decrease/inhibit HAAH expression and/or activity. In a specific embodiment, the antibody binds to the substrate binding domain or catalytic domain of HAAH and prevents HAAH activity. In another specific embodiment, the antibody binds to the extracellular domain of HAAH.

[00127] In a preferred embodiment, the HAAH antibody for use in methods of the invention is FB50, 8AC, 5C7, or 19B (see International Patent Publications WO 01/35102 and WO 02/092782). In another embodiment, the antibody for use in methods of the invention binds to an epitope bound by FB50, 8AC, 5C7, or 19B and/or competes for

HAAH binding with FB50, 8AC, 5C7, or 19B, *e.g.*, as assayed by ELISA or any other appropriate immunoassay. In other embodiments, the antibody for use in the methods of the invention immunospecifically binds to HAAH and decreases/inhibits HAAH activity (*e.g.*, enzymatic activity and/or or the ability of HAAH to alter expression levels of bcl-2, PCNA, p21/waf1, and p16). HAAH antibodies for use in the methods of the invention may or may not compete for binding with an HAAH substrate.

[00128] Hybridomas FB501, HA386A, HA15C7A, and HA219B which produce FB50, 8AC, 5C7, or 19B, respectively, have been deposited with the American Type Culture Collection (ATCC, P.O. Box 1549, Manassas, VA 20108) on May 17, 2001 under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedures, and assigned accession numbers _, respectively, and incorporated by reference herein. In a most preferred embodiment, the antibody is human or has been humanized.

[00129] Antibodies or antigen binding fragments thereof for use in the methods of the invention also encompass antibodies or antigen binding fragments thereof that immunospecifically bind to HAAH and decrease/inhibit HAAH expression and/or activity, said antibodies comprising a VH CDR having an amino acid sequence of any one of the VH CDRs of FB50, 8AC, 5C7, or 19B. Antibodies or antigen binding fragments thereof for use in the methods of the invention also encompass antibodies or antigen binding fragments thereof that immunospecifically bind to HAAH and decrease/inhibit HAAH expression and/or activity, said antibodies comprising a VL CDR having an amino acid sequence of any one of the VH CDRs of FB50, 8AC, 5C7, or 19B. Antibodies or antigen binding fragments thereof for use in the methods of the invention also encompass antibodies or antigen binding fragments thereof that immunospecifically bind to HAAH and decrease/inhibit HAAH expression and/or activity, said antibodies comprising one or more VH CDRs and one or more VL CDRs of FB50, 8AC, 5C7, or 19B. In particular, the invention encompasses the use of antibodies that immunospecifically bind to HAAH and decrease/inhibit HAAH expression and/or activity, said antibodies comprising a VH CDR1 and a VL CDR1; a VH CDR1 and a VL CDR2; a VH CDR1 and a VL CDR3; a VH CDR2 and a VL CDR1; VH CDR2 and VL CDR2; a VH CDR2 and a VL CDR3; a VH CDR3 and a VL CDR1; a VH CDR3 and a VL CDR2; a VH CDR3 and a VL CDR3; a VH1 CDR1, a VH CDR2 and a VL CDR1; a VH CDR1, a VH CDR2 and a VL CDR2; a VH CDR1, a VH CDR2 and a VL CDR3; a VH CDR2, a VH CDR3 and a VL CDR1, a VH CDR2, a VH CDR3 and a VL CDR2; a VH CDR2, a VH CDR3 and a VL CDR3; a VH1 CDR1, a VH

CDR3 and a VL CDR1; a VH CDR1, a VH CDR3 and a VL CDR2; a VH CDR1, a VH CDR3 and a VL CDR3; a VH CDR1, a VL CDR1 and a VL CDR2; a VH CDR1, a VL CDR1 and a VL CDR3; a VH CDR1, a VL CDR2 and a VL CDR3; a VH CDR2, a VL CDR1 and a VL CDR2; a VH CDR2, a VL CDR1 and a VL CDR3; a VH CDR2, a VL CDR2 and a VL CDR3; a VH CDR3, a VL CDR1 and a VL CDR2; a VH CDR3, a VL CDR1 and a VL CDR3; a VH CDR3, a VL CDR2 and a VL CDR3; a VH CDR1, a VH CDR2, a VH CDR3 and a VL CDR1; a VH CDR1, a VH CDR2, a VH CDR3 and a VL CDR2; a VH CDR1, a VH CDR2, a VH CDR3 and a VL CDR3; a VH CDR1, a VL CDR1, a VL CDR2 and a VL CDR3; a VH CDR2, a VL CDR1, a VL CDR2 and a VL CDR3; a VH CDR3, a VL CDR1, a VL CDR2 and a VL CDR3; a VH CDR1, a VH CDR2, a VL CDR1 and a VL CDR2; a VH CDR1, a VH CDR2, a VL CDR1 and a VL CDR3; a VH CDR1, a VH CDR2, a VL CDR2 and a VL CDR3; a VH CDR1, a VH CDR3, a VL CDR1 and a VL CDR2; a VH CDR1, a VH CDR3, a VL CDR1 and a VL CDR3; ; a VH CDR1, a VH CDR3, a VL CDR2 and a VL CDR3; a VH CDR2, a VH CDR3, a VL CDR1 and a VL CDR2; a VH CDR2, a VH CDR3, a VL CDR1 and a VL CDR3; a VH CDR2, a VH CDR3, a VL CDR2 and a VL CDR3; a VH CDR1, a VH CDR2, a VH CDR3, a VL CDR1 and a VL CDR2; a VH CDR1, a VH CDR2, a VH CDR3, a VL CDR1 and a VL CDR3; a VH CDR1, a VH CDR2, a VH CDR3, a VL CDR2 and a VL CDR3; a VH CDR1, a VH CDR2, a VL CDR1, a VL CDR2, and a VL CDR3; a VH CDR1, a VH CDR3, a VL CDR1, a VL CDR2, and a VL CDR3; a VH CDR2, a VH CDR3, a VL CDR1, a VL CDR2, and a VL CDR3; a VH CDR1, a VH CDR2, a VH CDR3, a VL CDR1, a VL CDR2, and a VL CDR3 or any combination thereof of the VH CDRs and VL CDRs of FB50, 8AC, 5C7, or 19B. The invention also encompasses the use of any of the foregoing with one, two, three, four, or five amino acid substitutions, additions, or deletions that bind HAAH.

[00130] The present invention also encompasses the use of antibodies or antigen binding fragments thereof that immunospecifically bind to HAAH and decrease/inhibit HAAH expression and/or activity, said antibodies or antibody fragments comprising an amino acid sequence of a variable light chain and/or variable heavy chain that is at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to the amino acid sequence of the variable light chain and/or heavy chain of FB50, 8AC, 5C7, or 19B. The present invention further encompasses the use of antibodies or antigen binding fragments thereof that immunospecifically bind to HAAH and decrease/inhibit HAAH expression and/or activity, said antibodies or antibody fragments comprising an amino acid

sequence of one or more CDRs that is at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to the amino acid sequence of one or more CDRs of FB50, 8AC, 5C7, or 19B. The determination of percent identity of two amino acid sequences can be determined by any method known to one skilled in the art, including BLAST protein searches.

[00131] The present invention further encompasses the use of antibodies or antigen binding fragments thereof that immunospecifically bind to HAAH and decrease/inhibit HAAH expression and/or activity, said antibodies or antibody fragments comprising an amino acid sequence of one or more CDRs comprising amino acid residue substitutions, deletions or additions as compared to the CDRs of FB50, 8AC, 5C7, or 19B. The antibody comprising the one or more CDRs comprising amino acid residue substitutions, deletions or additions may have substantially the same binding, better binding, or worse binding when compared to an antibody comprising one or more CDRs without amino acid residue substitutions, deletions or additions. In specific embodiments, one, two, three, four, or five amino acid residues of the CDR have been substituted, deleted or added (*i.e.,* mutated).

[00132] The present invention also encompasses the use of antibodies or antibody fragments that immunospecifically bind to HAAH and decrease/inhibit HAAH expression and/or activity, where said antibodies or antibody fragments are encoded by a nucleotide sequence that hybridizes to the nucleotide sequence of FB50, 8AC, 5C7, or 19B under stringent conditions. In one embodiment, the invention provides antibodies or antigen binding fragments thereof that immunospecifically bind to HAAH and decrease/inhibit HAAH expression and/or activity, said antibodies or antibody fragments comprising a variable light chain encoded by a nucleotide sequence that hybridizes under stringent conditions to the nucleotide sequence of the variable light chain of FB50, 8AC, 5C7, or 19B. In another embodiment, the invention provides antibodies or antigen binding fragments thereof that immunospecifically bind to HAAH and decrease/inhibit HAAH expression and/or activity, said antibodies or antibody fragments comprising a variable heavy chain encoded by a nucleotide sequence that hybridizes under stringent conditions to the nucleotide sequence of the variable heavy chain of FB50, 8AC, 5C7, or 19B.

[00133] In another embodiment, the invention provides the use of antibodies or antigen binding fragments thereof that immunospecifically bind to HAAH and decrease/inhibit HAAH expression and/or activity, said antibodies or antibody fragments comprising one or more CDRs encoded by a nucleotide sequence that hybridizes under

stringent conditions to the nucleotide sequence of one or more CDRs of FB50, 8AC, 5C7, or 19B. Stringent hybridization conditions include, but are not limited to, hybridization to filter-bound DNA in 6X sodium chloride/sodium citrate (SSC) at about 45°C followed by one or more washes in 0.2X SSC/0.1% SDS at about 50-65°C, highly stringent conditions such as hybridization to filter-bound DNA in 6X SSC at about 45°C followed by one or more washes in 0.1X SSC/0.2% SDS at about 60°C, or any other stringent hybridization conditions known to those skilled in the art (see, for example, Ausubel, F.M. et al., eds. 1989 Current Protocols in Molecular Biology, vol. 1, Green Publishing Associates, Inc. and John Wiley and Sons, Inc., NY at pages 6.3.1 to 6.3.6 and 2.10.3).

[00134] The present invention further encompasses the use of antibodies or antigen binding fragments thereof that immunospecifically bind to HAAH and decrease/inhibit HAAH expression and/or activity, said antibodies or antibody fragments comprising one or more CDRs encoded by a nucleotide sequence of one or more CDRs comprising nucleic acid residue substitutions, deletions or additions as compared to the CDRs of FB50, 8AC, 5C7, or 19B. The antibody comprising the one or more CDRs comprising nucleic acid residue substitutions, deletions or additions may have substantially the same binding, better binding, or worse binding when compared to an antibody comprising one or more CDRs without nucleic acid residue substitutions, deletions or additions. In specific embodiments, one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, or fifteen nucleic acid residues of the CDR have been substituted, deleted or added (*i.e.*, mutated). The nucleic acid substitutions may or may not change the amino acid sequence of the mutated CDR.

3.1.1.5. Intrabodies

[00135] In certain embodiments, the antibody to be used with the invention binds to an intracellular epitope, *i.e.*, is an intrabody. In particular, an intrabody used in the methods of the invention binds to the cytoplasmic domain of EphA2, PCDGF receptor, or HAAH. An intrabody comprises at least a portion of an antibody that is capable of immunospecifically binding an antigen and preferably does not contain sequences coding for its secretion. Such antibodies will bind antigen intracellularly. In one embodiment, the intrabody comprises a single-chain Fv ("sFv"). sFvs are antibody fragments comprising the VH and VL domains of antibody, wherein these domains are present in a single polypeptide chain. Generally, the sFv polypeptide further comprises a polypeptide linker between the VH and VL domains which enables the sFv to form the desired structure for antigen

binding. For a review of sFvs see Pluckthun in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenburg and Moore, eds. Springer-Verlag, New York, pp. 269-315 (1994). In a further embodiment, the intrabody preferably does not encode an operable secretory sequence and thus remains within the cell (see generally Marasco, WA, 1998, "Intrabodies: Basic Research and Clinical Gene Therapy Applications" Springer:New York).

[00136] Generation of intrabodies is well-known to the skilled artisan and is described, for example, in U.S. Patent Nos. 6,004,940; 6,072,036; 5,965,371, which are incorporated by reference in their entireties herein. Further, the construction of intrabodies is discussed in Ohage and Steipe, 1999, *J. Mol. Biol.* 291:1119-1128; Ohage et al., 1999, *J. Mol. Biol.* 291:1129-1134; and Wirtz and Steipe, 1999, *Protein Science* 8:2245-2250. Recombinant molecular biological techniques such as those described for recombinant production of antibodies (*e.g.*, Sections 5.1.1.6, 5.1.6, and 5.1.7) may also be used in the generation of intrabodies.

[00137] In one embodiment, intrabodies of the invention retain at least about 75% of the binding effectiveness of the complete antibody *(i.e.*, having the entire constant domain as well as the variable regions) to the antigen. More preferably, the intrabody retains at least 85% of the binding effectiveness of the complete antibody. Still more preferably, the intrabody retains at least 90% of the binding effectiveness of the complete antibody. Even more preferably, the intrabody retains at least 95% of the binding effectiveness of the complete antibody.

[00138] In producing intrabodies, polynucleotides encoding variable region for both the VH and VL chains of interest can be cloned by using, for example, hybridoma mRNA or splenic mRNA as a template for PCR amplification of such domains (Huse et al., 1989, *Science* 246:1276). In one preferred embodiment, the polynucleotides encoding the VH and VL domains are joined by a polynucleotide sequence encoding a linker to make a single chain antibody (sFv). The sFv typically comprises a single peptide with the sequence VH-linker-VL or VL-linker-VH. The linker is chosen to permit the heavy chain and light chain to bind together in their proper conformational orientation (see for example, Huston, et al., 1991, *Methods in Enzym.* 203:46-121). In a further embodiment, the linker can span the distance between its points of fusion to each of the variable domains (*e.g.*, 3.5 nm) to minimize distortion of the native Fv conformation. In such an embodiment, the linker is a polypeptide of at least 5 amino acid residues, at least 10 amino acid residues, at least 15 amino acid residues, or greater. In a further embodiment, the linker should not cause a

steric interference with the VH and VL domains of the combining site. In such an embodiment, the linker is 35 amino acids or less, 30 amino acids or less, or 25 amino acids or less. Thus, in a most preferred embodiment, the linker is between 15-25 amino acid residues in length. In a further embodiment, the linker is hydrophilic and sufficiently flexible such that the VH and VL domains can adopt the conformation necessary to detect antigen. Intrabodies can be generated with different linker sequences inserted between identical VH and VL domains. A linker with the appropriate properties for a particular pair of VH and VL domains can be determined empirically by assessing the degree of antigen binding for each. Examples of linkers include, but are not limited to, those sequences disclosed in Table 2.

Table 2

Sequence	**SEQ ID NO.**
$(Gly\ Gly\ Gly\ Gly\ Ser)_3$	SEQ ID NO:52
Glu Ser Gly Arg Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser	SEQ ID NO:53
Glu Gly Lys Ser Ser Gly Ser Gly Ser Glu Ser Lys Ser Thr	SEQ ID NO:54
Glu Gly Lys Ser Ser Gly Ser Gly Ser Glu Ser Lys Ser Thr Gln	SEQ ID NO:55
Glu Gly Lys Ser Ser Gly Ser Gly Ser Glu Ser Lys Val Asp	SEQ ID NO:56
Gly Ser Thr Ser Gly Ser Gly Lys Ser Ser Glu Gly Lys Gly	SEQ ID NO:57
Lys Glu Ser Gly Ser Val Ser Ser Glu Gln Leu Ala Gln Phe Arg Ser Leu Asp	SEQ ID NO:58
Glu Ser Gly Ser Val Ser Ser Glu Glu Leu Ala Phe Arg Ser Leu Asp	SEQ ID NO:59

[00139] In one embodiment, intrabodies are expressed in the cytoplasm. In other embodiments, the intrabodies are localized to various intracellular locations. In such embodiments, specific localization sequences can be attached to the intrabody polypeptide to direct the intrabody to a specific location. Intrabodies can be localized, for example, to the following intracellular locations: endoplasmic reticulum (Munro et al., 1987, *Cell* 48:899-907; Hangejorden et al., 1991, *J. Biol. Chem.* 266:6015); nucleus (Lanford et al., 1986, *Cell* 46:575; Stanton et al.,1986, *PNAS* 83:1772; Harlow et al., 1985, *Mol. Cell Biol.* 5:1605; Pap et al., 2002, *Exp. Cell Res.* 265:288-93); nucleolar region (Seomi et al., 1990, *J. Virology* 64:1803; Kubota et al., 1989, *Biochem. Biophys. Res. Comm.* 162:963; Siomi et al., 1998, *Cell* 55:197); endosomal compartment (Bakke et al., 1990, *Cell* 63:707-716); mitochondrial matrix (Pugsley, A. P., 1989, "Protein Targeting", Academic Press, Inc.);

Golgi apparatus (Tang et al., 1992, *J. Bio. Chem.* 267:10122-6); liposomes (Letourneur et al., 1992, *Cell* 69:1183); peroxisome (Pap et al., 2002, *Exp. Cell Res.* 265:288-93); trans Golgi network (Pap et al., 2002, *Exp. Cell Res.* 265:288-93); and plasma membrane (Marchildon et al., 1984, *PNAS* 81:7679-82; Henderson et al., 1987, *PNAS* 89:339-43; Rhee et al., 1987, *J. Virol.* 61:1045-53; Schultz et al., 1984, *J. Virol.* 133:431-7; Ootsuyama et al., 1985, *Jpn. J. Can. Res.* 76:1132-5; Ratner et al., 1985, *Nature* 313:277-84). Examples of localization signals include, but are not limited to, those sequences disclosed in Table 3.

Table 3

Localization	**Sequence**	**SEQ ID NO.**
endoplasmic reticulum	Lys Asp Glu Leu	SEQ ID NO: 60
endoplasmic reticulum	Asp Asp Glu Leu	SEQ ID NO: 61
endoplasmic reticulum	Asp Glu Glu Leu	SEQ ID NO: 62
endoplasmic reticulum	Gln Glu Asp Leu	SEQ ID NO: 63
endoplasmic reticulum	Arg Asp Glu Leu	SEQ ID NO: 64
nucleus	Pro Lys Lys Lys Arg Lys Val	SEQ ID NO: 65
nucleus	Pro Gln Lys Lys Ile Lys Ser	SEQ ID NO: 66
nucleus	Gln Pro Lys Lys Pro	SEQ ID NO: 67
nucleus	Arg Lys Lys Arg	SEQ ID NO: 68
nucleus	Lys Lys Lys Arg Lys	SEQ ID NO: 69
nucleolar region	Arg Lys Lys Arg Arg Gln Arg Arg Arg Ala His Gln	SEQ ID NO: 70
nucleolar region	Arg Gln Ala Arg Arg Asn Arg Arg Arg Arg Trp Arg Glu Arg Gln Arg	SEQ ID NO: 71
nucleolar region	Met Pro Leu Thr Arg Arg Arg Pro Ala Ala Ser Gln Ala Leu Ala Pro Pro Thr Pro	SEQ ID NO: 72
endosomal compartment	Met Asp Asp Gln Arg Asp Leu Ile Ser Asn Asn Glu Gln Leu Pro	SEQ ID NO: 73
mitochondrial matrix	Met Leu Phe Asn Leu Arg Xaa Xaa Leu Asn Asn Ala Ala Phe Arg His Gly His Asn Phe Met Val Arg Asn Phe Arg Cys Gly Gln Pro Leu Xaa	SEQ ID NO: 74

Localization	Sequence	SEQ ID NO.
peroxisome	Ala Lys Leu	SEQ ID NO: 75
trans golgi network	Ser Asp Tyr Gln Arg Leu	SEQ ID NO: 76
plasma membrane	Gly Cys Val Cys Ser Ser Asn Pro	SEQ ID NO: 77
plasma membrane	Gly Gln Thr Val Thr Thr Pro Leu	SEQ ID NO: 78
plasma membrane	Gly Gln Glu Leu Ser Gln His Glu	SEQ ID NO: 79
plasma membrane	Gly Asn Ser Pro Ser Tyr Asn Pro	SEQ ID NO: 80
plasma membrane	Gly Val Ser Gly Ser Lys Gly Gln	SEQ ID NO: 81
plasma membrane	Gly Gln Thr Ile Thr Thr Pro Leu	SEQ ID NO: 82
plasma membrane	Gly Gln Thr Leu Thr Thr Pro Leu	SEQ ID NO: 83
plasma membrane	Gly Gln Ile Phe Ser Arg Ser Ala	SEQ ID NO: 84
plasma membrane	Gly Gln Ile His Gly Leu Ser Pro	SEQ ID NO: 85
plasma membrane	Gly Ala Arg Ala Ser Val Leu Ser	SEQ ID NO: 86
plasma membrane	Gly Cys Thr Leu Ser Ala Glu Glu	SEQ ID NO: 87

[00140] VH and VL domains are made up of the immunoglobulin domains that generally have a conserved structural disulfide bond. In embodiments where the intrabodies are expressed in a reducing environment (*e.g.*, the cytoplasm), such a structural feature cannot exist. Mutations can be made to the intrabody polypeptide sequence to compensate for the decreased stability of the immunoglobulin structure resulting from the absence of disulfide bond formation. In one embodiment, the VH and/or VL domains of the intrabodies contain one or more point mutations such that their expression is stabilized in reducing environments (see Steipe et al., 1994, *J. Mol. Biol.* 240:188-92; Wirtz and Steipe, 1999, *Protein Science* 8:2245-50; Ohage and Steipe, 1999, *J. Mol. Biol.* 291:1119-28; Ohage et al., 1999, *J. Mol Biol.* 291:1129-34).

Intrabody Proteins as Therapeutics

[00141] In one embodiment, the recombinantly expressed intrabody protein is administered to a patient. Such an intrabody polypeptide must be intracellular to mediate a prophylactic or therapeutic effect. In this embodiment of the invention, the intrabody polypeptide is associated with a "membrane permeable sequence". Membrane permeable sequences are polypeptides capable of penetrating through the cell membrane from outside

of the cell to the interior of the cell. When linked to another polypeptide, membrane permeable sequences can also direct the translocation of that polypeptide across the cell membrane as well.

[00142] In one embodiment, the membrane permeable sequence is the hydrophobic region of a signal peptide (see, e.g., Hawiger, 1999, *Curr. Opin. Chem. Biol.* 3:89-94; Hawiger, 1997, *Curr. Opin. Immunol.* 9:189-94; U.S. Patent Nos. 5,807,746 and 6,043,339). The sequence of a membrane permeable sequence can be based on the hydrophobic region of any signal peptide. The signal peptides can be selected, *e.g.*, from the SIGPEP database (see *e.g.*, von Heijne, 1987, *Prot. Seq. Data Anal.* 1:41-2; von Heijne and Abrahmsen, 1989, *FEBS Lett.* 224:439-46). When a specific cell type is to be targeted for insertion of an intrabody polypeptide, the membrane permeable sequence is preferably based on a signal peptide endogenous to that cell type. In another embodiment, the membrane permeable sequence is a viral protein (*e.g.*, Herpes Virus Protein VP22) or fragment thereof (see *e.g.*, Phelan et al., 1998, *Nat. Biotechnol.* 16:440-3). A membrane permeable sequence with the appropriate properties for a particular intrabody and/or a particular target cell type can be determined empirically by assessing the ability of each membrane permeable sequence to direct the translocation of the intrabody across the cell membrane. Examples of membrane permeable sequences include, but are not limited to, those sequences disclosed in Table 4.

Table 4

Sequence	**SEQ ID NO.**
Ala Ala Val Ala Leu Leu Pro Ala Val Leu Leu Ala Leu Leu Ala Pro	SEQ ID NO:88
Ala Ala Val Leu Leu Pro Val Leu Leu Ala Ala Pro	SEQ ID NO:89
Val Thr Val Leu Ala Leu Gly Ala Leu Ala Gly Val Gly Val Gly	SEQ ID NO:90

[00143] In another embodiment, the membrane permeable sequence can be a derivative. In this embodiment, the amino acid sequence of a membrane permeable sequence has been altered by the introduction of amino acid residue substitutions, deletions, additions, and/or modifications. For example, but not by way of limitation, a polypeptide may be modified by, *e.g.*, glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. A derivative of a membrane permeable sequence polypeptide may be modified by chemical modifications using techniques known

to those of skill in the art, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, etc. Further, a derivative of a membrane permeable sequence polypeptide may contain one or more non-classical amino acids. In one embodiment, a polypeptide derivative possesses a similar or identical function as an unaltered polypeptide. In another embodiment, a derivative of a membrane permeable sequence polypeptide has an altered activity when compared to an unaltered polypeptide. For example, a derivative membrane permeable sequence polypeptide can translocate through the cell membrane more efficiently or be more resistant to proteolysis.

[00144] The membrane permeable sequence can be attached to the intrabody in a number of ways. In one embodiment, the membrane permeable sequence and the intrabody are expressed as a fusion protein. In this embodiment, the nucleic acid encoding the membrane permeable sequence is attached to the nucleic acid encoding the intrabody using standard recombinant DNA techniques (see *e.g.*, Rojas et al., 1998, *Nat. Biotechnol.* 16:370-5). In a further embodiment, there is a nucleic acid sequence encoding a spacer peptide placed in between the nucleic acids encoding the membrane permeable sequence and the intrabody. In another embodiment, the membrane permeable sequence polypeptide is attached to the intrabody polypeptide after each is separately expressed recombinantly (see *e.g.*, Zhang et al., 1998, *PNAS* 95:9184-9). In this embodiment, the polypeptides can be linked by a peptide bond or a non-peptide bond (*e.g.* with a crosslinking reagent such as glutaraldehyde or a thiazolidino linkage see *e.g.*, Hawiger, 1999, *Curr. Opin. Chem. Biol.* 3:89-94) by methods standard in the art.

[00145] The administration of the membrane permeable sequence-intrabody polypeptide can be by parenteral administration, *e.g.*, by intravenous injection including regional perfusion through a blood vessel supplying the tissues(s) or organ(s) having the target cell(s), or by inhalation of an aerosol, subcutaneous or intramuscular injection, topical administration such as to skin wounds and lesions, direct transfection into, *e.g.*, bone marrow cells prepared for transplantation and subsequent transplantation into the subject, and direct transfection into an organ that is subsequently transplanted into the subject. Further administration methods include oral administration, particularly when the complex is encapsulated, or rectal administration, particularly when the complex is in suppository form. A pharmaceutically acceptable carrier includes any material that is not biologically or otherwise undesirable, *i.e.*, the material may be administered to an individual along with the selected complex without causing any undesirable biological effects or interacting in a

deleterious manner with any of the other components of the pharmaceutical composition in which it is contained.

[00146] Conditions for the administration of the membrane permeable sequence-intrabody polypeptide can be readily be determined, given the teachings in the art (see *e.g.*, *Remington's Pharmaceutical Sciences*, 18th Ed., E. W. Martin (ed.), Mack Publishing Co., Easton, Pa. (1990)). If a particular cell type *in vivo* is to be targeted, for example, by regional perfusion of an organ or section of artery/blood vessel, cells from the target tissue can be biopsied and optimal dosages for import of the complex into that tissue can be determined *in vitro* to optimize the *in vivo* dosage, including concentration and time length. Alternatively, culture cells of the same cell type can also be used to optimize the dosage for the target cells *in vivo*.

Intrabody Gene Therapy as Therapeutic

[00147] In another embodiment, a polynucleotide encoding an intrabody is administered to a patient (*e.g.*, as in gene therapy). In this embodiment, methods as described in Section 5.9.1 can be used to administer the intrabody polynucleotide.

3.1.1.6. Methods Of Producing Antibodies

[00148] The antibodies or antigen binding fragments thereof for use in the methods of the invention can be produced by any method known in the art for the synthesis of antibodies, in particular, by chemical synthesis or preferably, by recombinant expression techniques.

[00149] Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, or a combination thereof. For example, monoclonal antibodies can be produced using hybridoma techniques including those known in the art and taught, for example, in Harlow et al., *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling, et al., in: *Monoclonal Antibodies and T-Cell Hybridomas* 563-681 (Elsevier, N.Y., 1981) (said references incorporated by reference in their entireties herein). The term "monoclonal antibody" as used herein is not limited to antibodies produced through hybridoma technology. The term "monoclonal antibody" refers to an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced.

[00150] Methods for producing and screening for specific antibodies using hybridoma technology are routine and well known in the art. Briefly, mice can be immunized with an EphA2, PCDGF, PCDGF receptor, or HAAH polypeptide (either the full length polypeptide or a domain thereof, *e.g.*, the extracellular domain, the ligand binding domain, receptor binding domain, the substrate binding domain, etc.) and once an immune response is detected, *e.g.,* antibodies specific for an EphA2, PCDGF, PCDGF receptor, or HAAH polypeptide are detected in the mouse serum, the mouse spleen is harvested and splenocytes isolated. The splenocytes are then fused by well known techniques to any suitable myeloma cells, for example cells from cell line SP20 available from the ATCC. Hybridomas are selected and cloned by limited dilution. Hybridoma clones are then assayed by methods known in the art for cells that secrete antibodies capable of binding a polypeptide of interest (*e.g.*, EphA2, PCDGF, PCDGF receptor, or HAAH). Ascites fluid, which generally contains high levels of antibodies, can be generated by immunizing mice with positive hybridoma clones.

[00151] Accordingly, monoclonal antibodies can be generated by culturing a hybridoma cell secreting an antibody of the invention wherein, preferably, the hybridoma is generated by fusing splenocytes isolated from a mouse immunized with an EphA2, PCDGF, PCDGF receptor, or HAAH polypeptide or fragment thereof with myeloma cells and then screening the hybridomas resulting from the fusion for hybridoma clones that secrete an antibody able to bind EphA2, PCDGF, PCDGF receptor, or HAAH polypeptide.

[00152] Antibody fragments which recognize specific EphA2, PCDGF, PCDGF receptor, or HAAH polypeptide epitopes may be generated by any technique known to those of skill in the art. For example, Fab and F(ab')2 fragments of the invention may be produced by proteolytic cleavage of immunoglobulin molecules, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')2 fragments). F(ab')2 fragments contain the variable region, the light chain constant region and the CH1 domain of the heavy chain. Further, the antibodies of the present invention can also be generated using various phage display methods known in the art.

[00153] In phage display methods, functional antibody domains are displayed on the surface of phage particles which carry the polynucleotide sequences encoding them. In particular, DNA sequences encoding VH and VL domains are amplified from animal cDNA libraries (*e.g.*, human or murine cDNA libraries of lymphoid tissues). The DNA encoding the VH and VL domains are recombined together with an scFv linker by PCR and cloned into a phagemid vector (*e.g.*, p CANTAB 6 or pComb 3 HSS). The vector is electroporated

in *E. coli* and the *E. coli* is infected with helper phage. Phage used in these methods are typically filamentous phage including fd and M13 and the VH and VL domains are usually recombinantly fused to either the phage gene III or gene VIII. Phage expressing an antigen binding domain that binds to the EphA2 epitope of interest can be selected or identified with antigen, *e.g.*, using labeled antigen or antigen bound or captured to a solid surface or bead. Examples of phage display methods that can be used to make the antibodies of the present invention include those disclosed in Brinkman et al., 1995, *J. Immunol. Methods* 182:41-50; Ames et al., 1995, *J. Immunol. Methods* 184:177; Kettleborough et al., 1994, *Eur. J. Immunol.* 24:952-958; Persic et al., 1997, *Gene* 187:9; Burton et al., 1994, *Advances in Immunology* 57:191-280; International Application No. PCT/GB91/01134; International Publication Nos. WO 90/02809, WO 91/10737, WO 92/01047, WO 92/18619, WO 93/1 1236, WO 95/15982, WO 95/20401, and WO97/13844; and U.S. Patent Nos. 5,698,426, 5,223,409, 5,403,484, 5,580,717, 5,427,908, 5,750,753, 5,821,047, 5,571,698, 5,427,908, 5,516,637, 5,780,225, 5,658,727, 5,733,743 and 5,969,108; each of which is incorporated herein by reference in its entirety.

[00154] Phage may be screened for EphA2, PCDGF, PCDGF receptor, or HAAH polypeptide binding. Ability to decrease EphA2, PCDGF, PCDGF receptor, or HAAH expression and/or activity or ability to decrease a cancer phenotype (*e.g.*, reducing colony formation in soft agar, tubular network formation in a three-dimensional basement membrane or extracellular matrix preparation, such as MATRIGEL™, etc.) may also be screened.

[00155] As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies, including human antibodies, or any other desired antigen binding fragment, and expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria, *e.g.*, as described below. Techniques to recombinantly produce Fab, Fab' and F(ab')2 fragments can also be employed using methods known in the art such as those disclosed in International Publication No. WO 92/22324; Mullinax et al., 1992, *BioTechniques* 12:864; Sawai et al., 1995, *AJRI* 34:26; and Better et al., 1988, *Science* 240:1041 (said references incorporated by reference in their entireties herein).

[00156] To generate whole antibodies, PCR primers including VH or VL nucleotide sequences, a restriction site, and a flanking sequence to protect the restriction site can be used to amplify the VH or VL sequences in scFv clones. Utilizing cloning techniques known to those of skill in the art, the PCR amplified VH domains can be cloned into vectors

expressing a VH constant region, *e.g.*, the human gamma 4 constant region, and the PCR amplified VL domains can be cloned into vectors expressing a VL constant region, *e.g.*, human kappa or lambda constant regions. Preferably, the vectors for expressing the VH or VL domains comprise an EF-1α promoter, a secretion signal, a cloning site for the variable domain, constant domains, and a selection marker such as neomycin. The VH and VL domains may also be cloned into one vector expressing the necessary constant regions. The heavy chain conversion vectors and light chain conversion vectors are then co-transfected into cell lines to generate stable or transient cell lines that express full-length antibodies, *e.g.*, IgG, using techniques known to those of skill in the art.

[00157] For some uses, including *in vivo* use of antibodies in humans and *in vitro* detection assays, it may be preferable to use human or chimeric antibodies. Completely human antibodies are particularly desirable for therapeutic treatment of human subjects. Human antibodies can be made by a variety of methods known in the art including phage display methods described above using antibody libraries derived from human immunoglobulin sequences. See also U.S. Patent Nos. 4,444,887 and 4,716,111; and International Publication Nos. WO 98/46645, WO 98/50433, WO 98/24893, WO 98/16654, WO 96/34096, WO 96/33735, and WO 91/10741; each of which is incorporated herein by reference in its entirety herein.

