
(21) International Application Number: PCT/US2004/038842

(22) International Filing Date: 19 November 2004 (19.11.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:


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(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, 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).

Declaration under Rule 4.17:

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

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

(54) Title: ANTI-IGF1R ANTIBODY THERAPEUTIC COMBINATIONS

(57) Abstract: The present invention provides combinations including a binding composition, such as an anti-IGF1R antibody, in association with a chemotherapeutic agent. Methods for using the combinations to treat medical conditions, such as cancer, are also provided.
ANTI-IGFR1 ANTIBODY THERAPEUTIC COMBINATIONS

This application claims the benefit of U.S. Provisional Patent Application No. 60/524,732; filed November 21, 2003 which is herein incorporated by reference in its entirety.

FIELD OF THE INVENTION

The present invention relates to therapeutic combinations comprising one or more anti-IGFR1 antibodies and one or more chemotherapeutic agents.

BACKGROUND OF THE INVENTION


Several lines of evidence indicate that IGF-I, IGF-II and their receptor IGFR1 are important mediators of the malignant phenotype. Plasma levels of IGF-I have been found to be the strongest predictor of prostate cancer risk (Chan, et al., (1998) Science 279:563) and similar epidemiological studies strongly link plasma IGF-I levels with breast, colon and lung cancer risk.

Overexpression of Insulin-like Growth Factor Receptor-I has also been demonstrated in several cancer cell lines and tumor tissues. IGFR1 is overexpressed in 40% of all breast cancer cell lines (Pandini, et al., (1999) Cancer Res. 5:1935) and in 15% of lung cancer cell lines. In breast cancer tumor tissue, IGFR1 is overexpressed 6-14 fold and IGFR1 exhibits 2-4 fold higher kinase activity as compared to normal tissue (Webster, et al., (1996) Cancer Res. 56:2781 and Pekonen, et al., (1998) Cancer Res. 48:1343).
Moreover, colorectal cancer tissue has been reported to exhibit strongly elevated IGFR1 levels (Weber et al., Cancer 95(10):2086-95 (2002)). Analysis of primary cervical cancer cell cultures and cervical cancer cell lines revealed 3- and 5-fold overexpression of IGFR1, respectively, as compared to normal ectocervical cells (Steller, et al., (1996) Cancer Res. 56:1762). Expression of IGFR1 in synovial sarcoma cells also correlated with an aggressive phenotype (i.e., metastasis and high rate of proliferation; Xie, et al., (1999) Cancer Res. 59:3588).


There are several antibodies, which are known in the art, which inhibit the activity of IGFR1. However, these are of relatively low therapeutic value. For example, α-IR3 (Kull, et al., (1983) J. Biol. Chem. 258:6561), 1H7 (Li et al., (1993) Biochem. Biophys. Res. Comm. 196.92-98 and Xiong et al., (1992) Proc. Natl. Acad. Sci., U.S.A. 89:5356-5360; Santa Cruz biotechnology, Inc.; Santa Cruz, CA) and MAB391 (R&D Systems; Minneapolis, MN) are mouse monoclonal antibodies which interact with IGFR1 and inhibit its activity. Since these are mouse antibodies, their therapeutic utility in humans is limited. When an immunocompetent human subject is administered a dose of a murine antibody, the subject produces antibodies against the mouse immunoglobulin sequences. These human anti-mouse antibodies (HAMA) neutralize the therapeutic antibodies and may induce acute toxicity (i.e., a HAMA response).

One method by which to avert a HAMA response is through the use of fully human antibodies which lack any foreign (e.g., mouse) amino acid sequences. Although the use of fully-human antibodies is an effective method by which to reduce or prevent human host immune rejection of the therapeutic antibody, rejection of the fully-human antibody can occur. Human rejection of human antibodies may be referred to as a human anti-human antibody response (HAHA response). HAHA response can be mediated by factors such as the presence of rare, low occurrence amino acid sequences in the fully-human antibodies. For this reason, therapeutic antibodies can also be optimized by the inclusion of non-immunogenic or only weakly immunogenic human antibody framework sequences. Preferably, the sequences occur frequently in other human antibodies.

Although anti-IGFR1 antibodies are an effective means by which to treat medical conditions mediated by the receptor (e.g., cancer or acromegaly), the efficacy of such treatments would be enhanced by use of one or more additional chemotherapeutic
agents. For example, an anti-IGFR1 antibody can be administered to a subject in association with a second anti-IGFR1 antibody or a small molecule IGFR1 antagonist. The present invention provides, *inter alia*, such treatments and compositions for use in the treatments.

**SUMMARY OF THE INVENTION**

The present invention provides a combination comprising (a) one or more binding compositions, such as any anti-IGFR1 antibody, preferably an isolated fully-human monoclonal antibody, preferably comprising a member selected from the group consisting of: (i) a light chain amino acid sequence which comprises CDR-L1 defined by SEQ ID NO: 5, CDR-L2 defined by SEQ ID NO: 6 and CDR-L3 defined by SEQ ID NO: 7; and (ii) a heavy chain amino acid sequence which comprises CDR-H1 defined by SEQ ID NO: 8, CDR-H2 defined by SEQ ID NO: 9 and CDR-H3 defined by SEQ ID NO: 10; in association with (b) one or more chemotherapeutic agents and, optionally, a pharmaceutically acceptable carrier.

