COMBINATION TREATMENT FOR NON-HEMATOLOGIC MALIGNANCIES USING AN ANTI-IGF-1R ANTIBODY

Background of the Invention

The present invention relates to a method of treatment for non-hematologic malignancies comprising the administration of anti-insulin-like growth factor I receptor (IGF-1R) antibodies, in conjunction with other therapeutic agents such as chemotherapeutic agents and hormonal therapy.

The insulin-like growth factor (IGF) signaling system plays an important role in the growth and development of many tissues and regulates overall growth. Insulin-like growth factor (IGF-1) is a 7.5-kD polypeptide that circulates in plasma in high concentrations and is detectable in most tissues. IGF-1 stimulates cell differentiation and cell proliferation, and is required by most mammalian cell types for sustained proliferation. These cell types include, among others, human diploid fibroblasts, epithelial cells, smooth muscle cells, T lymphocytes, neural cells, myeloid cells, chondrocytes, osteoblasts and bone marrow stem cells.

The first step in the transduction pathway leading to IGF-1-stimulated cellular proliferation or differentiation is binding of IGF-1 or IGF-2 (or insulin at supraphysiological concentrations) to the IGF-1 receptor. The IGF-1 receptor (IGF-1R) is composed of two types of subunits: an alpha subunit (a 130-135 kD protein that is entirely extracellular and functions in ligand binding) and a beta subunit (a 95-kD transmembrane protein, with transmembrane and cytoplasmic domains). IGF binding proteins (IGFBPs) have growth inhibiting effects by, at least in part, competitively binding IGFs and preventing their association with IGF-1F. The interactions between IGF-1, IGF-2, IGF1R, and IGFBPs affect many physiological and pathological processes such as development, growth and metabolic regulation.

The IGF-1R is initially synthesized as a single chain proreceptor polypeptide that is processed by glycosylation, proteolytic cleavage, and covalent bonding to assemble into a mature 460-kD heterotetramer comprising two alpha-subunits and two beta-subunits. The beta subunit(s) possesses ligand-activated tyrosine kinase activity. This activity is implicated in the signaling pathways mediating ligand action which involve autophosphorylation of the beta-subunit and phosphorylation of IGF-1R substrates.

There is considerable evidence for a role for IGF-1 and/or IGF-1R in the maintenance of tumor cells *in vitro* and *in vivo*. IGF-1R levels are elevated in tumors of lung (Kaiser et al., *J. Cancer Res. Clin. Oncol.* 119: 665-668, 1993; Moody et al., *Life Sciences* 52: 1161-1173, 1993; Macauley et al., *Cancer Res.*, 50: 2511-2517, 1990), breast (Pollack et al., *Cancer Lett.* 38: 223-230, 1987; Foekens et al., *Cancer Res.* 49: 7002-7009, 1989; Cullen et al., *Cancer Res.* 49: 7002-7009, 1990; Arteaga et al., *J. Clin. Invest.* 84: 1418-1423, 1989), prostate and colon (Remaole-Bennet et al., *J. Clin. Endocrinol. Metab.* 75: 609-616, 1992; Guo et al., *Gastroenterol.* 102: 1101-1108, 1992). In addition, IGF-1 appears to be an autocrine stimulator of human gliomas (Sandberg-Nordqvist et al., *Cancer Res.* 53: 2475-2478, 1993),

while IGF-1 stimulated the growth of fibrosarcomas that overexpressed IGF-1R (Butler et al., *Cancer Res.* 58: 3021-27, 1998). In addition, individuals with "high normal" levels of IGF-1 have an increased risk of common cancers compared to individuals with IGF-1 levels in the "low normal" range (Rosen et al., *Trends Endocrinol. Metab.* 10: 136-41, 1999). For a review of the role IGF-1/IGF-1 receptor interaction plays in the growth of a variety of human tumors, see Macaulay, *Br. J. Cancer*, 65: 311-320, 1992.

Numerous classes of antineoplastic agents are currently in use. Docetaxel, one of a group of drugs called "taxanes," which are derived from the bark and needles of yew trees, is the first anticancer agent to show a significantly higher response rate than doxorubicin, a very active agent and widely used chemotherapy in the first-line treatment of metastatic breast cancer. Docetaxel also is the first chemotherapy drug as a single agent to demonstrate increased survival among patients with advanced breast cancer compared to the combination of mitomycin C and vinblastine, a commonly used regimen in this patient population. Median time to progression and time to treatment failure were significantly longer for docetaxel than for mitomycin C in combination with vinblastine, and the one-year survival rate significantly greater. Promising results have also been recorded for docetaxel in other human malignancies, such as ovarian, lung, head and neck, gastric and pancreatic cancers.

Paclitaxel, also a taxane, binds to microtubules and prevents their molecular disassembly, thereby inhibiting mitosis (cell division). With the spindle still in place the cell cannot divide into daughter cells. Paclitaxel is most effective against ovarian carcinomas and advanced breast carcinomas.

Hormonal therapy can be very effective in lowering the risk of recurrence for women with hormone-receptor-positive breast cancer. Tamoxifen is the hormonal therapy that has been around the longest—nearly 30 years. It blocks the effect of estrogen on breast cancer cells, keeping the cells from growing. Tamoxifen can reduce recurrence by 40-50% in post-menopausal women, and by 30-50% in pre-menopausal women. It also lowers the risk of a new breast cancer developing in the unaffected breast, and can slow down the progression of advanced disease.

In recent years, aromatase inhibitors have been used as hormonal therapy. This type of therapy is recommended only for postmenopausal women with hormone-receptor-positive breast cancer. It works by blocking the production of estrogen in muscle and fat tissue, which is the main source of estrogen in women beyond menopause, after which the ovaries stop making significant levels of estrogen.

Prostate cancer is the most common cancer and the second cause of cancer death in men in the United States. About 10% of the initial cases of prostate cancer present with metastatic disease. However, in the rest, metastases will develop despite treatment with surgery, radiation or medical therapy, and those metastases will eventually become refractory

to hormonal treatment. The use of chemotherapy in hormone refractory (androgen independent) progressive prostate cancer (HRPC) has been characterized historically by poor efficacy and high toxicity. Newer regimens containing docetaxel have shown a survival benefit over previous palliative regimens. Despite this positive trend, the median survival of HRPC patients treated with docetaxel and prednisone is only 18.9 months; clearly, more effective regimens are required for the treatment of HRPC patients.

Although some currently available anti-cancer treatments have been successful, complete responses to these treatments are infrequently observed, and the patient population refractory to these treatments is still large. Thus, development of new therapeutic regimens, particularly those capable of augmenting or potentiating the anti-tumor activity of other anti-neoplastic agents, is necessary.

In view of the roles that IGF-1 and IGF-1R have in such disorders as cancer and other proliferative disorders when IGF-1 and/or IGF-1R are overexpressed, antibodies to IGF-1R have been produced that block binding of IGF-1 or IGF-2 to IGF-1R. Such antibodies are described, for example, in International Patent Application No. WO 02/053596, published July 11, 2002; International Patent Application Nos. WO 05/016967 and WO 05/016970, both published February 24, 2005; International Patent Application No. WO 03/106621, published December 24, 2003; International Patent Application No. WO 04/083248, published September 30, 2004; International Patent Application No. WO 03/100008, published December 4, 2003; International Patent Publication WO 04/087756, published October 14, 2004; and International Patent Application No WO 05/005635, published January 26, 2005. Because of their ability to block a tumor cell survival pathway, it is desirable to use such anti-IGF-1R antibodies to treat cancer, particularly non-hematological malignancies, in patients to obtain an improved clinical benefit relative to standard cancer treatment regimes alone.

Summary of the Invention

The present invention is directed to a method for the treatment of an advanced non-hematologic malignancy in a patient in need of such treatment comprising the step of administering to the patient a therapeutically effective amount of an anti-IGF-1R antibody.

More particularly, the present invention is directed to a method comprising the step of administering to the patient an antibody that specifically binds to IGF-1R in combination with a therapeutically effective amount of at least one agent selected from the group consisting of an alkylating agent, a folate antagonist, a pyrimidine antagonist, a cytotoxic antibiotic, a platinum compound, a taxane, a vinca alkaloid, a topoisomerase inhibitor, an EGFR inhibitor, and a hormonal therapy agent. Preferably the antibody is one that specifically binds to human IGF-1R.

In a preferred embodiment of the present invention, the anti-IGF-1R antibody has the following properties: (a) a binding affinity for human IGF-1R of K_d of 8 x 10^{-9} or less, and (b) inhibition of binding between human IGF-1R and IGF-1 with an IC_{50} of less than 100 nM.