[00158] Human antibodies can also be produced using transgenic mice which are incapable of expressing functional endogenous immunoglobulins, but which can express human immunoglobulin genes. For example, the human heavy and light chain immunoglobulin gene complexes may be introduced randomly or by homologous recombination into mouse embryonic stem cells. Alternatively, the human variable region, constant region, and diversity region may be introduced into mouse embryonic stem cells in addition to the human heavy and light chain genes. The mouse heavy and light chain immunoglobulin genes may be rendered non-functional separately or simultaneously with the introduction of human immunoglobulin loci by homologous recombination. In particular, homozygous deletion of the JH region prevents endogenous antibody production. The modified embryonic stem cells are expanded and microinjected into blastocysts to produce chimeric mice. The chimeric mice are then be bred to produce homozygous offspring which express human antibodies. The transgenic mice are immunized in the normal fashion with a selected antigen, *e.g.*, all or a portion of an EphA2, PCDGF, or HAAH polypeptide. Monoclonal antibodies directed against the antigen can be obtained from the immunized, transgenic mice using conventional hybridoma technology. The

human immunoglobulin transgenes harbored by the transgenic mice rearrange during B cell differentiation, and subsequently undergo class switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA, IgM and IgE antibodies. For an overview of this technology for producing human antibodies, see Lonberg and Huszar (1995, *Int. Rev. Immunol.* 13:65-93). For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, *see, e.g.*, International Publication Nos. WO 98/24893, WO 96/34096, and WO 96/33735; and U.S. Patent Nos. 5,413,923, 5,625,126, 5,633,425, 5,569,825, 5,661,016, 5,545,806, 5,814,318, and 5,939,598, which are incorporated by reference herein in their entirety. In addition, companies such as Abgenix, Inc. (Freemont, CA) and Medarex (Princeton, NJ) can be engaged to provide human antibodies directed against a selected antigen using technology similar to that described above.

[00159] A chimeric antibody is a molecule in which different portions of the antibody are derived from different immunoglobulin molecules such as antibodies having a variable region derived from a non-human antibody and a human immunoglobulin constant region. Methods for producing chimeric antibodies are known in the art. See *e.g.*, Morrison, 1985, *Science* 229:1202; Oi et al., 1986, *BioTechniques* 4:214; Gillies et al., 1989, *J. Immunol. Methods* 125:191-202; and U.S. Patent Nos. 6,311,415, 5,807,715, 4,816,567, and 4,816,397, which are incorporated herein by reference in their entirety. Chimeric antibodies comprising one or more CDRs from a non-human species and framework regions from a human immunoglobulin molecule can be produced using a variety of techniques known in the art including, for example, CDR-grafting (EP 239,400; International Publication No. WO 91/09967; and U.S. Patent Nos. 5,225,539, 5,530,101, and 5,585,089), veneering or resurfacing (EP 592,106; EP 519,596; Padlan, 1991, *Molecular Immunology* 28(4/5):489-498; Studnicka et al., 1994, *Protein Engineering* 7:805; and Roguska et al., 1994, *PNAS* 91:969), and chain shuffling (U.S. Patent No. 5,565,332). In one embodiment, a chimeric antibody for use in methods of the invention immunospecifically binds EphA2 and comprises one, two, or three VL CDRs having an amino acid sequence of any of the VL CDRs of Eph099B-102.147, Eph099B-208.261, Eph099B-210.248, Eph099B-233.152 within human framework regions. In a specific embodiment, a chimeric antibody for use in methods of the invention immunospecifically binds EphA2 and comprises a VL CDR having an amino acid sequence of SEQ ID NO: 2, 3, 4, 18, 19, 20, 34, 35, or 36. In another embodiment, a chimeric antibody for use in

methods of the invention immunospecifically binds EphA2 and comprises one, two, or three VH CDRs having an amino acid sequence of any of the VH CDRs of Eph099B-102.147, Eph099B-208.261, Eph099B-210.248, Eph099B-233.152, EA2, or EA5 within human framework regions. In a specific embodiment, a chimeric antibody for use in methods of the invention immunospecifically binds EphA2 and comprises a VH CDR having an amino acid sequence of SEQ ID NO:6, 7, 8, 22, 23, 24, 38, 39, or 40. In a preferred embodiment, a chimeric antibody for use in methods of the invention immunospecifically binds EphA2 and comprises one, two, or three VL CDRs having an amino acid sequence of any of the VL CDRs of Eph099B-102.147, Eph099B-208.261, Eph099B-210.248, Eph099B-233.152, EA2, or EA5 and further comprises one, two, or three VH CDRs having an amino acid sequence of any of the VH CDRs of Eph099B-102.147, Eph099B-208.261, Eph099B-210.248, Eph099B-233.152, EA2, or EA5 within human framework regions. In a specific preferred embodiment, a chimeric antibody for use in methods of the invention immunospecifically binds EphA2 and comprises a VL CDR having an amino acid sequence of SEQ ID NO: 2, 3, 4, 18, 19, 20, 34, 35, or 36 and further comprises a VH CDR having an amino acid sequence of SEQ ID NO:6, 7, 8, 22, 23, 24, 38, 39, or 40. In a more preferred embodiment, a chimeric antibody for use in methods of the invention immunospecifically binds EphA2 and comprises three VL CDRs having an amino acid sequence of any of the VL CDRs of Eph099B-102.147, Eph099B-208.261, Eph099B-210.248, Eph099B-233.152, EA2, or EA5 and three VH CDRs having an amino acid sequence of any of the VH CDRs of Eph099B-102.147, Eph099B-208.261, Eph099B-210.248, Eph099B-233.152, EA2, or EA5 within human framework regions. In an even more preferred embodiment, a chimeric antibody for use in methods of the invention immunospecifically binds EphA2 and comprises VL CDRs having an amino acid sequence selected from the group consisting of SEQ ID NO: 2, 3, 4, 18, 19, 20, 34, 35, or 36 and further comprises VH CDRs having an amino acid sequence selected from the group consisting of SEQ ID NO:6, 7, 8, 22, 23, 24, 38, 39, or 40.

[00160] Often, framework residues in the framework regions will be substituted with the corresponding residue from the CDR donor antibody to alter, preferably improve, antigen binding. These framework substitutions are identified by methods well known in the art, *e.g.*, by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. (See, *e.g.*, U.S. Patent No. 5,585,089;

and Riechmann et al., 1988, *Nature* 332:323, which are incorporated by reference in their entireties herein)

[00161] A humanized antibody is an antibody or its variant or fragment thereof which is capable of binding to a predetermined antigen and which comprises a framework region having substantially the amino acid sequence of a human immunoglobulin and a CDR having substantially the amino acid sequence of a non-human immunoglobulin. A humanized antibody comprises substantially all of at least one, and typically two, variable domains in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin (*i.e.*, donor antibody) and all or substantially all of the framework regions are those of a human immunoglobulin consensus sequence. Preferably, a humanized antibody also comprises at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. Ordinarily, the antibody will contain both the light chain as well as at least the variable domain of a heavy chain. The antibody also may include the CH1, hinge, CH2, CH3, and CH4 regions of the heavy chain. The humanized antibody can be selected from any class of immunoglobulins, including IgM, IgG, IgD, IgA and IgE, and any isotype, including IgG_1, IgG_2, IgG_3 and IgG_4. Usually the constant domain is a complement fixing constant domain where it is desired that the humanized antibody exhibit cytotoxic activity, and the class is typically IgG_1. Where such cytotoxic activity is not desirable, the constant domain may be of the IgG_2 class. The humanized antibody may comprise sequences from more than one class or isotype, and selecting particular constant domains to optimize desired effector functions is within the ordinary skill in the art. The framework and CDR regions of a humanized antibody need not correspond precisely to the parental sequences, *e.g.*, the donor CDR or the consensus framework may be mutagenized by substitution, insertion or deletion of at least one residue so that the CDR or framework residue at that site does not correspond to either the consensus or the import antibody. Such mutations, however, will not be extensive. Usually, at least 75% of the humanized antibody residues will correspond to those of the parental framework region (FR) and CDR sequences, more often 90%, and most preferably greater than 95%. Humanized antibodies can be produced using variety of techniques known in the art, including but not limited to, CDR-grafting (European Patent No. EP 239,400; International Publication No. WO 91/09967; and U.S. Patent Nos. 5,225,539, 5,530,101, and 5,585,089), veneering or resurfacing (European Patent Nos. EP 592,106 and EP 519,596; Padlan, 1991, *Molecular Immunology* 28(4/5):489-498; Studnicka et al., 1994, *Protein Engineering* 7(6):805-814; and Roguska et al., 1994, *PNAS* 91:969-973), chain

shuffling (U.S. Patent No. 5,565,332), and techniques disclosed in, *e.g.*, U.S. Patent Nos. 6,407,213, 5,766,886, 5,585,089, International Publication No. WO 9317105, Tan et al., 2002, *J. Immunol.* 169:1119-25, Caldas et al., 2000, *Protein Eng.* 13:353-60, Morea et al., 2000, *Methods* 20:267-79, Baca et al., 1997, *J. Biol. Chem.* 272:10678-84, Roguska et al., 1996, *Protein Eng.* 9:895-904, Couto et al., 1995, *Cancer Res.* 55 (23 Supp):5973s-5977s, Couto et al., 1995, *Cancer Res.* 55:1717-22, Sandhu, 1994, *Gene* 150:409-10, Pedersen et al., 1994, J. *Mol. Biol.* 235:959-73, Jones et al., 1986, *Nature* 321:522-525, Riechmann et al., 1988, *Nature* 332:323, and Presta, 1992, *Curr. Op. Struct. Biol.* 2:593-596. Often, framework residues in the framework regions will be substituted with the corresponding residue from the CDR donor antibody to alter, preferably improve, antigen binding. These framework substitutions are identified by methods well known in the art, *e.g.*, by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. (*See, e.g.*, Queen et al., U.S. Patent No. 5,585,089; and Riechmann et al., 1988, *Nature* 332:323, which are incorporated by reference in their entireties herein)

[00162] Further, the antibodies for use in the methods of the invention can, in turn, be utilized to generate anti-idiotype antibodies using techniques well known to those skilled in the art. (See, *e.g.*, Greenspan & Bona, 1989, *FASEB J.* 7:437-444; and Nissinoff, 1991, *J. Immunol.* 147:2429-2438). The invention provides methods employing the use of polynucleotides comprising a nucleotide sequence encoding an antibody of the invention or a fragment thereof.

3.1.2. EphA2 Ligand-Based Polypeptide Agents

[00163] In another embodiment, a polypeptide agent is an EphA2 ligand (e.g., Ephrin A1) or fragment thereof that is capable of binding EphA2 and decreasing or inhibiting EphA2 expression and/or activity (*e.g.*, agonizing EphA2, increases EphA2 cytoplasmic tail phosphorylation, increases EphA2 degradation, decreases survival of EphA2 expressing cells, increases EphA2 autophosphorylation, reduces EphA2 activity other than autophosphorylation, and/or decreases a pathology-causing cell phenotype). In a specific embodiment, a fragment of EphA2 ligand which retains its ability to bind and agonize EphA2 (*e.g.*, the Ephrin A1 extracellular domain) is used in the methods of the invention. In another specific embodiment, a fusion protein comprises the fragment of EphA2 ligand which retains its ability to bind and agonize EphA2 (*e.g.*, the extracellular domain of Ephrin

A1 fused to immunoglobulin heavy chain, see Pratt and Kinch, 2002, Oncogene 21:7690-9, which is incorporated herein by reference in its entirety). In a preferred embodiment, the EphA2 ligand fragment is soluble. Fragments of EphA2 ligand can be made (*e.g.*, using EphA2 ligand sequences known in the art such as the Ephrin A1 sequence of Genbank Accession No. BC032698, nucleic and amino acid sequence of Ephrin A1 is incorporated herein by reference in its entirety) and assayed for the ability to bind and agonize EphA2. In one embodiment, the fragment comprises amino acid residues 1 to approximately 400, 500, or 600 of EphA2. In a more specific embodiment, the fragment is amino acid residues 1-534 of EphA2. Any method known in the art to detect binging between proteins may be used including, but not limited to, affinity chromatography, size exclusion chromatography, electrophoretic mobility shift assay. Polypeptide agonistic agents of the invention that are EphA2 ligand fragments include polypeptides that are 100%, 98%, 95%, 90%, 85%, 80%, 75%, 70%, 65%, 60%, 55%, 50%, 45%, 40% identical to endogenous EphA2 ligand sequences. The determination of percent identity of two amino acid sequences can be determined by any method known to one skilled in the art, including BLAST protein searches.

3.1.3. PCDGF-Based or PCDGF Receptor-Based Polypeptide Agents

[00164] In another embodiment, the polypeptide agent is a fragment of PCDGF polypeptide or its receptor. Because PCDGF bound to its endogenous receptor (*e.g.*, Rse, see Genbank Accession Nos. BC051756, BC049368, and NM006293) causes an increase in cell growth or proliferation, any method that decreases the amount of PCDGF-PCDGF receptor binding is encompassed in the methods of the invention. In one embodiment, a fragment of PCDGF which can bind to but not activate its receptor is used in the methods of the invention to inhibit binding of endogenous PCDGF to its receptor. In a specific embodiment, a fusion protein comprises a fragment of PCDGF which can bind to but not activate its receptor (*e.g.*, the PCDGF fragment fused to the immunoglobulin heavy chain, see, *e.g.*, Carles-Kinch et al., 2002, Cancer Res. 62:2840-7). In another specific embodiment, the fragment is not part of a fusion protein. Fragments of PCDGF can be made (*e.g.*, using PCDGF sequences known in the art such as Genbank Accession Nos. AY124489, NM002087, and M75161) and assayed for the ability to bind the PCDGF receptor Rse. Any method known in the art to detect binging between proteins may be used including, but not limited to, affinity chromatography, size exclusion chromatography, electrophoretic mobility shift assay. Polypeptide agents of the invention that are PCDGF

fragments include polypeptides that are 100%, 98%, 95%, 90%, 85%, 80%, 75%, 70%, 65%, 60%, 55%, 50%, 45%, 40% identical to endogenous PCDGF sequences. The determination of percent identity of two amino acid sequences can be determined by any method known to one skilled in the art, including BLAST protein searches.

[00165] In another embodiment, a fragment of a PCDGF receptor (*e.g.*, Rse) which can bind PCDGF is used in the methods of the invention to inhibit binding of PCDGF to its endogenous, cell-bound receptor. In a specific embodiment, a fusion protein comprises a fragment of Rse which can bind PCDGF (*e.g.*, the Rse fragment fused to the immunoglobulin heavy chain, see, *e.g.*, Carles-Kinch et al., 2002, Cancer Res. 62:2840-7). In another specific embodiment, the fragment is soluble. Fragments of Rse can be made (*e.g.*, using Rse sequences known in the art such as Genbank Accession Nos. BC051756, BC049368, and NM006293) and assayed for the ability to bind the PCDGF. In one embodiment, the fragment comprises the extracellular domain of Rse. Any method known in the art to detect binging between proteins may be used including, but not limited to, affinity chromatography, size exclusion chromatography, electrophoretic mobility shift assay. Polypeptide agents of the invention that are Rse fragments include polypeptides that are 100%, 98%, 95%, 90%, 85%, 80%, 75%, 70%, 65%, 60%, 55%, 50%, 45%, 40% identical to endogenous Rse sequences. The determination of percent identity of two amino acid sequences can be determined by any method known to one skilled in the art, including BLAST protein searches.

3.1.4. HAAH-Based or HAAH Substrate-Based Polypeptide Agents

[00166] In another embodiment, the polypeptide agent is a HAAH polypeptide or fragment thereof that is not enzymatically active or a HAAH substrate or fragment thereof. Any method that decreases the amount of enzymatically active HAAH-HAAH endogenous substrate binding is encompassed in the methods of the invention. In one embodiment, a fragment of HAAH which can bind to its substrate but is not enzymatically active is used in the methods of the invention to inhibit binding of endogenous HAAH to its substrate. In a specific embodiment, a fusion protein comprises a fragment of HAAH which can bind to its substrate but is not enzymatically active (*e.g.*, the HAAH fragment fused to the immunoglobulin heavy chain, see, *e.g.*, Carles-Kinch et al., 2002, *Cancer Res.* 62:2840-7). In another specific embodiment, the fragment is not part of a fusion protein. Fragments of HAAH can be made (*e.g.*, using HAAH sequences known in the art such as Genbank Accession Nos. S83325, NM032466, NM032468, 004318, NM032467, and NM020164)

and assayed for the ability to bind to, but not hydroxylate, HAAH substrate. In one embodiment, the fragment does not comprise the complete or substantial portion of the catalytic domain (*e.g.*, amino acid residues 650-700 of Genbank Accession No. S83325).

[00167] In another embodiment, an enzymatically inactive (*i.e.*, dominant negative) HAAH which can bind to its substrate but is not enzymatically active is used in the methods of the invention to inhibit binding of endogenous HAAH to its substrate. In a specific embodiment, the enzymatically inactive HAAH has a deletion such that the catalytic domain (*e.g.*, amino acid residues 650-700 of Genbank Accession No. S83325) is not present or not functional. In another specific embodiment, the enzymatically inactive HAAH has one or more amino acid substitutions such that the catalytic domain is inactive (*e.g.*, by substituting the ferrous iron binding histidine residues such as residues 671, 675, 679, or 690 of Genbank Accession No. S83325 with a non-iron binding amino acid). In another specific embodiment, a fusion protein comprises an enzymatically inactive HAAH which can bind to its substrate but is not enzymatically active (*e.g.*, the HAAH fragment fused to the immunoglobulin heavy chain, see, *e.g.*, Carles-Kinch et al., 2002, Cancer Res. 62:2840-7). In another specific embodiment, the enzymatically inactive HAAH is not part of a fusion protein. Candidate enzymatically inactive HAAH can be made using standard molecular biological techniques (*e.g.*, using HAAH sequences known in the art such as Genbank Accession Nos. S83325, NM032466, NM032468, 004318, NM032467, and NM020164) and assayed for the ability to bind to, but not hydroxylate, HAAH substrate.

[00168] In another embodiment, a fragment of an HAAH substrate which can bind to HAAH, especially the substrate binding domain of HAAH, is used in the methods of the invention to inhibit binding of HAAH to its endogenous substrate. Preferably, the HAAH substrate fragment comprises an EGF-like domain. In a specific embodiment, a fusion protein comprises a fragment of an HAAH substrate which can bind to HAAH and prevent HAH from binding to its endogenous substrate (*e.g.*, the HAAH substrate fragment fused to the immunoglobulin heavy chain, see, *e.g.*, Carles-Kinch et al., 2002, Cancer Res. 62:2840-7). In another specific embodiment, the fragment is not part of a fusion protein. Fragments of HAAH substrates can be made (*e.g.*, based on the EGF-like domain consensus sequence $CX_7CX_4CX_{10}CXCX_8C$) and assayed for the ability to bind to bind HAAH and inhibit endogenous substrate hydroxylation.

[00169] Any method known in the art to detect binging between proteins may be used including, but not limited to, affinity chromatography, size exclusion chromatography, electrophoretic mobility shift assay. Polypeptide agents that are HAAH fragments or

enzymatically inactive HAAH include polypeptides that are 100%, 98%, 95%, 90%, 85%, 80%, 75%, 70%, 65%, 60%, 55%, 50%, 45%, 40% identical to endogenous HAAH sequences. Polypeptide agents that are HAAH substrate fragments include polypeptides that are 100%, 98%, 95%, 90%, 85%, 80%, 75%, 70%, 65%, 60%, 55%, 50%, 45%, 40% identical to endogenous HAAH substrate sequences. The determination of percent identity of two amino acid sequences can be determined by any method known to one skilled in the art, including BLAST protein searches.

3.1.5. Modified Polypeptide Agents

[00170] The polypeptide agents used in the methods of the invention (*e.g.*, antibodies, EphA2 polypeptide ligands, PCDGF receptor binding mimetics, soluble PCDGF receptor, dominant negative HAAH, or HAAH substrate mimetics) include derivatives that are modified, *i.e*, by the covalent attachment of any type of molecule to the polypeptide agent such that covalent attachment does not substantially alter the binding properties of the polypeptide agent. For example, but not by way of limitation, the polypeptide agent derivatives include polypeptide agents that have been modified, *e.g.*, by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, or therapeutic/detection moiety, etc. Any of numerous chemical modifications may be carried out by known techniques, including, but not limited to, specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, etc. Additionally, the derivative may contain one or more non-classical amino acids.

[00171] The methods of the present invention also encompass the use of polypeptide agents or fragments thereof that have half-lives (*e.g.*, serum half-lives) in a mammal, preferably a human, of greater than 15 days, preferably greater than 20 days, greater than 25 days, greater than 30 days, greater than 35 days, greater than 40 days, greater than 45 days, greater than 2 months, greater than 3 months, greater than 4 months, or greater than 5 months. The increased half-lives of the polypeptide agents in mammals, preferably humans, result in higher serum concentration of said polypeptide agents in the mammals, and thus, reduces the frequency of the administration of said polypeptide agents and/or reduces the amount of said polypeptide agents to be administered. Polypeptide agents having increased *in vivo* half-lives can be generated by techniques known to those of skill in the art. For example, antibody polypeptide agents with increased *in vivo* half-lives can be generated by modifying (*e.g.*, substituting, deleting or adding) amino acid residues

identified as involved in the interaction between the Fc domain and the FcRn receptor (see, *e.g.*, International Patent Publication No. WO 97/34631 and U.S. Patent Application No. 10/020,354 filed December 12, 2001 entitled "Molecules With Extended Half-Lives, Compositions and Uses Thereof," which are incorporated by reference in their entireties herein). Polypeptide agents with increased *in vivo* half-lives can be generated by attaching to said polypeptide agonistic agents polymer molecules such as high molecular weight polyethyleneglycol (PEG). PEG can be attached to said polypeptide agents with or without a multifunctional linker either through site-specific conjugation of the PEG to the N- or C-terminus of said polypeptide agonistic agents or via epsilon-amino groups present on lysine residues. Linear or branched polymer derivatization that results in minimal loss of biological activity will be used. The degree of conjugation will be closely monitored by SDS-PAGE and mass spectrometry to ensure proper conjugation of PEG molecules to the polypeptide agents. Unreacted PEG can be separated from polypeptide agent-PEG conjugates by, *e.g.*, size exclusion or ion-exchange chromatography.

[00172] The methods of the present invention also encompass the use of polypeptide agents or fragments thereof that are conjugated to a therapeutic or detection moiety (see Section 5.4).

3.1.6. Polynucleotides encoding Polypeptide Agents

[00173] Polynucleotides that encode polypeptide agents are meant to encompass polynucleotides that encode the polypeptide agents described in Sections 5.1.1, 5.1.2, 5.1.3, 5.1.4, and 5.1.5 as well as polynucleotides that hybridize to polynucleotides which encode polypeptides agents described in Sections 5.1.1, 5.1.2, 5.1.3, 5.1.4, and 5.1.5. Conditions for hybridization can be high stringency, intermediate stringency, or lower stringency. For example, conditions for stringent hybridization include, but are not limited to, hybridization to filter-bound DNA in 6X sodium chloride/sodium citrate (SSC) at about 45°C followed by one or more washes in 0.2X SSC/0.1% SDS at about 50-65°C, highly stringent conditions such as hybridization to filter-bound DNA in 6X SSC at about 45°C followed by one or more washes in 0.1X SSC/0.2% SDS at about 60°C, or any other stringent hybridization conditions known to those skilled in the art (see, for example, Ausubel, F.M. et al., eds. 1989 Current Protocols in Molecular Biology, vol. 1, Green Publishing Associates, Inc. and John Wiley and Sons, Inc., NY at pages 6.3.1 to 6.3.6 and 2.10.3).

[00174] The polynucleotides encoding polypeptide agents for use in the methods of the invention may be obtained, and the nucleotide sequence of the polynucleotides

determined, by any method known in the art. Such a polynucleotide encoding a polypeptide agent may be assembled from chemically synthesized oligonucleotides (*e.g.*, as described in Kutmeier et al., 1994, *BioTechniques* 17:242), which, briefly, involves the synthesis of overlapping oligonucleotides containing portions of the sequence encoding the polypeptide, annealing and ligating of those oligonucleotides, and then amplification of the ligated oligonucleotides by PCR. Alternatively, a polynucleotide encoding a polypeptide agent may be generated from nucleic acid from a suitable source. If a clone containing a nucleic acid encoding a particular polypeptide is not available, but the sequence of the polypeptide is known, a nucleic acid encoding the polypeptide may be chemically synthesized or obtained from a suitable source (*e.g.*, an antibody cDNA library, or a cDNA library generated from, or nucleic acid, preferably poly A+ RNA, isolated from, any tissue or cells expressing the polypeptide of interest, such as hybridoma cells selected to express an antibody, or cells expressing EphA2, an EphA2 ligand, PCDGF, PCDGF receptor, HAAH, or HAAH substrate) by PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of the sequence or by cloning using an oligonucleotide probe specific for the particular sequence to identify, *e.g.*, a cDNA clone from a cDNA library that encodes the polypeptide of interest. Amplified nucleic acids generated by PCR may then be cloned into replicable cloning vectors using any method well known in the art.

[00175] Once the nucleotide sequence of the polypeptide agent used in the methods of the invention is determined, the nucleotide sequence may be manipulated using methods well known in the art for the manipulation of nucleotide sequences, *e.g.*, recombinant DNA techniques, site directed mutagenesis, PCR, etc. (see, for example, the techniques described in Sambrook et al., 1990, Molecular Cloning, A Laboratory Manual, 2d Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY and Ausubel et al., eds., 1998, Current Protocols in Molecular Biology, John Wiley & Sons, NY, which are both incorporated by reference in their entireties herein) to generate polypeptides having a different amino acid sequence, for example to create amino acid substitutions, deletions, and/or insertions.

[00176] Standard techniques known to those skilled in the art can be used to introduce mutations in the nucleotide sequence encoding a polypeptide agent, or fragment thereof, including, *e.g.*, site-directed mutagenesis and PCR-mediated mutagenesis, which results in amino acid substitutions. Preferably, the derivatives include less than 15 amino acid substitutions, less than 10 amino acid substitutions, less than 5 amino acid substitutions, less than 4 amino acid substitutions, less than 3 amino acid substitutions, or less than 2 amino acid substitutions relative to the original polypeptide agent or fragment

thereof. In a preferred embodiment, the derivatives have conservative amino acid substitutions made at one or more predicted non-essential amino acid residues. In embodiments where the polypeptide agent is an antibody or fragment thereof, the amino acid sequence may be mutated (*e.g.*, one or more amino acid substitutions) in the framework or variable regions. Preferably, mutations in these antibodies maintain or enhance the avidity and/or affinity of the antibodies for the particular antigen to which they immunospecifically bind. Standard techniques known to those skilled in the art (*e.g.*, immunoassays or ELISA assays) can be used to assay the degree of binding between a mutated polypeptide agent and its binding partner.

3.1.7. Recombinant Production of Polypeptide Agents

[00177] Recombinant expression of a polypeptide agent (including, but not limited to derivatives, analogs or fragments thereof) requires construction of an expression vector containing a polynucleotide that encodes the polypeptide. Once a polynucleotide encoding a polypeptide agent has been obtained, a vector for the production of the polypeptide agent may be produced by recombinant DNA technology using techniques well known in the art. Methods which are well known to those skilled in the art can be used to construct expression vectors containing polypeptide coding sequences and appropriate transcriptional and translational control signals. Thus, methods for preparing a protein by expressing a polynucleotide containing are described herein. These methods include, for example, *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. The invention, thus, provides replicable vectors comprising a nucleotide sequence encoding an EphA2, PCDGF, HAAH polypeptide agent operably linked to a promoter. In embodiments where the polypeptide agent is an antibody, such vectors may include the nucleotide sequence encoding the constant region of the antibody molecule (see, *e.g.*, International Publication Nos. WO 86/05807 and WO 89/01036; and U.S. Patent No. 5,122,464) and the variable domain of the antibody may be cloned into such a vector for expression of the entire heavy, the entire light chain, or both the entire heavy and light chains.

[00178] The expression vector is transferred to a host cell by conventional techniques and the transfected cells are then cultured by conventional techniques to produce a polypeptide agent. Thus, the invention includes host cells containing a polynucleotide encoding a polypeptide agent or fragments thereof operably linked to a heterologous promoter.

[00179] A variety of host-expression vector systems may be utilized to express polypeptide agents (see, *e.g.*, U.S. Patent No. 5,807,715). Such host-expression systems represent vehicles by which the coding sequences of interest may be produced and subsequently purified, but also represent cells which may, when transformed or transfected with the appropriate nucleotide coding sequences, express a polypeptide agent of the invention *in situ*. These include but are not limited to microorganisms such as bacteria (*e.g.*, *E. coli* and *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing antibody coding sequences; yeast (*e.g.*, *Saccharomyces Pichia*) transformed with recombinant yeast expression vectors containing antibody coding sequences; insect cell systems infected with recombinant virus expression vectors (*e.g.*, baculovirus) containing polypeptide agonistic agent coding sequences; plant cell systems infected with recombinant virus expression vectors (*e.g.*, cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (*e.g.*, Ti plasmid) containing antibody coding sequences; or mammalian cell systems (*e.g.*, COS, CHO, BHK, 293, NS0, and 3T3 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (*e.g.*, metallothionein promoter) or from mammalian viruses (*e.g.*, the adenovirus late promoter; the vaccinia virus 7.5K promoter). Preferably, bacterial cells such as *Escherichia coli*, and more preferably, eukaryotic cells, especially for the expression of a whole recombinant polypeptide agent, are used for the expression of a polypeptide agent. For example, mammalian cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus is an effective expression system for polypeptide agents, especially antibody polypeptide agents (Foecking et al., 1986, *Gene* 45:101; and Cockett et al., 1990, *BioTechnology* 8:2). In a specific embodiment, the expression of nucleotide sequences encoding a polypeptide agent is regulated by a constitutive promoter, inducible promoter or tissue specific promoter.

[00180] In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the polypeptide agent being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of pharmaceutical compositions, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited to, the *E. coli* expression vector pUR278 (Ruther et al., 1983, *EMBO* 12:1791), in which the antibody coding sequence may be ligated individually into the vector in frame

with the lac Z coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, 1985, *Nucleic Acids Res.* 13:3101-3109; Van Heeke & Schuster, 1989, *J. Biol. Chem.* 24:5503-5509); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione 5-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption and binding to matrix glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

[00181] In an insect system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The polypeptide agent coding sequence may be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter).

[00182] In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the polypeptide coding sequence of interest may be ligated to an adenovirus transcription/translation control complex, *e.g.*, the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by *in vitro* or *in vivo* recombination. Insertion in a non-essential region of the viral genome (*e.g.*, region El or E3) will result in a recombinant virus that is viable and capable of expressing the polypeptide agonistic agent in infected hosts (*e.g.*, see Logan & Shenk, 1984, *PNAS* 8 1:355-359). Specific initiation signals may also be required for efficient translation of inserted polypeptide coding sequences. These signals include the ATG initiation codon and adjacent sequences. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see, *e.g.*, Bittner et al., 1987, *Methods in Enzymol.* 153:516-544).

[00183] In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (*e.g.*, glycosylation) and processing (*e.g.*, cleavage) of protein products may be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and

modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include but are not limited to CHO, VERO, BHK, HeLa, COS, MDCK, 293, 3T3, W138, BT483, Hs578T, HTB2, BT2O, T47D, NS1, NS0, CRL7O3O HsS78Bst cells.

[00184] For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express the polypeptide agent molecule may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (*e.g.*, promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines which express the polypeptide agent. Such engineered cell lines may be particularly useful in screening and evaluation of compositions that interact directly or indirectly with the polypeptide agent.

[00185] A number of selection systems may be used, including but not limited to, the herpes simplex virus thymidine kinase (Wigler et al., 1977, *Cell* 11:223), glutamine synthetase, hypoxanthine guanine phosphoribosyltransferase (Szybalska & Szybalski, 1992, Proc. Natl. Acad. Sci. USA 48:202), and adenine phosphoribosyltransferase (Lowy et al., 1980, *Cell* 22:8-17) genes can be employed in tk-, gs-, hgprt- or aprt- cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes: *dhfr*, which confers resistance to methotrexate (Wigler et al., 1980, *PNAS* 77:357; O'Hare et al., 1981, *PNAS* 78:1527); *gpt*, which confers resistance to mycophenolic acid (Mulligan & Berg, 1981, *PNAS* 78:2072); neo, which confers resistance to the aminoglycoside G-418 (Wu and Wu, 1991, *Biotherapy* 3:87; Tolstoshev, 1993, *Ann. Rev. Pharmacol. Toxicol.* 32:573; Mulligan, 1993, *Science* 260:926; and Morgan and Anderson, 1993, *Ann. Rev. Biochem.* 62: 191; May, 1993, *TIB TECH* 11:155-); and *hygro*, which confers resistance to hygromycin (Santerre et al., 1984, *Gene* 30:147). Methods commonly known in the art of

recombinant DNA technology may be routinely applied to select the desired recombinant clone, and such methods are described, for example, in Ausubel et al. (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, NY (1993); Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990); and in Chapters 12 and 13, Dracopoli et al. (eds), Current Protocols in Human Genetics, John Wiley & Sons, NY (1994); Colberre-Garapin et al., 1981, *J. Mol. Biol.* 150:1, which are incorporated by reference in their entireties herein.

[00186] The expression levels of a polypeptide agent can be increased by vector amplification (for a review, see Bebbington and Hentschel). The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells in DNA cloning, Vol.3. (Academic Press, New York, 1987)). When a marker in the vector system expressing polypeptide agent is amplifiable, increase in the level of inhibitor present in culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the nucleic acid sequence encoding the polypeptide agent, production of the polypeptide agent will also increase (Crouse et al., 1983, *Mol. Cell. Biol.* 3:257).

[00187] In embodiments where the polypeptide agent is an antibody, the host cell may be co-transfected with two expression vectors, the first vector encoding a heavy chain derived polypeptide and the second vector encoding a light chain derived polypeptide. The two vectors may contain identical selectable markers which enable equal expression of heavy and light chain polypeptides. Alternatively, a single vector may be used which encodes, and is capable of expressing, both heavy and light chain polypeptides. In such situations, the light chain should be placed before the heavy chain to avoid an excess of toxic free heavy chain (Proudfoot, 1986, *Nature* 322:52; and Kohler, 1980, *PNAS* 77:2197). The coding sequences for the heavy and light chains may comprise cDNA or genomic DNA. In some embodiments, the variable domain of an EphA2, PCDGF, PCDGF receptor, or HAAH antibody that is a polypeptide agent or portion thereof is cloned into vectors already containing the nucleotide sequence encoding the constant region of the antibody molecule (see, *e.g.*, US Patent Nos. 5,919,900; 5,747,296; 5,789,178; 5,591,639; 5,658,759; 5,849,522; 5,122,464; 5,770,359; 5,827,739; International Patent Publication Nos. WO 89/01036; WO 89/10404; Bebbington et al., 1992, *BioTechnology* 10:169).

[00188] Once a polypeptide agent has been produced by recombinant expression, it may be purified by any method known in the art for purification of a polypeptide, for example, by chromatography (*e.g.*, ion exchange, affinity, and sizing column

chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. Further, the polypeptide agents may be fused to heterologous polypeptide sequences described herein or otherwise known in the art to facilitate purification.