In one embodiment, a binding composition (e.g., an isolated fully-human monoclonal antibody) comprises a light chain amino acid sequence which comprises CDR-L1 defined by SEQ ID NO: 5, CDR-L2 defined by SEQ ID NO: 6 and CDR-L3 defined by SEQ ID NO: 7; and a heavy chain amino acid sequence which comprises CDR-H1 defined by SEQ ID NO: 8, CDR-H2 defined by SEQ ID NO: 9 and CDR-H3 defined by SEQ ID NO: 10. In an embodiment, a binding composition comprises a light chain immunoglobulin comprising mature LCF (amino acids 20-128 of SEQ ID NO: 2) and a heavy chain immunoglobulin comprising mature HCA (amino acids 20-137 of SEQ ID NO: 4).

A binding composition can be any binding composition (e.g., an isolated fully-human monoclonal antibody) set forth in U.S. Patent Application No. 10/443,466, filed May 22, 2003.

A chemotherapeutic agent can be one or more members selected from the group consisting of a taxane, a topoisomerase inhibitor, a signal transduction inhibitor, a cell cycle inhibitor, an IGF/IGFR1 system modulator, a farnesyl protein transferase (FPT) inhibitor, an epidermal growth factor receptor (EGFR) inhibitor, a HER2 inhibitor, a vascular epidermal growth factor (VEGF) receptor inhibitor, a mitogen activated protein (MAP) kinase inhibitor, a MEK inhibitor, an AKT inhibitor, a mTOR inhibitor, a pI3 kinase inhibitor, a Raf inhibitor, a cyclin dependent kinase (CDK) inhibitor, a microtubule
stabilizer, a microtubule inhibitor, a SERM/Antiestrogen, an aromatase inhibitor, an anthracycline, a proteasome inhibitor, an agent which inhibits insulin-like growth factor (IGF) production and/or an anti-sense inhibitor of IGFR1, IGF-1 or IGF2.

A taxane can be, for example, paclitaxel or docetaxel. A microtubule inhibitor can be, for example, vincristine, vinblastine, a podophyllotoxin, epothilone B, BMS-247550 or BMS-310705. An epidermal growth factor receptor (EGFR) inhibitor can be, for example, gefitinib, erlotinib, cetuximab, ABX-EGF, lapatanib, canertinib, EKB-569 or PKI-166. A farnesyl protein transferase inhibitor can be, for example, lonafarnib or tipifarnib (R155777). A selective estrogen receptor modulator (SERM)/antiestrogen can be, for example, tamoxifen, raloxifene, fulvestrant, acolbifene, pipendoxifene, arzoxifene, toremifene, lasofoxifene, bazedoxifene (TSE-424), idoxifene, HMR-3339 and ZK-186619. An anthracycline can be doxorubicin, daunorubicin or epirubicin. A HER2 inhibitor can be, for example, trastuzumab, HKI-272, CP-724714 or TAK-165. A topoisomerase inhibitor can be, for example, etoposide, topotecan, camptothecin or irinotecan.

In one embodiment, the present invention comprises a combination comprising: (a) one or more binding compositions (e.g., an isolated fully-human monoclonal antibody) comprising a light chain immunoglobulin comprising amino acids 20-128 of SEQ ID NO: 2 and a heavy chain immunoglobulin comprising amino acids 20-137 of SEQ ID NO: 4; in association with (b) one or more chemotherapeutic agents selected from:
Also provided by the present invention is a method for treating or preventing a medical condition in a subject, which medical condition is mediated by elevated expression or activity of Insulin-like Growth Factor Receptor-1, comprising administering (e.g., by a parenteral or non-parenteral route), to the subject, a composition comprising a therapeutically effective amount of (a) one or more binding compositions (e.g., an isolated fully-human monoclonal antibody), such as any anti-IGFR1 antibody, preferably comprising a member selected from the group consisting of: (i) a light chain amino acid sequence which comprises CDR-L1 defined by SEQ ID NO: 5, CDR-L2 defined by SEQ ID NO: 6 and CDR-L3 defined by SEQ ID NO: 7; and (ii) a heavy chain amino acid sequence which comprises CDR-H1 defined by SEQ ID NO: 8, CDR-H2 defined by SEQ ID NO: 9 and CDR-H3 defined by SEQ ID NO: 10; optionally in association with (b) a therapeutically effective amount of one or more chemotherapeutic agents and, optionally, a pharmaceutically acceptable carrier. In an embodiment of the invention, the medical condition is treated with a therapeutically effective amount of any isolated anti-IGFR antibody or antigen binding fragment thereof of the invention alone.
In one embodiment, the binding composition (e.g., an isolated fully-human monoclonal antibody) comprises a light chain immunoglobulin comprising amino acids 20-128 of SEQ ID NO: 2 and a heavy chain immunoglobulin comprising amino acids of 20-137 of SEQ ID NO: 4. In one embodiment, a chemotherapeutic agent is one or more members selected from the group consisting of:

and
In one embodiment, the medical condition treated by a method of the present invention is selected from the group consisting of Rheumatoid Arthritis, Grave's disease, Multiple Sclerosis, Systemic Lupus Erythematosus, Hashimoto's Thyroiditis, Myasthenia Gravis, Auto-Immune Thyroiditis, Bechet's disease, acromegaly, bladder cancer, Wilms' cancer, ovarian cancer, pancreatic cancer, benign prostatic hyperplasia, breast cancer, prostate cancer, bone cancer, lung cancer, colorectal cancer, cervical cancer, synovial sarcoma, diarrhea associated with metastatic carcinoid, vasoactive intestinal peptide secreting tumors, gigantism, psoriasis, atherosclerosis, smooth muscle restenosis of blood vessels and inappropriate microvascular proliferation.