In another preferred embodiment of the present invention, the anti-IGF-1R antibody comprises (a) a heavy chain comprising the amino acid sequences of CDR-1, CDR-2, and CDR-3 of an antibody selected from the group consisting of 2.12.1, 2.13.2, 2.14.3, 4.9.2, 4.17.3, and 6.1.1, and (b) a light chain comprising the amino acid sequences of CDR-1, CDR-2, and CDR-3 of an antibody selected from the group consisting of 2.12.1, 2.13.2, 2.14.3, 4.9.2, 4.17.3, and 6.1.1, or (c) sequences having changes from the CDR sequences of an antibody selected from the group consisting of 2.12.1, 2.13.2, 2.14.3, 4.9.2, 4.17.3, and 6.1.1, said sequences being selected from the group consisting of conservative changes, wherein the conservative changes are selected from the group consisting of replacement of nonpolar residues by other nonpolar residues, replacement of polar charged residues by other polar uncharged residues, replacement of polar charged residues by other polar charged residues, and substitution of structurally similar residues; and non-conservative substitutions, wherein the non-conservative substitutions are selected from the group consisting of substitution of polar charged residue for polar uncharged residues and substitution of nonpolar residues for polar residues, additions and deletions.

The present invention is also directed to a pharmaceutical composition for the treatment of a non-hematologic malignancy comprising (a) a therapeutically effective amount of an antibody that specifically binds IGF-1R, (b) a therapeutically effective amount of at least one agent selected from the group consisting of an alkylating agent, a folate antagonist, a pyrimidine antagonist, a cytotoxic antibiotic, a platinum compound, a taxane, a vinca alkaloid, a topoisomerase inhibitor, an EGFR inhibitor, and a hormonal therapy agent; and (c) a pharmaceutically acceptable carrier.

Detailed Description Of The Drawings

Figs. 1A-1C show alignments of the nucleotide sequences of the light chain variable regions from six human anti-IGF-1R antibodies to each other and to germline sequences. Fig. 1A shows the alignment of the nucleotide sequences of the variable region of the light chain (VL) of antibodies 2.12.1 (SEQ ID NO: 1) 2.13.2 (SEQ ID NO: 5), 2.14.3 (SEQ ID NO: 9) and 4.9.2 (SEQ ID NO: 13) to each other and to the germline Vκ A30 sequence (SEQ ID NO: 39). Fig. 1B shows the alignment of the nucleotide sequence of VL of antibody 4.17.3 (SEQ ID NO: 17) to the germline Vκ O12 sequence (SEQ ID NO: 41). Fig. 1C shows the alignment of the nucleotide sequence of VL of antibody 6.1.1 (SEQ ID NO: 21) to the germline Vκ A27 sequence (SEQ ID NO: 37). The alignments also show the CDR regions of the VL from each antibody. The consensus sequences for Figs. 1A-1C are shown in SEQ ID NOS: 53-55, respectively.

Figs. 2A-2D show alignments of the nucleotide sequences of the heavy chain variable regions from six human anti-IGF-1R antibodies to each other and to germline sequences. Fig. 2A shows the alignment of the nucleotide sequence of the VH of antibody 2.12.1 (SEQ ID NO: 3) to the germline VH DP-35 sequence (SEQ ID NO: 29). Fig. 2B shows the alignment of the nucleotide sequence of the VH of antibody 2.14.3 (SEQ ID NO: 11) to the germline VIV-4/4.35 sequence (SEQ ID NO: 43). Figs. 2C-1 and 2C-2 show the alignments of the nucleotide sequences of the VH of antibodies 2.13.2 (SEQ ID NO: 7), 4.9.2 (SEQ ID NO: 15) and 6.1.1 (SEQ ID NO: 23) to each other and to the germline VH DP-47 sequence (SEQ ID NO: 31). Fig. 2D shows the alignment of the nucleotide sequence of the VH of antibody 4.17.3 (SEQ ID NO: 19) to the germline VH DP-71 sequence (SEQ ID NO: 35). The alignment also shows the CDR regions of the antibodies. The consensus sequences for Figs. 2A-2D are shown in SEQ ID NOS: 56-59, respectively.

Fig. 3A shows the number of mutations in different regions of the heavy and light chains of 2.13.2 and 2.12.1 compared to the germline sequences. Figs. 3A-D show alignments of the amino acid sequences from the heavy and light chains of antibodies 2.13.2 and 2.12.1 with the germline sequences from which they are derived. Fig. 3B shows an alignment of the amino acid sequence of the heavy chain of antibody 2.13.2 (SEQ ID NO: 45) with that of germline sequence DP-47(3-23)/D6-19/JH6 (SEQ ID NO: 46). Fig. 3C shows an alignment of the amino acid sequence of the light chain of antibody 2.13.2 (SEQ ID NO: 47) with that of germline sequence A30/Jk2 (SEQ ID NO: 48). Fig. 3D shows an alignment of the amino acid sequence of the heavy chain of antibody 2.12.1 (SEQ ID NO: 49) with that of germline sequence DP-35(3-11)/D3-3/JH6 (SEQ ID NO: 50). Fig. 3E shows an alignment of the amino acid sequence of the light chain of antibody 2.12.1 (SEQ ID NO: 51) with that of germline sequence A30/Jk1 (SEQ ID NO: 52). For Figures 3B-E, the signal sequences are in italic, the CDRs are underlined, the constant domains are bold, the framework (FR) mutations are highlighted with a plus sign ("+") above the amino acid residue and CDR mutations are highlighted with an asterisk above the amino acid residue.

Figure 4 shows that anti-IGF-1R antibodies 2.13.2 and 4.9.2 reduce IGF-1R phosphotyrosine signal in 3T3-IGF-1R tumors.

Figure 5 shows that anti-IGF-1R antibody 2.13.2 inhibits 3T3-IGF-1R tumor growth *in vivo*.

Detailed Description of the Invention

The present invention are directed to the treatment of non-hematologic malignancies, including breast, lung, brain, skin, ovarian, prostate, head and neck, colorectal, gastric, bladder, renal, esophageal, and pancreatic cancers, as well as solid tumors of childhood. Treatment of both early stage and advanced (metastatic) cancers are within the scope of the

present invention. In preferred embodiments, the method of the present invention is used in the treatment of breast cancer, prostate cancer, and non-small cell lung cancer (NSCLC).

There are many classes of chemotherapeutic drugs currently in use for the treatment of non-hematological malignancies that are suitable for use in the combination therapy of the present invention. For example, alkylating agents are a class of drugs that alkylate DNA, restricting uncoiling and replication of strands. Alkylating agents include cyclophosphamide (CYTOXAN), ifosfamide (IFEX), mechlorethamine hydrochloride (MUSTARGEN), thiotepa (THIOPLEX), streptozotocin (ZANOSAR), carmustine (BICNU, GLIADEL WAFER), lomustine (CEENU), and dacarbazine (DTIC-DOME). A preferred alkylating agent for use in the methods of the present invention is cyclophosphamide.

Folate antagonists bind to dihydrofolate reductase (DHFR) and interfere with pyrimidine (thymidine) synthesis. Methotrexate (MATREX, FOLEX, TREXALL), trimetrexate (NEUTREXIN) and pemetrexed (ARIMTA) are folate antagonists suitable for use in the methods of the present invention. In addition to DHFR, pemetrexed also inhibits thymidylate synthase and glycinamide ribonucleotide formyl transferase, two other folate-dependent enzymes involved in thymidine synthesis.

Pyrimidine antagonists inhibit enzymes involved in pyrimidine synthesis. As pyrimidine analogs, they also interfere with DNA production by competing with normal nucleotides for incorporation into the DNA molecule. Pyrimidine antagonists suitable for use in the methods of the present invention include 5-fluorouracil (5-FU); capecitabine (XELODA), a prodrug of 5'-deoxy-5-fluorouridine (5'-FDUR), which is enzymatically converted to 5-FU *in vivo*; raltitrexed (TOMUDEX); tegafur-uracil (UFTORAL); and gemcitabine (GEMZAR).

Anthracycline antibiotics exert a cytotoxic effect by inhibiting the uncoiling of DNA by intercalation between DNA strands. Anthracyclines and anthracyclines derivatives include doxorubicin hydrochloride (ADRIAMYCIN, RUBEX, DOXIL), epirubicin hydrochloride (ELLENCE, PHARMORUBICIN), daunorubicin (CERUBIDINE, DAUNOXOME), nemorubicin, idarubicin hydrochloride (IDAMYCIN PFS, ZAVEDOS) and mitoxantrone (DHAD, NOVANTRONE). Preferred anthracyclines for use with the present invention include doxorubicin and epirubicin.