3.2. Polynucleotide Agents

In addition polypeptide agents, nucleic acid molecules can be used in methods of the invention. Nucleic acid molecules including, but not limited to, antisense, ribozymes, and dsRNA for mediating RNA interference can be used to decrease EphA2, PCDGF, PCDGF receptor and/or HAAH expression. Nucleotide agents can be administered to a patient according to methods described in Section 5.9.1.

3.2.1. Antisense

[00189] The present invention encompasses antisense nucleic acid molecules (*i.e.*, molecules which are complementary to all or part of a sense nucleic acid encoding a polypeptide of interest *e.g.*, complementary to the coding strand of a double-stranded cDNA molecule or complementary to an mRNA sequence) for use in the methods of the present invention. Accordingly, an antisense nucleic acid can hydrogen bond to a sense nucleic acid. The antisense nucleic acid can be complementary to an entire coding strand, or to only a portion thereof, *e.g.*, all or part of the protein coding region (or open reading frame). An antisense nucleic acid molecule can be antisense to all or part of a non-coding region of the coding strand of a nucleotide sequence encoding an EphA2, PCDGF, PCDGF receptor, or HAAH polypeptide. The non-coding regions ("5' and 3' untranslated regions") are the 5' and 3' sequences which flank the coding region and are not translated into amino acids.

[00190] In one embodiment, the antisense nucleic acid molecule is directed to EphA2 (see *e.g.*, Genbank Accession Nos. NM004431 and BC037166, nucleic and amino acid sequences of EphA2 are incorporated by reference in their entireties herein). In a specific embodiment, the EphA2 antisense molecule is

5'-CCA GCA GTA CCG CTT CCT TGC CCT GCG GCC G-3' (SEQ ID NO:91).

[00191] In another embodiment, the antisense molecule is directed to PCDGF (see *e.g.*, GenBank Accession Nos. AY124489, NM002087, and M75161). In a specific embodiment, the PCDGF antisense molecule is

5'-GGG TCC ACA TGG TCT GCC TGC-3' (SEQ ID NO:92) or

5'-GCC ACC AGC CCT GCT GTT AAG GCC-3' (SEQ ID NO:93).

[00192] In another embodiment, the antisense molecule is directed to a PCDGF receptor. In a specific embodiment, the PCDGF receptor is Rse (see *e.g.*, Genbank Accession Nos. BC051756, BC049368, and NM006293).

[00193] In another embodiment, the antisense molecule is directed to HAAH (see *e.g.*, GenBank Accession Nos. S83325, NM032466, NM032468, 004318, NM032467, and NM020164). In a specific embodiment, the HAAH antisense molecule is

5'-CAT TCT TAC GCT GGG CCA TT-3' (SEQ ID NO:94) or

5'-TTA CGC TGG GCC ATT GCA CG-3' (SEQ ID NO:95) or

5'-CTG GGC CAT TGC ACG GTC CG-3' (SEQ ID NO:96).

[00194] An antisense oligonucleotide can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 nucleotides in length. An antisense nucleic acid for use in the methods of the invention can be constructed using chemical synthesis and enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (*e.g.*, an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, *e.g.*, phosphorothioate derivatives and acridine substituted nucleotides can be used. Examples of modified nucleotides which can be used to generate the antisense nucleic acid include 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, β-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, β-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine. Alternatively, the antisense nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (*i.e.*, RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest, *i.e.*, EphA2, PCDGF, PCDGF receptor or HAAH).

[00195] The antisense nucleic acid molecules for use in the methods of the invention are typically administered to a subject or generated *in situ* such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding a selected EphA2, PCDGF, PCDGF receptor, or HAAH polypeptide to thereby inhibit expression, *e.g.*, by inhibiting transcription and/or translation. The hybridization can be by conventional nucleotide complementarity to form a stable duplex, or, for example, in the case of an antisense nucleic acid molecule which binds to DNA duplexes, through specific interactions in the major groove of the double helix. An example of a route of administration of antisense nucleic acid molecules includes direct injection at a tissue site. Alternatively, antisense nucleic acid molecules can be modified to target selected cells and then administered systemically. For example, for systemic administration, antisense molecules can be modified such that they specifically bind to receptors or antigens expressed on a selected cell surface, *e.g.*, by linking the antisense nucleic acid molecules to peptides or antibodies which bind to cell surface receptors or antigens. The antisense nucleic acid molecules can also be delivered to cells using the vectors described herein. To achieve sufficient intracellular concentrations of the antisense molecules, vector constructs in which the antisense nucleic acid molecule is placed under the control of a strong pol II or pol III promoter are preferred.

[00196] An antisense nucleic acid molecule of the invention can be an α-anomeric nucleic acid molecule. An α-anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β-units, the strands run parallel to each other (Gaultier et al., 1987, *Nucleic Acids Res.* 15:6625). The antisense nucleic acid molecule can also comprise a 2'-o-methylribonucleotide (Inoue et al., 1987, *Nucleic Acids Res.* 15:6131) or a chimeric RNA-DNA analogue (Inoue et al., 1987, *FEBS Lett.* 215:327).

3.2.2. Ribozymes

[00197] The invention also encompasses the use of ribozymes in the methods of the invention. Ribozymes are catalytic RNA molecules with ribonuclease activity which are capable of cleaving a single-stranded nucleic acid, such as an mRNA, to which they have a complementary region. Thus, ribozymes (*e.g.*, hammerhead ribozymes; described in Haselhoff and Gerlach, 1988, *Nature* 334:585-591) can be used to catalytically cleave mRNA transcripts to thereby inhibit translation of the protein encoded by the mRNA. A ribozyme having specificity for a nucleic acid molecule encoding EphA2, PCDGF, PCDGF receptor, or HAAH can be designed based upon the nucleotide sequence of EphA2,

PCDGF, PCDGF receptor, or HAAH. For example, a derivative of a *Tetrahymena* L-19 IVS RNA can be constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in U.S. Patent Nos. 4,987,071 and 5,116,742. Alternatively, an mRNA encoding a polypeptide of interest can be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. *See, e.g.*, Bartel and Szostak, 1993, *Science* 261:1411.

3.2.3. RNA Interference

[00198] In certain embodiments, an RNA interference (RNAi) molecule is used to decrease EphA2, PCDGF, PCDGF receptor or HAAH expression. RNA interference (RNAi) is the ability of double-stranded RNA (dsRNA) to suppress the expression of a gene corresponding to its own sequence (see, *e.g.*, Cogoni and Macino, 2000, *Genes Dev* 10: 638-643, Guru, 2000, *Nature* 404, 804-808, Hammond et al., 2001, *Nature Rev Gen* 2: 110-119, Shi, 2003, *Trends Genet.* 19:9-12, US Patent 6,506,559, each incorporated by reference in their entireties herein). RNAi is also called post-transcriptional gene silencing or PTGS. Since the only RNA molecules normally found in the cytoplasm of a cell are molecules of single-stranded mRNA, the cell has enzymes that recognize and cut dsRNA into fragments containing 21-25 base pairs (approximately two turns of a double helix). The antisense strand of the fragment separates enough from the sense strand so that it hybridizes with the complementary sense sequence on a molecule of endogenous cellular mRNA. This hybridization triggers cutting of the mRNA in the double-stranded region, thus destroying its ability to be translated into a polypeptide. Introducing dsRNA corresponding to a particular gene thus knocks out the cell's own expression of that gene in particular tissues and/or at a chosen time.

[00199] The current models of the RNAi mechanism includes both initiation and effector steps (Hutvagner and Zamore, 2002, *Curr Opin Genetics & Development* 12:225-32; Hammond et al., 2001, *Nature Rev Gen* 2: 110-9, each incorporated by reference in their entireties herein). In the initiation step, input dsRNA is digested into 21-23 nucleotide small interfering RNAs (siRNAs), which have also been called "guide RNAs" (Sharp, 2001, *Genes Dev* 15: 485-490). Evidence indicates that siRNAs are produced when the enzyme Dicer, a member of the RNase III family of dsRNA-specific ribonucleases, processively cleaves dsRNA (introduced directly or via a transgene or virus) in an ATP-dependent, processive manner. Successive cleavage events degrade the RNA to 19-21 base pair duplexes (siRNAs), each with 2-nucleotide 3' overhangs (Bernstein et al., 2001, *Nature*

409:363-366; Hutvagner and Zamore, 2002, *Curr Opin Genetics & Development* 12:225-232). In the effector step, the siRNA duplexes bind to a nuclease complex to form what is known as the RNA-induced silencing complex, or RISC. An ATP-depending unwinding of the siRNA duplex is required for activation of the RISC. The active RISC then targets the homologous transcript by base pairing interactions and cleaves the mRNA ~12 nucleotides from the 3' terminus of the siRNA. Although the mechanism of cleavage is at this date unclear, research indicates that each RISC contains a single siRNA and an RNase that appears to be distinct from Dicer (Hutvagner and Zamore, 2002, *Curr Opin Genetics & Development* 12:225-232). Because of the remarkable potency of RNAi in some organisms, an amplification step within the RNAi pathway has also been proposed. Amplification could occur by copying of the input dsRNAs, which would generate more siRNAs, or by replication of the siRNAs themselves. Alternatively or in addition, amplification could be effected by multiple turnover events of the RISC.

[00200] Elbashir and colleagues (Elbashir et al., 2001, *Nature* 411:494-498; Elbashir et al., 2001, *EMBO* 20:6877-6888) have suggested a procedure for designing siRNAs for inducing RNAi in mammalian cells. Briefly, find a 21 nucleotide sequence in the mRNA of interest that begins with an adenine-adenine (AA) dinucleotide as a potential siRNA target site. This strategy for choosing siRNA target sites is based on the observation that siRNAs with 3' overhanging UU dinucleotides are the most effective. This is also compatible with using RNA pol III to transcribe hairpin siRNAs because RNA pol III terminates transcription at 4-6 nucleotide poly(T) tracts creating RNA molecules with a short poly(U) tail. Although siRNAs with other 3' terminal dinucleotide overhangs have been shown to effectively induce RNAi, siRNAs with guanine residues in the overhang are not recommended because of the potential for the siRNA to be cleaved by RNase at single-stranded guanine residues. In addition to beginning with an AA dinucleotide, the siRNA target site should have a guanosine and cytidine residue percentage within the range of 30-70%. The chosen siRNA target sequence should then be subjected to a BLAST search against the EST database to ensure that only the desired gene is targeted. Various products are commercially available to aid in the preparation and use of siRNA (*e.g.*, Ambion, Inc., Austin, Texas).

[00201] Double-stranded (ds) RNA can be used to interfere with gene expression in mammals (Brummelkamp et al., *Science* 296:550-3, Krichevsky and Kosik, 2002, *PNAS* 99:11926-9, Paddison et al., 2002, *PNAS* 99:1443-8, Wianny & Zernicka-Goetz, 2000, *Nature Cell Biology* 2:70-75, European Patent 1144623, International Patent Publication

Nos. WO 02/055693, WO 02/44321, WO 03/006,477; each incorporated by reference in their entireties herein). dsRNA is used as inhibitory RNA or RNAi of the function of EphA2, PCDGF, or HAAH to produce a phenotype that is the same as that of a null mutant of EphA2, PCDGF, PCDGF receptor, or HAAH.

3.3. Small Molecule Agents

In addition polypeptide agents and nucleic acid agents, small molecules can be used in methods of the invention. Small molecules can be an organic or inorganic compound that is usually less than 1000 daltons. Small molecule agents can be derived from any known organism (including, but not limited to, animals, plants, bacteria, fungi, and protista, or viruses) or from a library of synthetic molecules. Any known method known in the art can be used to isolate EphA2, PCDGF, or HAAH small molecule agents (see *e.g.*, Section 5.6).

[00202] Candidate small molecule agents encompass numerous chemical classes, though typically they are organic molecules, preferably small organic compounds having a molecular weight of less than about 2,500 daltons, with molecules preferably ranging from about 100 to about 1,000 daltons being preferred. Candidate small molecule agents comprise functional groups necessary for structural interaction with proteins, particularly hydrogen bonding, and typically include at least one of an amine, carbonyl, hydroxyl or carboxyl group, preferably at least two of the functional chemical groups. The candidate agents often comprise cyclical carbon or heterocyclic structures and/or aromatic or polyaromatic structures substituted with one or more of the above functional groups. Candidate agents are also found among biomolecules including peptides, saccharides, lipids, fatty acids, steroids, purines, pyrimidines, derivatives, structural analogs or combinations thereof.

[00203] Candidate small molecule agents are obtained from a wide variety of sources including libraries of synthetic or natural compounds. For example, numerous means are available for random and directed synthesis of a wide variety of organic compounds and biomolecules. Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant and animal extracts are available or readily produced. In addition, new libraries or species of candidate agents can be made by feeding precursor molecules (*e.g.* chemical scaffolds) to microorganisms (including bacteria, yeast, etc.) or other organisms (plants, actinomycetes, fungi, etc.) to generate new chemicals or difficult to artificially synthesize

chemicals/molecules. In a preferred embodiment, the candidate bioactive agents are organic chemical moieties, a wide variety of which are available in the literature.

[00204] In a preferred embodiment, a library of different candidate bioactive agents are used. Preferably, the library should provide a sufficiently structurally diverse population of randomized agents to effect a probabilistically sufficient range of diversity to allow binding to a particular polypeptide of interest. Accordingly, an interaction library should be large enough so that at least one of its members will have a structure that gives it affinity for the target.

[00205] Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt et al., 1993, *PNAS* 90:6909; Erb et al., 1994, *PNAS* 91:11422; Zuckermann et al., 1994, *J. Med. Chem.* 37:2678; Cho et al., 1993, *Science* 261:1303; Carrell et al., 1994, *Angew. Chem. Int. Ed. Engl.* 33:2059; Carell et al., 1994, *Angew. Chem. Int. Ed. Engl.* 33:2061; and Gallop et al., 1994, *J. Med. Chem.* 37:1233, each of which is incorporated in their entireties by reference herein.

[00206] Libraries of compounds may be presented, *e.g.*, presented in solution (*e.g.*, Houghten, 1992, *BioTechniques* 13:412-421), or on beads (Lam, 1991, *Nature* 354:82-84), chips (Fodor, 1993, *Nature* 364:555-556), bacteria (U.S. Patent No. 5,223,409), spores (Patent Nos. 5,571,698; 5,403,484; and 5,223,409), plasmids (Cull et al., 1992, *PNAS* 89:1865-1869) or phage (Scott and Smith, 1990, *Science* 249:386-390; Devlin, 1990, Science 249:404-406; Cwirla et al., 1990, *PNAS* 87:6378-6382; and Felici, 1991, *J. Mol. Biol.* 222:301-310), each of which is incorporated herein in its entirety by reference.

[00207] As will be appreciated by those in the art, there are a wide variety of possible small molecules that can be uses in the methods of the invention. As will be appreciated by those in the art, there are a wide variety of delivery methods available, including the use of vesicles and other vehicles such as liposomes, organic solutions, dispersions, suspensions, electroporation, etc. (see e.g., Section 5.8).

3.4. Targeting Of Therapeutics

[00208] The present invention encompasses the use of a targeting moiety (*e.g.*, antibody) to specifically target therapeutic agents to cells involved in the hyperproliferative disorder to be treated (*e.g.*, cancer cells). Such therapeutic agents are recombinantly fused or chemically conjugated (including both covalent and non-covalent conjugations) to a targeting moiety such as, but not limited to, antibodies or antigen binding fragments thereof. Conjugated targeting moieties can be used to target therapeutic agents to particular cell

types associated with the disorder to be treated. Such targeting can improve the efficacy by increasing the concentration of targeted agent at the desired site. Also, toxicity or side effects of treatment can be minimized by reducing systemic exposure to the agent.

[00209] A conjugated agent's relative efficacy in comparison to the free agent can depend on a number of factors. For example, rate of uptake of the antibody-agent into the cell (*e.g.*, by endocytosis), rate/efficiency of release of the agent from the antibody, rate of export of the agent from the cell, etc. can all effect the action of the agent. Antibodies used for targeted delivery of agents can be assayed for the ability to be endocytosed by the relevant cell type (*i.e.*, the cell type associated with the disorder to be treated) by any method known in the art. Additionally, the type of linkage used to conjugate an agent to an antibody should be assayed by any method known in the art such that the agent action within the target cell is not impeded.

[00210] In some embodiments, antibodies can be fused or conjugated to liposomes, wherein the liposomes are used to encapsulate therapeutic agents (see *e.g.*, Park et al., 1997, *Can. Lett.* 118:153-160; Lopes de Menezes et al., 1998, *Can. Res.* 58:3320-30; Tseng et al., 1999, *Int. J. Can.* 80:723-30; Crosasso et al., 1997, *J. Pharm. Sci.* 86:832-9). In a preferred embodiment, the pharmokinetics and clearance of liposomes are improved by incorporating lipid derivatives of PEG into liposome formulations (see *e.g.*, Allen et al., 1991, *Biochem Biophys Acta* 1068:133-41; Huwyler et al., 1997, *J. Pharmacol. Exp. Ther.* 282:1541-6).

[00211] Therapeutic agents can be conjugated to antibodies by any method known in the art, including, but not limited to aldehyde/Schiff linkage, sulphydryl linkage, acid-labile linkage, cis-aconityl linkage, hydrazone linkage, enzymatically degradable linkage (see generally Garnett, 2002, *Adv. Drug Deliv. Rev.* 53:171-216). Additional techniques for conjugating therapeutic moieties to antibodies are well known, see, *e.g.*, Arnon et al., "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in Monoclonal Antibodies And Cancer Therapy, Reisfeld et al. (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom et al., "Antibodies For Drug Delivery", in Controlled Drug Delivery (2nd Ed.), Robinson et al. (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in Monoclonal Antibodies '84: Biological And Clinical Applications, Pinchera et al. (eds.), pp. 475-506 *(1985);* "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin et al. (eds.), pp. 303-16 (Academic Press 1985), and Thorpe et al., 1982, *Immunol. Rev.* 62:119-58. Methods for fusing or conjugating

antibodies to polypeptide agents are known in the art. See, *e.g.*, U.S. Patent Nos. 5,336,603, 5,622,929, 5,359,046, 5,349,053, 5,447,851, and 5,112,946; EP 307,434; EP 367,166; International Publication Nos. WO 96/04388 and WO 91/06570; Ashkenazi et al., 1991, *PNAS* 88: 10535-10539; Zheng et al., 1995, *J. Immunol.* 154:5590-5600; and Vil et al., 1992, *PNAS* 89:11337- 11341. The fusion of an antibody to a agent does not necessarily need to be direct, but may occur through linker sequences. Such linker molecules are commonly known in the art and described in Denardo et al., 1998, *Clin Cancer Res.* 4:2483-90; Peterson et al., 1999, *Bioconjug. Chem.* 10:553; Zimmerman et al., 1999, *Nucl. Med. Biol.* 26:943-50; Garnett, 2002, *Adv. Drug Deliv. Rev.* 53:171-216.

[00212] In other embodiments, antibody properties can be altered as desired (*e.g.*, antibodies or antigen binding fragments thereof with higher affinities and lower dissociation rates) through the techniques of gene-shuffling, motif-shuffling, exon-shuffling, and/or codon-shuffling (collectively referred to as "DNA shuffling"). See, generally, U.S. Patent Nos. 5,605,793; 5,811,238; 5,830,721; 5,834,252; and 5,837,458, and Patten et al., 1997, *Curr. Opinion Biotechnol.* 8:724-33; Harayama, 1998, *Trends Biotechnol.* 16:76; Hansson, et al., 1999, *J. Mol. Biol.* 287:265; and Lorenzo and Blasco, 1998, *BioTechniques* 24:308. Antibodies or antigen binding fragments thereof, or the encoded antibodies or antigen binding fragments thereof, may be altered by being subjected to random mutagenesis by error-prone PCR, random nucleotide insertion or other methods prior to recombination. One or more portions of a polynucleotide encoding an antibody or antibody fragment, which portions immunospecifically bind to an antigen expressed on a cell associated with a particular disorder may be recombined with one or more components, motifs, sections, parts, domains, fragments, etc. of one or more heterologous molecules.

[00213] In other embodiments, the antibodies or antigen binding fragments thereof can be fused to marker sequences, such as a peptide, to facilitate purification. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., Chatsworth, CA), among others, many of which are commercially available (see *e.g.*, Gentz et al., 1989, *PNAS* 86:821) Other peptide tags useful for purification include, but are not limited to, the hemagglutinin (HA) tag, which corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson et al., 1984, *Cell* 37:767) and the "flag" tag. Any purification method known in the art can be used (see *e.g.*, International Publication WO 93/21232; EP 439,095; Naramura et al., 1994, *Immunol. Lett.* 39:91-99; U.S. Patent 5,474,981; Gillies et al., 1992, *PNAS* 89:1428-1432; and Fell et al., 1991, *J. Immunol.* 146:2446-2452).

[00214] In one embodiment, the antibody used to target the therapeutic is an EphA2 antibody, a PCDGF antibody, a PCDGF receptor antibody, or an HAAH antibody. Malignant cancer cells overexpress EphA2, PCDGF, PCDGF receptor and/or HAAH polypeptides and thus are good candidates to use to target therapeutic agents to cancer cells rather than non-cancer cells. When HAAH is overexpressed (as in malignant cancer cells), the polypeptide is expressed on the cell surface in addition to intracellularly. An antibody or antigen binding fragment thereof may be conjugated to a therapeutic moiety such as a cytotoxin, *e.g.*, a cytostatic or cytocidal agent, a therapeutic agent or a radioactive metal ion, *e.g.*, alpha-emitters. A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include paclitaxel, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, puromycin, epirubicin, and cyclophosphamide and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (*e.g.*, methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (*e.g.*, mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BCNU) and lomustine (CCNU), cyclothosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cisdichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (*e.g.*, daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (*e.g.*, dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (*e.g.*, vincristine and vinblastine).

[00215] Further, an EphA2 antibody, a PCDGF antibody, PCDGF receptor antibody, or an HAAH antibody or antigen binding fragment thereof may be conjugated to a therapeutic agent or drug moiety that modifies a given biological response. Therapeutic agents or drug moieties are not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a molecule (*e.g.*, protein, polypeptide, nucleic acid, etc.) possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, cholera toxin, or diphtheria toxin; a protein such as tumor necrosis factor, α-interferon, β-interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator, an apoptotic agent, *e.g.*, TNF-α, TNF-β, AIM I (see, International Publication No. WO 97/33899), AIM II (see, International Publication No. WO 97/34911), Fas Ligand (Takahashi et al., 1994, *J. Iminunol.*, 6:1567), and VEGI (see, International Publication No. WO 99/23105), a

thrombotic agent or an anti-angiogenic agent, *e.g.*, angiostatin or endostatin; or, a biological response modifier such as, for example, a lymphokine (*e.g.*, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophage colony stimulating factor ("GM-CSF"), and granulocyte colony stimulating factor ("G-CSF")), or a growth factor (*e.g.*, growth hormone ("GH")). In other embodiments, the moiety possessing a desired biological activity is an EphA2 agent, a PCDGF agent, and/or an HAAH agent.

[00216] Moreover, an antibody can be conjugated to therapeutic moieties such as a radioactive materials or macrocyclic chelators useful for conjugating radiometal ions (see above for examples of radioactive materials). In certain embodiments, the macrocyclic chelator is 1,4,7,10-tetraazacyclododecane-N,N',N'',N''-tetraacetic acid (DOTA) which can be attached to the antibody via a linker molecule. Such linker molecules are commonly known in the art and described in Denardo et al., 1998, *Clin Cancer Res.* 4:2483-90; Peterson et al., 1999, *Bioconjug. Chem.* 10:553; and Zimmerman et al., 1999, *Nucl. Med. Biol.* 26:943-50 each incorporated by reference in their entireties herein.

[00217] Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980.

[00218] In other embodiments, antibodies of the invention or fragments or variants thereof can be conjugated to a diagnostic or detectable agent. Such antibodies can be useful for monitoring or prognosing the development or progression of a cancer as part of a clinical testing procedure, such as determining the efficacy of a particular therapy. Such diagnosis and detection can accomplished by coupling the antibody to detectable substances including, but not limited to various enzymes, such as but not limited to horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; prosthetic groups, such as but not limited to streptavidin/biotin and avidin/biotin; fluorescent materials, such as but not limited to, umbelliferone, fluorescein, fluorescein isothiocynate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; luminescent materials, such as but not limited to, luminol; bioluminescent materials, such as but not limited to, luciferase, luciferin, and aequorin; radioactive materials, such as but not limited to, bismuth (^{213}Bi), carbon (^{14}C), chromium (^{51}Cr), cobalt (^{57}Co), fluorine (^{18}F), gadolinium (^{153}Gd, ^{159}Gd), gallium (^{68}Ga, ^{67}Ga), germanium (^{68}Ge), holmium (^{166}Ho), indium (^{115}In, ^{113}In, ^{112}In, ^{111}In), iodine (^{131}I, ^{125}I, ^{123}I, ^{121}I), lanthanium (^{140}La), lutetium (^{177}Lu), manganese (^{54}Mn), molybdenum (^{99}Mo), palladium (^{103}Pd), phosphorous (^{32}P), praseodymium (^{142}Pr), promethium (^{149}Pm), rhenium (^{186}Re, ^{188}Re), rhodium (^{105}Rh), ruthemium (^{97}Ru), samarium (^{153}Sm), scandium (^{47}Sc), selenium (^{75}Se), strontium (^{85}Sr),

sulfur (^{35}S), technetium (^{99}Tc), thallium (^{201}Ti), tin (^{113}Sn, ^{117}Sn), tritium (^{3}H), xenon (^{133}Xe), ytterbium (^{169}Yb, ^{175}Yb), yttrium (^{90}Y), zinc (^{65}Zn); positron emitting metals using various positron emission tomographies, and nonradioactive paramagnetic metal ions.

[00219] In another embodiment, EphA2 agents, PCDGF agents, or HAAH agents can be conjugated to an antibody that does not immunospecifically bind an EphA2, PCDGF, PCDGF receptor or HAAH polypeptide but targets cancer cells by immunospecifically binding to an epitope only expressed or overexpressed on cancer cells. Examples of such monoclonal antibodies that immunospecifically bind tumor-associated antigens expressed at a higher density on malignant cells relative to non-cancer cells can be found in the art, *e.g.*, listed in Table 5.

Table 5

Cancer	Antibody	Antigen	Reference
colorectal	MAb 17-1A	epithelial	Kufer et al, 1997, *Cancer Immunol Immunother* 45:193
breast	antiHER2 MAb	HER2	Pegram et al., 1998, *J. Clin. Oncol.* 16:2659
breast	MAb CT-M-01	polyepithelial mucin	Hinman et al., 1993, *Can. Res.* 53:3336
carcinomas of lung, breast, colon	BR96	Le^y-related tumor antigen	Trail et al., 1993, *Science* 261:212; Trail et al., 1995, *Drug Dev Res* 34:196
carcinomas of lung, breast, colon	B3	Le^y-related tumor antigen	Pai et al., 1996, *Nat. Med.* 2:350
ovarian	260F9		Pirker et al., 1985, *J Clin Invest* 76:1261
ovarian	454C11		Pirker et al., 1985, *J Clin Invest* 76:1261
	VITAXIN™	Integrin $\alpha_v\beta_3$	International Publication Nos. WO 98/33919 and WO 00/78815

3.5. Prophylactic/Therapeutic Methods

[00220] The present invention encompasses methods for treating, preventing, or managing a disease or disorder associated with overexpression of an EphA2, PCDGF, PCDGF receptor and/or HAAH polypeptide or cell hyperproliferative disorders, preferably cancer, in a subject comprising administering one or more EphA2 agents, PCDGF agents, and/or HAAH agents. In one embodiment, one or more EphA2 agents are administered with one or more PCDGF agents. In another embodiment, one or more EphA2 agents are administered with one or more HAAH agents. In another embodiment one or more PCDGF agents are administered with one or more HAAH agents. In another embodiment, one or more EphA2 agents are administered with one or more PCDGF agents and one or more HAAH agents. In a specific embodiment, the disorder to be treated, prevented, or managed is malignant cancer. In another specific embodiment, the disorder to be treated, prevented, or managed is a non-cancer hyperproliferative disorder. In more specific embodiments, the non-cancer hyperproliferative disorder is asthma, COPD, lung fibrosis, bronchial hyper responsiveness, psoriasis, seborrheic dermatitis, cystic fibrosis, restenosis, hyperproliferative vascular disease, Behcet's Syndrome, atherosclerosis, or macular degeneration. In another specific embodiment, the disorder to be treated, prevented, or managed is a pre-cancerous condition associated with cells that overexpress an EphA2, PCDGF, PCDGF receptor and/or HAAH polypeptide. In more specific embodiments, the pre-cancerous condition is ductal carcinoma in situ (DCIS) of the breast, fibroadenoma of the breast, fibrocystic disease, cervix dysplasia, squamous intraepithelial lesions (SIL), adenomatous polyps, Barrett's esophageal dysplasia, hepatocellular carcinoma, adenomatous hyperplasia, atypical adenomatous hyperplasia (AAH) of the lung, lymphomatoid granulomatosis, pancreatic ductal lesions, pancreatic hyperplasias, pancreatic dysplasias, prostatic intraepithelial neoplasia (PIN), xeroderma pigmentosum, carcinoma in situ of the skin, squamous cell carcinoma, solar keratosis, compound nevi, actinic cheilitis, leukoplakia, or Bowen's disease.

[00221] In some embodiments, the one or more EphA2, PCDGF, and/or HAAH agents for use in the methods of the invention are antibodies. In preferred embodiments, the EphA2 antibodies are one or more of Eph099B-102.147, Eph099B-208.261, Eph099B-210.248, Eph099B-233.152, EA2, or EA5. In other preferred embodiments, the HAAH antibodies are FB50, 8AC, 5C7, or 19B. In more preferred embodiments, the EphA2, PCDGF, PCDGF receptor, and/or HAAH antibodies for use in the methods of the invention have been humanized. In other embodiments, variants of EphA2, PCDGF, PCDGF

receptor, and/or HAAH antibodies *e.g.*, with one or more amino acid substitutions, particularly in the variable domain, are provided that have increased activity, binding ability, etc., as compared to non-variant EphA2, PCDGF, PCDGF receptor, and/or HAAH antibodies are used in the methods of the invention.

[00222] In another specific embodiment, the therapeutic and prophylactic methods of the invention comprise administration of an agent of the invention that inhibits expression of EphA2, PCDGF, PCDGF receptor, or HAAH. Such agents include but are not limited to, antisense nucleic acids specific for EphA2, PCDGF, PCDGF receptor or HAAH, double stranded EphA2, PCDGF, PCDGF receptor or HAAH RNA that mediates RNAi, anti-EphA2, PCDGF, PCDGF receptor or HAAH ribozymes, etc. (see Section 5.2 *infra*) or a small molecule inhibitor of EphA2, PCDGF, PCDGF receptor or HAAH activity.

[00223] The EphA2, PCDGF, and/or HAAH agents for use in the methods of the invention are administered concurrently with one another. The term "concurrently" is not limited to the administration of EphA2, PCDGF, and/or HAAH prophylactic or therapeutic agents at exactly the same time, but rather it is meant that the EphA2, PCDGF, and/or HAAH agents are administered to a subject in a sequence and within a time interval such that the agents can act together with one another to provide an increased benefit than if they were administered otherwise. For example, each prophylactic or therapeutic agent may be administered at the same time or sequentially in any order at different points in time; however, if not administered at the same time, they should be administered sufficiently close in time so as to provide the desired therapeutic or prophylactic effect. Each therapeutic agent can be administered separately, in any appropriate form and by any suitable route. In some embodiments, the EphA2, PCDGF, and/or HAAH agents for use in the methods of the invention can be administered in combination with one or more other prophylactic or therapeutic agents useful in the treatment, prevention or management of diseases or disorders associated with EphA2, PCDGF, PCDGF receptor and/or HAAH overexpression, hyperproliferative disorders, and/or cancer. In certain embodiments, one or more EphA2, PCDGF, and/or HAAH agents are administered to a mammal, preferably a human, concurrently with one or more other therapeutic agents useful for the treatment of cancer. In other embodiments, the EphA2, PCDGF, and/or HAAH agents are administered before, concurrently or after surgery. Preferably the surgery completely removes localized tumors or reduces the size of large tumors. Surgery can also be done as a preventive measure or to relieve pain.

[00224] In various embodiments, the prophylactic or therapeutic agents are administered less than 1 hour apart, at about 1 hour apart, at about 1 hour to about 2 hours apart, at about 2 hours to about 3 hours apart, at about 3 hours to about 4 hours apart, at about 4 hours to about 5 hours apart, at about 5 hours to about 6 hours apart, at about 6 hours to about 7 hours apart, at about 7 hours to about 8 hours apart, at about 8 hours to about 9 hours apart, at about 9 hours to about 10 hours apart, at about 10 hours to about 11 hours apart, at about 11 hours to about 12 hours apart, no more than 24 hours apart or no more than 48 hours apart. In preferred embodiments, two or more components are administered within the same patient visit.

[00225] The dosage amounts and frequencies of administration provided herein are encompassed by the terms therapeutically effective and prophylactically effective. The dosage and frequency further will typically vary according to factors specific for each patient depending on the specific therapeutic or prophylactic agents administered, the severity and type of cancer, the route of administration, as well as age, body weight, response, and the past medical history of the patient. Suitable regimens can be selected by one skilled in the art by considering such factors and by following, for example, dosages reported in the literature and recommended in the *Physician's Desk Reference* (56^{th} ed., 2002).

3.5.1. Patient Population

[00226] The invention provides methods for treating, preventing, and managing a hyperproliferative cell disorder, particularly cancer, by administrating to a subject in need thereof a therapeutically or prophylactically effective amount of one or more EphA2 agents in combination with one or more PCDGF agents and/or one or more HAAH agents. In one embodiment, the agents of the invention can be administered in combination with one or more other therapeutic agents useful in the treatment, prevention or management of hyperproliferative disorders, especially cancer, that are not EphA2-, PCDGF-, or HAAH-based. The subject is preferably a mammal such as non-primate (*e.g.*, cows, pigs, horses, cats, dogs, rats, etc.) and a primate (*e.g.*, monkey, such as a cynomolgous monkey and a human). In a preferred embodiment, the subject is a human.

[00227] In one embodiment, the methods of the invention comprise the administration of one or more EphA2 agents in combination with one or more PCDGF agents and/or one or more HAAH agents to patients suffering from cancer. Examples of patients having specific cancers that can be treated by the methods encompassed by the

invention include, but are not limited to, cancers that overexpress an EphA2, PCDGF, PCDGF receptor, and/or HAAH polypeptide. In a further embodiment, the patient has a cancer of an epithelial origin. Examples of such cancers are cancer of the lung, colon, prostate, breast, skin, bladder, and pancreas, and renal cell carcinoma and melanoma. Additional cancers are listed by example and not by limitation in the following Section 5.5.1.1. In particular embodiments, methods of the invention can be used to treat patients having metastasis from primary tumors.