An embodiment of the present invention includes a method for treating or preventing a medical condition in a subject (e.g., rheumatoid arthritis, Grave's disease, multiple sclerosis, systemic lupus erythematosus, Hashimoto's Thyroiditis, Myasthenia Gravis, auto-immune thyroiditis, Bechet's disease, acromegaly, bladder cancer, Wilms' cancer, ovarian cancer, pancreatic cancer, benign prostatic hyperplasia, breast cancer, prostate cancer, bone cancer, lung cancer, colorectal cancer, cervical cancer, synovial sarcoma, diarrhea associated with metastatic carcinoid, vasoactive intestinal peptide secreting tumors, gigantism, psoriasis, atherosclerosis, smooth muscle restenosis of blood vessels or inappropriate microvascular proliferation) comprising administering a combination comprising: (a) a therapeutically effective amount of one or more binding compositions (e.g., an isolated fully-human monoclonal antibody) comprising a light chain immunoglobulin comprising amino acids 20-128 of SEQ ID NO: 2 and a heavy chain immunoglobulin comprising amino acids 20-137 of SEQ ID NO: 4; in association with (b) a therapeutically effective amount of one or more chemotherapeutic agents selected from:
to the subject.

Also provided by the present invention is a method for inhibiting the growth or
proliferation of any cell (e.g., a cell in vitro or a cell in vivo (e.g., in the body of a subject)),
for example a malignant cell, including, but not limited to, an NCI-H322 cell, an A2780
cell, an MCF7 cell, a non-small cell carcinoma lung cancer cell, a breast cancer cell, an ovarian cancer cell, a colorectal cancer cell, a prostate cancer cell, a pediatric cancer or a pancreatic cancer cell, comprising contacting the cell with a combination comprising (a) one or more binding compositions, such as any isolated anti-IGFR1 antibody, preferably an isolated fully-human monoclonal antibody, preferably comprising a member selected from the group consisting of: (i) a light chain amino acid sequence which comprises CDR-L1 defined by SEQ ID NO: 5, CDR-L2 defined by SEQ ID NO: 6 and CDR-L3 defined by SEQ ID NO: 7; and (ii) a heavy chain amino acid sequence which comprises CDR-H1 defined by SEQ ID NO: 8, CDR-H2 defined by SEQ ID NO: 9 and CDR-H3 defined by SEQ ID NO: 10; in association with (b) one or more chemotherapeutic agents and, optionally, a pharmaceutically acceptable carrier. In one embodiment, a binding composition comprises a light chain immunoglobulin comprising amino acids 20-128 of SEQ ID NO: 2 and a heavy chain immunoglobulin comprising amino acids of 20-137 of SEQ ID NO: 4. In one embodiment, a chemotherapeutic agent is one or more members selected from the group consisting of:
The present invention also provides a kit comprising (a) one or more binding compositions (e.g., an isolated fully-human monoclonal antibody) comprising a member selected from the group consisting of: a light chain amino acid sequence which comprises CDR-L1 defined by SEQ ID NO: 5, CDR-L2 defined by SEQ ID NO: 6 and CDR-L3 defined by SEQ ID NO: 7; and a heavy chain amino acid sequence which comprises CDR-H1 defined by SEQ ID NO: 8 or 12, CDR-H2 defined by SEQ ID NO: 9 and CDR-H3 defined by SEQ ID NO: 10; in association with (b) one or more chemotherapeutic agents. The binding composition can be in a separate container from the chemotherapeutic agent.

**DETAILED DESCRIPTION OF THE INVENTION**

The present invention provides combinations and methods for treating medical conditions that are characterized by a high level of IGFR1 expression, ligand binding or activity or a high level of IGF-1 or IGF-2, such as cancer. The combinations of the invention, which can be used to treat the medical conditions, include one or more anti-IGFR1 antibodies (e.g., an isolated fully-human monoclonal antibody) in association with one or more chemotherapeutic agents.

The combinations of the invention include the binding composition component and chemotherapeutic agent component "in association" with one another. The term "in
association" indicates that the components of the combinations of the invention can be formulated into a single composition for simultaneous delivery or formulated separately into two or more compositions (e.g., a kit). Furthermore, each component of a combination of the invention can be administered to a subject at a different time than when the other component is administered; for example, each administration may be given non-simultaneously at several intervals over a given period of time. Moreover, the separate components may be administered to a subject by the same or by a different route (e.g., orally, intravenously, intratumorally).

The compositions of the invention provide a particularly effective means for treating diseases mediated by IGFR1, IGF-1 and/or IGF-2. The therapeutic efficacy of both the binding composition of the invention and the chemotherapeutic agent(s), when administered in association, is far superior to that of either component alone.