Other cytotoxic antibiotics are useful as cancer chemotherapeutic agents and suitable for use in the present invention. These include dactinomycin (actinomycin D, COSMEGEN), plicamycin (MITHRACIN), mitomycin (MUTAMYCIN), and bleomycin (BLENOXANE). Dactinomycin is particularly preferred.

Platinum compounds exert their anti-neoplastic effect by intercalation and intracalation between DNA strands, which inhibits uncoiling of the DNA. Platinum compounds useful in the methods of the present invention include cisplatin (PLATINOL) and carboplatin (PARAPLATIN).

Taxanes promote assembly of microtubules while inhibiting their disassembly into tubulin, thereby blocking a cell's ability to break down the mitotic spindle during mitosis. They have demonstrated significant activity against many solid tumors as single agent therapy and in combination with other chemotherapy agents. One embodiment of the combination therapy of the present invention includes the use of one or more taxanes in combination with the IGF-1R antibody. Suitable taxanes for use in combination with the IGF-1R antibody include docetaxel (TAXOTERE) and paclitaxel (TAXOL).

Vinca alkaloids, like taxanes, are "spindle poisons," acting on the microtubules that form the mitotic spindle. They inhibit mitosis by interfering with microtubule assembly, keeping the spindle from being formed. Vinca alkaloids include vindesine (ELDISINE), vinblastine sulfate (VELBAN), vincristine sulfate (ONCOVIN) and vinorelbine tartrate (NAVELBINE). A preferred vinca alkaloid for use in the methods of the present invention is vinorelbine.

The camptothecin analogs act through inhibition of topoisomerase I, an enzyme critical for DNA replication and packaging. Levels of topoisomerase I are higher in tumor cells than in normal tissue. Camptothecin analogs useful in the methods of the present invention include irinotecan (CAMPTOSAR) and topotecan (HYCAMTIN). Irinotecan is particularly preferred.

Inhibitors of topoisomerase II interfere with the normal DNA breakage resealing process (as do inhibitors of topoisomerase I), and they also interfere with the separation of newly replicated chromosomes, resulting in clastogenic mutation and potential cell death. The anthracyline antibiotics discussed above exhibit topoisomerase II inhibitory activity. Derivatives of podophyllotoxin, an extract of the mayapple that is an antimitotic glucoside) are also topoisomerase II inhibitors. Podophyllotoxin derivatives suitable for use in the present invention include etoposide (VEPESID), etoposide phosphate (ETOPOPHOS), and teniposide (VUMON). Etoposide is particularly preferred.

Compounds directed at inhibition of epidermal growth factor receptor (EGFR) tyrosine kinase (TK) represent a relatively new class of antineoplastic drugs that are useful in the method of the present invention. Many human cancers express members of the EGFR family on the cell surface. When a ligand binds to EGFR, it sets off a cascade of cellular reactions that result in increased cell division and influence other aspects of cancer development and progression, including angiogenesis, metastatic spread, and inhibition of apoptosis. EGFR-TK inhibitors may selectively target one of the members of the EGFR family (EGFR (also known as HER1 or ErbB-1), HER2/neu (also known as ErbB-2), HER3 (also known as ErbB-3), or HER4 (also known as ErbB-4)), or may target two or more of them. EGFR-TK inhibitors suitable for use in the present invention include gefitinib (IRESSA), erlotinib (TARCEVA), trastuzumab (HERCEPTIN), panitumumab (ABX-EGF; Abgenix/Amgen), lapatinib

(GlaxoSmithKline), CI-1033 (Pfizer), GW2016 (GlaxoSmithKline), EKB-569 (Wyeth), PKI-166 (Novartis), CP-724,714 (Pfizer), and BIBX-1382 (Boeringer-Ingelheim). Additional EGFR-TK inhibitors are described in United States Patent Publication No. US 2002-0169165A1, published November 14, 2002.

Another embodiment of the combination therapy of the present invention includes the use of hormonal therapy in combination with the IGF-1R antibody, particularly anti-estrogens in the treatment of breast cancer. Some hormonal treatments compete with estrogen for binding sites in breast tissue. These include tamoxifen citrate (NOLVADEX) and fulvestrant (FASLODEX). Similarly, anti-androgens block testosterone receptors and therefore are useful in the treatment of androgen-dependent prostate cancer.

Other hormone treatments include aromatase inhibitors. This class of hormonal agents inactivate aromatase, the enzyme which converts androgens to estrogens. Examples of aromatase inhibitors suitable for use in combination with the IGF-1R antibody include anastrozole (ARIMIDEX), letrozole (FEMARA), exemestane (AROMASIN), and fadrozole hydrochloride. Exemestane is a particularly preferred aromatase inhibitor for use in the methods of the present invention.

Co-administration of the antibody with an additional therapeutic agent (combination therapy) encompasses administering a pharmaceutical composition comprising both the anti-IGF-1R antibody and one or more additional therapeutic agents, and administering two or more separate pharmaceutical compositions, one comprising the anti-IGF-1R antibody and the other(s) comprising the additional therapeutic agent(s). Further, although co-administration or combination (conjoint) therapy generally mean that the antibody and additional therapeutic agents are administered at the same time as one another, it also encompasses simultaneous, sequential or separate dosing of the individual components of the treatment.

The present invention also encompasses the administration of other therapeutic agents in addition to the first and second components, either concurrently with one or more of those components, or sequentially. Such therapeutic agents include analgesics, cancer vaccines, anti-vascular agents, anti-proliferative agents, and anti-emetic agents. Preferred anti-emetic agents include aprepitant, ondansetron hydrochloride, granisetron hydrochloride, and metoclopramide.

Each administration may vary in its duration from a rapid administration to a continuous perfusion. As a result, for the purposes of the present invention, the combinations are not exclusively limited to those that are obtained by physical association of the constituents, but also to those that permit a separate administration, which can be simultaneous or spaced out over a period of time. The compositions according to the invention are preferably compositions which can be administered parentally. However, these

compositions may be administered orally or intraperitoneally in the case of localized regional therapies.

As will be appreciated by one of skill in the art, the choice of therapeutic agents to be used in combination with IGF-1R antibodies, and the timing of their use, will be determined in part by the type and stage of the cancer that is being treated. For example, in early breast cancer (where the cancer has not spread outside the breast), surgery and radiation are generally followed by adjuvant chemotherapy or adjuvant hormonal therapy, either of which may be combined with IGF-1R antibodies in the methods of the present invention. Typical adjuvant chemotherapy for early breast cancer includes cyclophosphamide, methotrexate and 5-FU ("CMF"); 5-FU, doxorubicin, and cyclophosphamide ("FAC"); docetaxel, doxorubicin, and cyclophosphamide ("TAC"); doxorubicin and cyclophosphamide ("AC"); doxorubicin and cyclophosphamide followed by paclitaxel ("AC and T"); and 5-FU, epirubicin, and cyclophosphamide ("FEC"). Tamoxifen is a preferred hormonal treatment at this stage.

In locally advanced breast cancer, wherein the cancer has spread only to nearby tissues or lymph nodes, the patient is often given chemotherapy prior to surgery and radiation, which are then followed by adjuvant hormonal therapy. Alternatively, surgery/radiation is followed by adjuvant chemotherapy, then adjuvant hormonal therapy. IGF-1R antibodies may be administered in conjunction with the chemotherapeutic or hormonal therapy agents whether they are used either before or after surgery/radiation. Typical chemotherapy regimes for locally advanced breast cancer include FAC, AC, FEC, and doxorubicin plus docetaxel ("AT").

Metastatic breast cancer has spread to other parts of the body from the breast in which it started. Chemotherapy optionally may be preceded by hormonal therapy. First line hormonal therapy currently includes tamoxifen and anastrozole. First line chemotherapy regimens currently include FAC, TAC, docetaxel plus epirubicin, docetaxel, paclitaxel, capecitabine, vinorelbine, and trastuzumab. Second line chemotherapy treatments include docetaxel, alone or in combination with capecitabine. The methods of the present invention are suitable for use both as first line therapy and second line therapy.

In the United States, the combination of paclitaxel and carboplatin has become accepted as the standard of care for first line treatment of inoperable Stage IIIB (*i.e.* cancer has spread to structures near the lung, to lymph nodes in the mediastinum, or to lymph nodes on the other side of the chest or in the lower neck) and Stage IV (*i.e.* cancer has spread to other parts of the body or to another lobe of the lungs) non-small cell lung cancer (NSCLC). But the overall response rate is only approximately 28% for patients with performance status 0-1 in efficacy studies with a predominantly Stage IV population. In Europe, first line treatment for NSCLC is gemcitabine and cisplatin. Other treatment regimens for NSCLC include paclitaxel alone or with cisplatin or gemcitabine; docetaxel alone or with cisplatin or

gemcitabine; vinorelbine alone or with gemcitabine; irinotecan alone or with gemcitabine; pemetrexed; and gefitinib.