[00228] The methods and compositions of the invention may be used as a first line or second line cancer treatment. Included in the invention is also the treatment of patients undergoing cancer therapies that are not EphA2-, PCDGF-, or HAAH-based and the methods of the invention can be used before any adverse effects or intolerance of these other cancer therapies occurs. Cancer therapies that are not EphA2-, PCDGF-, or HAAH-based include, but are not limited to, chemotherapy, radiation therapy, hormonal therapy, biological therapy/immunotherapy, surgery (see *e.g.*, Section 5.5.2).

[00229] The invention also encompasses methods for administering one or more EphA2 agents in combination with one or more PCDGF agents and/or one or more HAAH agents to treat or ameliorate symptoms in patients refractory to one or more cancer therapies that are not EphA2-, PCDGF-, or HAAH-based. In a certain embodiment, that a cancer is refractory to a therapy means that at least some significant portion of the cancer cells are not killed or their cell division arrested. The determination of whether the cancer cells are refractory can be made either *in vivo* or *in vitro* by any method known in the art for assaying the effectiveness of treatment on cancer cells, using the art-accepted meanings of "refractory" in such a context. In various embodiments, a cancer is refractory where the number of cancer cells has not been significantly reduced, or has increased. Among these patients are refractory patients and those with cancer despite treatment with existing cancer therapies. In particular embodiments, one or more EphA2 agents in combination with one or more PCDGF agents and/or one or more HAAH agents are administered to reverse resistance or reduced sensitivity of cancer cells to certain hormonal, radiation and chemotherapeutic agents, particularly tamoxifen, thereby resensitizing the cancer cells to one or more of these agents, which can then be administered (or continue to be administered) to treat or manage cancer, including to prevent metastasis.

[00230] In another embodiment, the methods and compositions of the invention comprise the administration of one or more EphA2 agents in combination with one or more PCDGF agents and/or one or more HAAH agents to patients expected to suffer from cancer,

e.g., have a genetic predisposition for a particular type of cancer, have been exposed to a carcinogen, or are in remission from a particular cancer. Such patients may or may not have been previously treated for cancer. In other embodiments, the patients have been treated previously for cancer and currently have no disease activity. One or more EphA2 agents in combination with one or more PCDGF agents and/or one or more HAAH agents are administered to prevent the recurrence of cancer.

[00231] In other embodiments, the invention provides methods of treating, preventing and managing non-cancer hyperproliferative cell disorders, particularly those associated with overexpression of EphA2, PCDGF, PCDGF receptor and/or HAAH, including but not limited to, asthma, COPD, lung fibrosis, bronchial hyper responsiveness, psoriasis, seborrheic dermatitis, cystic fibrosis, restenosis, hyperproliferative vascular disease, Behcet's Syndrome, atherosclerosis, and macular degeneration. These methods include methods analogous to those described above for treating, preventing and managing cancer, for example, by administering one or more EphA2 agents in combination with one or more PCDGF agents and/or one or more HAAH agents.

[00232] In other embodiments, patients with a pre-cancerous condition associated with cells that overexpress EphA2, PCDGF, PCDGF receptor and/or HAAH can be administered one or more EphA2 agents in combination with one or more PCDGF agents and/or one or more HAAH to treat the disorder and prevent, delay, or decrease the likelihood that it will progress to cancer. In a specific embodiment, the disorder to be treated, prevented, or managed is a pre-cancerous condition associated with cells that overexpress EphA2, PCDGF, PCDGF receptor and/or HAAH. In more specific embodiments, the pre-cancerous condition is ductal carcinoma in situ (DCIS) of the breast, fibroadenoma of the breast, fibrocystic disease, cervix dysplasia, squamous intraepithelial lesions (SIL), adenomatous polyps, Barrett's esophageal dysplasia, hepatocellular carcinoma, adenomatous hyperplasia, atypical adenomatous hyperplasia (AAH) of the lung, lymphomatoid granulomatosis, pancreatic ductal lesions, pancreatic hyperplasias, pancreatic dysplasias, prostatic intraepithelial neoplasia (PIN), xeroderma pigmentosum, carcinoma in situ of the skin, squamous cell carcinoma, solar keratosis, compound nevi, actinic cheilitis, leukoplakia, or Bowen's disease. These methods include methods analogous to those described above for treating, preventing and managing cancer, for example, by administering one or more EphA2 agents in combination with one or more PCDGF agents and/or one or more HAAH agents.

3.5.1.1. **Cancers**

[00233] Cancers and related disorders that can be treated or prevented by methods and compositions of the present invention include but are not limited to cancers overexpressing an EphA2, PCDGF, PCDGF receptor and/or HAAH polypeptide and/or are of an epithelial cell origin. Examples of such cancers include the following: leukemias (*e.g.*, acute leukemia, acute lymphocytic leukemia, acute myelocytic leukemias, myeloblastic, promyelocytic, myelomonocytic, monocytic, erythroleukemia leukemia, myelodysplastic syndrome, chronic myelocytic/granulocytic leukemia, chronic lymphocytic leukemia, hairy cell leukemia, polycythemia vera); lymphomas (*e.g.*, Hodgkin's disease, non-Hodgkin's disease); multiple myelomas (*e.g.*, smoldering multiple myeloma, nonsecretory myeloma, osteosclerotic myeloma, plasma cell leukemia, solitary plasmacytoma and extramedullary plasmacytoma, Waldenström's macroglobulinemia, monoclonal gammopathy of undetermined significance, benign monoclonal gammopathy, heavy chain disease); bone and connective tissue sarcomas (*e.g.*, bone sarcoma, osteosarcoma, osteogenic sarcoma, chondrosarcoma, Ewing's sarcoma, malignant giant cell tumor, fibrosarcoma of bone, chordoma, periosteal sarcoma, soft-tissue sarcomas, angiosarcoma (hemangiosarcoma), fibrosarcoma, Kaposi's sarcoma, leiomyosarcoma, liposarcoma, lymphangiosarcoma, neurilemmoma, rhabdomyosarcoma, synovial sarcoma); brain tumors (*e.g.*, glioma, astrocytoma, brain stem glioma, ependymoma, oligodendroglioma, nonglial tumor, acoustic neurinoma, craniopharyngioma, medulloblastoma, meningioma, pineocytoma, pineoblastoma, primary brain lymphoma); breast cancer (*e.g.*, adenocarcinoma, lobular/small cell carcinoma, intraductal carcinoma, medullary breast cancer, mucinous breast cancer, tubular breast cancer, papillary breast cancer, Paget's disease, inflammatory breast cancer); adrenal cancers (*e.g.*, pheochromocytom and adrenocortical carcinoma); thyroid cancers (*e.g.*, papillary or follicular thyroid cancer, medullary thyroid cancer and anaplastic thyroid cancer); pancreatic cancers (*e.g.*, insulinoma, gastrinoma, glucagonoma, vipoma, somatostatin-secreting tumor, carcinoid or islet cell tumor); pituitary cancers (*e.g.*, Cushing's disease, prolactin-secreting tumor, acromegaly, and diabetes insipius); eye cancers (*e.g.*, ocular melanoma such as iris melanoma, choroidal melanoma, and cilliary body melanoma, retinoblastoma); vaginal cancers (*e.g.*, squamous cell carcinoma, adenocarcinoma, and melanoma); vulvar cancer (*e.g.*, squamous cell carcinoma, melanoma, adenocarcinoma, basal cell carcinoma, sarcoma, Paget's disease); cervical cancers (*e.g.*, squamous cell carcinoma, adenocarcinoma); uterine cancers (*e.g.*, endometrial carcinoma and uterine

sarcoma); ovarian cancers (*e.g.*, ovarian epithelial carcinoma, borderline tumor, germ cell tumor, and stromal tumor); esophageal cancers (*e.g.*, squamous cancer, adenocarcinoma, adenoid cyctic carcinoma, mucoepidermoid carcinoma, adenosquamous carcinoma, sarcoma, melanoma, plasmacytoma, verrucous carcinoma, and oat cell/small cell carcinoma); stomach cancers (*e.g.*, adenocarcinoma, fungating/polypoid, ulcerating, superficial spreading, diffusely spreading, malignant lymphoma, liposarcoma, fibrosarcoma, carcinosarcoma); colon cancers; rectal cancers; liver cancers (*e.g.*, hepatocellular carcinoma, hepatoblastoma); gallbladder cancers (*e.g.*, adenocarcinoma); cholangiocarcinomas (*e.g.*, pappillary, nodular, diffuse); lung cancers (*e.g.*, non-small cell lung cancer, squamous cell carcinoma, epidermoid carcinoma, adenocarcinoma, large-cell carcinoma and small-cell lung cancer, bronchogenic carcinoma); testicular cancers (*e.g.*, germinal tumor, seminoma, anaplastic, spermatocytic, nonseminoma, embryonal carcinoma, teratoma carcinoma, choriocarcinoma/yolk-sac tumor); prostate cancers (*e.g.*, adenocarcinoma, leiomyosarcoma, rhabdomyosarcoma); oral cancers (*e.g.*, squamous cell carcinoma, basal cancers, salivary gland cancers, mucoepidermoid carcinoma, adenoidcystic carcinoma); pharynx cancers (*e.g.*, squamous cell cancer, verrucous); skin cancers (*e.g.*, basal cell carcinoma, squamous cell carcinoma, melanoma, superficial spreading melanoma, nodular melanoma, lentigo malignant melanoma, acral lentiginous melanoma, xeroderma pigmentosum, keratoactanthoma); kidney cancers (*e.g.*, renal cell carcinoma, adenocarcinoma, hypernephroma, fibrosarcoma, transitional cell cancer, Wilms' tumor); bladder cancers (*e.g.*, transitional cell carcinoma, squamous cell cancer, adenocarcinoma, carcinosarcoma); myxosarcoma; endotheliosarcoma; lymphangioendotheliosarcoma; mesothelioma; synovioma; hemangioblastoma; cystadenocarcinoma; sebaceous gland carcinoma (for a review of such disorders, see Fishman et al., 1985, *Medicine*, 2d Ed., J.B. Lippincott Co., Philadelphia and Murphy et al., 1997, *Informed Decisions: The Complete Book of Cancer Diagnosis, Treatment, and Recovery*, Viking Penguin, Penguin Books U.S.A., Inc., United States of America). Additionally, cancers associated with or caused by aberrations in apoptosis can be treated by the methods of the present invention. Such cancers may include but not be limited to follicular lymphomas; carcinomas with p53 mutations; hormone dependent tumors of the breast, prostate and ovary; pre-cancerous lesions such as familial adenomatous polyposis, and myelodysplastic syndromes. The absence of, decrease in or resistance to apoptosis in cancer cells or abnormal cells can readily be determined by techniques known in the art.

[00234] In preferred embodiments, the methods and compositions of the invention are used for the treatment and/or prevention of breast, colon, ovarian, lung, and prostate cancers and melanoma and are provided below by example rather than by limitation.

3.5.1.2. Treatment of Breast Cancer

[00235] In specific embodiments, patients with breast cancer are administered an effective amount of one or more EphA2 agents in combination with one or more PCDGF agents and/or one or more HAAH. In another embodiment, the agents of the invention can be administered in combination with an effective amount of one or more other agents useful for breast cancer therapy including but not limited to: doxorubicin, epirubicin, the combination of doxorubicin and cyclophosphamide (AC), the combination of cyclophosphamide, doxorubicin and 5-fluorouracil (CAF), the combination of cyclophosphamide, epirubicin and 5-fluorouracil (CEF), herceptin, tamoxifen, the combination of tamoxifen and cytotoxic chemotherapy, taxanes (such as docetaxel and paclitaxel). In a further embodiment, agents of the invention can be administered with taxanes plus standard doxorubicin and cyclophosphamide for adjuvant treatment of node-positive, localized breast cancer.

[00236] In a specific embodiment, patients with pre-cancerous fibroadenoma of the breast or fibrocystic disease are administered an EphA2, PCDGF, and/or HAAH agent to treat the disorder and prevent, delay, or decrease the likelihood that it will progress to malignant breast cancer. In another specific embodiment, patients refractory to treatment, particularly hormonal therapy, more particulatly tamoxifen therapy, are administered an EphA2, PCDGF, and/or HAAH agent to treat the cancer and/or render the patient non-refractory or responsive.

3.5.1.3. Treatment of Colon Cancer

[00237] In specific embodiments, patients with colon cancer are administered an effective amount of one or more EphA2 agents of the invention in combination with one or more PCDGF agents of the invention and/or one or more HAAH agents of the invention. In another embodiment, the agents of the invention can be administered in combination with an effective amount of one or more other agents useful for colon cancer therapy including but not limited to: the combination of 5-FU and leucovorin, the combination of 5-FU and levamisole, irinotecan (CPT-11) or the combination of irinotecan, 5-FU and leucovorin (IFL).

3.5.1.4. Treatment of Prostate Cancer

[00238] In specific embodiments, patients with prostate cancer are administered an effective amount of one or more EphA2 agents of the invention in combination with one or more PCDGF agents of the invention and/or one or more HAAH agents of the invention. In another embodiment, the agents of the invention can be administered in combination with an effective amount of one or more other agents useful for prostate cancer therapy including but not limited to: external-beam radiation therapy, interstitial implantation of radioisotopes (*i.e.*, I^{125}, palladium, iridium), leuprolide or other LHRH agonists, non-steroidal antiandrogens (flutamide, nilutamide, bicalutamide), steroidal antiandrogens (cyproterone acetate), the combination of leuprolide and flutamide, estrogens such as DES, chlorotrianisene, ethinyl estradiol, conjugated estrogens U.S.P., DES-diphosphate, radioisotopes, such as strontium-89, the combination of external-beam radiation therapy and strontium-89, second-line hormonal therapies such as aminoglutethimide, hydrocortisone, flutamide withdrawal, progesterone, and ketoconazole, low-dose prednisone, or other chemotherapy regimens reported to produce subjective improvement in symptoms and reduction in PSA level including docetaxel, paclitaxel, estramustine/docetaxel, estramustine/etoposide, estramustine/vinblastine, and estramustine/paclitaxel.

[00239] In a specific embodiment, patients with pre-cancerous high-grade prostatic intraepithelial neoplasia (PIN) are administered an EphA2, PCDGF, and/or HAAH agent to treat the disorder and decrease the likelihood that it will progress to malignant prostate cancer.

3.5.1.5. Treatment of Melanoma

[00240] In specific embodiments, patients with melanoma are administered an effective amount of one or more EphA2 agents of the invention in combination with one or more PCDGF agents of the invention and/or one or more HAAH agents of the invention. In another embodiment, the agents of the invention can be administered in combination with an effective amount of one or more other agents useful for melanoma cancer therapy including but not limited to: dacarbazine (DTIC), nitrosoureas such as carmustine (BCNU) and lomustine (CCNU), agents with modest single agent activity including vinca alkaloids, platinum compounds, and taxanes, the Dartmouth regimen (cisplatin, BCNU, and DTIC), interferon alpha (IFN-A), and interleukin-2 (IL-2). In a specific embodiment, an effective amount of one or more agonistic monoclonal antibodies of the invention can be

administered in combination with isolated hyperthermic limb perfusion (ILP) with melphalan (L-PAM), with or without tumor necrosis factor-alpha (TNF-alpha) to patients with multiple brain metastases, bone metastases, and spinal cord compression to achieve symptom relief and some shrinkage of the tumor with radiation therapy.

[00241] In a specific embodiment, patients with pre-cancerous compound nevi are administered an EphA2, PCDGF, and/or HAAH agent to treat the disorder and decrease the likelihood that it will progress to malignant melanoma.

3.5.1.6. Treatment of Ovarian Cancer

[00242] In specific embodiments, patients with ovarian cancer are administered an effective amount of one or more EphA2 agents of the invention in combination with one or more PCDGF agents of the invention and/or one or more HAAH agents of the invention. In another embodiment, the agents of the invention can be administered in combination with an effective amount of one or more other agents useful for ovarian cancer therapy including but not limited to: intraperitoneal radiation therapy, such as P^{32} therapy, total abdominal and pelvic radiation therapy, cisplatin, the combination of paclitaxel (Taxol) or docetaxel (Taxotere) and cisplatin or carboplatin, the combination of cyclophosphamide and cisplatin, the combination of cyclophosphamide and carboplatin, the combination of 5-FU and leucovorin, etoposide, liposomal doxorubicin, gemcitabine or topotecan. Included is the treatment of patients with refractory ovarian cancer including administration of: ifosfamide in patients with disease that is platinum-refractory, hexamethylmelamine (HMM) as salvage chemotherapy after failure of cisplatin-based combination regimens, and tamoxifen in patients with detectable levels of cytoplasmic estrogen receptor on their tumors.

3.5.1.7. Treatment of Lung Cancers

[00243] In specific embodiments, patients with small lung cell cancer are administered an effective amount of one or more EphA2 agents of the invention in combination with one or more PCDGF agents of the invention and/or one or more HAAH agents of the invention. In another embodiment, the agents of the invention can be administered in combination with an effective amount of one or more other agents useful for lung cancer therapy including but not limited to: thoracic radiation therapy, cisplatin, vincristine, doxorubicin, and etoposide, alone or in combination, the combination of cyclophosphamide, doxorubicin, vincristine/etoposide, and cisplatin (CAV/EP), local palliation with endobronchial laser therapy, endobronchial stents, and/or brachytherapy.

[00244] In other specific embodiments, patients with non-small lung cell cancer are administered an effective amount of one or more EphA2 agents of the invention in combination with one or more PCDGF agents of the invention and/or one or more HAAH agents of the invention in combination with an effective amount of one or more other agents useful for lung cancer therapy including but not limited to: palliative radiation therapy, the combination of cisplatin, vinblastine and mitomycin, the combination of cisplatin and vinorelbine, paclitaxel, docetaxel or gemcitabine, the combination of carboplatin and paclitaxel, interstitial radiation therapy for endobronchial lesions or stereotactic radiosurgery.

3.5.2. Other Prophylactic/Therapeutic Agents

[00245] In some embodiments, the invention encompasses methods for administering the agents of the invention (*i.e.*, one or more EphA2 agents in combination with one or more PCDGF agents and/or one or more HAAH agents) in combination with cancer therapies that are not EphA2-, PCDGF-, or HAAH-based (such as, but not limited to, chemotherapies, radiation therapies, hormonal therapies, and/or biological therapies/immunotherapies) to treat or ameliorate cancer in patients. In some specific embodiments, dosages of cancer therapies that are not EphA2-, PCDGF-, or HAAH-based can be reduced due to combination therapy with agents of the invention to decrease adverse effects or intolerance of these other cancer therapies occurs. In other embodiments, the invention encompasses methods for administering the agents of the invention (*i.e.*, one or more EphA2 agents in combination with one or more PCDGF agents and/or one or more HAAH agents) to patients suffering from cancer who are refractory to one or more cancer therapies that are not EphA2-, PCDGF-, or HAAH-based to treat or ameliorate cancer in patients.

[00246] In a specific embodiment, the methods of the invention encompass administration of one or more EphA2 agents, PCDGF agents, and/or HAAH agents in combination with the administration of one or more prophylactic/therapeutic agents that are inhibitors of kinases such as, but not limited to, ABL, ACK, AFK, AKT (*e.g.*, AKT-1, AKT-2, and AKT-3), ALK, AMP-PK, ATM, Auroral, Aurora2, bARK1, bArk2, BLK, BMX, BTK, CAK, CaM kinase, CDC2, CDK, CK, COT, CTD, DNA-PK, EGF-R, ErbB-1, ErbB-2, ErbB-3, ErbB-4, ERK (*e.g.*, ERK1, ERK2, ERK3, ERK4, ERK5, ERK6, ERK7), ERT-PK, FAK, FGR (*e.g.*, FGF1R, FGF2R), FLT (*e.g.*, FLT-1, FLT-2, FLT-3, FLT-4), FRK, FYN, GSK (*e.g.*, GSK1, GSK2, GSK3-alpha, GSK3-beta, GSK4, GSK5), G-protein

coupled receptor kinases (GRKs), HCK, HER2, HKII, JAK (*e.g.*, JAK1, JAK2, JAK3, JAK4), JNK (*e.g.*, JNK1, JNK2, JNK3), KDR, KIT, IGF-1 receptor, IKK-1, IKK-2, INSR (insulin receptor), IRAK1, IRAK2, IRK, ITK, LCK, LOK, LYN, MAPK, MAPKAPK-1, MAPKAPK-2, MEK, MET, MFPK, MHCK, MLCK, MLK3, NEU, NIK, PDGF receptor alpha, PDGF receptor beta, PHK, PI-3 kinase, PKA, PKB, PKC, PKG, PRK1, PYK2, p38 kinases, p135tyk2, p34cdc2, p42cdc2, p42mapk, p44mpk, RAF, RET, RIP, RIP-2, RK, RON, RS kinase, SRC, SYK, S6K, TAK1, TEC, TIE1, TIE2, TRKA, TXK, TYK2, UL13, VEGFR1, VEGFR2, YES, YRK, ZAP-70, and all subtypes of these kinases (see *e.g.*, Hardie and Hanks (1995) The Protein Kinase Facts Book, I and II, Academic Press, San Diego, Calif.). In preferred embodiments, one or more EphA2 agents, PCDGF agents, and/or HAAH agents are administered in combination with the administration of one or more prophylactic/therapeutic agents that are inhibitors of Eph receptor kinases (*e.g.*, EphA4).

[00247] In another specific embodiment, the methods of the invention encompass administration of one or more EphA2 agents, PCDGF agents, and/or HAAH agents in combination with the administration of one or more prophylactic/therapeutic agents that are angiogenesis inhibitors such as, but not limited to: Angiostatin (plasminogen fragment); antiangiogenic antithrombin III; Angiozyme; ABT-627; Bay 12-9566; Benefin; Bevacizumab; BMS-275291; cartilage-derived inhibitor (CDI); CAI; CD59 complement fragment; CEP-7055; Col 3; Combretastatin A-4; Endostatin (collagen XVIII fragment); fibronectin fragment; Gro-beta; Halofuginone; Heparinases; Heparin hexasaccharide fragment; HMV833; Human chorionic gonadotropin (hCG); IM-862; Interferon alpha/beta/gamma; Interferon inducible protein (IP-10); Interleukin-12; Kringle 5 (plasminogen fragment); Marimastat; Metalloproteinase inhibitors (TIMPs); 2-Methoxyestradiol; MMI 270 (CGS 27023A); MoAb IMC-1C11; Neovastat; NM-3; Panzem; PI-88; Placental ribonuclease inhibitor; Plasminogen activator inhibitor; Platelet factor-4 (PF4); Prinomastat; Prolactin 16kD fragment; Proliferin-related protein (PRP); PTK 787/ZK 222594; Retinoids; Solimastat; Squalamine; SS 3304; SU 5416; SU6668; SU11248; Tetrahydrocortisol-S; tetrathiomolybdate; thalidomide; Thrombospondin-1 (TSP-1); TNP-470; Transforming growth factor-beta (TGF-β); Vasculostatin; Vasostatin (calreticulin fragment); ZD6126; ZD6474; farnesyl transferase inhibitors (FTI); and bisphosphonates.

[00248] In another specific embodiment, the methods of the invention encompass administration of one or more EphA2 agents, PCDGF agents, and/or HAAH agents in

combination with the administration of one or more prophylactic/therapeutic agents that are anti-cancer agents such as, but not limited to: acivicin, aclarubicin, acodazole hydrochloride, acronine, adozelesin, aldesleukin, altretamine, ambomycin, ametantrone acetate, aminoglutethimide, amsacrine, anastrozole, anthramycin, asparaginase, asperlin, azacitidine, azetepa, azotomycin, batimastat, benzodepa, bicalutamide, bisantrene hydrochloride, bisnafide dimesylate, bizelesin, bleomycin sulfate, brequinar sodium, bropirimine, busulfan, cactinomycin, calusterone, caracemide, carbetimer, carboplatin, carmustine, carubicin hydrochloride, carzelesin, cedefingol, chlorambucil, cirolemycin, cisplatin, cladribine, crisnatol mesylate, cyclophosphamide, cytarabine, dacarbazine, dactinomycin, daunorubicin hydrochloride, decarbazine, decitabine, dexormaplatin, dezaguanine, dezaguanine mesylate, diaziquone, docetaxel, doxorubicin, doxorubicin hydrochloride, droloxifene, droloxifene citrate, dromostanolone propionate, duazomycin, edatrexate, eflornithine hydrochloride, elsamitrucin, enloplatin, enpromate, epipropidine, epirubicin hydrochloride, erbulozole, esorubicin hydrochloride, estramustine, estramustine phosphate sodium, etanidazole, etoposide, etoposide phosphate, etoprine, fadrozole hydrochloride, fazarabine, fenretinide, floxuridine, fludarabine phosphate, fluorouracil, flurocitabine, fosquidone, fostriecin sodium, gemcitabine, gemcitabine hydrochloride, hydroxyurea, idarubicin hydrochloride, ifosfamide, ilmofosine, interleukin 2 (including recombinant interleukin 2, or rIL2), interferon alpha-2a, interferon alpha-2b, interferon alpha-n1, interferon alpha-n3, interferon beta-I a, interferon gamma-I b, iproplatin, irinotecan hydrochloride, lanreotide acetate, letrozole, leuprolide acetate, liarozole hydrochloride, lometrexol sodium, lomustine, losoxantrone hydrochloride, masoprocol, maytansine, mechlorethamine hydrochloride, megestrol acetate, melengestrol acetate, melphalan, menogaril, mercaptopurine, methotrexate, methotrexate sodium, metoprine, meturedepa, mitindomide, mitocarcin, mitocromin, mitogillin, mitomalcin, mitomycin, mitosper, mitotane, mitoxantrone hydrochloride, mycophenolic acid, nitrosoureas, nocodazole, nogalamycin, ormaplatin, oxisuran, paclitaxel, pegaspargase, peliomycin, pentamustine, peplomycin sulfate, perfosfamide, pipobroman, piposulfan, piroxantrone hydrochloride, plicamycin, plomestane, porfimer sodium, porfiromycin, prednimustine, procarbazine hydrochloride, puromycin, puromycin hydrochloride, pyrazofurin, riboprine, rogletimide, safingol, safingol hydrochloride, semustine, simtrazene, sparfosate sodium, sparsomycin, spirogermanium hydrochloride, spiromustine, spiroplatin, streptonigrin, streptozocin, sulofenur, talisomycin, tecogalan sodium, tegafur, teloxantrone hydrochloride, temoporfin, teniposide, teroxirone, testolactone, thiamiprine, thioguanine, thiotepa,

tiazofurin, tirapazamine, toremifene citrate, trestolone acetate, triciribine phosphate, trimetrexate, trimetrexate glucuronate, triptorelin, tubulozole hydrochloride, uracil mustard, uredepa, vapreotide, verteporfin, vinblastine sulfate, vincristine sulfate, vindesine, vindesine sulfate, vinepidine sulfate, vinglycinate sulfate, vinleurosine sulfate, vinorelbine tartrate, vinrosidine sulfate, vinzolidine sulfate, vorozole, zeniplatin, zinostatin, zorubicin hydrochloride. Other anti-cancer drugs include, but are not limited to: 20-epi-1,25 dihydroxyvitamin D3, 5-ethynyluracil, abiraterone, aclarubicin, acylfulvene, adecypenol, adozelesin, aldesleukin, ALL-TK antagonists, altretamine, ambamustine, amidox, amifostine, aminolevulinic acid, amrubicin, amsacrine, anagrelide, anastrozole, andrographolide, angiogenesis inhibitors, antagonist D, antagonist G, antarelix, anti-dorsalizing morphogenetic protein-1, antiandrogens, antiestrogens, antineoplaston, aphidicolin glycinate, apoptosis gene modulators, apoptosis regulators, apurinic acid, ara-CDP-DL-PTBA, arginine deaminase, asulacrine, atamestane, atrimustine, axinastatin 1, axinastatin 2, axinastatin 3, azasetron, azatoxin, azatyrosine, baccatin III derivatives, balanol, batimastat, BCR/ABL antagonists, benzochlorins, benzoylstaurosporine, beta lactam derivatives, beta-alethine, betaclamycin B, betulinic acid, bFGF inhibitor, bicalutamide, bisantrene, bisaziridinylspermine, bisnafide, bistratene A, bizelesin, breflate, bropirimine, budotitane, buthionine sulfoximine, calcipotriol, calphostin C, camptothecin derivatives, canarypox IL-2, capecitabine, carboxamide-amino-triazole, carboxyamidotriazole, CaRest M3, CARN 700, cartilage derived inhibitor, carzelesin, casein kinase inhibitors (ICOS), castanospermine, cecropin B, cetrorelix, chloroquinoxaline sulfonamide, cicaprost, cis-porphyrin, cladribine, clomifene analogues, clotrimazole, collismycin A, collismycin B, combretastatin A4, combretastatin analogue, conagenin, crambescidin 816, crisnatol, cryptophycin 8, cryptophycin A derivatives, curacin A, cyclopentanthraquinones, cycloplatam, cypemycin, cytarabine ocfosfate, cytolytic factor, cytostatin, dacliximab, decitabine, dehydrodidemnin B, deslorelin, dexamethasone, dexifosfamide, dexrazoxane, dexverapamil, diaziquone, didemnin B, didox, diethylnorspermine, dihydro-5-azacytidine, dihydrotaxol, dioxamycin, diphenyl spiromustine, docetaxel, docosanol, dolasetron, doxifluridine, droloxifene, dronabinol, duocarmycin SA, ebselen, ecomustine, edelfosine, edrecolomab, eflornithine, elemene, emitefur, epirubicin, epristeride, estramustine analogue, estrogen agonists, estrogen antagonists, etanidazole, etoposide phosphate, exemestane, fadrozole, fazarabine, fenretinide, filgrastim, finasteride, flavopiridol, flezelastine, fluasterone, fludarabine, fluorodaunorunicin hydrochloride, forfenimex, formestane, fostriecin, fotemustine,

gadolinium texaphyrin, gallium nitrate, galocitabine, ganirelix, gelatinase inhibitors, gemcitabine, glutathione inhibitors, hepsulfam, heregulin, hexamethylene bisacetamide, hypericin, ibandronic acid, idarubicin, idoxifene, idramantone, ilmofosine, ilomastat, imidazoacridones, imiquimod, immunostimulant peptides, insulin-like growth factor-1 receptor inhibitor, interferon agonists, interferons, interleukins, iobenguane, iododoxorubicin, ipomeanol, iroplact, irsogladine, isobengazole, isohomohalicondrin B, itasetron, jasplakinolide, kahalalide F, lamellarin-N triacetate, lanreotide, leinamycin, lenograstim, lentinan sulfate, leptolstatin, letrozole, leukemia inhibiting factor, leukocyte alpha interferon, leuprolide+estrogen+progesterone, leuprorelin, levamisole, liarozole, linear polyamine analogue, lipophilic disaccharide peptide, lipophilic platinum compounds, lissoclinamide 7, lobaplatin, lombricine, lometrexol, lonidamine, losoxantrone, lovastatin, loxoribine, lurtotecan, lutetium texaphyrin, lysofylline, lytic peptides, maitansine, mannostatin A, marimastat, masoprocol, maspin, matrilysin inhibitors, matrix metalloproteinase inhibitors, menogaril, merbarone, meterelin, methioninase, metoclopramide, MIF inhibitor, mifepristone, miltefosine, mirimostim, mismatched double stranded RNA, mitoguazone, mitolactol, mitomycin analogues, mitonafide, mitotoxin fibroblast growth factor-saporin, mitoxantrone, mofarotene, molgramostim, monoclonal antibody, human chorionic gonadotrophin, monophosphoryl lipid A+myobacterium cell wall sk, mopidamol, multiple drug resistance gene inhibitor, multiple tumor suppressor 1-based therapy, mustard anticancer agent, mycaperoxide B, mycobacterial cell wall extract, myriaporone, N-acetyldinaline, N-substituted benzamides, nafarelin, nagrestip, naloxone+pentazocine, napavin, naphterpin, nartograstim, nedaplatin, nemorubicin, neridronic acid, neutral endopeptidase, nilutamide, nisamycin, nitric oxide modulators, nitroxide antioxidant, nitrullyn, O6-benzylguanine, octreotide, okicenone, oligonucleotides, onapristone, ondansetron, ondansetron, oracin, oral cytokine inducer, ormaplatin, osaterone, oxaliplatin, oxaunomycin, paclitaxel, paclitaxel analogues, paclitaxel derivatives, palauamine, palmitoylrhizoxin, pamidronic acid, panaxytriol, panomifene, parabactin, pazelliptine, pegaspargase, peldesine, pentosan polysulfate sodium, pentostatin, pentrozole, perflubron, perfosfamide, perillyl alcohol, phenazinomycin, phenylacetate, phosphatase inhibitors, picibanil, pilocarpine hydrochloride, pirarubicin, piritrexim, placetin A, placetin B, plasminogen activator inhibitor, platinum complex, platinum compounds, platinum-triamine complex, porfimer sodium, porfiromycin, prednisone, propyl bis-acridone, prostaglandin J2, proteasome inhibitors, protein A-based immune modulator, protein kinase C inhibitors, microalgal, protein tyrosine phosphatase inhibitors, purine

nucleoside phosphorylase inhibitors, purpurins, pyrazoloacridine, pyridoxylated hemoglobin polyoxyethylene conjugate, raf antagonists, raltitrexed, ramosetron, ras farnesyl protein transferase inhibitors, ras inhibitors, ras-GAP inhibitor, retelliptine demethylated, rhenium Re 186 etidronate, rhizoxin, ribozymes, RII retinamide, rogletimide, rohitukine, romurtide, roquinimex, rubiginone B1, ruboxyl, safingol, saintopin, SarCNU, sarcophytol A, sargramostim, Sdi 1 mimetics, semustine, senescence derived inhibitor 1, sense oligonucleotides, signal transduction inhibitors, signal transduction modulators, single chain antigen binding protein, sizofiran, sobuzoxane, sodium borocaptate, sodium phenylacetate, solverol, somatomedin binding protein, sonermin, sparfosic acid, spicamycin D, spiromustine, splenopentin, spongistatin 1, squalamine, stem cell inhibitor, stem-cell division inhibitors, stipiamide, stromelysin inhibitors, sulfinosine, superactive vasoactive intestinal peptide antagonist, suradista, suramin, swainsonine, synthetic glycosaminoglycans, tallimustine, tamoxifen methiodide, tauromustine, taxol, tazarotene, tecogalan sodium, tegafur, tellurapyrylium, telomerase inhibitors, temoporfin, temozolomide, teniposide, tetrachlorodecaoxide, tetrazomine, thaliblastine, thalidomide, thiocoraline, thioguanine, thrombopoietin, thrombopoietin mimetic, thymalfasin, thymopoietin receptor agonist, thymotrinan, thyroid stimulating hormone, tin ethyl etiopurpurin, tirapazamine, titanocene bichloride, topsentin, toremifene, totipotent stem cell factor, translation inhibitors, tretinoin, triacetyluridine, triciribine, trimetrexate, triptorelin, tropisetron, turosteride, tyrosine kinase inhibitors, tyrphostins, UBC inhibitors, ubenimex, urogenital sinus-derived growth inhibitory factor, urokinase receptor antagonists, vapreotide, variolin B, vector system, erythrocyte gene therapy, velaresol, veramine, verdins, verteporfin, vinorelbine, vinxaltine, vitaxin, vorozole, zanoterone, zeniplatin, zilascorb, zinostatin stimalamer, 5-fluorouracil, leucovorin, angiostatin (plasminogen fragment); antiangiogenic antithrombin III; Angiozyme; ABT-627; Bay 12-9566; Benefin; Bevacizumab; BMS-275291; cartilage-derived inhibitor (CDI); CAI; CD59 complement fragment; CEP-7055; Col 3; Combretastatin A-4; Endostatin (collagen XVIII fragment); fibronectin fragment; Gro-beta; Halofuginone; Heparinases; Heparin hexasaccharide fragment; HMV833; Human chorionic gonadotropin (hCG); IM-862; Interferon alpha/beta/gamma; Interferon inducible protein (IP-10); Interleukin-12; Kringle 5 (plasminogen fragment); Marimastat; Metalloproteinase inhibitors (TIMPs); 2-Methoxyestradiol; MMI 270 (CGS 27023A); MoAb IMC-1C11; Neovastat; NM-3; Panzem; PI-88; Placental ribonuclease inhibitor; Plasminogen activator inhibitor; Platelet factor-4 (PF4); Prinomastat; Prolactin 16kD fragment; Proliferin-related protein (PRP);

PTK 787/ZK 222594; Retinoids; Solimastat; Squalamine; SS 3304; SU 5416; SU6668; SU11248; Tetrahydrocortisol-S; tetrathiomolybdate; thalidomide; Thrombospondin-1 (TSP-1); TNP-470; Transforming growth factor-beta (TGF-b); Vasculostatin; Vasostatin (calreticulin fragment); ZD6126; ZD 6474; farnesyl transferase inhibitors (FTI); and bisphosphonates. Radiation therapy can comprise the use of x-rays, gamma rays and other sources of radiation to destroy the cancer cells. In preferred embodiments, the radiation treatment is administered as external beam radiation or teletherapy wherein the radiation is directed from a remote source. In other preferred embodiments, the radiation treatment is administered as internal therapy or brachytherapy wherein a radioactive source is placed inside the body close to cancer cells or a tumor mass.