The present invention includes any isolated nucleic acid or isolated polypeptide (e.g., an isolated fully-human monoclonal antibody) which comprises one or more (e.g., 1, 2, 3, 4, 5, 6, 7 or 8) of any of the nucleic acids or polypeptides (including mature fragments thereof) set forth, below, in Table 1.

<table>
<thead>
<tr>
<th>Sequence</th>
<th>Sequence Identifier</th>
</tr>
</thead>
<tbody>
<tr>
<td>19D12/15H12 light chain F (LCF) variable region polynucleotide sequence</td>
<td>SEQ ID NO: 1</td>
</tr>
<tr>
<td>19D12/15H12 light chain F variable region polypeptide sequence</td>
<td>SEQ ID NO: 2</td>
</tr>
<tr>
<td>19D12/15H12 heavy chain A (HCA) variable region polynucleotide sequence</td>
<td>SEQ ID NO: 3</td>
</tr>
<tr>
<td>19D12/15H12 heavy chain A variable region polypeptide sequence</td>
<td>SEQ ID NO: 4</td>
</tr>
<tr>
<td>19D12/15H12 light chain F CDR-L1 polypeptide sequence</td>
<td>SEQ ID NO: 5</td>
</tr>
<tr>
<td>19D12/15H12 light chain F CDR-L2 polypeptide sequence</td>
<td>SEQ ID NO: 6</td>
</tr>
<tr>
<td>19D12/15H12 light chain F CDR-L3 polypeptide sequence</td>
<td>SEQ ID NO: 7</td>
</tr>
<tr>
<td>19D12/15H12 heavy chain A CDR-H1 polypeptide sequence</td>
<td>SEQ ID NO: 8</td>
</tr>
<tr>
<td>19D12/15H12 heavy chain A CDR-H2 polypeptide sequence</td>
<td>SEQ ID NO: 9</td>
</tr>
<tr>
<td>19D12/15H12 heavy chain A CDR-H3 polypeptide sequence</td>
<td>SEQ ID NO: 10</td>
</tr>
<tr>
<td>Amino acid sequence of Insulin-like Growth Factor Receptor-1 (IGFR1)</td>
<td>SEQ ID NO: 11</td>
</tr>
<tr>
<td>Alternative 19D12/15H12 heavy chain A</td>
<td>SEQ ID NO: 12</td>
</tr>
</tbody>
</table>

A "polynucleotide", "nucleic acid" or "nucleic acid molecule" may refer to the phosphate ester polymeric form of ribonucleosides (adenosine, guanosine, uridine or cytidine; "RNA molecules") or deoxyribonucleosides (deoxyadenosine, deoxyguanosine, deoxycytidine, or deoxycytidine; "DNA molecules"), or any phosphoester analogs thereof, such as phosphorothioates and thioesters, in single stranded form, double-stranded form or otherwise.

A "polynucleotide sequence", "nucleic acid sequence" or "nucleotide sequence" is a series of nucleotide bases (also called "nucleotides") in a nucleic acid, such as DNA or RNA, and means any chain of two or more nucleotides.

A "coding sequence" or a sequence "encoding" an expression product, such as a RNA, polypeptide, protein, or enzyme, is a nucleotide sequence that, when expressed, results in production of the product.

The term "gene" means a DNA sequence that codes for or corresponds to a particular sequence of ribonucleotides or amino acids which comprise all or part of one or more RNA molecules, proteins or enzymes, and may or may not include regulatory DNA sequences, such as promoter sequences, which determine, for example, the conditions
under which the gene is expressed. Genes may be transcribed from DNA to RNA which may or may not be translated into an amino acid sequence.

"Amplification" of DNA as used herein may denote the use of polymerase chain reaction (PCR) to increase the concentration of a particular DNA sequence within a mixture of DNA sequences. For a description of PCR see Saiki, et al., Science (1988) 239: 487. In a specific embodiment, the present invention includes a nucleic acid, which encodes an anti-IGFR1 antibody, an anti-IGFR1 antibody heavy or light chain, an anti-IGFR1 antibody heavy or light chain variable region, an anti-IGFR1 antibody heavy or light chain constant region or anti-IGFR1 antibody CDR (e.g., CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2 or CDR-H3) which can be amplified by PCR.

As used herein, the term "oligonucleotide" refers to a nucleic acid, generally of at least 10 (e.g., 10, 11, 12, 13 or 14), preferably at least 15 (e.g., 15, 16, 17, 18 or 19), and more preferably at least 20 nucleotides (e.g., 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30), preferably no more than 100 nucleotides (e.g., 40, 50, 60, 70, 80 or 90), that may be hybridizable to a genomic DNA molecule, a cDNA molecule, or an mRNA molecule encoding a gene, mRNA, cDNA, or other nucleic acid of interest. Oligonucleotides can be labeled, e.g., by incorporation of $^{32}$P-nucleotides, $^3$H-nucleotides, $^{14}$C-nucleotides, $^{35}$S-nucleotides or nucleotides to which a label, such as biotin, has been covalently conjugated. In one embodiment, a labeled oligonucleotide can be used as a probe to detect the presence of a nucleic acid. In another embodiment, oligonucleotides (one or both of which may be labeled) can be used as PCR primers, either for cloning full length or a fragment of the gene, or to detect the presence of nucleic acids. Generally, oligonucleotides are prepared synthetically, preferably on a nucleic acid synthesizer.