It is known that signaling through IGF-1R is required for the tumorgenicity of cell lines and has been shown to decrease the cytotoxicity of chemotherapy, and that blocking IGF-1R activity enhances the effectiveness of current therapies and prevents tumor progression in animal models. It was therefore expected that an inhibitor of IGF-1R such as the antibodies of the present invention would reduce tumor cell survival and enhance the efficacy of chemotherapy when given in combination.

When incubated with cells, fully human monoclonal antibodies that are highly specific and potent inhibitors of IGF-1-induced receptor autophosphorylation induced down-regulation of IGF-1R by receptor internalization. The doses that down-regulated IGF-1R in solid tumor *ex vivo* models (31.25-125 µg) corresponded to antibody concentrations of 8-40 µg/ml at Day 1 and 2-20 µg/ml at Day 9. Intraperitoneal administration of the anti-IGF-1R antibodies to athymic mice bearing tumors of the transfectant IGF-1R over-expressing NIH-3T3 cell line resulted in a dose dependent inhibition of tumor growth. The serum concentration of anti-IGF-1R antibodies that led to 50% growth inhibition was 20 µg/ml at Day 1, and 13 µg/ml at Day 9. Similar anti-tumor studies were extended to human tumor xenograft models. As a single agent, anti-IGF-1R antibodies inhibited the growth of several xenograft models including breast, lung and colorectal carcinomas.

The combination of anti-IGF-1R antibodies with paclitaxel or carboplatin was tested in the H460 and EBC-1 human NSCLC tumor xenograft models. Combination of anti-IGF-1R antibodies with those agents increased their tumor growth inhibition compared to each agent alone.

Unless otherwise defined herein, scientific, technical, and medical terms used in connection with the present invention shall have the meanings that are commonly understood by those of ordinary skill in the art. Generally, nomenclatures used in connection with, and techniques of, cell and tissue culture, molecular biology, immunology, microbiology, genetics and protein and nucleic acid chemistry described herein are those well known and commonly used in the art.

The following terms, unless otherwise indicated, shall be understood to have the following meanings:

An "antibody" refers to an intact immunoglobulin or to an antigen-binding portion thereof that competes with the intact antibody for specific binding. Antigen-binding portions may be produced by recombinant DNA techniques or by enzymatic or chemical cleavage of intact antibodies. Antigen-binding portions include, *inter alia*, Fab, Fab', $F(ab')_2$, Fv, dAb, and complementarity determining region (CDR) fragments, single-chain antibodies (scFv),

chimeric antibodies, diabodies and polypeptides that contain at least a portion of an immunoglobulin that is sufficient to confer specific antigen binding to the polypeptide.

Immunoglobulin chains exhibit the same general structure of relatively conserved framework regions (FR) joined by three hypervariable regions, also called complementarity determining regions or CDRs. The CDRs from the two chains of each pair are aligned by the framework regions, enabling binding to a specific epitope. From N-terminus to C-terminus, both light and heavy chains comprise the domains FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. The assignment of amino acids to each domain is in accordance with the definitions of Kabat, *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md. (1987 and 1991)), or Chothia & Lesk, *J. Mol. Biol.* 196:901-917 (1987); Chothia et al., *Nature* 342:878-883 (1989).

An "isolated antibody" is an antibody that (1) is not associated with naturally-associated components, including other naturally-associated antibodies, that accompany it in its native state, (2) is free of other proteins from the same species, (3) is expressed by a cell from a different species, or (4) does not occur in nature. Examples of isolated antibodies include an anti-IGF-1R antibody that has been affinity purified using IGF-1R is an isolated antibody, an anti-IGF-1R antibody that has been synthesized by a hybridoma or other cell line *in vitro*, and a human anti-IGF-1R antibody derived from a transgenic mouse.

The term "chimeric antibody" refers to an antibody that contains one or more regions from one antibody and one or more regions from one or more other antibodies. In a preferred embodiment, one or more of the CDRs are derived from a human anti-IGF-1R antibody. In a more preferred embodiment, all of the CDRs are derived from a human anti-IGF-1R antibody. In another preferred embodiment, the CDRs from more than one human anti-IGF-1R antibodies are mixed and matched in a chimeric antibody. Further, the framework regions may be derived from one of the same anti-IGF-1R antibodies, from one or more different antibodies, such as a human antibody, or from a humanized antibody.

The term “epitope” includes any protein determinant capable of specific binding to an immunoglobulin or T-cell receptor. Epitopic determinants usually consist of chemically active surface groupings of molecules such as amino acids or sugar sides chains and usually have specific three dimensional structural characteristics, as well as specific charge characteristics. An antibody is said to specifically bind an antigen when the dissociation constant is ≤ 1 μM, preferably ≤ 100 nM and most preferably ≤ 10 nM.

As applied to polypeptides, the term "substantial identity" means that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 75% or 80% sequence identity, preferably at least 90% or 95% sequence identity, even more preferably at least 98% or 99% sequence identity. Preferably, residue positions that are not identical differ by conservative amino acid substitutions. A

"conservative amino acid substitution" is one in which an amino acid residue is substituted by another amino acid residue having a side chain (R group) with similar chemical properties (*e.g.*, charge or hydrophobicity). In general, a conservative amino acid substitution will not substantially change the functional properties of a protein. In cases where two or more amino acid sequences differ from each other by conservative substitutions, the percent sequence identity or degree of similarity may be adjusted upwards to correct for the conservative nature of the substitution. Means for making this adjustment are well-known to those of skill in the art. See, *e.g.*, Pearson, *Methods Mol. Biol.* 24: 307-31 (1994). Examples of groups of amino acids that have side chains with similar chemical properties include 1) aliphatic side chains: glycine, alanine, valine, leucine and isoleucine; 2) aliphatic-hydroxyl side chains: serine and threonine; 3) amide-containing side chains: asparagine and glutamine; 4) aromatic side chains: phenylalanine, tyrosine, and tryptophan; 5) basic side chains: lysine, arginine, and histidine; and 6) sulfur-containing side chains are cysteine and methionine. Conservative amino acids substitution groups include: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, glutamate-aspartate, and asparagine-glutamine.

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

The phrase "in combination with" encompasses simultaneous, sequential or separate dosing of the individual components of the treatment. For example, the antibody may be administered once every three days, while the additional therapeutic agent is administered once daily. The antibody may be administered prior to or subsequent to treatment of the disorder with the additional therapeutic agent. Similarly, the anti-IGF-1R antibody may be administered prior to or subsequent to other therapy, such as radiotherapy, chemotherapy, photodynamic therapy, surgery or other immunotherapy.

The terms “concurrently” and “simultaneously” are used interchangeably and mean the compounds of the combination therapy of the present invention are administered (1) simultaneously in time, or (2) at different times during the course of a common treatment schedule. The term “sequentially” as used herein means administration of the a first

component, followed by administration of a second component. Anti-IGF-1R antibodies may be the first component or the second component. After administration of one component, the second component can be administered substantially immediately after the first component, or the second component can be administered an effective time period after the first component; the effective time period is the amount of time given for realization of maximum benefit from the administration of the first component.

The term "patient" includes mammals. In a preferred embodiment, the mammal is a human.

The term "treating," as used herein, unless otherwise indicated, means reversing, alleviating, inhibiting the progress of, or preventing the disorder or condition to which such term applies, or one or more symptoms of such disorder or condition. The term "treatment," as used herein, unless otherwise indicated, refers to the act of treating as "treating" is defined immediately above.

Human antibodies avoid certain of the problems associated with antibodies that possess mouse or rat variable and/or constant regions. More preferred are fully human anti-human IGF-1R antibodies. Fully human anti-IGF-1R antibodies are expected to minimize the immunogenic and allergic responses intrinsic to mouse or mouse-derivatized monoclonal antibodies (Mabs) and thus to increase the efficacy and safety of the administered antibodies. The use of fully human antibodies can be expected to provide a substantial advantage in the treatment of chronic and recurring human diseases, such as inflammation and cancer, which may require repeated antibody administrations. In another embodiment, the invention provides an anti-IGF-1R antibody that does not bind complement.

In another aspect of the invention, the anti-IGF-1R antibodies bind to IGF-1R with high affinity. In one embodiment, the anti-IGF-1R antibody binds to IGF-1R with a K_d of 1 x 10^{-8} M or less. In a more preferred embodiment, the antibody binds to IGF-1R with a K_d or 1 x 10^{-9} M or less. In an even more preferred embodiment, the antibody binds to IGF-1R with a K_d or 5 x 10^{-10} M or less. In another preferred embodiment, the antibody binds to IGF-1R with a K_d or 1 x 10^{-10} M or less. In another preferred embodiment, the antibody binds to IGF-1R with substantially the same K_d as an antibody selected from 2.12.1, 2.13.2, 2.14.3, 3.1.1, 4.9.2, 4.17.3 or 6.1.1. In another preferred embodiment, the antibody binds to IGF-1R with substantially the same K_d as an antibody that comprises one or more CDRs from an antibody selected from 2.12.1, 2.13.2, 2.14.3, 3.1.1, 4.9.2, 4.17.3 or 6.1.1.