[00249] Cancer therapies (*e.g.*, chemotherapies, hormonal therapies, biological therapies/immunotherapies, radiation therapies) and their dosages, routes of administration and recommended usage are known in the art and have been described in such literature as the *Physician's Desk Reference* (56th ed., 2002).

3.6. Identification Of Agents Of The Invention

[00250] The invention provides methods of assaying and screening for agents of the invention by incubating candidate agents with cells, particularly cancer cells, that express or bind to a polypeptide of interest (*e.g.*, EphA2 for EphA2 agents, PCDGF or PCDGF receptor for PCDGF agents or HAAH for HAAH agents) and then assaying for a desirable change in cell phenotype. Additionally, animal models of hyperproliferative disorders, particularly cancer, can be used to screen for agents of the invention.

3.6.1. EphA2 Agents

[00251] The invention provides methods of assaying and screening for EphA2 agents that decrease EphA2 expression and/or activity. In one embodiment, an EphA2 agent of the invention decreases EphA2 expression level (*e.g.*, decreases mRNA transcription or translation etc.). Any method known in the art for assaying EphA2 expression can be used including, but not limited to, RT-PCR, northern blot analysis, western blot analysis, and ELISA. In a specific embodiment, the EphA2 agent of the invention that decreases EphA2 expression is an EphA2 agonist. Such agents of the invention increase EphA2 phosphorylation and/or EphA2 degradation when contacting cells expressing EphA2, particularly cells overexpressing EphA2 such as cancer cells. Any method known in the art

to assay either the level of EphA2 phosphorylation, activity, or expression can be used to assay EphA2 agents to determine their agonistic activity (see, *e.g.*, Section 6, *infra*).

[00252] In another embodiment, when the EphA2 agent is an antibody (preferably a monoclonal antibody), the EphA2 antibody preferably binds to an EphA2 epitope exposed in malignant cells (*e.g.*, cells overexpressing EphA2 and/or cells with EphA2 unbound to ligand). In this embodiment, antibodies of the invention are antibodies directed to an EphA2 epitope not normally exposed in non-cancer cells but exposed in malignant cells. Differences in EphA2 membrane distribution between non-cancer cells and malignant cancer cells expose certain epitopes on malignant cells that are not exposed normally. For example, normally EphA2 is bound to its ligand, EphrinA1, and localizes at areas of cell-cell contacts. However, malignant cells generally display decreased cell-cell contacts as well as overexpress EphA2 in excess of its ligand. Thus, in malignant cells, there is an increased amount of unbound EphA2 that is not localized to cell-cell contacts. As such, any antibody identified that preferentially binds this unbound, unlocalized form of EphA2 is antibody of the invention. Any method known in the art to determine antibody binding/localization on a cell can be used to screen candidate antibodies for desirable binding properties. In a specific embodiment, immunofluorescence is used to determine the binding characteristics of an antibody. In this embodiment, antibodies that bind poorly to EphA2 when it is bound to its ligand and localized to cell-cell contacts but bind well to free EphA2 on a cell are encompassed by the invention.

[00253] In another embodiment, when the EphA2 agent is an antibody (preferably a monoclonal antibody), the EphA2 antibody has a low K_{off} rate. The binding affinity of a antibody of the invention to EphA2 or a fragment thereof and the off-rate of a monoclonal antibody-EphA2 interaction can be determined by competitive binding assays. One example of a competitive binding assay is a radioimmunoassay comprising the incubation of labeled EphA2 (*e.g.*, ^{3}H or ^{125}I) with the antibody of interest in the presence of increasing amounts of unlabeled EphA2, and the detection of the monoclonal antibody bound to the labeled EphA2. The affinity of a monoclonal antibody for a EphA2 and the binding off-rates can be determined from the data by scatchard plot analysis. Competition with a second antibody can also be determined using radioimmunoassays. In this case, EphA2 is incubated with a monoclonal antibody conjugated to a labeled compound (*e.g.*, ^{3}H or ^{125}I) in the presence of increasing amounts of a second unlabeled antibody.

[00254] In a preferred embodiment, BIACORE™ kinetic analysis is used to determine the binding on and off rates of antibodies to EphA2. BIACORE™ kinetic

analysis comprises analyzing the binding and dissociation of a monoclonal antibody from chips with immobilized EphA2 or fragment thereof on their surface.

[00255] An antibody that immunospecifically binds to EphA2 preferably has a K_{off} rate (antibody (Ab) + antigen (Ag) $\xleftarrow{K_{off}}$ Ab-Ag) of less than $3x10^{-3}$ s^{-1}, less than 10^{-3} s^{-1}, less than 10^{-4} s^{-1}, less than $5x10^{-4}$ s^{-1}, less than 10^{-5} s^{-1}, less than $5x10^{-5}$ s^{-1}, less than 10^{-6} s^{-1}, less than $5x10^{-6}$ s^{-1}, less than 10^{-7} s^{-1}, less than $5x10^{-7}$ s^{-1}, less than 10^{-8} s^{-1}, less than $5x10^{-8}$ s^{-1}, less than 10^{-9} s^{-1}, less than $5x10^{-9}$ s^{-1}, or less than 10^{-10} s.

3.6.2. PCDGF Agents

[00256] The invention provides methods of assaying and screening candidate agents for those agents that decrease PCDGF or PCDGF receptor expression, secretion, and/or activity. In one embodiment, a PCDGF agent decreases PCDGF or PCDGF receptor expression levels (*e.g.*, decreases mRNA transcription or translation etc.). Any method known in the art for assaying PCDGF or PCDGF receptor expression can be used including, but not limited to, RT-PCR, northern blot analysis, western blot analysis, and ELISA.

[00257] In another embodiment, a PCDGF agent of the invention decreases/inhibits secretion of PCDGF or PCDGF receptor. Any method known in the art can be used to assay for candidate agents that decrease PCDGF or PCDGF receptor secretion. In a specific embodiment, conditioned medium from cells expressing PCDGF can be used for ELISA or western blot analysis or immunoprecipitation. In another specific embodiment, cells which express PCDGF receptor can be used for immunofluorescence or FACS analysis to assay if the PCDGF receptor extracellular domain is expressed on the surface of the cell.. In a more specific embodiment, the PCDGF agent that decreases PCDGF or PCDGF receptor secretion is an intrabody.

[00258] In another embodiment, the PCDGF agent inhibits/decreases binding of PCDGF to its receptor. In one embodiment, a PCDGF agent is a competitive inhibitor, non-competitive inhibitor, or un-competitive inhibitor of PCDGF. In another embodiment, a PCDGF agent neutralizers PCDGF such that PCDGF cannot bind its receptor (see *e.g.*, Sections 5.1.2 and 5.1.3). In a specific embodiment, the PCDGF agent is a neutralizing antibody, preferably monoclonal antibody. Such neutralizing antibodies can be utilized to generate anti-idiotype antibodies that "mimic" PCDGF polypeptides using techniques well known to those skilled in the art (*see, e.g.*, Greenspan & Bona, 1993, *FASEB* 17:437-44; Nissinoff, 1991, *J. Immunol.* 147:2429-38). For example, PCDGF antibodies which bind to

PCDGF and competitively inhibit the binding of PCDGF to its receptor can be used to generate anti-idiotypes that "mimic" the PCDGF ligand/receptor-binding domain and, as a consequence, bind to and neutralize PCDGF receptors. For example, such anti-idiotypic antibodies can be used to bind PCDGF ligands/receptors, and thereby block PCDGF-mediated biological activity. Alternatively, anti-idiotypes that "mimic" a PCDGF binding domain may bind to PCDGF receptors and block PCDGF from binding thus inhibiting receptor mediated signaling.

[00259] In another embodiment, the PCDGF agent inhibits/decreases a biological effect normally observed when PCDGF binds its endogenous binding partner (*e.g.*, receptor such as Rse). In a specific embodiment, the biological activity of PCDGF is increased cell proliferation. Any method known in the art to measure cell proliferation can be used. In another specific embodiment, the biological activity of PCDGF is activation of mitogen-activated protein (MAP) kinase, phosphatidylinositol 3' kinase (PI3K), and/or focal adhesion kinase (FAK). Any method known in the art can be used to determine MAP, PI3K, or FAK activation. In another specific embodiment, the biological activity of PCDGF is increased expression of cyclin D1, matrix metalloproteinase (MMP) 13, and/or MMP 17. Any method known in the art can be used to determine levels of cyclin D1, MMP13, or MMP17 including, but not limited to, RT-PCR, northern blot analysis, western blot analysis, and ELISA. In another specific embodiment, the biological activity of PCDGF is increased phosphorylation of pRB. Any method known in the art can be used to determine phosphorylation levels of pRB. For example, cell lysates from cells incubated with PCDGF and a candidate agent can be immunoprecipitated with a pRB-specific antibody and then resolved by SDS-PAGE before being subjected to western blot analysis (see Taya et al., 2003, *Methods Mol Biol.* 223:17-26.).

[00260] In some embodiments, the antibody binds PCDGF with a K_{off} of less than 10^{-3} s^{-1}, less than, less than 9 X 10^{-4} s^{-1}, less than 8 X 10^{-4} s^{-1}, less than 7 X 10^{-4} s^{-1}, less than 5 X 10^{-4} s^{-1}, less than 10^{-4} s^{-1}, less than 9 X 10^{-5} s^{-1}, less than 5 X 10^{-5} s^{-1}, less than 10^{-5} s^{-1}, less than 5 X 10^{-6} s^{-1}, less than 10^{-6} s^{-1}, less than 5 X 10^{-7} s^{-1}, less than 10^{-7} s^{-1}, less than 5 X 10^{-8} s^{-1}, less than 10^{-8} s^{-1}, less than 5 X 10^{-9} s^{-1}, less than 10^{-9} s^{-1}, or less than 10^{-10} s^{-1}.

[00261] In other embodiments, the antibody binds PCDGF receptor with a K_{off} of less than 3 X 10^{-3} s^{-1}, less than 10^{-3} s^{-1}, less than, less than, less than 9 X 10^{-4} s^{-1}, less than 8 X 10^{-4} s^{-1}, less than 7 X 10^{-4} s^{-1}, less than 5 X 10^{-4} s^{-1}, less than 10^{-4} s^{-1}, less than 9 X 10^{-5} s^{-1}, less than 5 X 10^{-5} s^{-1}, less than 10^{-5} s^{-1}, less than 5 X 10^{-6} s^{-1}, less than 10^{-6} s^{-1}, less than 5 X

10^{-7} s^{-1}, less than 10^{-7} s^{-1}, less than 5 X 10^{-8} s^{-1}, less than 10^{-8} s^{-1}, less than 5 X 10^{-9} s^{-1}, less than 10^{-9} s^{-1}, or less than 10^{-10} s^{-1}.

3.6.3. HAAH Agents

[00262] The invention provides methods of assaying and screening for HAAH agents that decrease HAAH expression and/or activity. In one embodiment, an HAAH agent is an antagonist of HAAH. In another embodiment, an HAAH agent of the invention decreases HAAH expression level (*e.g.*, decreases mRNA transcription or translation etc.). Any method known in the art for assaying HAAH expression can be used including, but not limited to, RT-PCR, northern blot analysis, western blot analysis, and ELISA.

[00263] In another embodiment, an HAAH agent of the invention inhibits/decreases binding of HAAH to its substrate (see *e.g.*, Section 5.1.4). In one embodiment, an HAAH agent is a competitive inhibitor, non-competitive inhibitor, or un-competitive inhibitor of HAAH. HAAH hydroxylates aspartic acid or asparagine residues in polypeptides with EGF-like domains (consensus sequence of EGF-like domains $CX_7CX_4CX_{10}CXCX_8C$). In a specific embodiment, peptides comprising EGF-like domains are HAAH competitive inhibitor agents of the invention. Any method known in the art to detect binding between HAAH and its substrate can be used to assay for candidate agents that prevent such binding. In another specific embodiment, the HAAH agent can bind to the carboxy terminal catalytic domain of HAAH and prevents binding to its substrate. In a more specific embodiment, the HAAH agent of the invention is an intrabody.

[00264] In another embodiment, an HAAH agent of the invention inhibits/decreases a biological effect normally observed when HAAH binds its endogenous binding partner (*e.g.*, substrate). In a specific embodiment, the biological effect is HAAH hydroxylase activity. Candidate compounds can be screened for the ability to inhibit/decrease the hydroxylase activity of HAAH. Any method known in the art to measure hydroxylase activity can be used, *see e.g.*, Lavaissiere et al., 1996, *J. Clin. Invest.* 98:1313-23; Jia et al., 1992, *J. Biol. Chem.* 267:14322-7; Wang et al., 1991, *J. Biol. Chem.* 266:14004-10; Gronke et al., 1990, *J. Biol. Chem.* 265:8558-65; Zhang, et al., 1999, *Anal. Biochem.* 271:137-42.

[00265] In another specific embodiment, the biological effect is alteration in expression of certain genes and their corresponding proteins. For example, in cells overexpressing HAAH, bcl-2 and proliferating cell nuclear antigen (PCNA) expression are increased while p21/waf1 and p16 expression are decreased relative to cells not overexpressing HAAH. Any method known in the art can be used to assay the expression

level of the genes of interest and their corresponding proteins including, but not limited to, RT-PCR, northern blot analysis, western blot analysis, ELISA.

[00266] In some embodiments, the antibody binds HAAH with a K_{off} of less than 3 X 10^{-3} s^{-1}, less than 10^{-3} s^{-1}, less than, less than, less than 9 X 10^{-4} s^{-1}, less than 8 X 10^{-4} s^{-1}, less than 7 X 10^{-4} s^{-1}, less than 5 X 10^{-4} s^{-1}, less than 10^{-4} s^{-1}, less than 9 X 10^{-5} s^{-1}, less than 5 X 10^{-5} s^{-1}, less than 10^{-5} s^{-1}, less than 5 X 10^{-6} s^{-1}, less than 10^{-6} s^{-1}, less than 5 X 10^{-7} s^{-1}, less than 10^{-7} s^{-1}, less than 5 X 10^{-8} s^{-1}, less than 10^{-8} s^{-1}, less than 5 X 10^{-9} s^{-1}, less than 10^{-9} s^{-1}, or less than 10^{-10} s^{-1}.

3.6.4. Cancer Cell Phenotype Inhibiting Agents

[00267] Agents of the invention may reduce (and preferably inhibit) cancer cell phenotypes such as colony formation in soft agar, tubular network formation in a three-dimensional basement membrane or extracellular matrix preparation, or hyperproliferation. EphA2 agents, PCDGF agents, and/or HAAH agents can also be agents that inhibit a cancer cell phenotype. One of skill in the art can assay candidate agents for their ability to inhibit such behavior. In one embodiment, the cancer cell phenotype is colony formation in soft agar. Metastatic tumor cells suspended in soft agar form colonies while benign tumors cells do not. Colony formation in soft agar can be assayed as described in Zelinski et al. (2001, *Cancer Res.* 61:2301-6). Agents to be assayed for inhibitory activity can be included in bottom and top agar solutions. Metastatic tumor cells can be suspended in soft agar and allowed to grow. Cancer cell phenotype inhibiting agents will inhibit colony formation. In addition to inhibiting cancer cell colony formation, cancer cell phenotype inhibiting agents may also cause a reduction or elimination of colonies when added to already established colonies of cancer cells by cell killing, *e.g.*, by necrosis or apoptosis. Methods for assaying for necrosis and apoptosis are well known in the art.

[00268] In another embodiment, the cancer cell phenotype is tubular network formation within a three-dimensional microenvironment, such as MATRIGEL™. Normally, cancer cells quickly assemble into tubular networks that progressively invade all throughout the MATRIGEL™. In the presence of a cancer cell phenotype inhibiting agent, cancer cells assemble into spherical structures that resemble the behavior of differentiated, non-cancerous cells. Accordingly, cancer cell phenotype inhibiting agents can be identified by their ability to inhibit tubular network formation of cancer cells.

[00269] In another embodiment, the cancer cell phenotype is hyperproliferation. Many assays well-known in the art can be used to assess survival and/or growth; for

example, cell proliferation can be assayed by measuring (^{3}H)-thymidine incorporation, by direct cell count, by detecting changes in transcription, translation or activity of known genes such as cell cycle markers (Rb, cdc2, cyclin A, D1, D2, D3, E, etc). The levels of such protein and mRNA and activity can be determined by any method well known in the art. For example, protein can be quantitated by known immunodiagnostic methods such as western blotting or immunoprecipitation using commercially available antibodies (for example, many cell cycle marker antibodies are from Santa Cruz Inc.). mRNA can be quantitated by methods that are well known and routine in the art, for example by northern analysis, RNase protection, the polymerase chain reaction in connection with the reverse transcription, etc. Cell viability can be assessed by using trypan-blue staining or other cell death or viability markers known in the art.

[00270] The present invention provides for cell cycle and cell proliferation analysis by a variety of techniques known in the art, including but not limited to the following:

[00271] As one example, bromodeoxyuridine (BRDU) incorporation may be used as an assay to identify proliferating cells. The BRDU assay identifies a cell population undergoing DNA synthesis by incorporation of BRDU into newly synthesized DNA. Newly synthesized DNA may then be detected using an anti-BRDU antibody (*see* Hoshino et al., 1986, *Int. J. Cancer* 38:369; Campana et al., 1988, *J. Immunol. Meth.* 107:79).

[00272] Cell proliferation may also be examined using (^{3}H)-thymidine incorporation (*see e.g.,* Chen, 1996, *Oncogene* 13:1395-403; Jeoung, 1995, J. *Biol. Chem.* 270:18367-73). This assay allows for quantitative characterization of S-phase DNA synthesis. In this assay, cells synthesizing DNA will incorporate (^{3}H)-thymidine into newly synthesized DNA. Incorporation may then be measured by standard techniques in the art such as by counting of radioisotope in a Scintillation counter (*e.g.* Beckman LS 3800 Liquid Scintillation Counter).

[00273] Detection of proliferating cell nuclear antigen (PCNA) may also be used to measure cell proliferation. PCNA is a 36 kilodalton protein whose expression is elevated in proliferating cells, particularly in early G1 and S phases of the cell cycle and therefore may serve as a marker for proliferating cells. Positive cells are identified by immunostaining using an anti-PCNA antibody (*see* Li et al., 1996, *Curr. Biol.* 6:189-99; Vassilev et al., 1995, *J. Cell Sci.* 108:1205-15).

[00274] Cell proliferation may be measured by counting samples of a cell population over time (*e.g.* daily cell counts). Cells may be counted using a hemacytometer and light microscopy (*e.g.* HyLite hemacytometer, Hausser Scientific). Cell number may be plotted

against time in order to obtain a growth curve for the population of interest. In a preferred embodiment, cells counted by this method are first mixed with the dye Trypan-blue (Sigma), such that living cells exclude the dye, and are counted as viable members of the population.

[00275] DNA content and/or mitotic index of the cells may be measured, for example, based on the DNA ploidy value of the cell. For example, cells in the G1 phase of the cell cycle generally contain a 2N DNA ploidy value. Cells in which DNA has been replicated but have not progressed through mitosis (*e.g.* cells in S-phase) will exhibit a ploidy value higher than 2N and up to 4N DNA content. Ploidy value and cell-cycle kinetics may be further measured using propidum iodide assay (*see e.g.* Turner, et al., 1998, *Prostate* 34:175-81). Alternatively, the DNA ploidy may be determined by quantitation of DNA Feulgen staining (which binds to DNA in a stoichiometric manner) on a computerized microdensitometrystaining system (*see e.g.,* Bacus, 1989, *Am. J. Pathol.*135:783-92). In an another embodiment, DNA content may be analyzed by preparation of a chromosomal spread (Zabalou, 1994, *Hereditas.*120:127-40; Pardue, 1994, *Meth. Cell Biol.* 44:333-351).

[00276] The expression of cell-cycle proteins (e.g., CycA. CycB, CycE, CycD, cdc2, Cdk4/6, Rb, p21, p27, etc.) provide crucial information relating to the proliferative state of a cell or population of cells. For example, identification in an anti-proliferation signaling pathway may be indicated by the induction of $p21^{cip1}$. Increased levels of p21 expression in cells results in delayed entry into G1 of the cell cycle (Harper et al., 1993, *Cell* 75:805-816; Li et al., 1996, *Curr. Biol.* 6:189-199). p21 induction may be identified by immunostaining using a specific anti-p21 antibody available commercially (*e.g.* Santa Cruz). Similarly, cell-cycle proteins may be examined by western blot analysis using commercially available antibodies. In another embodiment, cell populations are synchronized prior to detection of a cell cycle protein. Cell cycle proteins may also be detected by FACS (fluorescence-activated cell sorter) analysis using antibodies against the protein of interest.

[00277] EphA2, PCDGF, or HAAH agents for use in the methods of the invention can also be identified by their ability to change the length of the cell cycle or speed of cell cycle so that cell proliferation is decreased or inhibited. In one embodiment the length of the cell cycle is determined by the doubling time of a population of cells (*e.g.*, using cells contacted or not contacted with one or more candidate EphA2, PCDGF, or HAAH agents). In another embodiment, FACS analysis is used to analyze the phase of cell cycle progression, or purify G1, S, and G2/M fractions (*see e.g.,* Delia et al., 1997, *Oncogene* 14:2137-47).

3.7. Characterization And Demonstration Of Therapeutic Or Prophylactic Utility

[00278] Toxicity and efficacy of the prophylactic and/or therapeutic protocols of the instant invention can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD_{50}/ED_{50}. Prophylactic and/or therapeutic agents that exhibit large therapeutic indices are preferred. While prophylactic and/or therapeutic agents that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such agents to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

[00279] The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage of the prophylactic and/or therapeutic agents for use in humans. The dosage of such agents lies preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any agent used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC_{50} (*i.e.*, the concentration of the test compound that achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

[00280] The anti-cancer activity of the therapies used in accordance with the present invention also can be determined by using various experimental animal models for the study of cancer such as the SCID mouse model or transgenic mice where a mouse gene of interest (*e.g.*, EphA2, PCDGF, PCDGF receptor, and/ HAAH) is replaced with the corresponding human gene or portion thereof, nude mice with human xenografts, animal models described in Section 6 *infra*, or any animal model (including hamsters, rabbits, etc.) known in the art and described in *Relevance of Tumor Models for Anticancer Drug Development* (1999, eds. Fiebig and Burger); *Contributions to Oncology* (1999, Karger); *The Nude Mouse in Oncology Research* (1991, eds. Boven and Winograd); and *Anticancer*

Drug Development Guide (1997 ed. Teicher), incorporated by reference in their entireties herein.

3.8. Demonstration Of Prophylactic/Therapeutic Utility

[00281] The protocols and compositions of the invention are preferably tested *in vitro*, and then *in vivo*, for the desired therapeutic or prophylactic activity, prior to use in humans. For example, *in vitro* assays which can be used to determine whether administration of a specific therapeutic protocol is indicated, include *in vitro* cell culture assays in which a patient tissue sample is grown in culture, and exposed to or otherwise administered a protocol, and the effect of such protocol upon the tissue sample is observed, *e.g.*, decreased expression and/or activity of the polypeptide of interest (*e.g.*, EphA2, PCDGF, PCDGF receptor and/or HAAH). A lower level of proliferation or survival of the contacted cells indicates that the therapeutic agent is effective to treat the condition in the patient. Alternatively, instead of culturing cells from a patient, therapeutic agents and methods may be screened using cells of a tumor or malignant cell line. Many assays standard in the art can be used to assess such survival and/or growth; for example, cell proliferation can be assayed by measuring ^{3}H-thymidine incorporation, by direct cell count, by detecting changes in transcriptional activity of known genes such as proto-oncogenes (*e.g.*, fos, myc) or cell cycle markers; cell viability can be assessed by trypan blue staining, differentiation can be assessed visually based on changes in morphology, decreased expression and/or activity of the polypeptide of interest (*e.g.*, EphA2, PCDGF, PCDGF receptor, and/or HAAH), decreased growth and/or colony formation in soft agar or tubular network formation in three-dimensional basement membrane or extracellular matrix preparation, etc.

[00282] Agents for use in therapy can be tested in suitable animal model systems prior to testing in humans, including but not limited to in rats, mice, chicken, cows, monkeys, rabbits, hamsters, etc., for example, the animal models described above. The agents can then be used in the appropriate clinical trials.

[00283] Further, any assays known to those skilled in the art can be used to evaluate the prophylactic and/or therapeutic utility of the combinatorial therapies disclosed herein for treatment or prevention of cancer.

3.9. PHARMACEUTICAL COMPOSITIONS

[00284] The compositions of the invention include bulk drug compositions useful in the manufacture of pharmaceutical compositions (*e.g.*, impure or non-sterile compositions) and pharmaceutical compositions (*i.e.*, compositions that are suitable for administration to a subject or patient) which can be used in the preparation of unit dosage forms. Such compositions comprise a prophylactically or therapeutically effective amount of a prophylactic and/or therapeutic agent disclosed herein or a combination of those agents and a pharmaceutically acceptable carrier. Preferably, compositions of the invention comprise a prophylactically or therapeutically effective amount of one or more EphA2 agents of the invention in combination with one or more PCDGF agents of the invention and/or one or more HAAH agents of the invention and a pharmaceutically acceptable carrier. In a further embodiment, the composition of the invention further comprises an additional cancer therapeutic that is not EphA2-, PCDGF-, or HAAH-based.

[00285] In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant (*e.g.*, Freund's adjuvant (complete and incomplete) or, more preferably, MF59C.1 adjuvant available from Chiron, Emeryville, CA), excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like.

[00286] Generally, the ingredients of compositions of the invention are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or

sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

[00287] The compositions of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

[00288] Various delivery systems are known and can be used to administer an agonistic monoclonal antibody of the invention or the combination of an agonistic monoclonal antibody of the invention and a prophylactic agent or therapeutic agent useful for preventing or treating cancer, *e.g.*, encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the antibody or antibody fragment, receptor-mediated endocytosis (see, *e.g.*, Wu and Wu, 1987, *J. Biol. Chem.* 262:4429-4432), construction of a nucleic acid as part of a retroviral or other vector, etc. Methods of administering a prophylactic or therapeutic agent of the invention include, but are not limited to, parenteral administration (*e.g.*, intradermal, intramuscular, intraperitoneal, intravenous and subcutaneous), epidural, and mucosal (*e.g.*, intranasal, inhaled, and oral routes). In a specific embodiment, prophylactic or therapeutic agents of the invention are administered intramuscularly, intravenously, or subcutaneously. The prophylactic or therapeutic agents may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.*, oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local.

[00289] In a specific embodiment, it may be desirable to administer the prophylactic or therapeutic agents of the invention locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion, by injection, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers.

[00290] In yet another embodiment, the prophylactic or therapeutic agent can be delivered in a controlled release or sustained release system. In one embodiment, a pump may be used to achieve controlled or sustained release (see Langer, *supra*; Sefton, 1987,

CRC Crit. Ref. Biomed. Eng. 14:20; Buchwald et al., 1980, *Surgery* 88:507; Saudek et al., 1989, *N. Engl. J. Med.* 321:574). In another embodiment, polymeric materials can be used to achieve controlled or sustained release of the antibodies of the invention or fragments thereof (see *e.g.*, Medical Applications of Controlled Release, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida (1974); Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, 1983, *J. Macromol. Sci. Rev. Macromol. Chem.* 23:61; see also Levy et al., 1985, *Science* 228:190; During et al., 1989, *Ann. Neurol.* 25:351; Howard et al., 1989, *J. Neurosurg.* 7 1:105); U.S. Patent Nos. 5,679,377; 5,916,597; 5,912,015; 5,989,463; 5,128,326; International Publication Nos. WO 99/15154 and WO 99/20253. Examples of polymers used in sustained release formulations include, but are not limited to, poly(2-hydroxy ethyl methacrylate), poly(methyl methacrylate), poly(acrylic acid), poly(ethylene-co-vinyl acetate), poly(methacrylic acid), polyglycolides (PLG), polyanhydrides, poly(N-vinyl pyrrolidone), poly(vinyl alcohol), polyacrylamide, poly(ethylene glycol), polylactides (PLA), poly(lactide-co-glycolides) (PLGA), and polyorthoesters. In a preferred embodiment, the polymer used in a sustained release formulation is inert, free of leachable impurities, stable on storage, sterile, and biodegradable. In yet another embodiment, a controlled or sustained release system can be placed in proximity of the prophylactic or therapeutic target, thus requiring only a fraction of the systemic dose (see, *e.g.*, Goodson, in Medical Applications of Controlled Release, supra, vol. 2, pp. 115-138 (1984)).

[00291] Controlled release systems are discussed in the review by Langer (1990, Science 249:1527-1533). Any technique known to one of skill in the art can be used to produce sustained release formulations comprising one or more therapeutic agents of the invention. See, *e.g.*, U.S. Patent No. 4,526,938; International Publication Nos. WO 91/05548 and WO 96/20698; Ning et al., 1996, *Radiotherapy & Oncology* 39:179-189; Song et al., 1995, *PDA Journal of Pharmaceutical Science & Technology* 50:372-397; Cleek et al., 1997, *Pro. Int'l. Symp. Control. Rel. Bioact. Mater.* 24:853-854; and Lam et al., 1997, *Proc. Int'l. Symp. Control Rel. Bioact. Mater.* 24:759-760.

3.9.1. Gene Therapy

[00292] In a specific embodiment, nucleic acids (*e.g.*, antisense nucleic acids specific for EphA2, double stranded EphA2 RNA that mediates RNAi, anti-EphA2 ribozymes, nucleotide encoding an EphA2 intrabody, antisense nucleic acids specific for PCDGF, double stranded PCDGF RNA that mediates RNAi, anti-PCDGF ribozymes, nucleotide

encoding a PCDGF intrabody, antisense nucleic acids specific for PCDGF receptor, double stranded PCDGF receptor RNA that mediates RNAi, anti-PCDGF receptor ribozymes, nucleotide encoding a PCDGF receptor intrabody, antisense nucleic acids specific for HAAH, double stranded HAAH RNA that mediates RNAi, anti-HAAH ribozymes, nucleotide encoding an HAAH intrabody etc.) that reduce expression of a polypeptide of interest (*e.g.*, EphA2, PCDGF, PCDGF receptor and/or HAAH) are administered to treat, prevent or manage cancer by way of gene therapy. Gene therapy refers to therapy performed by the administration to a subject of an expressed or expressible nucleic acid. In this embodiment of the invention, the antisense nucleic acids are produce and mediate a prophylactic or therapeutic effect.

[00293] Any of the methods for gene therapy available in the art can be used according to the present invention. Exemplary methods are described below.

[00294] For general reviews of the methods of gene therapy, see Goldspiel et al., 1993, *Clinical Pharmacy* 12:488; Wu and Wu, 1991, *Biotherapy* 3:87; Tolstoshev, 1993, *Ann. Rev. Pharmacol. Toxicol.* 32:573; Mulligan, 1993, *Science* 260:926-932; and Morgan and Anderson, 1993, *Ann. Rev. Biochem.* 62:191; May, 1993, *TIBTECH* 11:155. Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel et al. (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, NY (1993); and Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990).

[00295] In a preferred aspect, a composition of the invention comprises nucleic acid agents for use in the methods of the invention, said nucleic acids being part of an expression vector that expresses the nucleic acid in a suitable host. In particular, such nucleic acids have promoters, preferably heterologous promoters, said promoter being inducible or constitutive, and, optionally, tissue-specific. In another particular embodiment, nucleic acid molecules are used in which the nucleic acid agent is flanked by regions that promote homologous recombination at a desired site in the genome, thus providing for intrachromosomal expression of the nucleic acids that reduce EphA2, PCDGF, PCDGF receptor and/or HAAH expression (Koller and Smithies, 1989, *PNAS* 86:8932; Zijlstra et al., 1989, *Nature* 342:435).