The sequence of any nucleic acid (e.g., a nucleic acid encoding an IGFR1 gene or a nucleic acid encoding an anti-IGFR1 antibody or a fragment or portion thereof) may be determined by any method known in the art (e.g., chemical sequencing or enzymatic sequencing). "Chemical sequencing" of DNA may denote methods such as that of Maxam and Gilbert (1977) (Proc. Natl. Acad. Sci. USA 74:560), in which DNA is randomly cleaved using individual base-specific reactions. "Enzymatic sequencing" of DNA may denote methods such as that of Sanger (Sanger, et al., (1977) Proc. Natl. Acad. Sci. USA 74:5463).

The nucleic acids herein may be flanked by natural regulatory (expression control) sequences, or may be associated with heterologous sequences, including promoters, internal ribosome entry sites (IRES) and other ribosome binding site sequences,
enhancers, response elements, suppressors, signal sequences, polyadenylation sequences, introns, 5'- and 3'- non-coding regions, and the like.

A "promoter" or "promoter sequence" is a DNA regulatory region capable of binding an RNA polymerase in a cell (e.g., directly or through other promoter-bound proteins or substances) and initiating transcription of a coding sequence (e.g., LCF or HCA). A promoter sequence is, in general, bounded at its 3' terminus by the transcription initiation site and extends upstream (5' direction) to include the minimum number of bases or elements necessary to initiate transcription at any level. Within the promoter sequence may be found a transcription initiation site (conveniently defined, for example, by mapping with nuclease S1), as well as protein binding domains (consensus sequences) responsible for the binding of RNA polymerase. The promoter may be operably associated with other expression control sequences, including enhancer and repressor sequences or with a nucleic acid of the invention. Promoters which may be used to control gene expression include, but are not limited to, cytomegalovirus (CMV) promoter (U.S. Patent Nos. 5,385,839 and 5,168,062), the SV40 early promoter region (Benoist, et al., (1981) Nature 290:304-310), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto, et al., (1980) Cell 22:787-797), the herpes thymidine kinase promoter (Wagner, et al., (1981) Proc. Natl. Acad. Sci. USA 78:1441-1445), the regulatory sequences of the metallothionein gene (Brinster, et al., (1982) Nature 296:39-42); prokaryotic expression vectors such as the β-lactamase promoter (Villa-Komaroff, et al., (1978) Proc. Natl. Acad. Sci. USA 75:3727-3731), or the tac promoter (DeBoer, et al., (1983) Proc. Natl. Acad. Sci. USA 80:21-25); see also "Useful proteins from recombinant bacteria" in Scientific American (1980) 242:74-94; and promoter elements from yeast or other fungi such as the Gal 4 promoter, the ADC (alcohol dehydrogenase) promoter, PGK (phosphoglycerol kinase) promoter or the alkaline phosphatase promoter.

A coding sequence is "under the control of", "functionally associated with" or "operably associated with" transcriptional and translational control sequences in a cell when the sequences direct RNA polymerase mediated transcription of the coding sequence into RNA, preferably mRNA, which then may be trans-RNA spliced (if it contains introns) and, optionally, translated into a protein encoded by the coding sequence.

The terms "express" and "expression" mean allowing or causing the information in a gene, RNA or DNA sequence to become manifest; for example, producing a protein by activating the cellular functions involved in transcription and translation of a corresponding
gene. A DNA sequence is expressed in or by a cell to form an "expression product" such as an RNA (e.g., mRNA) or a protein. The expression product itself may also be said to be "expressed" by the cell.

The terms "vector", "cloning vector" and "expression vector" mean the vehicle (e.g., a plasmid) by which a DNA or RNA sequence can be introduced into a host cell, so as to transform the host and, optionally, promote expression and/or replication of the introduced sequence.

The term "transfection" or "transformation" means the introduction of a nucleic acid into a cell. These terms may refer to the introduction of a nucleic acid encoding an anti-IGFR1 antibody or fragment thereof into a cell. The introduced gene or sequence may be called a "clone". A host cell that receives the introduced DNA or RNA has been "transformed" and is a "transformant" or a "clone". The DNA or RNA introduced to a host cell can come from any source, including cells of the same genus or species as the host cell, or cells of a different genus or species.

The term "host cell" means any cell of any organism that is selected, modified, transfected, transformed, grown, or used or manipulated in any way, for the production of a substance by the cell, for example the expression or replication, by the cell, of a gene, a DNA or RNA sequence, a protein or an enzyme.

The term "expression system" means a host cell and compatible vector which, under suitable conditions, can express a protein or nucleic acid which is carried by the vector and introduced to the host cell. Common expression systems include E. coli host cells and plasmid vectors, insect host cells and Baculovirus vectors, and mammalian host cells and vectors. In a specific embodiment, IGFR1 or an antibody and antigen-binding fragment of the invention may be expressed in human embryonic kidney cells (HEK293).