The invention also employs an anti-IGF-1R antibody that binds the same antigen or epitope as a human anti-IGF-1R antibody. The invention may also employ an anti-IGF-1R antibody that cross-competes with a human anti-IGF-1R antibody. In a preferred embodiment, the human anti-IGF-1R antibody is 2.12.1, 2.13.2, 2.14.3, 3.1.1, 4.9.2, 4.17.3 or

6.1.1. In another preferred embodiment, the human anti-IGF-1R comprises one or more CDRs from an antibody selected from 2.12.1, 2.13.2, 2.14.3, 3.1.1, 4.9.2, 4.17.3 or 6.1.1

The invention can also be practiced using an anti-IGF-1R antibody that comprises variable sequences encoded by a human κ gene. In a preferred embodiment, the variable sequences are encoded by either the Vκ A27, A30 or O12 gene family. In a preferred embodiment, the variable sequences are encoded by a human Vκ A30 gene family. In a more preferred embodiment, the light chain comprises no more than ten amino acid substitutions from the germline Vκ A27, A30 or O12, preferably no more than six amino acid substitutions, and more preferably no more than three amino acid substitutions. In a preferred embodiment, the amino acid substitutions are conservative substitutions.

In a preferred embodiment, the VL of the anti-IGF-1R antibody contains the same amino acid substitutions, relative to the germline amino acid sequence, as any one or more of the VL of antibodies 2.12.1, 2.13.2, 2.14.3, 3.1.1, 4.9.2, 4.17.3 or 6.1.1.

In another preferred embodiment, the light chain comprises an amino acid sequence that is the same as the amino acid sequence of the VL of 2.12.1, 2.13.2, 2.14.3, 3.1.1, 4.9.2, 4.17.3 or 6.1.1. In another highly preferred embodiment, the light chain comprises amino acid sequences that are the same as the CDR regions of the light chain of 2.12.1, 2.13.2, 2.14.3, 3.1.1, 4.9.2, 4.17.3 or 6.1.1. In another preferred embodiment, the light chain comprises an amino acid sequence from at least one CDR region of the light chain of 2.12.1, 2.13.2, 2.14.3, 3.1.1, 4.9.2, 4.17.3 or 6.1.1.

The present invention can also be carried out using an anti-IGF-1R antibody or portion thereof comprising a human heavy chain or a sequence derived from a human heavy chain. In one embodiment, the heavy chain amino acid sequence is derived from a human V_H DP-35, DP-47, DP-70, DP-71 or VIV-4/4.35 gene family. In a preferred embodiment, the heavy chain amino acid sequence is derived from a human V_H DP-47 gene family. In a more preferred embodiment, the heavy chain comprises no more than eight amino acid changes from germline V_H DP-35, DP-47, DP-70, DP-71 or VIV-4/4.35, more preferably no more than six amino acid changes, and even more preferably no more than three amino acid changes.

In a preferred embodiment, the VH of the anti-IGF-1R antibody contains the same amino acid substitutions, relative to the germline amino acid sequence, as any one or more of the VH of antibodies 2.12.1, 2.13.2, 2.14.3, 3.1.1, 4.9.2, 4.17.3 or 6.1.1. In another embodiment, the amino acid substitutions are made in the same position as those found in any one or more of the VH of antibodies 2.12.1, 2.13.2, 2.14.3, 3.1.1, 4.17.3., 4.9.2 or 6.1.1, but conservative amino acid substitutions are made rather than using the same amino acid.

In another preferred embodiment, the heavy chain comprises an amino acid sequence that is the same as the amino acid sequence of the VH of 2.12.1, 2.13.2, 2.14.3, 3.1.1, 4.9.2, 4.17.3 or 6.1.1. In another highly preferred embodiment, the heavy chain

comprises amino acid sequences that are the same as the CDR regions of the heavy chain of 2.12.1, 2.13.2, 2.14.3, 3.1.1, 4.9.2, 4.17.3 or 6.1.1. In another preferred embodiment, the heavy chain comprises an amino acid sequence from at least one CDR region of the heavy chain of 2.12.1, 2.13.2, 2.14.3, 3.1.1, 4.9.2, 4.17.3 or 6.1.1. In another preferred embodiment, the heavy chain comprises amino acid sequences from CDRs from different heavy chains. In a more preferred embodiment, the CDRs from different heavy chains are obtained from 2.12.1, 2.13.2, 2.14.3, 3.1.1, 4.9.2, 4.17.3 or 6.1.1.

In another embodiment, the invention employs an anti-IGF-1R antibody that inhibits the binding of IGF-1 to IGF-1R or the binding of IGF-2 to IGF-1R. In a preferred embodiment, the IGF-1R is human. In another preferred embodiment, the anti-IGF-1R antibody is a human antibody. In another embodiment, the antibody or portion thereof inhibits binding between IGF-1R and IGF-1 with an IC_{50} of no more than 100 nM. In a preferred embodiment, the IC_{50} is no more than 10 nM. In a more preferred embodiment, the IC_{50} is no more than 5 nM. The IC_{50} can be measured by any method known in the art. Typically, an IC_{50} can be measured by ELISA or RIA. In a preferred embodiment, the IC_{50} is measured by RIA.

In another embodiment, the invention employs an anti-IGF-1R antibody that prevents activation of the IGF-1R in the presence of IGF-i. In another aspect of the invention, the antibody causes the downregulation of IGF-1R from a cell treated with the antibody. In a preferred embodiment, the antibody is selected 2.12.1, 2.13.2, 2.14.3, 3.1.1, 4.9.2, or 6.1.1, or comprises a heavy chain, light chain or antigen-binding region thereof.

Human antibodies can be produced by immunizing a non-human animal comprising of some or all of the human immunoglobulin locus with an IGF-1R antigen. In a preferred embodiment, the non-human animal is a XENOMOUSE™, which is an engineered mouse strain that comprises large fragments of the human immunoglobulin loci and is deficient in mouse antibody production. *See, e.g.*, Green et al. *Nature Genetics* 7:13-21 (1994) and United States Patent Nos. 5,916,771, 5,939,598, 5,985,615, 5,998,209, 6,075,181, 6,091,001, 6,114,598, and 6,130,364. *See also* International Patent Application Nos. WO 91/10741, published July 25, 1991; WO 94/02602, published February 3, 1994; WO 96/34096 and WO 96/33735, both published October 31, 1996; WO 98/16654, published April 23, 1998; WO 98/24893, published June 11, 1998; WO 98/50433, published November 12, 1998; WO 99/45031, published September 10, 1999; WO 99/53049, published October 21, 1999; WO 00/09560, published February 24, 2000; and WO 00/037504, published June 29, 2000. The XENOMOUSE ™ produces an adult-like human repertoire of fully human antibodies, and generates antigen-specific human monoclonal antibodies. A second generation XENOMOUSE™ contains approximately 80% of the human antibody repertoire through introduction of megabase sized, germline configuration YAC fragments of the human heavy

chain loci and κ light chain loci. See Mendez et al. *Nature Genetics* 15:146-156 (1997), Green and Jakobovits *J. Exp. Med.* 188:483-495 (1998).

The IGF-1R antigen can be administered with an adjuvant to stimulate the immune response. Such adjuvants include complete or incomplete Freund's adjuvant, RIBI (muramyl dipeptides) or ISCOM (immunostimulating complexes). Such adjuvants may protect the polypeptide from rapid dispersal by sequestering it in a local deposit, or they may contain substances that stimulate the host to secrete factors that are chemotactic for macrophages and other components of the immune system.

The nucleic acid molecule encoding the variable region of the light chain may be derived from the A30, A27 or O12 Vκ gene. In a preferred embodiment, the light chain is derived from the A30 Vκ gene. In an even more preferred embodiment, the nucleic acid molecule encoding the light chain contains no more than ten amino acid changes from the germline A30 Vκ gene, preferably no more than six amino acid changes, and even more preferably no more than three amino acid changes.

In one embodiment, the antibody contains no greater than ten amino acid changes in either the VH or VL regions of the mutated anti-IGF-1R antibody compared to the anti-IGF-1R antibody prior to mutation. In a more preferred embodiment, there are no more than five amino acid changes in either the VH or VL regions of the mutated anti-IGF-1R antibody, more preferably no more than three amino acid changes. In another embodiment, there are no more than fifteen amino acid changes in the constant domains, more preferably, no more than ten amino acid changes, even more preferably, no more than five amino acid changes.