[00296] Delivery of the nucleic acids into a subject may be either direct, in which case the subject is directly exposed to the nucleic acid or nucleic acid-carrying vectors, or indirect, in which case, cells are first transformed with the nucleic acids in vitro, then transplanted into the subject. These two approaches are known, respectively, as *in vivo* or

ex vivo gene therapy. In a specific embodiment, the nucleic acid sequences are directly administered *in vivo*. This can be accomplished by any of numerous methods known in the art, *e.g.*, by constructing them as part of an appropriate nucleic acid expression vector and administering it so that they become intracellular, *e.g.*, by infection using defective or attenuated retrovirals or other viral vectors (see U.S. Patent No. 4,980,286), or by direct injection of naked DNA, or by use of microparticle bombardment (*e.g.*, a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, encapsulation in liposomes, microparticles, or microcapsules, or by administering them in linkage to a peptide which is known to enter the nucleus, by administering it in linkage to a ligand subject to receptor-mediated endocytosis (see, *e.g.*, Wu and Wu, 1987, J. *Biol. Chem.* 262:4429) (which can be used to target cell types specifically expressing the receptors), etc. In another embodiment, nucleic acid-ligand complexes can be formed in which the ligand comprises a fusogenic viral peptide to disrupt endosomes, allowing the nucleic acid to avoid lysosomal degradation. In yet another embodiment, the nucleic acid can be targeted *in vivo* for cell specific uptake and expression, by targeting a specific receptor (see, *e.g.*, International Publication Nos. WO 92/06180; WO 92/22635; W092/203 16; W093/14188, WO 93/20221). Alternatively, the nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination (Koller and Smithies, 1989, *PNAS* 86:8932; and Zijlstra et al., 1989, *Nature* 342:435).

[00297] In a specific embodiment, viral vectors that contain the nucleic acid sequences that reduce EphA2 expression are used. For example, a retroviral vector can be used (see Miller et al., 1993, *Meth. Enzymol.* 217:581). These retroviral vectors contain the components necessary for the correct packaging of the viral genome and integration into the host cell DNA. The nucleic acid sequences to be used in gene therapy are cloned into one or more vectors, which facilitates delivery of the nucleic acid into a subject. More detail about retroviral vectors can be found in Boesen et al., 1994, *Biotherapy* 6:291-302, which describes the use of a retroviral vector to deliver the mdr 1 gene to hematopoietic stem cells in order to make the stem cells more resistant to chemotherapy. Other references illustrating the use of retroviral vectors in gene therapy are: Clowes et al., 1994, *J. Clin. Invest.* 93:644-651; Klein et al., 1994, *Blood* 83:1467-1473; Salmons and Gunzberg, 1993, *Human Gene Therapy* 4:129-141; and Grossman and Wilson, 1993, *Curr. Opin. in Genetics Devel.* 3:110-114.

[00298] Adenoviruses are other viral vectors that can be used in gene therapy. Adenoviruses are especially attractive vehicles for delivering genes to respiratory epithelia.

Adenoviruses naturally infect respiratory epithelia where they cause a mild disease. Adenoviruses have the advantage of being capable of infecting non-dividing cells. Kozarsky and Wilson, 1993, *Current Opinion in Genetics Development* 3:499 present a review of adenovirus-based gene therapy. Bout et al., 1994, *Human Gene Therapy* 5:3-10 demonstrated the use of adenovirus vectors to transfer genes to the respiratory epithelia of rhesus monkeys. Other instances of the use of adenoviruses in gene therapy can be found in Rosenfeld et al., 1991, *Science* 252:431; Rosenfeld et al., 1992, *Cell* 68:143; Mastrangeli et al., 1993, *J. Clin. Invest.* 91:225; International Publication No. W094/12649; and Wang et al., 1995, *Gene Therapy* 2:775. In a preferred embodiment, adenovirus vectors are used.

[00299] Adeno-associated virus (AAV) has also been proposed for use in gene therapy (Walsh et al., 1993, *Proc. Soc. Exp. Biol. Med.* 204:289-300; and U.S. Patent No. 5,436,146).

[00300] Another approach to gene therapy involves transferring a gene to cells in tissue culture by such methods as electroporation, lipofection, calcium phosphate mediated transfection, or viral infection. Usually, the method of transfer includes the transfer of a selectable marker to the cells. The cells are then placed under selection to isolate those cells that have taken up and are expressing the transferred gene. Those cells are then delivered to a subject.

[00301] In this embodiment, the nucleic acid is introduced into a cell prior to administration *in vivo* of the resulting recombinant cell. Such introduction can be carried out by any method known in the art, including but not limited to transfection, electroporation, microinjection, infection with a viral or bacteriophage vector containing the nucleic acid sequences, cell fusion, chromosome-mediated gene transfer, microcell mediated gene transfer, spheroplast fusion, etc. Numerous techniques are known in the art for the introduction of foreign genes into cells (see, *e.g.*, Loeffler and Behr, 1993, *Meth. Enzymol.* 217:599; Cohen et al., 1993, *Meth. Enzymol.* 217:618) and may be used in accordance with the present invention, provided that the necessary developmental and physiological functions of the recipient cells are not disrupted. The technique should provide for the stable transfer of the nucleic acid to the cell, so that the nucleic acid is expressible by the cell and preferably heritable and expressible by its cell progeny.

[00302] The resulting recombinant cells can be delivered to a subject by various methods known in the art. The amount of cells envisioned for use depends on the desired effect, patient state, etc., and can be determined by one skilled in the art.

3.9.2. Formulations

[00303] Pharmaceutical compositions for use in accordance with the present invention may be formulated in conventional manner using one or more physiologically acceptable carriers or excipients.

[00304] Thus, the agents of the invention and their physiologically acceptable salts and solvates may be formulated for administration by inhalation or insufflation (either through the mouth or the nose) or oral, parenteral or mucosal (such as buccal, vaginal, rectal, sublingual) administration. In a preferred embodiment, local or systemic parenteral administration is used.

[00305] For oral administration, the pharmaceutical compositions may take the form of, for example, tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (*e.g.*, pregelatinised maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (*e.g.*, lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (*e.g.*, magnesium stearate, talc or silica); disintegrants (*e.g.*, potato starch or sodium starch glycolate); or wetting agents (*e.g.*, sodium lauryl sulphate). The tablets may be coated by methods well known in the art. Liquid preparations for oral administration may take the form of, for example, solutions, syrups or suspensions, or they may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (*e.g.*, sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (*e.g.*, lecithin or acacia); non-aqueous vehicles (*e.g.*, almond oil, oily esters, ethyl alcohol or fractionated vegetable oils); and preservatives (*e.g.*, methyl or propyl-p-hydroxybenzoates or sorbic acid). The preparations may also contain buffer salts, flavoring, coloring and sweetening agents as appropriate.

[00306] Preparations for oral administration may be suitably formulated to give controlled release of the active compound.

[00307] For buccal administration the compositions may take the form of tablets or lozenges formulated in conventional manner.

[00308] For administration by inhalation, the prophylactic or therapeutic agents for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, *e.g.*, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized

aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of *e.g.*, gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

[00309] The prophylactic or therapeutic agents may be formulated for parenteral administration by injection, *e.g.*, by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, *e.g.*, in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, *e.g.*, sterile pyrogen-free water, before use.

[00310] The prophylactic or therapeutic agents may also be formulated in rectal compositions such as suppositories or retention enemas, *e.g.*, containing conventional suppository bases such as cocoa butter or other glycerides.

[00311] In addition to the formulations described previously, the prophylactic or therapeutic agents may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the prophylactic or therapeutic agents may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

[00312] The invention also provides that a prophylactic or therapeutic agent is packaged in a hermetically sealed container such as an ampoule or sachette indicating the quantity. In one embodiment, the prophylactic or therapeutic agent is supplied as a dry sterilized lyophilized powder or water free concentrate in a hermetically sealed container and can be reconstituted, *e.g.*, with water or saline to the appropriate concentration for administration to a subject.

[00313] In a preferred embodiment of the invention, the formulation and administration of various cancer therapies that are not EphA2-, PCDGF-, or HAAH- based (such as chemotherapeutic, biological/immunotherapeutic and hormonal therapeutic agents) are known in the art and often described in the *Physician's Desk Reference, 56th ed.* (2002).

[00314] In other embodiments of the invention, radiation therapy agents such as radioactive isotopes can be given orally as liquids in capsules or as a drink. Radioactive

isotopes can also be formulated for intravenous injections. The skilled oncologist can determine the preferred formulation and route of administration.

[00315] In certain embodiments the agents of the invention, are formulated at 1 mg/ml, 5 mg/ml, 10 mg/ml, and 25 mg/ml for intravenous injections and at 5 mg/ml, 10 mg/ml, and 80 mg/ml for repeated subcutaneous administration and intramuscular injection.

[00316] The compositions may, if desired, be presented in a pack or dispenser device that may contain one or more unit dosage forms containing the active ingredient. The pack may for example comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration.

3.9.3. Dosages

[00317] The amount of the composition of the invention which will be effective in the treatment, prevention or management of cancer can be determined by standard research techniques. For example, the dosage of the composition which will be effective in the treatment, prevention or management of cancer can be determined by administering the composition to an animal model such as, *e.g.*, the animal models disclosed herein or known to those skilled in the art. In addition, *in vitro* assays may optionally be employed to help identify optimal dosage ranges.

[00318] Selection of the preferred effective dose can be determined (*e.g.*, via clinical trials) by a skilled artisan based upon the consideration of several factors which will be known to one of ordinary skill in the art. Such factors include the disease to be treated or prevented, the symptoms involved, the patient's body mass, the patient's immune status and other factors known by the skilled artisan to reflect the accuracy of administered pharmaceutical compositions.

[00319] The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the cancer, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model test systems.

[00320] For antibodies, the dosage administered to a patient is typically 0.1 mg/kg to 100 mg/kg of the patient's body weight. Preferably, the dosage administered to a patient is between 0.1 mg/kg and 20 mg/kg of the patient's body weight, more preferably 1 mg/kg to 10 mg/kg of the patient's body weight. Generally, human and humanized antibodies have a longer half-life within the human body than antibodies from other species due to the

immune response to the foreign polypeptides. Thus, lower dosages of human antibodies and less frequent administration is often possible.

[00321] For cancer therapeutic agents that are not EpA2-, PCDGF-, or HAAH-based, the typical doses of various cancer therapeutics are known in the art. Given the invention, certain preferred embodiments will encompass the administration of lower dosages in combination treatment regimens than dosages recommended for the administration of single agents.

[00322] The invention provides for any method of administrating lower doses of known prophylactic or therapeutic agents than previously thought to be effective for the prevention, treatment, management or amelioration of cancer. Preferably, lower doses of known anti-cancer therapies are administered in combination with agents of the invention.

3.10. KITS

[00323] The invention provides a pharmaceutical pack or kit comprising one or more containers filled with agents of the invention. In some embodiments, the EphA2 agent is in a container that is separate from the PCDGF or HAAH agent. Additionally, one or more prophylactic or therapeutic agent useful for the treatment of a cancer that is not EphA2-, PCDGF-, or HAAH-based can also be included in the pharmaceutical pack or kit. The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

4. EXAMPLES

4.1. Preparation Of Monoclonal Antibodies

Immunization and Fusion

[00324] Monoclonal antibodies against the extracellular domain of EphA2 were generated using the fusion protein EphA2-Fc. This fusion protein consisted of the extracellular domain of human EphA2 linked to human immunoglobulin Fc domain to facilitate secretion of the fusion protein.

[00325] Two groups of 5 mice each (either Balb/c mice (group A) or SJL mice (group B)) were injected with 5 μg of EphA2-Fc in TiterMax Adjuvant (total volume 100μl)

in the left metatarsal region at days 0 and 7. Mice were injected with 10 μg of EphA2-Fc in PBS (total volume 100μl) in the left metatarsal region at days 12 and 14. On day 15, the popliteal and inguinal lymph nodes from the left leg and groin were removed and somatically fused (using PEG) with P3XBcl-2-13 cells.

[00326] Hybridomas producing Eph099B-102.147, Eph099B-208.261, Eph099B-210.248, and Eph099B-233.152 antibodies were isolated from fusions of lymph nodes from immunized SJL mice (see co-pending US Patent Application Serial No. 10/436,782, filed May 12, 2003).

Antibody Screening

[00327] Supernatants from bulk culture hybridomas were screened for immunoreactivity against EphA2 (Table 6, column 4) using standard molecular biological techniques (*e.g.*, ELISA immunoassay). Supernatants were further screened for the ability to inhibit an EphA2 monoclonal antibody (EA2; ATCC deposit no. PTA-4380; see co-pending US Patent Application Serial No. _, entitled "EphA2 Agonistic Monoclonal Antibodies and Methods of Use Thereof" filed May 12, 2003 as Attorney Docket No. 10271-107-999) from binding to EphA2. Briefly, the ability of labeled EA2 to bind EphA2-Fc was assayed by competitive ELISA in presence of either unlabeled EA2 or unlabeled Eph099B-208.261 (FIG. 1). Both antibodies could decrease the amount of labeled EA2 binding to EphA2-Fc with increasing concentrations of unlabeled antibody added. Additionally, many of the other antibodies could inhibit EA2 binding to EphA2 as well (Table 6, column 3).

[00328] Monoclonal antibodies to PCDGF or HAAH are made using the same techniques.

4.2. Epha2 Monoclonal Antibodies Decrease Metastatic Properties Of Tumor Cells

4.2.1. EphA2 Phosphorylation and Degradation

[00329] EphA2 antibodies promoted tyrosine phosphorylation and degradation of EphA2 in MDA-MB-231 cells. Monolayers of cells were incubated in the presence of EphA2 antibodies or control at 37°C. Cell lysates were then immunoprecipitated with an EphA2-specific antibody (D7, purchased from Upstate Biologicals, Inc., Lake Placid, NY and deposited with the American Type Tissue Collection on December 8, 2000, and

assigned ATCC number PTA 2755), resolved by SDS-PAGE and subjected to western blot analysis with a phosphotyrosine-specific antibody (PY20 or 4G10, purchased from Upstate Biologicals, Inc., Lake Placid, NY). The membranes were stripped and re-probed with the EphA2-specific antibody used in the immunoprecipitation (D7) as a loading control. Both Eph099B-208.261 and EA2 increased EphA2 phosphorylation (FIG. 2). Additionally, other EphA2 antibodies of the invention were also found to increase EphA2 phosphorylation (Table 5, column 5) including Eph099B-102.147 and Eph099B-210.248 (data not shown).

[00330] Monolayers of MDA-MB-231 cells were incubated in the presence of Eph099B-208.261 EA2 at 37°C. Cell lysates were then resolved by SDS-PAGE and subjected to western blot analysis with a EphA2-specific antibody (D7). The membranes were stripped and re-probed with a β-catenin-specific antibody as a loading control. Both Eph099B-208.261 and EA2 decreased EphA2 protein level (FIG. 3). Additionally, other EphA2 antibodies of the invention were also found to decrease EphA2 protein levels (Table 5, columns 6 and 7) including Eph099B-102.147 and Eph099B-210.248 (data not shown). Decreased EphA2 expression is due, in part, to deceased mRNA expression levels in response to EphA2 protein degradation caused by agonistic antibody binding (data not shown).

[00331] Western blot analyses and immunoprecipitations were performed as described previously (Zantek et al., 1999, *Cell Growth Diff.* 10:629-38). Briefly, detergent extracts of cell monolayers were extracted in Tris-buffered saline containing 1% Triton X-100 (Sigma, St. Louis, MO). After measuring protein concentrations (BioRad, Hercules, CA), 1.5 mg of cell lysate was immunoprecipitated, resolved by SDS-PAGE and transferred to nitrocellulose (Protran, Schleicher and Schuell, Keene, NH). Antibody binding was detected by enhanced chemiluminescence (Pierce, Rockford, IL) and autoradiography (Kodak X-OMAT; Rochester, NY).

4.2.2. Growth in Soft Agar

[00332] The ability of the antibodies of the invention to inhibit cancer cell formation in soft agar was assayed as described in Zelinski et al. (2001, *Cancer Res.* 61:2301-6). Briefly, cells were suspended in soft agar for 7 days at 37°C in the presence of purified antibody or control solution (PBS). Antibodies were administered at the time of suspension in both bottom and top agar solutions. Colony formation was scored microscopically using an Olympus CK-3 inverted phase-contrast microscope outfitted with a 40x objective. Clusters containing at least three cells were scored as a positive. Both Eph099B-208.261

and EA2 inhibited colony growth in soft agar (FIG. 4). Additionally, other antibodies of the invention can inhibit colony formation in soft agar (Table 6, column 9) including Eph099B-102.147 and Eph099B-210.248 (data not shown).

[00333] The ability of the antibodies of the invention to eliminate cancer cell colonies already formed in soft agar was assayed. Assay methods were similar to those described above except that antibodies were not added to the cancer cells until the third day of growth in soft agar. Some of the antibodies of the invention can kill cancer cells already growing in colonies in soft agar while other antibodies can slow or reduce cancer cell colony growth in soft agar (Table 6, column 10) including Eph099B-102.147, Eph099B-208.261, Eph099B-210.248, and Eph099B-233.152 (data not shown).

4.2.3. Tubular Network Formation in MATRIGEL™

[00334] Tumor cell behavior within a three-dimensional microenvironment, such as MATRIGEL™, can reliably predict the differentiation state and aggressiveness of breast epithelial cells. Monolayer cultures of benign (MCF-10A) or malignant (MDA-MB-231) breast epithelial cells are incubated on MATRIGEL™ in the presence of EphA2 antibodies (10 μg/ml) or control solution (PBS). The behavior of cells on MATRIGEL™ is analyzed as described in Zelinski et al. (2001, *Cancer Res.* 61:2301-6). Briefly, tissue culture dishes are coated with MATRIGEL™ (Collaborative Biomedical Products, Bedford, MA) at 37°C before adding 1 x 10^5 MDA-MB-231 or MCF-10A cells previously incubated on ice for 1 hour with the EphA2 antibody or control solution (PBS). Cells are incubated on MATRIGEL™ for 24 hours at 37°C, and cell behavior is assessed using an Olympus IX-70 inverted light microscope. All images are recorded onto 35 mm film (T-Max-400. Kodak, Rochester, NY).

4.2.4. Growth in vivo

[00335] The ability of the antibodies of the invention to inhibit tumor cancer growth *in vivo* was assayed. Eph099B-233.152 can inhibit tumor cell growth *in vivo* and extend survival time of tumor-bearing mice. Briefly, 5x10^6 MDA-MB-231 breast cancer cells were implanted subcutaneously into athymic mice. After the tumors had grown to an average volume of 100mm^3, mice were administered 6mg/ml Eph099B-233.152 or PBS control intraperitoneally twice a week for 3 weeks. Tumor growth was assessed and expressed as a ratio of the tumor volume divided by initial tumor volume (100 mm^3). After 30 days, mice administered Eph099B-233.152 had smaller tumors than mice administered

PBS (FIG. 5A). Tumor growth was allowed to proceed until tumor volume reached 1000mm^3. Survival of the mice was assessed by scoring the percent of mice living each day post treatment. A greater percentage of mice survived at each time point examined in the group administered Eph099B-233.152 (FIG. 5B). By day 36, all of the mice in the control group had died in contrast with only 70% of the mice admixture Eph099B-233.152.

[00336] Additionally, EA2 and Eph099B-208.261 can also inhibit tumor cell growth *in vivo*. 5x10^6 MDA-MB-231 breast cancer cells were implanted orthotopically or subcutaneously and 5x10^6 A549 lung cancer cells were implanted subcutaneously into athymic mice. After the tumors had grown to an average volume of 100mm^3, mice were administered 6mg/kg of an EphA2 agonistic antibody or negative control (PBS or 1A7 antibody) intraperitoneally twice a week for 3 weeks. Animals were generally sacrificed at least two weeks after the last treatment or when tumors exceeded 2000 mm^3. Tumor growth was assessed and expressed either as a ratio of the tumor volume divided by initial tumor volume (100 mm^3) or as total tumor volume. Growth of MDA-MB-231 cells implanted orthotopically was inhibited by EA2 (FIG. 6A). Growth of MDA-MB-231 cells implanted subcutaneously was inhibited by EA2, Eph099B-208.261, and Eph099B-233.152 (FIG. 6B, D). Growth of A549 cells implanted subcutaneously was inhibited by EA2, or Eph099B-208.261 (FIG. 6C).

4.3. Estrogen Dependence In Breast Cancer Cells

[00337] Estrogen-sensitive breast cancer cells, MCF-7 cells, were transfected with and stably overexpressed human EphA2 (MCF-7^{EphA2}) (pNeoMSV-EphA2 provided by Dr. T. Hunter, Scripps Institute). Western blot analyses confirmed the ectopic overexpression of EphA2 in transfected cells relative to matched controls.

[00338] EphA2 overexpression increased malignant growth (FIGS. 7A-7B). Growth assays were conducted as follows. MCF-7^{neo} (control cells) or MCF7^{EphA2} cells were seeded in 96-well plates. Cell growth was measured with Alamar blue (Biosource International, Camarillo, CA) following the manufacture's suggestion. Colony formation in soft agar was performed as previously described (Zelinski et al., 2001, *Cancer Res.* 61:2301-6) and scored microscopically, defining clusters of at least three cells as a positive. The data demonstrated that represent the average of ten separate high-power microscopic fields from each sample and representative of at least three separate experiments. Error bars represent the standard error of the mean of at least three different experiments as determined using Microsoft Excel software.

[00339] Although MCF-7 control cells were largely unable to colonize soft agar (an average of 0.1 colony/field), MCF-7^{EphA2} cells formed larger and more numerous colonies (4.7 colonies/field; $P < 0.01$) that persisted for at least three weeks (FIG. 7A and data not shown). Despite increased colonization of soft agar, the growth of MCF-7^{EphA2} cells in monolayer culture did not differ from matched controls (FIG. 7B), thus indicating that the growth promoting activities of EphA2 were most apparent using experimental conditions that model anchorage-independent (malignant) cell growth.

[00340] Consistent with increased soft agar colonization, orthotopically implanted MCF-7^{EphA2} cells formed larger, more rapidly growing tumors *in vivo*. Six to eight week-old athymic (*nu/nu*) mice were purchased from Harlan Sprague Dawley (Indianapolis, IN). When indicated, a controlled release estradiol pellet (0.72 mg 17β-estradiol, 60-day formulation) was injected subcutaneously via a sterile 14-gauge trocar 24 hours prior to tumor implantation and pellets were replaced every 60 days for those experiments spanning > 60 days in duration. $1x10^6$ MCF-7^{neo} or MCF7^{EphA2} cells were injected into the mammary fat pad under direct visualization. When indicated, tamoxifen (1 mg) was administered by oral gavage 6 days per week.

[00341] In the presence of supplemental estrogen (17β-estradiol purchased from Sigma), the MCF-7^{EphA2} cells demonstrated a two-fold increase in tumor volume relative to matched controls (FIG. 8A). EphA2-overexpressing tumors differed phenotypically from control tumors in that they were more vascular and locally invasive at the time of resection (data not shown). To confirm that these tumors expressed EphA2, whole cell lysates of resected tumors were subjected to western blot analyses with EphA2-specific antibodies (FIG. 8B). The membranes were then stripped and reprobed with β-catenin antibodies to verify equal sample loading. The relative amount of EphA2 was higher in tumor samples than in the input cells (prior to implantation), suggesting that tumors arose from cells with high levels of EphA2. Comparable findings with *in vitro* and *in vivo* models indicate that EphA2 overexpression results in a more aggressive phenotype.

[00342] Parallel studies were performed in the absence of exogenous estrogen. Experimental deprivation of estrogen amplified differences between the cellular behaviors of control and MCF-7^{EphA2} cells. While MCF-7^{EphA2} cells continued to colonize soft agar more efficiently than matched controls (FIG. 9A), these cells did grow in the absence of exogenous estrogen (FIG. 9B). In contrast, supplemental estrogen was required for monolayer growth of control cells (FIG. 9B). Additionally, MCF-7^{EphA2} cells retained

tumorigenic potential in the absence of supplemental estrogen. While control MCF-7 cells rarely formed palpable tumors, the MCF-7^{EphA2} cells formed tumors that persisted for over 12 weeks (FIG. 9C and data not shown). Thus, both *in vitro* and *in vivo* assay systems confirm that EphA2 overexpression decreases the need for exogenous estrogen.

[00343] Sensitivity of MCF-7^{EphA2} cells to tamoxifen was measured. Tamoxifen (4-hydroxy tamoxifen purchased from Sigma) reduced soft agar colonization of control MCF-7 cells by at least 60%. The inhibitory actions of tamoxifen on MCF-7^{EphA2} cells were less pronounced (25% inhibition, FIG. 10A). Notably, excess estradiol overcame the inhibitory effects of tamoxifen, which provided additional evidence for the specificity of this finding (FIG. 10A). Similarly, the tumorigenic potential of MCF-7^{EphA2} cells was less sensitive to tamoxifen as compared with control (MCF-7^{neo}) cells (FIG. 10B).

[00344] Since tamoxifen sensitivity often relates to estrogen receptor expression, estrogen receptor expression and activity was assayed in MCF-7^{EphA2}. Western blot analyses revealed comparable levels of ERα and ERβ in control and MCF-7^{EphA2} cells (FIGS. 11A-11B) (ERα and ERβ antibodies were purchased from Chemicon, Temecula, CA). Moreover, comparable levels of estrogen receptor activity were detected in control and MCF-7^{EphA2} cells and this enzymatic activity remained sensitive to tamoxifen (FIGS. 11E-11F). Estrogen receptor activity was measured using ERE-TK-CAT vector (which encodes a single ERE; a generous gift from Dr. Nakshatri, Indiana University School of Medicine) in the unstimulated state, after estradiol (10^{-8} M) stimulation and tamoxifen (10^{-6} M) inhibition. Cells were plated in phenol red free, charcoal stripped sera for 2 days and transfected with ERE-TK-CAT (5 μg) using calcium phosphate method. The β-galactosidase expression vector RSV/β–galactosidase (2 μg, Dr. Nakshatri's gift) was cotransfected as a control. Fresh media including the appropriate selection drugs were added 24 hours after transfection. Cells were harvested after 24 hours and CAT activity was evaluated as described (Nakshatri et al., 1997, *Mol. Cell. Biol.* 17:3629-39). These results indicate that the estrogen receptor in MCF-7^{EphA2} cells is expressed and remains sensitive to tamoxifen, thus suggesting that the defect which renders MCF-7^{EphA2} less dependent on estrogen lies downstream of estrogen signaling.

[00345] Growth MCF-7^{EphA2} cells which had decreased EphA2 expression levels was assayed in soft agar. The EphA2 monoclonal antibody EA2 induced EphA2 activation and subsequent degradation. Decreased levels of EphA2 expression were observed within two hours of EA2 treatment and EphA2 remained undetectable for at least the following 24

hours (FIG. 12A). The soft agar colonization of control MCF-7 cells was sensitive to tamoxifen (FIG. 12C) and EA2 did not further alter this response (since these cells lack of endogenous EphA2). The MCF-7^{EphA2} cells were less sensitive to tamoxifen (25% inhibition by tamoxifen) as compared to the matched controls (75% inhibition by tamoxifen). Whereas EA2 decreased soft agar colonization (by 19%), the combination of EA2 and tamoxifen caused a much more dramatic (>80%) decrease in soft agar colonization. Thus, EA2 treatment restored a phenotype that was comparable to control MCF-7 cells. These findings suggest that antibody targeting of EphA2 can serve to re-sensitize the breast tumor cells to tamoxifen.

[00346] All statistical analyses were performed using Student's t-test using Microsoft Excel (Seattle, WA), defining $P \leq 0.05$ as significant. *In vivo* tumor growth analyses were performed using GraphPad Software (San Diego, CA).

4.4. Kinetic Analysis Of Epha2 Antibodies

[00347] The BIACORE™ assay was used to measure the K_{off} rates of the monoclonal antibodies of the invention. IgG present in the hybridoma supernatant was used for measurement.

Immobilization of EphA2

[00348] EphA2-Fc was immobilize to a surface on a CM5 sensorchip using a standard amine (70µl of a 1:1 mix of NHS/EDC) coupling chemistry. Briefly, a 400nM solution of EphA2-Fc in 10mM NaOAc, pH4, was then injected over the activated surface to a density of 1000-1100 RU's. Unused reactive esters were subsequently "capped" with a 70µl injection of 1M Et-NH2. Similarly, an activated and "capped" control surface was prepared on the same sensor chip without protein to serve as a reference surface.

Binding Experiments

[00349] A 250 µl injection of each of the EphA2 hybridoma supernatants was made over both the EphA2-Fc and control surfaces, and the binding responses were recorded. These supernatants were used undiluted. Following each injection, at least 10 min. of dissociation phase data was collected. Purified EphA2 monoclonal antibody EA2 was prepared to serve as a positive control (at 1µg, 5µg and 25µg per 250µl of growth medium). A negative control monoclonal antibody that does not bind EphA2 was also prepared at 5µg/250µl growth medium. Control injections of growth medium across these surfaces

were also made. Following each binding cycle, the EphA2-Fc surface was regenerated with a single 1 min. pulse (injection) of 1M NaCl-50mM NaOH.

Data Evaluation

[00350] The binding data was corrected by subtracting out both artifactual noise (blank medium injections) and non-specific binding (control surface), in a technique known as "double-referencing." Thus the sensorgram overlays represent "net" binding curves. Eph099B-208.261 and Eph099B-233.152 (see Table 6) have slower K_{off} rates than EA2 (FIG. 13). Additionally, other antibodies of the invention have slow K_{off} rates (Table 6, column 8) including Eph099B-102.147 and Eph099B-210.248 (data not shown).

[00351] Table 6 summarizes the characterization of EphA2 monoclonal antibodies as described herein.

Table 6

Clone	*Subclone*	*Specificity*		*EphA2 Phosphorylation*	*EphA2 Degradation 4hrs*	*EphA2 Degradation 24 hrs*	*Off Rate*	*Colony Inhibition in Soft Agar*	*Colony Elimination in Soft Agar*
		Inhibits EA2 Binding	**Binds EphA2**						
A-Group									
101		yes	yes	nd	moderate	nd	very slow	nd	nd
102		yes	yes	nd	low-mod	nd	very slow	nd	nd
201		yes	yes	nd	no	nd	slow	nd	nd
B-Group									
101		nd	yes	weak	moderate	no	nd	strong	nd
102		yes	yes	yes	Strong	strong	ultra slow	strong	mod-strong
103		yes	yes	weak	Strong	strong	nd	moderate-strong	nd
108		nd	yes	nd	low-mod	nd	nd	strong	nd
201		yes	yes	nd	no	nd	very slow	strong	low
203		yes	yes	nd	low-mod	nd	nd	strong	nd
204		yes	yes	strong	strong	strong	nd	none	moderate
208		yes	yes	yes	strong	nd	nd	moderate	moderate
	103			nd	strong	strong	nd	nd	strong
	108			nd	strong	nd	nd	nd	nd
	117			nd	strong	strong	nd	nd	very strong
	177			nd	strong	nd	nd	nd	nd

Clone	*Subclone*	*Specificity*		*EphA2 Phosphorylation*	*EphA2 Degradation 4hrs*	*EphA2 Degradation 24 hrs*	*Off Rate*	*Colony Inhibition in Soft Agar*	*Colony Elimination in Soft Agar*
		Inhibits EA2 Binding	Binds EphA2						
	205			nd	strong	no	nd	nd	nd
	222			nd	strong	nd	nd	nd	nd
	234			nd	strong	nd	nd	nd	nd
	235			nd	strong	moderate	nd	nd	nd
	238			nd	strong	nd	nd	nd	nd
209		nd	yes	nd	low	nd	nd	strong	nd
210		yes	yes	yes	strong	no	nd	strong	moderate
211		no	yes	nd	no	nd	moderate	strong	nd
219		yes	yes	nd	low	nd	slow	strong	nd
220		yes	yes	nd	no	nd	ultra slow	strong	very strong
221		yes	yes	nd	no	nd	ultra slow	strong	very strong
223		yes	yes	strong	strong	moderate	slow	none	moderate
229		yes	yes	nd	no	nd	very slow	strong	nd
230		yes	yes	nd	no	nd	very slow	strong	nd
231		yes	yes	yes	strong	no	very slow	strong	moderate
233		yes	yes	weak	strong	strong	very slow	none	moderate
301		no	yes	nd	no	nd	very slow	strong	none
302		no	yes	nd	low	nd	nd	strong	nd
307		no	yes	weak	moderate	no	slow	strong	nd

Clone	*Subclone*	*Specificity*		*EphA2 Phosphorylation*	*EphA2 Degradation 4hrs*	*EphA2 Degradation 24 hrs*	*Off Rate*	*Colony Inhibition in Soft Agar*	*Colony Elimination in Soft Agar*
		Inhibits EA2 Binding	**Binds EphA2**						
308		no	yes	nd	low	nd	nd	strong	nd
309		yes	yes	nd	no	nd	ultra slow	strong	very strong
310		nd	yes	nd	no	nd	nd	strong	nd
311		yes	yes	nd	low	nd	very slow	strong	nd
312		no	yes	nd	low-moderate	nd	nd	strong	nd
313		yes	yes	nd	low	nd	very slow	strong	nd
314		yes	yes	nd	low	nd	ultra slow	strong	moderate
315		yes	yes	nd	low	nd	ultra slow	strong	moderate
316		yes	yes	nd	no	nd	very slow	strong	nd
317		yes	yes	nd	no	nd	slow	strong	nd
401		no	yes	nd	no	nd	nd	strong	nd
402		nd	yes	nd	low	nd	nd	strong	nd
404		nd	yes	yes	moderate	no	nd	nd	nd
406		no	yes	nd	no	nd	nd	nd	nd
407		no	yes	nd	no	nd	slow	nd	nd
408		no	yes	nd	no	nd	slow	nd	nd
409		nd	yes	nd	no	nd	nd	nd	nd
410		no	yes	strong	moderate	no	fast	nd	nd

4.5. Decreased Epha2 Levels Using Epha2 Antisense Oligonucleotides

[00353] An antisense oligonucleotide-based approach that decreased EphA2 expression in tumor cells independent of EphA2 activation was developed. To decrease EphA2 protein levels, MDA-MB-231 breast carcinoma cells were transiently transfected with phosphorothioate-modified antisense oligonucleotides that corresponded to a sequence that was found to be unique to EphA2 as determined using a sequence evaluation of GenBank (5'-CCAGCAGTACCGCTTCCTTGCCCTGCGGCCG-3'; SEQ ID NO:91). Inverted antisense oligonucleotides (5'-GCCGCGTCCCGTTCCTTCACCATGACGACC-3'; SEQ ID NO:97) provided a control. The cells were transfected with oligonucleotides (2 μg/ml) using Lipofectamine PLUS Reagent (Life Technologies, Inc.) according to the manufacturer's protocol. Twenty-four hours post-transfection, the cells were divided. Half of the cells were seeded into soft agar, and the remaining cells were extracted and subjected to western blot analysis.

[00354] Western blot analyses and immunoprecipitations were performed as described previously (Zantek et al., 1999, *Cell Growth Diff.* 10:629-38). Briefly, detergent extracts of cell monolayers were extracted in Tris-buffered saline containing 1% Triton X-100 (Sigma, St. Louis, MO). After measuring protein concentrations (BioRad, Hercules, CA), 1.5 mg of cell lysate was immunoprecipitated, resolved by SDS-PAGE and transferred to nitrocellulose (Protran, Schleicher and Schuell, Keene, NH). EphA2 was detected with an EphA2-specific antibody (D7, purchased from Upstate Biologicals, Inc., Lake Placid, NY). To control for sample loading, the membranes were stripped and re-probed with paxillin antibodies (a gift from Dr. K. Burridge at the University of North Carolina). Antibody binding was detected by enhanced chemiluminescence (Pierce, Rockford, IL) and autoradiography (Kodak X-OMAT; Rochester, NY).