The present invention contemplates any superficial or slight modification to the amino acid or nucleotide sequences which correspond to the antibodies or antigen-
binding fragments of the invention. In particular, the present invention contemplates sequence conservative variants of the nucleic acids which encode the antibodies or antigen-binding fragments of the invention. "Sequence-conservative variants" of a polynucleotide sequence are those in which a change of one or more nucleotides in a given codon results in no alteration in the amino acid encoded at that position. Function-conservative variants of the antibodies of the invention are also contemplated by the present invention. "Function-conservative variants" are those in which one or more amino acid residues in a protein or enzyme have been changed without altering the overall conformation and function of the polypeptide, including, but, by no means, limited to, replacement of an amino acid with one having similar properties. Amino acids with similar properties are well known in the art. For example, polar/hydrophilic amino acids which may be interchangeable include asparagine, glutamine, serine, cysteine, threonine, lysine, arginine, histidine, aspartic acid and glutamic acid; nonpolar/hydrophobic amino acids which may be interchangeable include glycine, alanine, valine, leucine, isoleucine, proline, tyrosine, phenylalanine, tryptophan and methionine; acidic amino acids which may be interchangeable include aspartic acid and glutamic acid and basic amino acids which may be interchangeable include histidine, lysine and arginine.

The present invention includes anti-IGFR1 antibodies and fragments thereof which are encoded by nucleic acids as described in Table 1 as well as nucleic acids which hybridize thereto. Preferably, the nucleic acids hybridize under low stringency conditions, more preferably under moderate stringency conditions and most preferably under high stringency conditions and, preferably, exhibit IGFR1 binding activity. A nucleic acid molecule is "hybridizable" to another nucleic acid molecule, such as a cDNA, genomic DNA, or RNA, when a single stranded form of the nucleic acid molecule can anneal to the other nucleic acid molecule under the appropriate conditions of temperature and solution ionic strength (see Sambrook, *et al.*, *supra*). The conditions of temperature and ionic strength determine the "stringency" of the hybridization. Typical low stringency hybridization conditions include 55°C, 5X SSC, 0.1% SDS and no formamide; or 30% formamide, 5X SSC, 0.5% SDS at 42°C. Typical, moderate stringency hybridization conditions are similar to the low stringency conditions except the hybridization is carried out in 40% formamide, with 5X or 6X SSC and 0.1% SDS at 42°C. High stringency hybridization conditions are similar to low stringency conditions except the hybridization conditions are carried out in 50% formamide, 5X or 6X SSC at 42°C or, optionally, at a higher temperature (e.g., 57 °C, 59 °C, 60 °C, 62 °C, 63 °C, 65°C or 68 °C). In general,
SSC is 0.15M NaCl and 0.015M Na-citrate. Hybridization requires that the two nucleic acids contain complementary sequences, although, depending on the stringency of the hybridization, mismatches between bases are possible. The appropriate stringency for hybridizing nucleic acids depends on the length of the nucleic acids and the degree of complementation, variables well known in the art. The greater the degree of similarity or homology between two nucleotide sequences, the higher the stringency under which the nucleic acids may hybridize. For hybrids of greater than 100 nucleotides in length, equations for calculating the melting temperature have been derived (see Sambrook, et al., supra, 9.50-9.51). For hybridization with shorter nucleic acids, i.e., oligonucleotides, the position of mismatches becomes more important, and the length of the oligonucleotide determines its specificity (see Sambrook, et al., supra, 11.7-11.8).

Also included in the present invention are nucleic acids comprising nucleotide sequences and polypeptides comprising amino acid sequences which are at least about 70% identical, preferably at least about 80% identical, more preferably at least about 90% identical and most preferably at least about 95% identical (e.g., 95%, 96%, 97%, 98%, 99%, 100%) to the reference nucleotide and amino acid sequences of Table 1 when the comparison is performed by a BLAST algorithm wherein the parameters of the algorithm are selected to give the largest match between the respective sequences over the entire length of the respective reference sequences. Polypeptides comprising amino acid sequences which are at least about 70% similar, preferably at least about 80% similar, more preferably at least about 90% similar and most preferably at least about 95% similar (e.g., 95%, 96%, 97%, 98%, 99%, 100%) to any of the reference amino acid sequences of Table 1 when the comparison is performed with a BLAST algorithm wherein the parameters of the algorithm are selected to give the largest match between the respective sequences over the entire length of the respective reference sequences, are also included in the present invention.

Sequence identity refers to exact matches between the nucleotides or amino acids of two sequences which are being compared. Sequence similarity refers to both exact matches between the amino acids of two polypeptides which are being compared in addition to matches between nonidentical, biochemically related amino acids. Biochemically related amino acids which share similar properties and may be interchangeable are discussed above.