SEQ ID NOS: 2, 6, 10, 14, 18 and 22 provide the amino acid sequences of the variable regions of six anti-IGF-1R κ light chains. SEQ ID NOS: 4, 8, 12, 16, 20 and 24 provide the amino acid sequences of the variable regions of six anti-IGF-1R heavy chains. SEQ ID NO: 26 depicts the amino acid sequence and SEQ ID NO: 25 depicts the nucleic acid sequence encoding the constant region of the light chain of the anti-IGF-1R antibodies 2.12.1, 2.13.2, 2.14.3, 3.1.1, 4.9.2, 4.17.3 and 6.1.1. SEQ ID NO: 28 depicts the amino acid sequence and SEQ ID NO: 27 depicts the nucleic acid sequence encoding the constant region of the heavy chain of the anti-IGF-1R antibodies 2.12.1, 2.13.2, 2.14.3, 3.1.1, 4.9.2, 4.17.3 and 6.1.1. SEQ ID NOS: 30, 32, 34, 36 and 44 provide the amino acid sequences of the germline heavy chains DP-35, DP-47, DP-70, DP-71 and VIV-4, respectively. SEQ ID NO: 33 provides the nucleotide sequence of the germline heavy chain DP-70. SEQ ID NOS: 38, 40 and 42 provide the amino acid sequences of the three germline κ light chains from which the six anti-IGF-1R κ light chains are derived.

The anti-IGF-1R antibodies can be incorporated into pharmaceutical compositions suitable for administration to a subject. Typically, the pharmaceutical composition comprises an antibody and a pharmaceutically acceptable carrier. As used herein, "pharmaceutically

acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. Examples of pharmaceutically acceptable carriers include water, saline, phosphate buffered saline, dextrose, glycerol, ethanol and the like, as well as combinations thereof. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Minor amounts of auxiliary substances such as wetting or emulsifying agents, preservatives or buffers, which enhance the shelf life or effectiveness of the antibody or antibody portion, may also be included.

The pharmaceutical compositions may be in a variety of forms. These include, for example, liquid, semi-solid and solid dosage forms, such as liquid solutions (e.g., injectable and infusible solutions), dispersions or suspensions, tablets, pills, powders, liposomes and suppositories. The preferred form depends on the intended mode of administration and therapeutic application. Typical preferred compositions are in the form of injectable or infusible solutions, such as compositions similar to those used for passive immunization of humans with other antibodies. A preferred mode of administration is parenteral (e.g., intravenous, subcutaneous, intraperitoneal, intramuscular, or infusion). In a preferred embodiment, the antibody is administered by intravenous infusion or injection. In another preferred embodiment, the antibody is administered by intramuscular or subcutaneous injection. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results.

Therapeutic compositions typically must be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, dispersion, liposome, or other ordered structure suitable to high drug concentration. Sterile injectable solutions can be prepared by incorporating the anti-IGF-1R antibody in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. The proper fluidity of a solution can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prolonged absorption of injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin.

In certain embodiments, the active compound may be prepared with a carrier that will protect the compound against rapid release, such as a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Many methods for the preparation of such formulations are patented or generally known to those skilled in the art. See, *e.g.,* Sustained and Controlled Release Drug Delivery Systems, J. R. Robinson, ed., Marcel Dekker, Inc., New York, 1978.

The pharmaceutical composition may include a "therapeutically effective amount" or a "prophylactically effective amount" of an antibody or antibody portion of the invention. A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result. A therapeutically effective amount of the antibody or antibody portion may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the antibody or antibody portion to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the antibody or antibody portion are outweighed by the therapeutically beneficial effects. A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount will be less than the therapeutically effective amount.

Dosage regimens may be adjusted to provide the optimum desired response. For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. Pharmaceutical composition comprising the antibody or comprising a combination therapy comprising the antibody and one or more additional therapeutic agents may be formulated for single or multiple doses. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic or prophylactic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals. A particularly useful formulation is 5

mg/ml anti-IGF-1R antibody in a buffer of 20mM sodium citrate, pH 5.5, 140mM NaCl, and 0.2mg/ml polysorbate 80.

The antibody, with or without an additional agent, may be administered once, or more than once for at least the period of time until the condition is treated, palliated or cured. The antibody generally will be administered for as long as the tumor is present provided that the antibody causes the tumor or cancer to stop growing or to decrease in weight or volume. The antibody will generally be administered as part of a pharmaceutical composition as described *supra*. The dosage of antibody will generally be in the range of 0.025-100 mg/kg, more preferably 0.05-50 mg/kg, more preferably 0.05-20 mg/kg, and even more preferably 0.1-10 mg/kg. It is to be noted that dosage values may vary with the type and severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that dosage ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition.

The antibody may be administered from three times daily to once every six months. The administration may be on a schedule such as three times daily, twice daily, once daily, once every two days, once every three days, once weekly, once every two weeks, once every month, once every two months, once every three months and once every six months. The antibody may be administered via an oral, mucosal, buccal, intranasal, inhalable, intravenous, subcutaneous, intramuscular, parenteral, intratumor or topical route.

The antibody may be administered at a site distant from the site of the tumor. The antibody may also be administered continuously via a minipump.

In certain embodiments, the antibody may be administered in an aerosol or inhalable form. Dry aerosol in the form of finely divided solid particles that are not dissolved or suspended in a liquid are also useful in the practice of the present invention. The pharmaceutical formulations of the present invention may be administered in the form of an aerosol spray using for example, a nebulizer such as those described in U.S. Patent Nos. 4,624,251; 3,703,173; 3,561,444; and 4,635,627.

The serum concentration of the antibody may be measured by any method known in the art. The antibody may also be administered prophylactically in order to prevent a cancer or tumor from occurring. This may be especially useful in patients that have a "high normal" level of IGF-1 because these patients have been shown to have a higher risk of developing common cancers. See Rosen et al., *supra*.

The antibody employed in the method of the invention can be labeled. This can be done by incorporation of a detectable marker, *e.g.*, incorporation of a radiolabeled amino acid or attachment to a polypeptide of biotinyl moieties that can be detected by marked avidin

(*e.g.*, streptavidin containing a fluorescent marker or enzymatic activity that can be detected by optical or colorimetric methods). In certain situations, the label or marker can also be therapeutic. Various methods of labeling polypeptides and glycoproteins are known in the art and may be used. Examples of labels for polypeptides include, but are not limited to, the following: radioisotopes or radionuclides (*e.g.*, ^{3}H, ^{14}C, ^{15}N, ^{35}S, ^{90}Y, ^{99}Tc, ^{111}In, ^{125}I, ^{131}I), fluorescent labels (*e.g.*, FITC, rhodamine, lanthanide phosphors), enzymatic labels (*e.g.*, horseradish peroxidase, β-galactosidase, luciferase, alkaline phosphatase), chemiluminescent, biotinyl groups, predetermined polypeptide epitopes recognized by a secondary reporter (*e.g.*, leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags). In some embodiments, labels are attached by spacer arms of various lengths to reduce potential steric hindrance.

The antibodies employed in the present invention are preferably derived from cells that express human immunoglobulin genes. Use of transgenic mice is known in the art to produce such "human" antibodies. One such method is described in U.S. Patent Application Serial No. 08/759,620, filed December 3, 1996. *See also* Mendez et al. *Nature Genetics* 15:146-156 (1997); Green and Jakobovits *J. Exp. Med.* 188:483-495 (1998); European Patent No. EP 0 463 151 (grant published June 12, 1996); and International Patent Application Nos. WO 94/02602, published February 3, 1994; WO 96/34096, published October 31, 1996; and WO 98/24893, published June 11, 1998.

As noted above, the invention encompasses use of antibody fragments. Antibody fragments, such as Fv, $F(ab')_2$ and Fab may be prepared by cleavage of the intact protein, e.g. by protease or chemical cleavage. Alternatively, a truncated gene is designed. For example, a chimeric gene encoding a portion of the $F(ab')_2$ fragment would include DNA sequences encoding the CH1 domain and hinge region of the H chain, followed by a translational stop codon to yield the truncated molecule.

In one approach, consensus sequences encoding the heavy and light chain J regions may be used to design oligonucleotides for use as primers to introduce useful restriction sites into the J region for subsequent linkage of V region segments to human C region segments. C region cDNA can be modified by site directed mutagenesis to place a restriction site at the analogous position in the human sequence.