[00355] Western blot analyses confirmed that antisense oligonucleotides selectively decreased EphA2 expression in MDA-MB-231 cells whereas an inverted antisense control (IAS) did not (FIG. 14A).

[00356] MDA-MB-231 cells were suspended in soft agar. Colony formation in soft agar was performed as described in Zelinski et al. (2001, *Cancer Res.* 61:2301-6). Antibodies or a control solution (PBS) was included in bottom and top agar solutions. Colony formation was scored microscopically using an Olympus CK-3 inverted phase-contrast microscope outfitted with a 40x objective. Clusters containing at least three cells were scored as a positive. The average number of colonies per high-powered field is

shown. Ten separate high-power microscopic fields were averaged in each experiment, and the results shown are representative of at least three separate experiments.

[00357] EphA2 antisense oligonucleotides decreased soft agar colonization by at least 60% as compared to matched controls (FIG. 14B). Consistent results with EphA2 antibodies and antisense oligonucleotides thus indicate that decreased EphA2 expression is sufficient to decrease tumor cell growth.

4.6. TREATMENT OF PATIENTS WITH METASTATIC CANCER

[00358] A study is designed to assess pharmacokinetics and safety of agents of the invention in patients with metastatic breast cancer. Cancer patients currently receive Taxol or Taxotere. Patients currently receiving treatment are permitted to continue these medications.

[00359] Patients are administered a single IV dose of an EphA2 agent of the invention in combination with either a PCDGF agent of the invention or an HAAH agent of the invention. Four weeks later, the patients are analyzed following administration of repeated weekly IV doses of the combination therapy at the same dose over a period of 12 weeks. The safety of treatment with the agents of the invention is assessed as well as potential changes in disease activity over 26 weeks of IV dosing. Different groups of patients are treated and evaluated similarly but receive doses of 1 mg/kg, 2 mg/kg, 4 mg/kg, or 8 mg/kg.

[00360] Agents of the invention are formulated at 5 mg/ml and 10 mg/ml for IV injection. A formulation of 80 mg/ml is required for repeated subcutaneous administration. The agents of the invention are also formulated at 100 mg/ml for administration for the purposes of the study.

[00361] Changes are measured or determined by the progression of tumor growth.

5. EQUIVALENTS

[00362] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

[00363] All publications, patents and patent applications mentioned in this specification are herein incorporated by reference into the specification to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference. In particular, U.S.

Provisional Application No. 60/489,036, filed on July 21, 2003, is incorporated herein by reference in its entirety.

We claim:

1. A method of treating cancer in a subject suffering from cancer, said method comprising administering to the subject a therapeutically effective amount of an EphA2 agent in combination with a therapeutically effective amount of a PCDGF agent.

2. The method of claim 1 wherein said administration increases EphA2 phosphorylation in a cancer cell relative to the level of EphA2 phosphorylation in an untreated cancer cell.

3. The method of claim 1 wherein said administration decreases EphA2 expression in a cancer cell relative to the level of EphA2 expression in an untreated cancer cell.

4. The method of claim 1 wherein said administration decreases PCDGF or PCDGF receptor expression in a cancer cell relative to the level of PCDGF or PCDGF receptor expression in an untreated cancer cell.

5. The method of claim 4 wherein said PCDGF agent is a PCDGF or PCDGF receptor antisense nucleic acid molecule.

6. The method of claim 1 wherein said EphA2 agent or said PCDGF agent inhibits colony formation in soft agar or tubular network formation in a three-dimensional basement membrane or extracellular matrix preparation.

7. The method of claim 1 wherein said EphA2 agent is an EphA2 agonistic antibody, EphA2 cancer cell phenotype inhibiting antibody, exposed EphA2 epitope antibody, or antibody that binds EphA2 with a K_{off} of less than 3×10^{-3} s^{-1}.

8. The method of claim 1 wherein said EphA2 agent is Eph099B-102.147, Eph099B-208.261, Eph099B-210.248, or Eph099B-233.152.

9. The method of claim 1 wherein said EphA2 agent is an antibody that competes for EphA2 binding with Eph099B-102.147, Eph099B-208.261, Eph099B-210.248, or Eph099B-233.152.

10. The method of claim 1 wherein said EphA2 agent is an EphA2 agonistic antibody, EphA2 cancer cell phenotype inhibiting antibody, exposed EphA2 epitope antibody, or antibody that binds EphA2 with a K_{off} of less than 3 X 10^{-3} s^{-1} that has been humanized.

11. The method of claim 1 wherein said EphA2 agent is Eph099B-102.147, Eph099B-208.261, Eph099B-210.248, or Eph099B-233.152 that has been humanized.

12. The method of claim 1 comprising the administration of one or more HAAH agents.

13. The method of claim 1 wherein said cancer is of an epithelial cell origin.

14. The method of claim 1 wherein said cancer is a cancer of the skin, lung, colon, breast, prostate, bladder or pancreas or is a renal cell carcinoma or a melanoma.

15. The method of claim 1 wherein said cancer is a metastatic cancer.

16. The method of claim 1 wherein said cancer comprises cells that overexpress EphA2 relative to non-cancer cells having the tissue type of said cancer cells.

17. The method of claim 1 wherein said cancer comprises cells that overexpress PCDGF or PCDGF receptor relative to non-cancer cells having the tissue type of said cancer cells.

18. A method of treating a cancer that is fully or partially refractory to a first treatment in a subject in need thereof, said method comprising administering to said subject a second treatment comprising administration of a therapeutically effective amount of an EphA2 agent in combination with a therapeutically amount of a PCDGF agent.

19. A pharmaceutical composition comprising a therapeutically effective amount of an EphA2 agent in combination with a therapeutically effective amount of a PCDGF agent and a pharmaceutically acceptable carrier.

20. The pharmaceutical composition of claim 19 wherein said EphA2 agent is Eph099B-102.147, Eph099B-208.261, Eph099B-210.248, or Eph099B-233.152.

21. The pharmaceutical composition of claim 19 wherein said EphA2 agent is an antibody that competes for EphA2 binding with Eph099B-102.147, Eph099B-208.261, Eph099B-210.248, or Eph099B-233.152.

22. A method of treating cancer in a subject suffering from cancer, said method comprising administering to the subject a therapeutically effective amount of an EphA2 agent in combination with a therapeutically effective amount of an HAAH agent.

23. The method of claim 22 wherein said administration increases EphA2 phosphorylation in a cancer cell relative to the level of EphA2 phosphorylation in an untreated cancer cell.

24. The method of claim 22 wherein said administration decreases EphA2 expression in a cancer cell relative to the level of EphA2 expression in an untreated cancer cell.

25. The method of claim 22 wherein said administration decreases HAAH expression in a cancer cell relative to the level of HAAH expression in an untreated cancer cell.

26. The method of claim 25 wherein said HAAH agent is an HAAH antisense nucleic acid molecule.

27. The method of claim 22 wherein said EphA2 agent or said HAAH agent inhibits colony formation in soft agar or tubular network formation in a three-dimensional basement membrane or extracellular matrix preparation.

28. The method of claim 22 wherein said EphA2 agent is an EphA2 agonistic antibody, EphA2 cancer cell phenotype inhibiting antibody, exposed EphA2 epitope antibody, or antibody that binds EphA2 with a K_{off} of less than $3 \times 10^{-3}\ s^{-1}$.

29. The method of claim 22 wherein said EphA2 agent is Eph099B-102.147, Eph099B-208.261, Eph099B-210.248, or Eph099B-233.152.

30. The method of claim 22 wherein said EphA2 agent is an antibody that competes for EphA2 binding with Eph099B-102.147, Eph099B-208.261, Eph099B-210.248, or Eph099B-233.152.

31. The method of claim 22 wherein said EphA2 agent is an EphA2 agonistic antibody, EphA2 cancer cell phenotype inhibiting antibody, exposed EphA2 epitope antibody, or antibody that binds EphA2 with a K_{off} of less than 3 X 10^{-3} s^{-1} that has been humanized.

32. The method of claim 22 wherein said EphA2 agent is Eph099B-102.147, Eph099B-208.261, Eph099B-210.248, or Eph099B-233.152 that has been humanized.

33. The method of claim 22 comprising the administration of one or more PCDGF agents.

34. The method of claim 22 wherein said cancer is of an epithelial cell origin.

35. The method of claim 22 wherein said cancer is a cancer of the skin, lung, colon, breast, prostate, bladder or pancreas or is a renal cell carcinoma or a melanoma.

36. The method of claim 22 wherein said cancer is a metastatic cancer.

37. The method of claim 22 wherein said cancer comprises cells that overexpress EphA2 relative to non-cancer cells having the tissue type of said cancer cells.

38. The method of claim 22 wherein said cancer comprises cells that overexpress HAAH relative to non-cancer cells having the tissue type of said cancer cells.

39. A method of treating a cancer that is fully or partially refractory to a first treatment in a subject in need thereof, said method comprising administering to said subject a second treatment comprising administration of a therapeutically effective amount of an EphA2 agent in combination with a therapeutically effective amount of an HAAH agent.

40. A pharmaceutical composition comprising a therapeutically effective amount of an EphA2 agent in combination with a therapeutically effective amount of an HAAH agent and a pharmaceutically acceptable carrier.

41. The pharmaceutical composition of claim 40 wherein at least one of said EphA2 agents is Eph099B-102.147, Eph099B-208.261, Eph099B-210.248, or Eph099B-233.152.

42. The pharmaceutical composition of claim 40 wherein at least one of said EphA2 agents is an antibody that competes for EphA2 binding with Eph099B-102.147, Eph099B-208.261, Eph099B-210.248, or Eph099B-233.152.

43. A method of identifying a PCDGF agent that potentiates the activity of an EphA2 agent to inhibit colony formation of a cancer cell in soft agar or tubular network formation of a cancer in a three-dimensional basement membrane or extracellular matrix preparation comprising:

a) contacting a cell responsive to said EphA2 agent expressing PCDGF and EphA2, which cell is cultured in soft agar or a three-dimensional basement membrane or extracellular matrix preparation, with a candidate PCDGF agent and said EphA2 agent; and

b) determining the ability of said cell to form colonies in soft agar or tubular networks in said three-dimensional basement membrane or extracellular matrix preparation,

wherein detecting a decrease in the ability of said cell to form colonies or tubular networks which is greater than the decrease in the ability of a cell expressing PCDGF and EphA2 contacted by said EphA2 agent alone to form colonies or tubular networks indicates a PCDGF agent which potentiates said EphA2 agent.

44. A method of identifying a PCDGF agent that potentiates the activity of an EphA2 agent to inhibit colony formation of a cancer cell in soft agar or tubular network formation of a cancer in a three-dimensional basement membrane or extracellular matrix preparation comprising:

a) contacting a cell responsive to said EphA2 agent expressing a PCDGF receptor and EphA2, which cell is cultured in soft agar or a three-dimensional basement membrane or extracellular matrix preparation, with PCDGF, a candidate PCDGF agent and said EphA2 agent; and

b) determining the ability of said cell to form colonies in soft agar or tubular networks in said three-dimensional basement membrane or extracellular matrix preparation,

wherein detecting a decrease in the ability of said cell to form colonies or tubular networks which is greater than the decrease in the ability of a cell expressing said PCDGF receptor and EphA2 contacted by PCDGF and said EphA2 agent to form colonies or tubular networks indicates a PCDGF agent which potentiates said EphA2 agent.

45. A method of identifying a PCDGF agent that potentiates the activity of an EphA2 agent to reduce the amount of colonies of cancer cells in soft agar or tubular networks of cancer cells in a three-dimensional basement membrane or extracellular matrix preparation comprising:

a) contacting cancer cells responsive to said EphA2 agent expressing PCDGF and EphA2, which cancer cells have formed colonies in soft agar or a tubular network in a three-dimensional basement membrane or extracellular matrix preparation, with a candidate PCDGF agent and said EphA2 agent; and

b) determining the amount of said colonies in soft agar or said tubular networks in said three-dimensional basement membrane or extracellular matrix preparation,

wherein detecting a reduction in the amount of said colonies or said tubular networks which is greater than the reduction in the amount of colonies in soft agar or tubular networks in a three-dimensional basement membrane or extracellular matrix preparation of cancer cells expressing PCDGF and EphA2 contacted by said EphA2 agent alone indicates a PCDGF agent which potentiates said EphA2 agent.

46. A method of identifying a PCDGF agent that potentiates the activity of an EphA2 agent to reduce the amount of colonies of cancer cells in soft agar or tubular networks of cancer cells in a three-dimensional basement membrane or extracellular matrix preparation comprising:

a) contacting cancer cells responsive to said EphA2 agent expressing a PCDGF receptor and EphA2, which cancer cells have formed colonies in soft agar or a tubular network in a three-dimensional basement membrane or extracellular matrix preparation, with PCDGF, a candidate PCDGF agent and said EphA2 agent; and

b) determining the amount of said colonies in soft agar or said tubular networks in said three-dimensional basement membrane or extracellular matrix preparation,

wherein detecting a reduction in the amount of said colonies or said tubular networks which is greater than the reduction in the amount of colonies in soft agar or

tubular networks in a three-dimensional basement membrane or extracellular matrix preparation of cancer cells expressing said PCDGF receptor and EphA2 contacted by PCDGF and said EphA2 agent indicates a PCDGF agent which potentiates said EphA2 agent.

47. A method of identifying an HAAH agent that potentiates the activity of an EphA2 agent to inhibit colony formation of a cancer cell in soft agar or tubular network formation of a cancer in a three-dimensional basement membrane or extracellular matrix preparation comprising:

a) contacting a cell responsive to said EphA2 agent expressing HAAH and EphA2, which cell is cultured in soft agar or a three-dimensional basement membrane or extracellular matrix preparation, with a candidate HAAH agent and said EphA2 agent; and

b) determining the ability of said cell to form colonies in soft agar or tubular networks in said three-dimensional basement membrane or extracellular matrix preparation,

wherein detecting a decrease in the ability of said cell to form colonies or tubular networks which is greater than the decrease in the ability of a cell expressing HAAH and EphA2 contacted by said EphA2 agent alone to form colonies or tubular networks indicates an HAAH agent which potentiates said EphA2 agent.

48. A method of identifying an HAAH agent that potentiates the activity of an EphA2 agent to reduce the amount of colonies of cancer cells in soft agar or tubular networks of cancer cells in a three-dimensional basement membrane or extracellular matrix preparation comprising:

a) contacting cancer cells responsive to said EphA2 agent expressing HAAH and EphA2, which cancer cells have formed colonies in soft agar or a tubular network in a three-dimensional basement membrane or extracellular matrix preparation, with a candidate HAAH agent and said EphA2 agent; and

b) determining the amount of said colonies in soft agar or said tubular networks in said three-dimensional basement membrane or extracellular matrix preparation,

wherein detecting a reduction in the amount of said colonies or said tubular networks which is greater than the reduction in the amount of colonies in soft agar or tubular networks in a three-dimensional basement membrane or extracellular matrix preparation of cancer cells expressing HAAH and EphA2 contacted by said EphA2 agent alone indicates An HAAH agent which potentiates said EphA2 agent.

49. A method of diagnosing, prognosing or monitoring the efficacy of therapy for cancer in a subject known to or suspected to have cancer, said method comprising:

a) contacting cells of said subject with an EphA2 antibody and a HAAH antibody under conditions appropriate for antibody binding; and

b) detecting said EphA2 antibody and said HAAH antibody binding to said cells,

wherein detecting a higher level of binding of said EphA2 antibody and said HAAH antibody than in a control subject that does not have cancer indicates that said subject has cancer.

50. The method of claim 49, wherein said cells are from whole blood, sputum, urine, serum or fine needle aspirates of tumor cell tissue.

51. The method of claim 49 wherein said cells are in frozen or fixed tissue or cells from said subject.

52. The method of claim 49 wherein said detecting comprises imaging of said antibody binding in said subject.

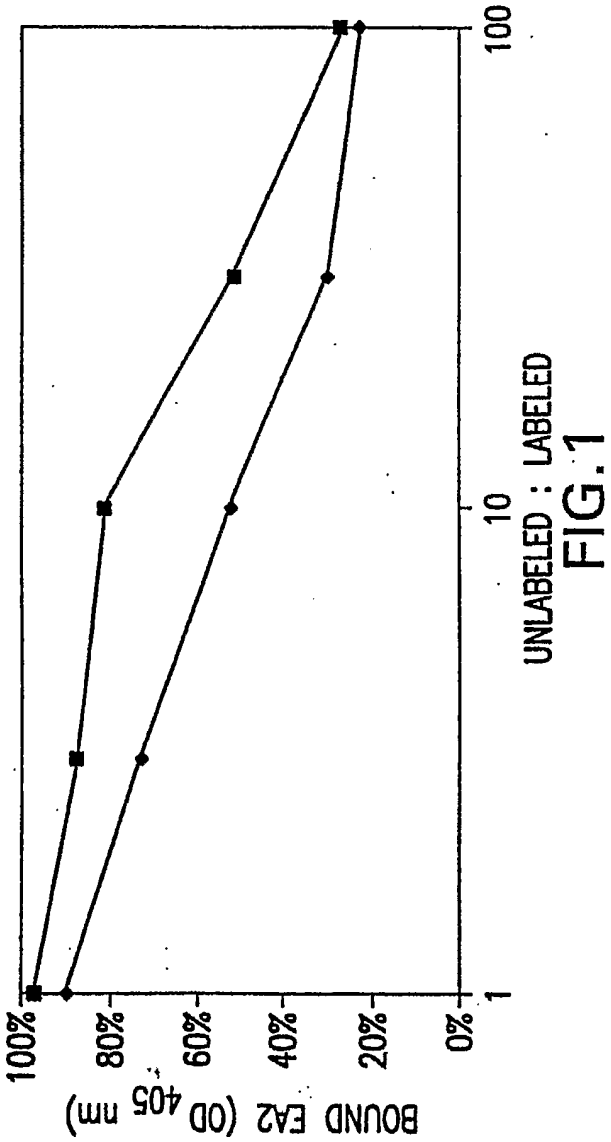


FIG.1

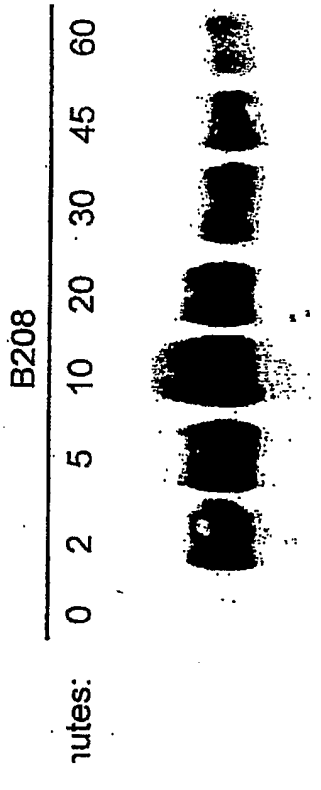


FIG.2A

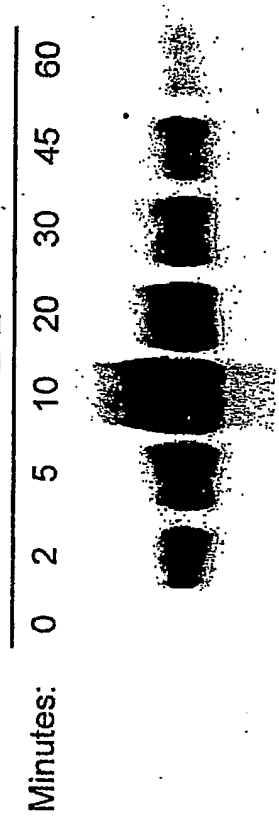


FIG.2B

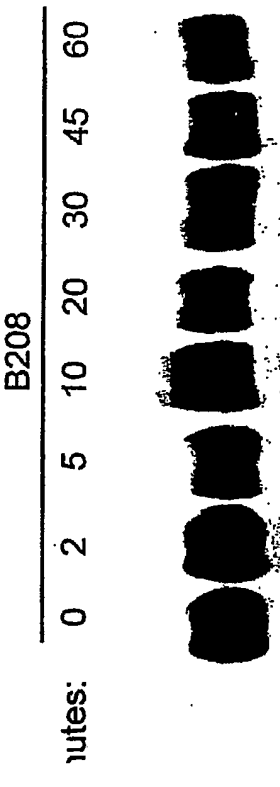


FIG.2C

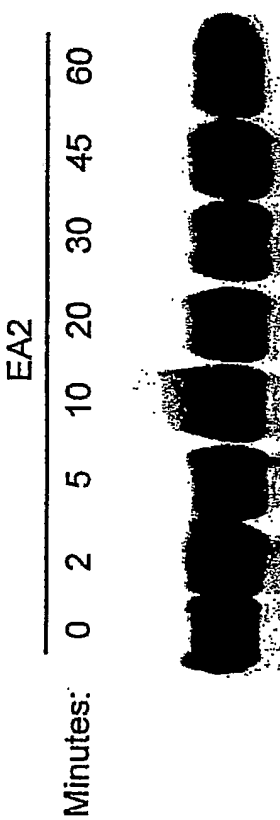


FIG.2D

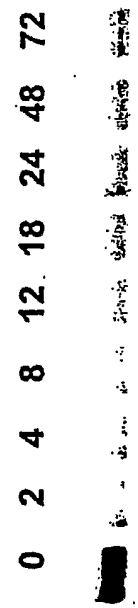


FIG.3A

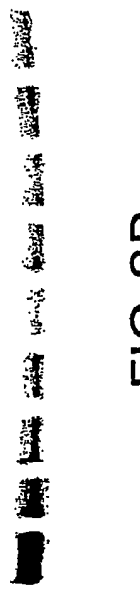


FIG.3B

FIG.3D

FIG.3D

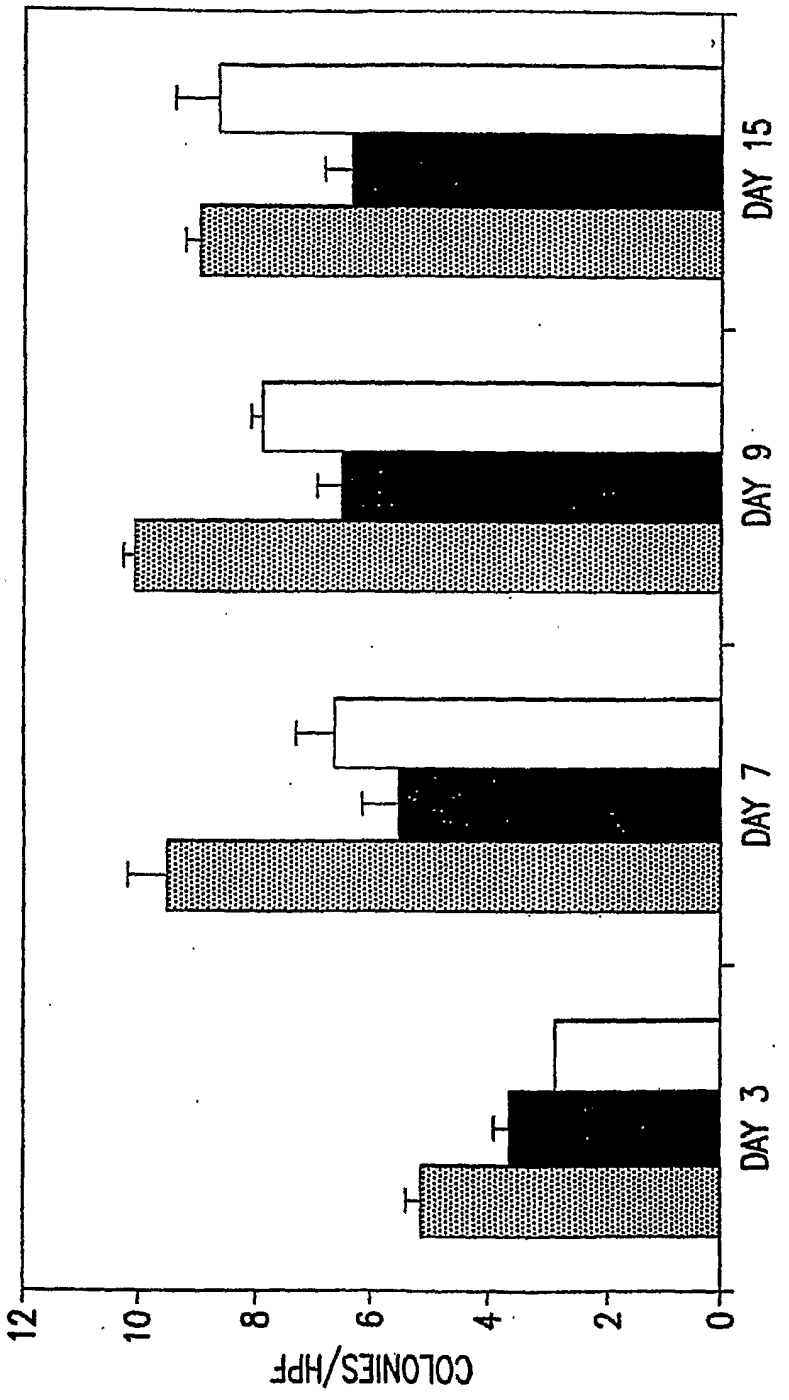


FIG.4

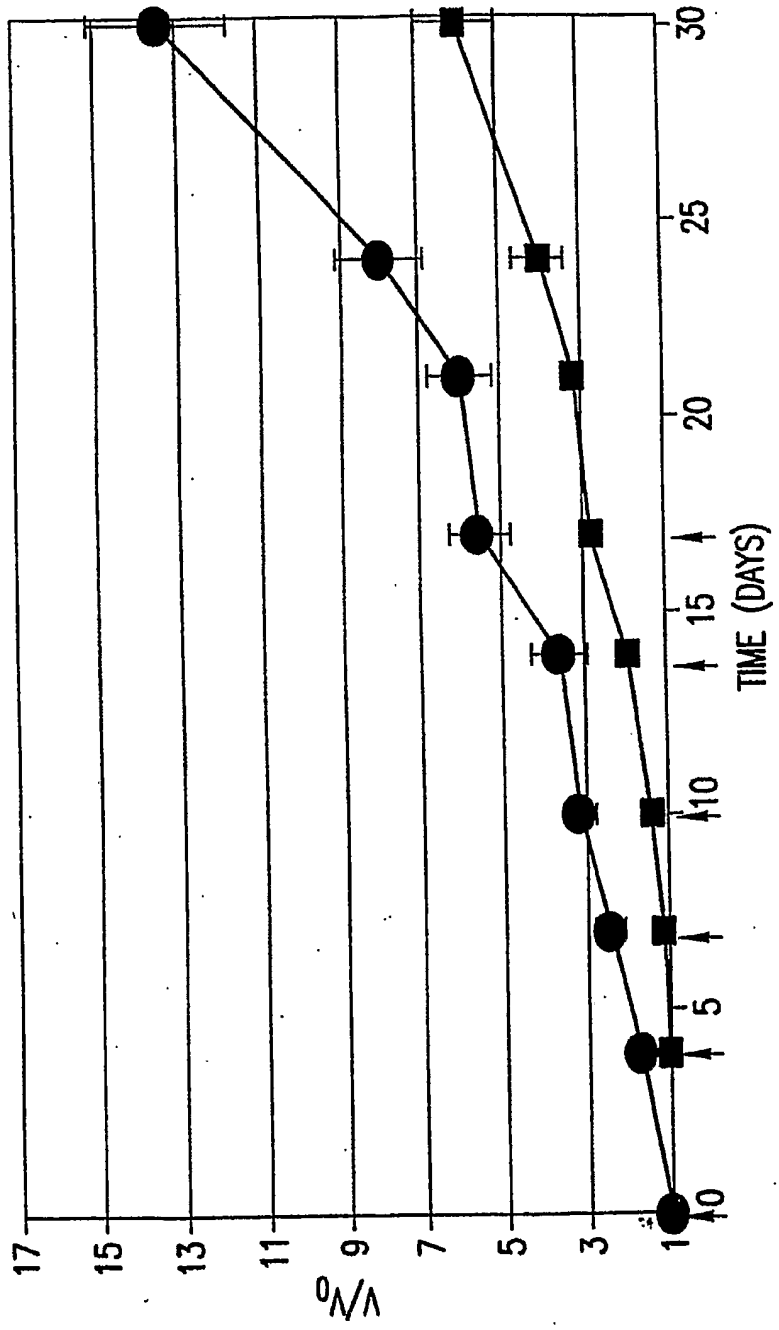


FIG. 5A

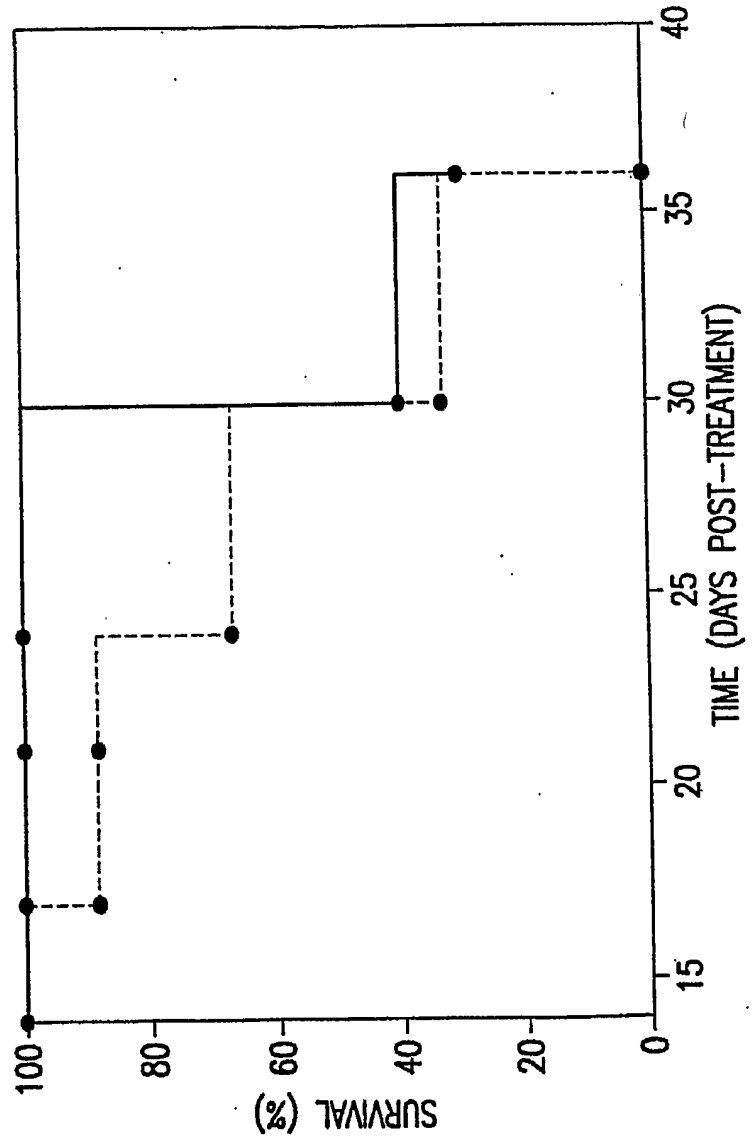

SURVIVAL (%)
100
80
60
40
20
0
15
20
25
30
35
40
TIME (DAYS POST-TREATMENT)