The following references regarding the BLAST algorithm are herein incorporated by reference: **BLAST ALGORITHMS**: Altschul, S.F., et al., (1990) J. Mol. Biol. 215:403-


Antibody Structure

In general, the basic antibody structural unit is known to comprise a tetramer. Each tetramer includes two identical pairs of polypeptide chains, each pair having one "light" (about 25 kDa) and one "heavy" chain (about 50-70 kDa). The amino-terminal portion of each chain may include a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The carboxy-terminal portion of each chain may define a constant region primarily responsible for effector function. Typically, human light chains are classified as kappa and lambda light chains. Furthermore, human heavy chains are typically classified as mu, delta, gamma, alpha, or epsilon, and define the antibody's isotype as IgM, IgD, IgG, IgA, and IgE, respectively. Within light and heavy chains, the variable and constant regions are joined by a "J" region of about 12 or more amino acids, with the heavy chain also including a "D" region of about 10 more amino acids. See generally, Fundamental Immunology Ch. 7 (Paul, W., ed., 2nd ed. Raven Press, N.Y. (1989)) (incorporated by reference in its entirety for all purposes).
The variable regions of each light/heavy chain pair form the antibody binding site. Thus, in general, an intact IgG antibody has two binding sites. Except in bifunctional or bispecific antibodies, the two binding sites are, in general, the same.


**Binding Compositions**

The binding compositions of the combinations of the present invention include any composition which binds specifically to IGFR1. A binding composition or agent refers to a molecule that binds with specificity to IGFR1, *e.g.*, in a ligand-receptor type fashion or an antibody-antigen interaction, *e.g.*, proteins which specifically associate with IGFR1, *e.g.*, in a natural physiologically relevant protein-protein interaction, either covalent or non-covalent. The term "binding composition" includes small organic molecules, nucleic acids and polypeptides, such as a full antibody (preferably an isolated monoclonal human antibody) or antigen-binding fragment thereof of the present invention (*e.g.*, antibody 19D12/15H12, antibody 19D12/15H12 LCF/HCA or any peptide set forth, above, in Table 1).

Antibodies and antigen binding fragments thereof, include, but are not limited to, monoclonal antibodies, polyclonal antibodies, bispecific antibodies, Fab antibody fragments, F(ab)2 antibody fragments, Fv antibody fragments (*e.g.*, VH or VL), single chain Fv antibody fragments and dsFv antibody fragments. Furthermore, antibodies of the invention may be fully human antibodies or chimeric antibodies.

The combinations of the present invention include any antibody or antigen binding fragment thereof or any polynucleotide encoding such antibody or antigen-binding fragment thereof as set forth in U.S. Patent Application No. 10/443,466, filed May 22,
2003 and in WO 03/100008. Preferably, the antibody molecules are isolated monoclonal, fully human antibodies. Preferably the antibodies of the invention comprise one or more, more preferably all 6 CDRs comprising an amino acid sequence set forth in any one of SEQ ID NOs: 5-10. Preferably, an antibody of the invention includes mature 19D12/15H12 light chain F (LCF) (see SEQ ID NO: 2) paired with mature 19D12/15H12 heavy chain A (HCA) (see SEQ ID NO: 4) (e.g., the monoclonal, fully-human antibody 19D12/15H12 LCF/HCA).

The amino acid and nucleotide sequences of preferred antibody chains are shown below. Dotted, underscored type indicates the signal peptide. Solid underscored type indicates the CDRs. Plain type indicates the framework regions. In one embodiment, the antibody chains are mature fragments which lack the signal peptide.

19D12/15H12 Light Chain-F (LCF; SEQ ID NO: 1)

```
ATG TCG CCA TCA CAA CTC ATT GSG TTT CTG CTC TGG GTT CCA GCC TCC
AGG GGT GAA ATT GTG CTG ACT CAG AGC CCA GGT ACC CTG TCT GTG TCT CCA
GTC GAG AGA GCC ACC CTC TCC TGC CGG GCC AGT CAG AGC ATT GGT AGT AGC
TTA CAC TGG TAC CAG CAG AAA CCA GGT CAG GCT CCA AGG CTT CTC ATC AGG
TAT GCA TCC CAG TCC CTC TCA GGG ATC CCC GAT AGG TTC AGT GGC AGT GGA
TCT GGG ACA GAT TTC ACC CTC ACC ATC AGT AGA CTG GAG CCT GAA GAT TCC
GCA GTG TAT TAC TGT CAT CAG AGT AGT GTG TTA CCT CAC ACT TCC GGC CAA
GGG ACC AAG GTG GAG ATC AAA GTG ACA
```

(SEQ ID NO: 2)

```
M S P S Q L I G F L L L W V P A S
R G H E I V L T Q S P G T L S V S P
G E R A T L S C R A S Q S I G S S
L H W Y Q Q K P G Q A P R L L I K
Y A S Q S L S G I P D R F S G S G
S G T D F T L T I S R L E P E D F
A V Y Y C H Q S R L P H T F G Q
G T K V E I K R T
```
19D12/15H12 heavy chain-A (HCA; SEQ ID NO: 3)