Expression vectors for use in obtaining the antibodies employed in the invention include plasmids, retroviruses, cosmids, YACs, EBV derived episomes, and the like. A convenient vector is normally one that encodes a functionally complete human CH or CL immunoglobulin sequence, with appropriate restriction sites engineered so that any VH or VL sequence can be easily inserted and expressed. In such vectors, splicing usually occurs between the splice donor site in the inserted J region and the splice acceptor site preceding the human C region, and also at the splice regions that occur within the human CH exons.

Polyadenylation and transcription termination occur at native chromosomal sites downstream of the coding regions. The resulting chimeric antibody may be joined to any strong promoter, including retroviral LTRs, *e.g.* SV-40 early promoter (Okayama et al. *Mol. Cell. Bio.* 3:280 (1983)), Rous sarcoma virus LTR (Gorman et al. *Proc. Natl. Acad. Sci.* 79:6777 (1982)), and moloney murine leukemia virus LTR (Grosschedl et al. *Cell* 41:885 (1985)); native Ig promoters, etc.

Antibodies that are generated for use in the invention need not initially possess a particular desired isotype. Rather, the antibody as generated can possess any isotype and can be isotype switched thereafter using conventional techniques. These include direct recombinant techniques (*see e.g.*, U.S. Patent No. 4,816,397), and cell-cell fusion techniques (*see e.g.,* U.S. Patent No. 5,916,771).

As noted above, the effector function of the antibodies of the invention may be changed by isotype switching to an IgG1, IgG2, IgG3, IgG4, IgD, IgA, IgE, or IgM for various therapeutic uses. Furthermore, dependence on complement for cell killing can be avoided through the use of bispecifics, immunotoxins, or radiolabels, for example.

Bispecific antibodies can be generated that comprise (i) two antibodies: one with a specificity for IGF-1R and the other for a second molecule (ii) a single antibody that has one chain specific for IGF-1R and a second chain specific for a second molecule, or (iii) a single chain antibody that has specificity for IGF-1R and the other molecule. Such bispecific antibodies can be generated using well known techniques, *e.g.,* Fanger et al. *Immunol. Methods* 4:72-81 (1994); Wright and Harris, *supra*; and Traunecker et al. *Int. J. Cancer* (Suppl.) 7:51-52 (1992).

Antibodies for use in the invention also include "kappabodies" (Ill et al. *Protein Eng.* 10:949-57 (1997)), "minibodies" (Martin et al. *EMBO J.* 13:5303-9 (1994)), "diabodies" (Holliger et al. *Proc. Natl. Acad. Sci. (USA)* 90:6444-6448 (1993)), and "janusins" (Traunecker et al. *EMBO J.* 10:3655-3659 (1991) and Traunecker et al. *Int. J. Cancer Suppl.* 7:51-52 (1992)) may also be prepared.

The antibodies employed can be modified to act as immunotoxins by conventional techniques. *See e.g.,* Vitetta *Immunol. Today* 14:252 (1993). *See also* U.S. Patent No. 5,194,594. Radiolabeled antibodies can also be prepared using well-known techniques. *See e.g.,* Junghans et al. in *Cancer Chemotherapy and Biotherapy* 655-686 (2d edition, Chafner and Longo, eds., Lippincott Raven (1996)). *See also* U.S. Patent Nos. 4,681,581, 4,735,210, 5,101,827, 5,102,990 (Re. 35,500), 5,648,471, and 5,697,902.

Particular antibodies useful in practice of the invention include those described in International Patent Application No. WO 02/053596, which further describes antibodies 2.12.1, 2.13.2., 2.14.3, 3.1.1, 4.9.2, and 4.17.3. As disclosed in that published application, hybridomas producing these antibodies were deposited in the American Type Culture

Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110-2209, on December 12, 2000 with the following deposit numbers:

Hybridoma	Deposit No.
2.12.1	PTA-2792
2.13.2	PTA-2788
2.14.3	PTA-2790
3.1.1	PTA-2791
4.9.2	PTA-2789
4.17.3	PTA-2793

These antibodies are either fully human IgG2 or IgG4 heavy chains with human kappa light chains. In particular the invention concerns use of antibodies having amino acid sequences of these antibodies.

Antibodies employed in the invention preferably possess very high affinities, typically possessing Kds of from about 10^{-9} through about 10^{-11} M, when measured by either solid phase or solution phase.

Antibodies used in the present invention can be expressed in cell lines other than hybridoma cell lines. Sequences encoding the cDNAs or genomic clones for the particular antibodies can be used for transformation of suitable mammalian or nonmammalian host cells. Transformation can be by any known method for introducing polynucleotides into a host cell, including, for example packaging the polynucleotide in a virus (or into a viral vector) and transducing a host cell with the virus (or vector) or by transfection procedures known in the art, as exemplified by U.S. Patent Nos. 4,399,216, 4,912,040, 4,740,461, and 4,959,455. Methods for introduction of heterologous polynucleotides into mammalian cells are well known in the art and include, but are not limited to, dextran-mediated transfection, calcium phosphate precipitation, polybrene mediated transfection, protoplast fusion, electroporation, particle bombardment, encapsulation of the polynucleotide(s) in liposomes, peptide conjugates, dendrimers, and direct microinjection of the DNA into nuclei.

Mammalian cell lines available as hosts for expression are well known in the art and include many immortalized cell lines available from the American Type Culture Collection (ATCC), including but not limited to Chinese hamster ovary (CHO) cells, NSO_0, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), and human hepatocellular carcinoma cells (e.g., Hep G2). Non-mammalian cells can also be employed, including bacterial, yeast, insect, and plant cells. Site directed mutagenesis of the antibody CH2 domain to eliminate glycosylation may be preferred in order to prevent changes in either the immunogenicity, pharmacokinetic, and/or effector functions resulting from non-human glycosylation. The glutamine synthase system of expression is discussed in whole or part in

connection with European Patent Nos. 0 216 846, 0 256 055, and 0 323 997, and European Patent Application No. 89303964.4.

Antibodies for use in the invention can also be produced transgenically through the generation of a mammal or plant that is transgenic for the immunoglobulin heavy and light chain sequences of interest and production of the antibody in a recoverable form therefrom. Transgenic antibodies can be produced in, and recovered from, the milk of goats, cows, or other mammals. *See, e.g.,* U.S. Patent Nos. 5,827,690, 5,756,687, 5,750,172, and 5,741,957.

The advantages of the present invention can be further appreciated by reference to the following examples. These examples serve intended to illustrate preferred embodiments of the invention and are by no means intended to limit the effective scope of the claims.

EXAMPLE I:

Anti-IGF-1R Antibodies in Combination with Docetaxel in the Treatment of Advanced Non-Hematologic Malignancies

Patients with advanced-stage non-hematologic malignancies (measurable disease defined by at least one lesion that can be accurately measured and whose size is ≥2 cm x 1 cm by conventional computed tomography (CT) scan or ≥1 cm x 1 cm by spiral CT scan) received a standard dose of docetaxel (TAXOTERE) (up to 75 mg/m^2, using actual body weight to calculate body surface area (BSA)) by intravenous (IV) infusion over 1 hour on Day 1 only of each cycle. After the docetaxel infusion was completed, anti-IGF-1R antibodies as described herein were administered intravenously in a 5 mg/ml liquid formulation at a dose between 0.1 mg/kg and 10 mg/kg. The treatment regimen was repeated after 21 days, with escalation of the anti-IGF-1R antibody dose, and every 21 days thereafter until disease progression or unacceptable toxicity develops for a minimum of 2 cycles and a maximum of 17 cycles. The pre-medication regimen for docetaxel included oral dexamethasone 8 mg twice daily for three days starting one day prior to docetaxel administration. Prophylactic anti-emetics were provided as appropriate.

Dose escalation used an accelerated titration design utilizing a dose-doubling schema with 3-6 subjects per dose level (cohort). Within each new cohort there was no required waiting period between subjects. Subsequent cohorts were not opened until the first subject at the current dose level had been observed for 21 days and subsequent subjects had been observed for 14 days.

The following endpoints were measured: safety, tolerability, pharmacokinetic (PK) parameters of the anti-IGF-1R antibody; human anti-human antibody response (HAHA); response rate and time to progression; and number of circulating tumor cells (CTC) and circulating soluble IGF-1R.