FIG. 5B

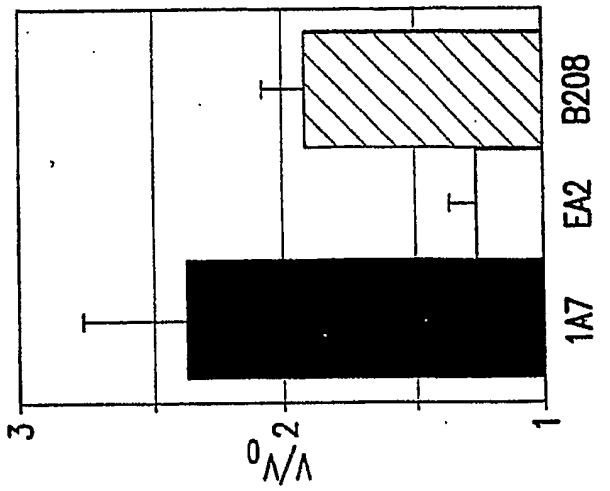


FIG. 6C

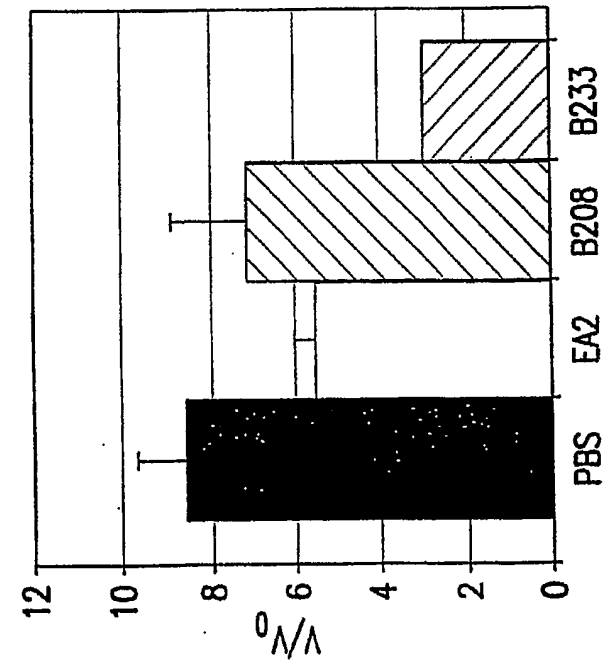


FIG. 6B

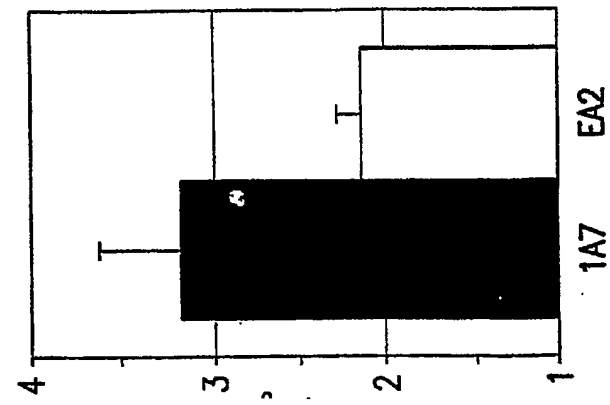


FIG. 6A

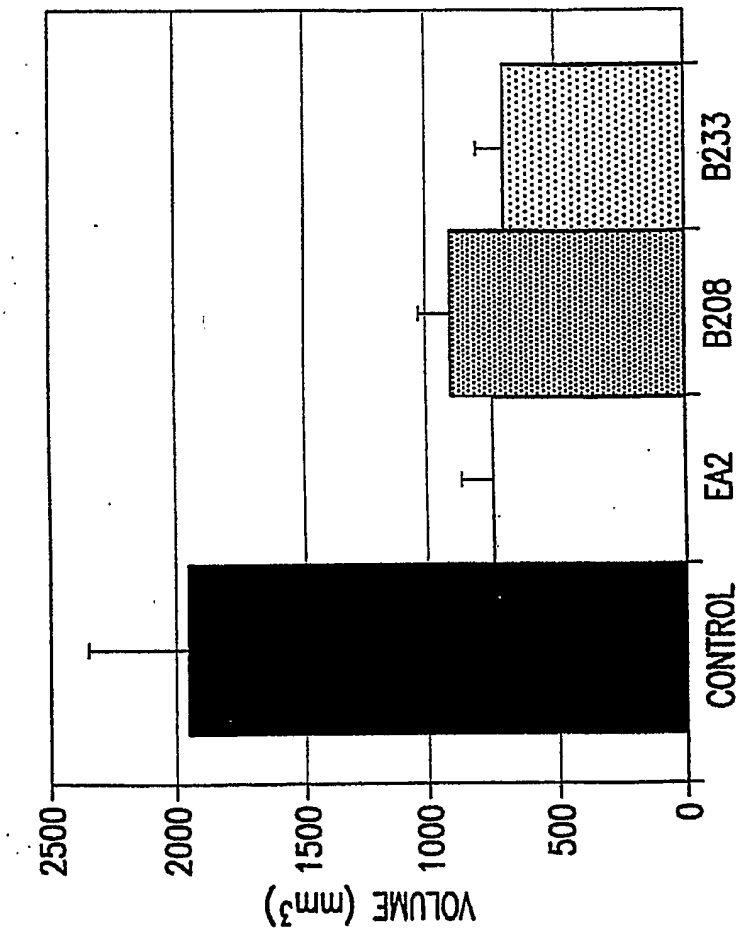


FIG. 6D

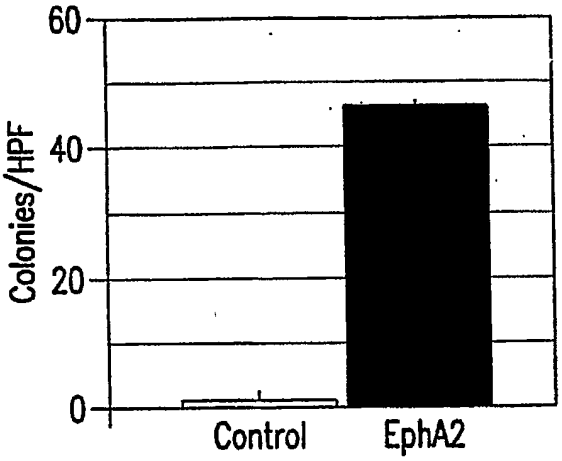

60
40
20
0
Colonies/HPF
Control
EphA2

FIG. 7A

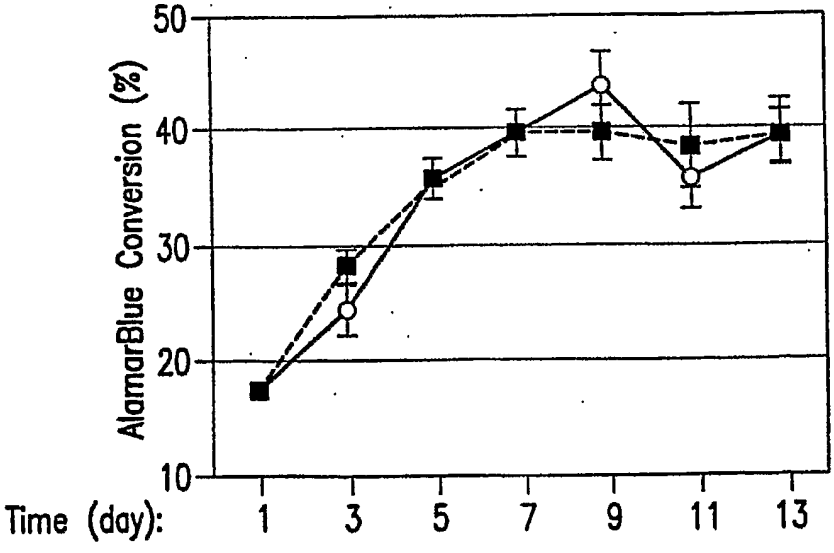

50
40
30
20
10
AlamarBlue Conversion (%)
Time (day):
1
3
5
7
9
11
13

FIG. 7B

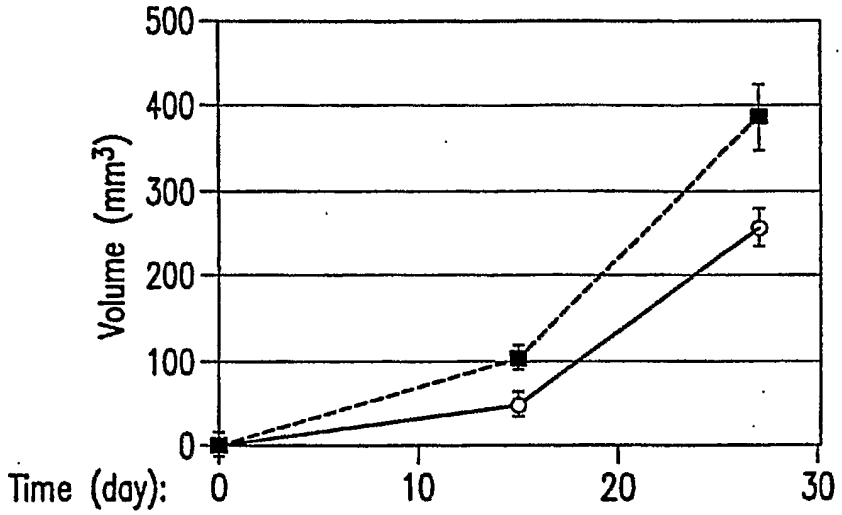


FIG. 8A

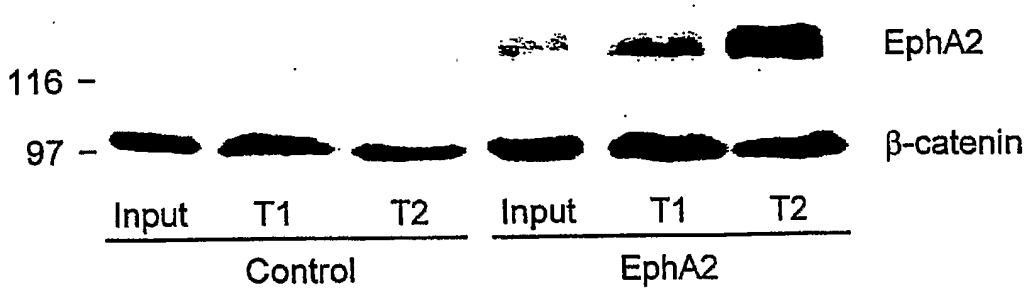


FIG. 8B

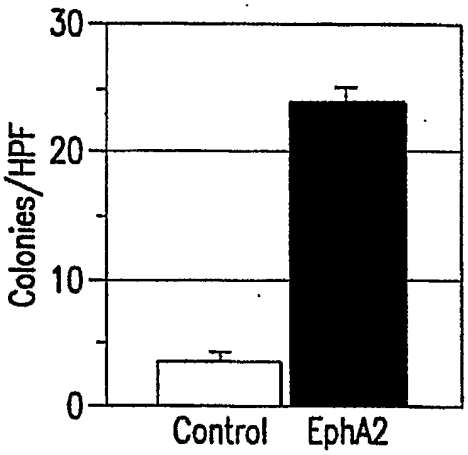

30
20
10
0
Colonies/HPF
Control
EphA2

FIG. 9A

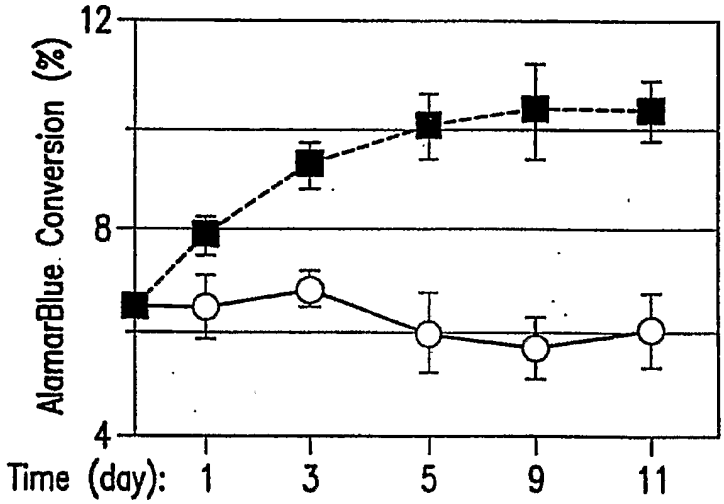
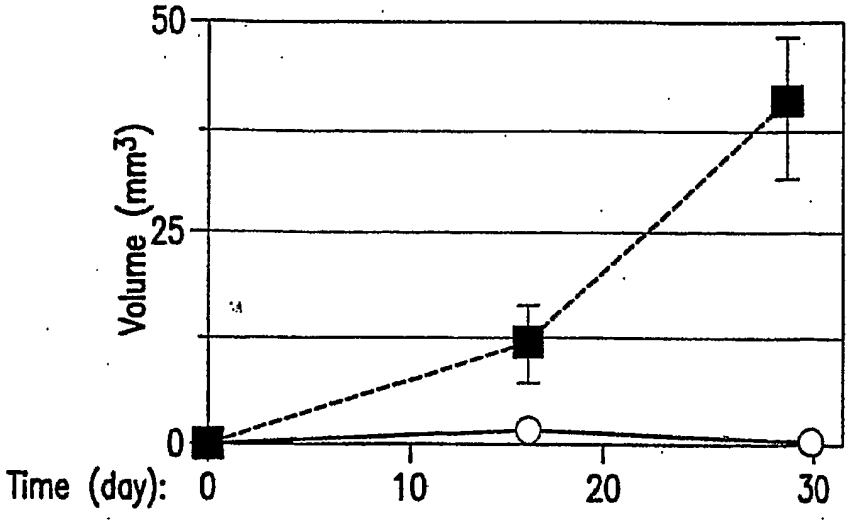

12
8
4
AlamarBlue Conversion (%)
Time (day): 1 3 5 9 11

FIG. 9B


50
25
0
Volume (mm3)
Time (day): 0 10 20 30

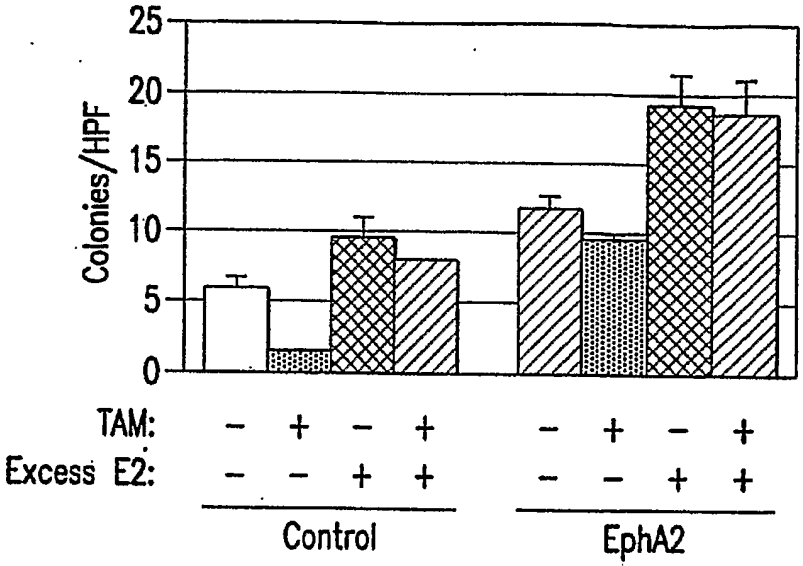


FIG. 10A

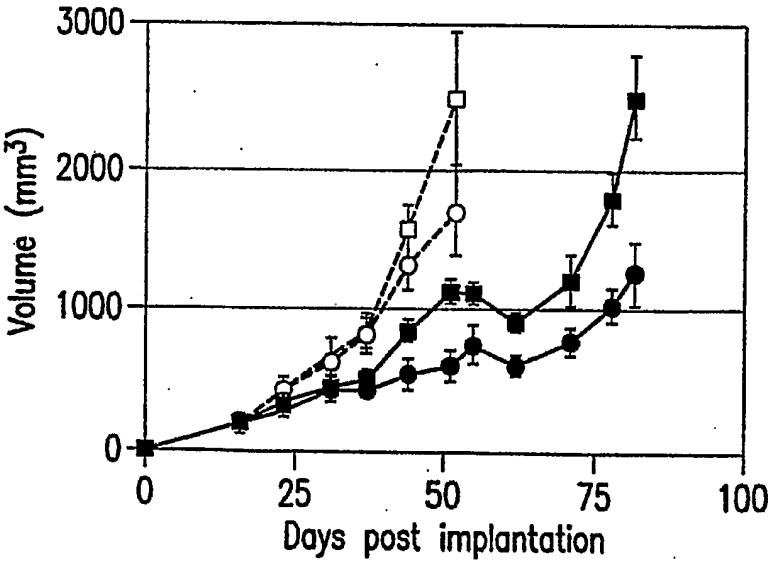


FIG. 10B

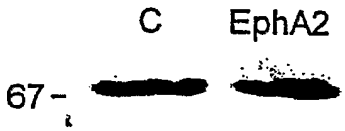


FIG. 11A

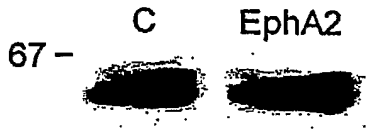


FIG. 11B

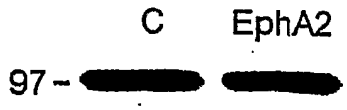


FIG. 11C

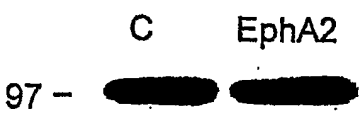


FIG. 11D

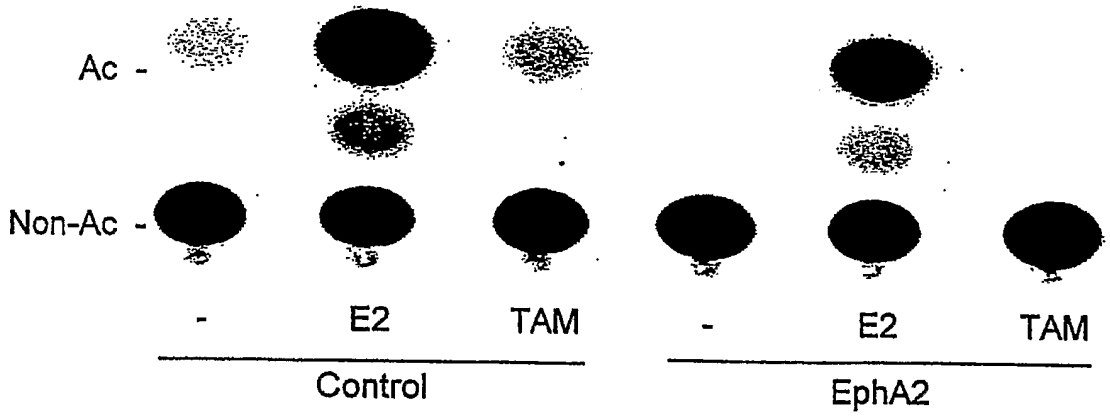


FIG. 11E

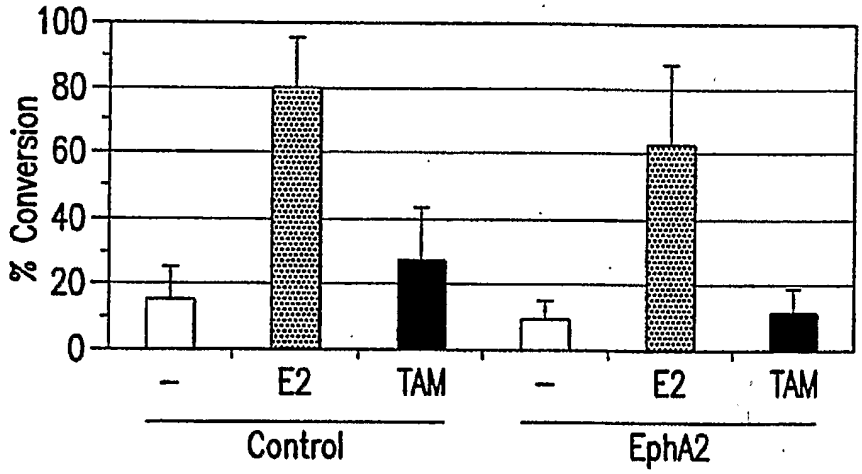


FIG. 11F

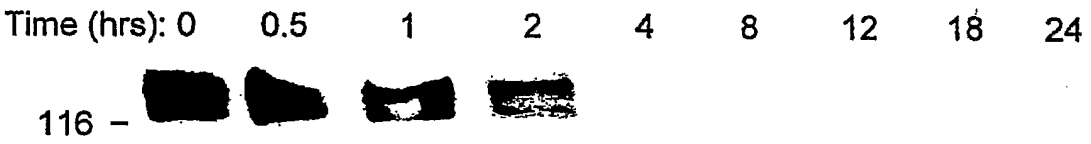


FIG. 12A

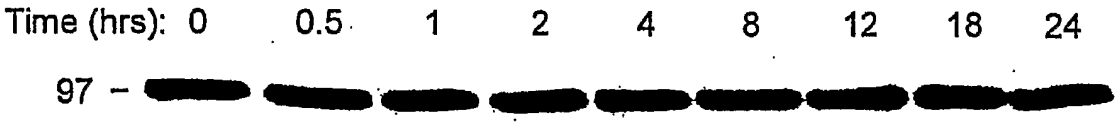


FIG. 12B

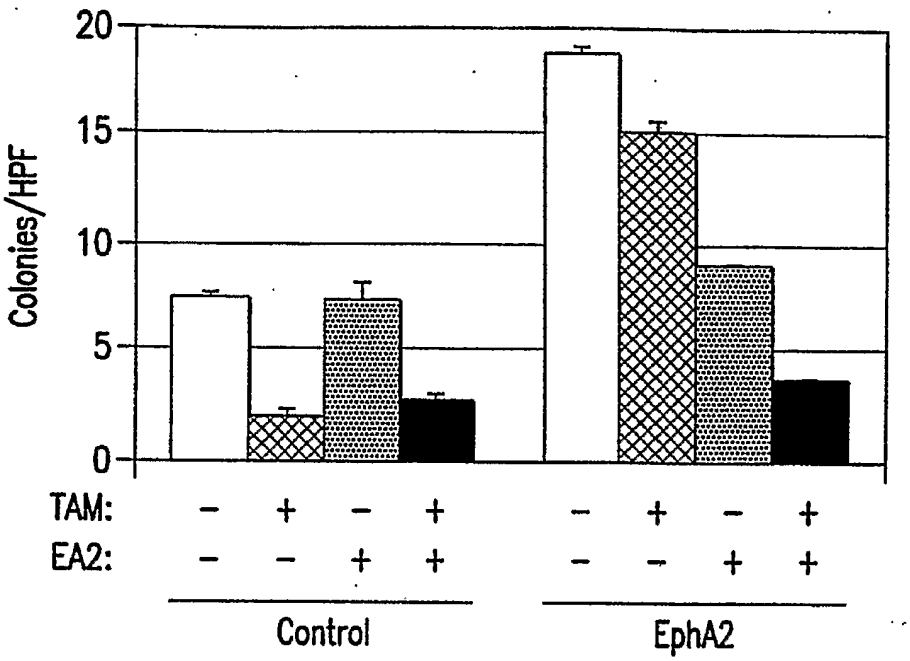


FIG. 12C

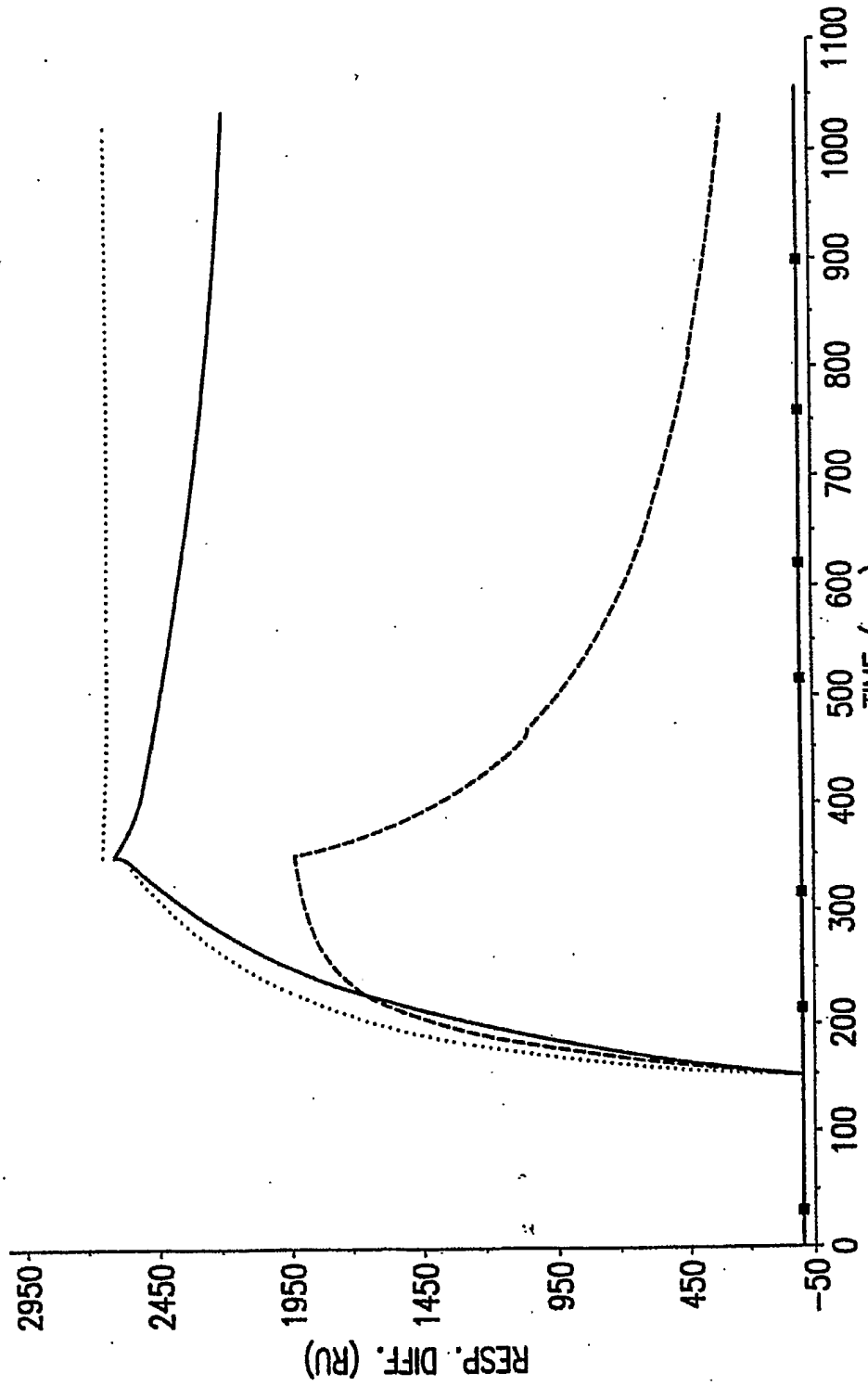

RESP. DIFF. (RU)
2950
2450
1950
1450
950
450
-50
0
100
200
300
400
500
600
700
800
900
1000
1100
TIME (sec.)

FIG. 13

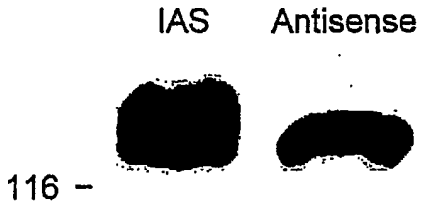


FIG.14A

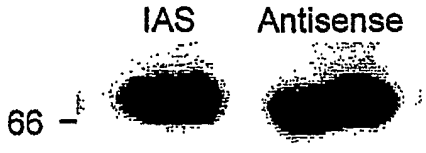


FIG.14B

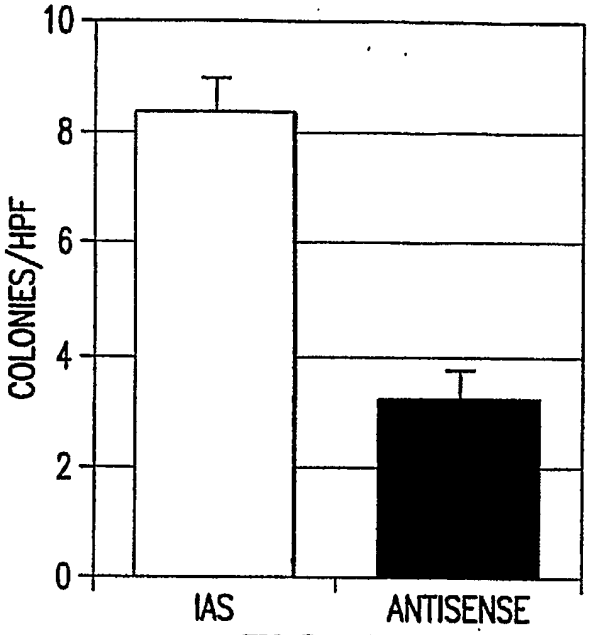


FIG.14C

208 Variable Light Chain

```
caa att gtt ctc acc cag tct cca gca ctc atg tct gca tct cca ggg      48
Gln Ile Val Leu Thr Gln Ser Pro Ala Leu Met Ser Ala Ser Pro Gly
1               5                  10                  15
                                        CDR1
                                   ____________________
gag aag gtc acc atg acc tgc agt gcc agc tca agt gta agt tac atg      96
Glu Lys Val Thr Met Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met
            20                  25                  30

___
tac tgg tac cag cag aag cca aga tcc tcc ccc aaa ccc tgg att tat     144
Tyr Trp Tyr Gln Gln Lys Pro Arg Ser Ser Pro Lys Pro Trp Ile Tyr
        35                  40                  45
      CDR2
_____________
ctc aca acc aac ctg gct tct gga gtc cct gct cgc ttc agt ggc agt     192
Leu Thr Thr Asn Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser
    50                  55                  60

ggg tct ggg acc tct tac tct ctc aca atc agc agc atg gag gct gaa     240
Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Ser Met Glu Ala Glu
65                  70                  75                  80
                                        CDR3
                                   ____________________
gat gct gcc act tat tac tgc cag cag tgg agt agt aac cca ttc acg     288
Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Phe Thr
                85                  90                  95

ttc ggc tcg ggg aca aag ttg gaa ata aga                            318
Phe Gly Ser Gly Thr Lys Leu Glu Ile Arg
            100                 105
```

FIG. 15A

208 Variable heavy Chain

```
cag gtc caa ctg cag cag cct ggg gct gag ctg gta aag cct ggg gct      48
Gln Val Gln Leu Gln Gln Pro Gly Ala Glu Leu Val Lys Pro Gly Ala
1               5                  10                  15
                                          CDR1
tca gtg aag ttg tcc tgc aag gct tct ggc tac act ttc acc agc tac      96
Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
            20                  25                  30

tgg atg cac tgg gtg aaa caa agg cct gga caa ggc ctt gag tgg att     144
Trp Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
        35                  40                  45
                    CDR2
ggg atg att cat cct aat agt ggt agt act aac tac aat gag aag ttc     192
Gly Met Ile His Pro Asn Ser Gly Ser Thr Asn Tyr Asn Glu Lys Phe
    50                  55                  60

aag agc aag gcc aca ctg act gta gac aaa tcc tcc agc aca gcc tac     240
Lys Ser Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
65                  70                  75                  80

atg cga ctc agc agc ctg aca tct gag gac tct gcg gtc tat tac tgt     288
Met Arg Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
                85                  90                  95
                    CDR3
gca aga ggg ggt aac atg gta ggg ggg ggc tac tgg ggc caa ggc acc     336
Ala Arg Gly Gly Asn Met Val Gly Gly Gly Tyr Trp Gly Gln Gly Thr
            100                 105                 110

act ctc aca gtc tcc tca                                            354
Thr Leu Thr Val Ser Ser
        115
```

FIG. 15B

233 Variable Light Chain

```
gat att gtg cta act cag tct cca gcc acc ctg tct gtg act cca gga      48
Asp Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Val Thr Pro Gly
1               5                  10                  15
                                        CDR1
gat agc gtc aat ctt tcc tgc agg gcc agc caa agt att agc aac aac      96
Asp Ser Val Asn Leu Ser Cys Arg Ala Ser Gln Ser Ile Ser Asn Asn
            20                  25                  30

cta cac tgg tat caa caa aaa tca cat gag tct cca agg ctt ctc atc     144
Leu His Trp Tyr Gln Gln Lys Ser His Glu Ser Pro Arg Leu Leu Ile
        35                  40                  45
                CDR2
aag tat gtt ttc cag tcc atc tct ggg atc ccc tcc agg ttc agt ggc     192
Lys Tyr Val Phe Gln Ser Ile Ser Gly Ile Pro Ser Arg Phe Ser Gly
    50                  55                  60

agt gga tca ggg aca gat ttc act ctc agt atc aac agt gtg gag act     240
Ser Gly Ser Gly Thr Asp Phe Thr Leu Ser Ile Asn Ser Val Glu Thr
65                  70                  75                  80
                                        CDR3
gaa gat ttt gga atg tat ttc tgt caa cag agt aac agc tgg ccg ctc     288
Glu Asp Phe Gly Met Tyr Phe Cys Gln Gln Ser Asn Ser Trp Pro Leu
                85                  90                  95

acg ttc ggt gct ggg acc aag ctg gag ctg aaa                        321
Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys
            100                 105
```

FIG. 15C

233 Variable Heavy Chain

```
gag gtg aag ctg gtg gag tct gga gga ggc ttg gta cag cct ggg ggt      48
Glu Val Lys Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                  10                  15

                                                  CDR1
tct ctg agt ctc tcc tgt gca gct tct gga ttc acc ttc act gat tac      96
Ser Leu Ser Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Thr Asp Tyr
            20                  25                  30

tcc atg aac tgg gtc cgc cag cct cca ggg aag gca ctt gag tgg ttg     144
Ser Met Asn Trp Val Arg Gln Pro Pro Gly Lys Ala Leu Glu Trp Leu
        35                  40                  45
                  CDR2
ggt ttt att aga aac aaa gct aat gat tac aca aca gag tac agt gca     192
Gly Phe Ile Arg Asn Lys Ala Asn Asp Tyr Thr Thr Glu Tyr Ser Ala
    50                  55                  60

tct gtg aag ggt cgg ttc acc atc tcc aga gat aat tcc caa agc atc     240
Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Gln Ser Ile
65                  70                  75                  80

ctc tat ctt caa atg aat gcc ctg aga gct gag gac agt gcc act tat     288
Leu Tyr Leu Gln Met Asn Ala Leu Arg Ala Glu Asp Ser Ala Thr Tyr
                85                  90                  95
                          CDR3
tac tgt gta aga tac cct agg tat cat gct atg gac tcc tgg ggt caa     336
Tyr Cys Val Arg Tyr Pro Arg Tyr His Ala Met Asp Ser Trp Gly Gln
            100                 105                 110

gga acc tca gtc acc gtc tcc tca                                    360
Gly Thr Ser Val Thr Val Ser Ser
        115                 120
```

FIG. 15D

EA2 Variable Light Chain

```
gac atc aag atg acc cag tct cca tct tcc atg tat gca tct cta gga      48
Asp Ile Lys Met Thr Gln Ser Pro Ser Ser Met Tyr Ala Ser Leu Gly
1               5                  10                  15
                                   CDR1
gag aga gtc act atc act tgc aag gcg agt cag gac att aat aac tat      96
Glu Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asp Ile Asn Asn Tyr
            20                  25                  30

tta agc tgg ttc cag cag aaa cca ggg aaa tct cct aag acc ctg atc     144
Leu Ser Trp Phe Gln Gln Lys Pro Gly Lys Ser Pro Lys Thr Leu Ile
        35                  40                  45
    CDR2
tat cgt gca aac aga ttg gta gat ggg gtc cca tca agg ttc agt ggc     192
Tyr Arg Ala Asn Arg Leu Val Asp Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

agt gga tct ggg caa gat tat tct ctc acc atc agc agc ctg gag tat     240
Ser Gly Ser Gly Gln Asp Tyr Ser Leu Thr Ile Ser Ser Leu Glu Tyr
65                  70                  75                  80
                                   CDR3
gaa gat atg gga att tat tat tgt ctg aaa tat gat gag ttt ccg tac     288
Glu Asp Met Gly Ile Tyr Tyr Cys Leu Lys Tyr Asp Glu Phe Pro Tyr
                85                  90                  95

acg ttc gga ggg ggg acc aag ctg gaa ata aaa                        321
Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
            100                 105
```

FIG. 15E

EA2 Variable Heavy Chain

```
gac gtg aag ctg gtg gag tct ggg gga ggc tta gtg aag cct gga ggg      48
Asp Val Lys Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
1               5                  10                  15
                                          CDR1
tcc ctg aaa ctc tcc tgt gca gcc tct gga ttc act ttc agt agc tat      96
Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
            20                  25                  30

acc atg tct tgg gtt cgc cag act ccg gag aag agg ctg gag tgg gtc     144
Thr Met Ser Trp Val Arg Gln Thr Pro Glu Lys Arg Leu Glu Trp Val
        35                  40                  45
                                CDR2
gca acc att agt agt ggt ggt act tac acc tac tat cca gac agt gtg     192
Ala Thr Ile Ser Ser Gly Gly Thr Tyr Thr Tyr Tyr Pro Asp Ser Val
    50                  55                  60

aag ggc cga ttc acc atc tcc aga gac aat gcc aag aac acc ctg tac     240
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65                  70                  75                  80

ctg caa atg agc agt ctg aag tct gag gac aca gcc atg tat tac tgt     288
Leu Gln Met Ser Ser Leu Lys Ser Glu Asp Thr Ala Met Tyr Tyr Cys
                85                  90                  95
            CDR3
aca aga gaa gct atc ttt act tac tgg ggc caa ggg act ctg gtc act     336
Thr Arg Glu Ala Ile Phe Thr Tyr Trp Gly Gln Gly Thr Leu Val Thr
            100                 105                 110

gtc tct gca                                                        345
Val Ser Ala
        115
```

FIG. 15F