EXAMPLE II:

Anti-IGF-1R Antibodies in Combination with Paclitaxel and Carboplatin in the Treatment of Advanced Non-Small Cell Lung Cancer

In Part 1 of the study, patients with Stage IIIB or Stage IV or recurrent (after surgery/radiation), measurable, non-small cell lung cancer (NSCLC) who have received no prior chemotherapy received paclitaxel (TAXOL) at a standard dose of 200 mg/m^2 by IV infusion over 3 hours. Prior to receiving paclitaxel, all patients received prophylactic anti-allergic/emetic medicines. Carboplatin (PARAPLATIN) was administered by IV infusion over 15-30 minutes; the dose was calculated based on the Calvert formula with a target area under the curve (AUC) of 6 mg/ml x min. After the carboplatin infusion was completed, anti-IGF-1R antibodies as described herein were administered intravenously in a 5 mg/ml formulation at a dose between 0.05 mg/kg and 10 mg/kg. The treatment regimen was repeated after 21 days, with escalation of the anti-IGF-1R antibody dose, and every 21 days thereafter until disease progression or unacceptable toxicity develops, for a minimum of 1 cycle and a maximum of 6 cycles.

Doses were escalated using an accelerated titration design utilizing a dose-doubling schema with 3-6 subjects per cohort. Within each new cohort there was no required waiting period between subjects. Subsequent cohorts were not opened until the first subject at the current dose level has been observed for 21 days and subsequent subjects have been observed for 14 days.

Once at least six patients have been observed for 21 days (i.e., completed one cycle), the randomized second portion of the study will begin.

Part 2 of the study is a two-arm randomized, non-comparative study of anti-IGF-1R antibody in combination with paclitaxel and carboplatin (Arm A) and of paclitaxel and carboplatin alone (Arm B). On Day 1 of Part 2, the patients in both arms are given the same dosages of paclitaxel and carboplatin, over the same time periods, as in the first part. After administration of carboplatin, patients in Arm A are also given the same anti-IGF-1R antibody dose they were given in Part 1. The dose is determined in view of the safety and tolerability demonstrated in Part 1. The treatment is repeated after 21 days, and every 21 days thereafter, until progression or unacceptable toxicity occurs for a minimum of 2 cycles and a maximum of 6.

The following endpoints are measured: PK parameters of the anti-IGF-1R antibody, HAHA, response rate and time to progression, CTC, circulating IGF-1, IGFBPs, and soluble circulating IGF-1R.

EXAMPLE III:

Anti-IGF-1R in Combination with Docetaxel and Epirubicin in Metastatic Breast Cancer

Patients having metastatic breast cancer with at least one lesion that can be accurately measured in two dimensions and whose size is ≥2 cm x 1 cm by conventional CT scan or ≥1 cm x 1 cm by spiral CT scan are given epirubicin 75 mg/m^2 as a single 15 minute infusion. After a one hour pause, docetaxel (TAXOTERE) 75 mg/m^2 is administered as a single IV infusion, followed by IV infusion of anti-IGF-1R antibodies as described herein at a dose between 0.05 mg/kg and 10 mg/kg. Prophylactic anti-emetics are given as appropriate. The treatment is repeated after 21 days with escalation of the anti-IGF-1R antibody dose, and every 21 days thereafter until disease progression or unacceptable toxicity develops for a minimum of 2 cycles and a maximum of 6.

Doses are escalated using an accelerated titration design utilizing a dose-doubling schema with 3-6 subjects per cohort. Within each new cohort there is no required waiting period between subjects. Subsequent cohorts may not be opened until the first subject at the current dose level has been observed for 21 days and subsequent subjects have been observed for 14 days.

The following endpoints are measured: PK parameters, HAHA, response rate and time to progression. Time to progression and overall survival are calculated using the Kaplan-Meier product limit method.

EXAMPLE IV: Anti-IGF-1R in Combination with Docetaxel and Prednisone in Hormone-Refractory Prostate Cancer

Subjects are patients with metastatic adenocarcinoma of the prostate who, after at least one hormonal treatment (orchiectomy, estrogens, LHRH therapy, etc.), have testosterone levels less than 50 ng/dL, prostate-specific antigen (PSA) above 20 ng/mL, and an increase in PSA > 50% over nadir value on hormonal therapy measured on 3 successive occasions at least 1 week apart. A pre-medication regimen for docetaxel includes oral dexamethasone 8 mg twice a day given for 3 days starting one day prior to docetaxel administration . A 75 mg/m^2 dose of docetaxel (TAXOTERE) (using actual body weight to calculate BSA) is administered by IV infusion over 1 hour on Day 1 only of each cycle. After the docetaxel infusion is completed, anti-IGF-1R antibodies as described herein are administered intravenously in a 5 mg/ml liquid formulation. Prednisone is given daily in two oral 5 mg doses per day, starting on Day 1. Prophylactic anti-emetics may be given as appropriate. The treatment regimen is repeated every 21 days (±3 days) until disease progression or unacceptable toxicity develops, for a maximum of 10 cycles.

The following endpoints are measured: PSA response, population PK parameters of the anti-IGF-1R antibody, HAHA, total number of CTCs and CTCs expressing IGF-1R.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

CLAIMS

1. A method for the treatment of a non-hematologic malignancy in a patient in need of such treatment comprising the step of administering to the patient a therapeutically effective amount of an antibody that specifically binds to IGF-1R in combination with a therapeutically effective amount of at least one agent selected from the group consisting of an alkylating agent, a folate antagonist, a pyrimidine antagonist, a cytotoxic antibiotic, a platinum compound, a taxane, a vinca alkaloid, a topoisomerase inhibitor, an EGFR inhibitor, and a hormonal therapy agent.

2. The method of claim 1, wherein the agent is a taxane.

3. The method of claim 2, wherein the taxane is docetaxel.

4. The method of claim 2, wherein the taxane is paclitaxel.

5. The method of any one of claims 3 and 4, wherein the antibody and the taxane are administered in combination with an additional therapeutic agent selected from the group consisting of carboplatin, cisplatin, gemcitabine, capecitabine, epirubicin and prednisone.

6. The method of claim 5, wherein the additional therapeutic agent is carboplatin.

7. The method of claim 5, wherein the additional therapeutic agent is epirubicin.

8. The method of claim 5, wherein the additional therapeutic agent is prednisone.

9. The method of any one of claims 1-8, wherein the non-hematological malignancy is breast cancer.

10. The method of any one of claims 1-8, wherein the non-hematological malignancy is lung cancer.

11. The method of any one of claims 1-8, wherein the non-hematological malignancy is prostate cancer.

12. A pharmaceutical composition for the treatment of a non-hematologic malignancy according to the method of any one of claims 1-11, comprising:

a therapeutically effective amount of an antibody that specifically binds IGF-1R,

a therapeutically effective amount of at least one agent selected from the group consisting of an alkylating agent, a folate antagonist, a pyrimidine antagonist, a cytotoxic antibiotic, a platinum compound, a taxane, a vinca alkaloid, a topoisomerase inhibitor, an EGFR inhibitor, and a hormonal therapy agent; and

a pharmaceutically acceptable carrier.

13. The composition of claim 12 wherein the antibody has the following properties:

a binding affinity for human IGF-1R of K_d of 8×10^{-9} or less; and

inhibition of binding between human IGF-1R and IGF-1 with an IC_{50} of less than 100 nM.

14. The composition of any one of claims 12 or 13 wherein the antibody comprises at least one of the group consisting of:

(a) a heavy chain comprising the amino acid sequences of CDR-1, CDR-2, and CDR-3 of an antibody selected from the group consisting of 2.12.1, 2.13.2, 2.14.3, 4.9.2, 4.17.3, and 6.1.1;

(b) a light chain comprising the amino acid sequences of CDR-1, CDR-2, and CDR-3 of an antibody selected from the group consisting of 2.12.1, 2.13.2, 2.14.3, 4.9.2, 4.17.3, and 6.1.1; and

(c) sequences having changes from the CDR sequences of an antibody selected from the group consisting of 2.12.1, 2.13.2, 2.14.3, 4.9.2, 4.17.3, and 6.1.1, said sequences being selected from the group consisting of conservative changes, wherein the conservative changes are selected from the group consisting of replacement of nonpolar residues by other nonpolar residues, replacement of polar charged residues by other polar uncharged residues, replacement of polar charged residues by other polar charged residues, and substitution of structurally similar residues; and non-conservative substitutions, wherein the non-conservative substitutions are selected from the group consisting of substitution of polar charged residue for polar uncharged residues and substitution of nonpolar residues for polar residues, additions and deletions.

15. The composition of any one of claims 12-14, wherein the antibody comprises a heavy chain comprising the amino acid sequences of CDR-1, CDR-2, and CDR-3, and a light chain comprising the amino acid sequences of CDR-1, CDR-2, and CDR-3, of an antibody selected from the group consisting of 2.12.1, 2.13.2, 2.14.3, 4.9.2, 4.17.3, and 6.1.1.

16. The composition of any one of claims 12-15, wherein the antibody is selected from the group consisting of an antibody comprising a heavy chain amino acid sequence derived from human gene DP-47 and a light chain amino acid sequence derived from human gene A30.