<table>
<thead>
<tr>
<th>Codon</th>
</tr>
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<tbody>
<tr>
<td>ATG GAG TTT GGG CTG AGC TGG GGT TCC CTT GCT GTA TTA AAA GGT GTC</td>
</tr>
<tr>
<td>CAG GGT GAT GAG CTC GTG GAG GCA GCG TCT GAA AAG CCT GGG</td>
</tr>
<tr>
<td>GGG TCC CTG AGA CTC TCC TGT GCA GCC TCT GGA TTC ACC TTC AGT AGC TTT</td>
</tr>
<tr>
<td>GCT ATG CAC TGG GGT GGC CAG GCT CCA GGA AAA GGT CTG GAG TGG ATA TCA</td>
</tr>
<tr>
<td>GTT ATT GAT ACT CTT GGT GCC ACA TAT GCA GAC TCC GIG AAG GGC GCA</td>
</tr>
<tr>
<td>TTC ACC ATC TCC AGA GAC AAT GCC AAG AAC TCC TTG TAT CTT CAA ATG AAC</td>
</tr>
<tr>
<td>AAG CTG AGA GCC GAG GAC ACT GCT GTG TAT TAC TGT GCA AAG CTG GGG AAC</td>
</tr>
<tr>
<td>TTC TAC TAC GGT ATG GAC GTC TGG GGC CAA GGG ACC ACG GTC ACC GTC TCC</td>
</tr>
<tr>
<td>TCA</td>
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</table>

(SEQ ID NO: 4)

<table>
<thead>
<tr>
<th>Amino Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Met Glu Phe Gly Leu Ser Trp Val Phe Leu Val Ala Ile Leu Lys Gly Val Gln Cys Glu Val Gln Leu Val Gln Ser Gly Gly Leu Val Lys Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Phe Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile Ser Val Ile Asp Thr Arg Gly Ala Thr Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Cys Ala Arg Leu Gly Asp Phe Tyr Tyr Gly Met Asp Val Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser</td>
</tr>
</tbody>
</table>

Three plasmids comprising a CMV promoter operably linked to the 15H12/19D12 LCF (κ) (variable region sequence set forth in SEQ ID NOs: 1 and 2), to the 15H12/19D12 HCA (γ4) (variable region sequence set forth in SEQ ID NOs: 3 and 4) or to the 15H12/19D12 HCA (γ1) (variable region sequence set forth in SEQ ID NOs: 3 and 4) has been deposited at the American Type Culture Collection (ATCC); 10801 University Boulevard; Manassas, Virginia 20110-2209 on May 21, 2003. The deposit name and the ATCC accession numbers for the plasmids are set forth below:

CMV promoter-15H12/19D12 HCA (γ4)-

Deposit name: “15H12/19D12 HCA (γ4)”;

ATCC accession No.: PTA-5214;
CMV promoter-15H12/19D12 HCA (γ1)-
   Deposit name: “15H12/19D12 HCA (γ4)”;  
   ATCC accession No.: PTA-5216;
CMV promoter-15H12/19D12 LCF (κ)-
   Deposit name: “15H12/19D12 LCF (κ)”;  
   ATCC accession No.: PTA-5220.

All restrictions on access to the plasmids deposited in ATCC will be removed upon grant of a patent.

Each of the above-referenced plasmids constitutes part of the present invention.

Further, the nucleic acid located within each expression cassette, along with the immunoglobulin variable region therein, along with the mature, processed version thereof (i.e., lacking the signal sequence), particularly, SEQ ID NO: 3, mature HCA (nucleotides 58-411 of SEQ ID NO: 3), SEQ ID NO: 1 or mature LCF (nucleotides 58-384 of SEQ ID NO: 1), optionally including an immunoglobulin constant region, along with any polypeptide encoded by any of the foregoing nucleic acids, including mature or unprocessed chains, optionally including an immunoglobulin constant region, is a part of the present invention. Moreover, any antibody or antigen-binding fragment thereof comprising one of the encoded polypeptides is part of the present invention.

The scope of the present invention includes antibody variable regions of the present invention (e.g., any variable region, mature or unprocessed, indicated in Table 1) linked to any immunoglobulin constant region. If a light chain variable region is linked to a constant region, preferably it is a κ chain. If a heavy chain variable region is linked to a constant region, preferably it is a γ1, γ2, γ3 or γ4 constant region, more preferably, γ1, γ2 or γ4 and even more preferably γ1 or γ4.

The anti-IGFR1 antibody molecules of the invention preferably recognize human IGFR1, preferably a soluble fragment of IGFR1 (i.e., sIGFR1) such as amino acids 30-902 or SEQ ID NO: 11; however, the present invention includes antibody molecules which recognize IGFR1 from different species, preferably mammals (e.g., mouse, rat, rabbit, sheep or dog).

The present invention also includes an anti-IGFR1 antibody (e.g., LCF/HCA) or antigen-binding fragments thereof which are complexed with IGFR1 or any fragment thereof (e.g., sIGFR1, such as amino acids 30-902 of SEQ ID NO: 11) or with any cell which is expressing IGFR1 or any portion or fragment thereof on the cell surface (e.g., HEK293 cells stably transformed with human IGFR1 or MCF7 (e.g., ATCC Cell Line No.
HTB-22). Such complexes may be made by contacting the antibody or antibody fragment with IGFR1 or the IGFR1 fragment.


Preferably, the mice will be 6-16 weeks of age upon the first immunization. For example, a purified preparation of IGFR1 or sIGFR1 can be used to immunize the HuMab mice intraperitoneally. The mice can also be immunized with whole HEK293 cells which are stably transfected with an IGFR1 gene. An “antigenic IGFR1 polypeptide” may refer to an IGFR1 polypeptide of any fragment thereof, preferably amino acids 30-902 of SEQ ID NO: 11, which elicits an anti-IGFR1 immune response, preferably in HuMab mice.

In general, HuMab transgenic mice respond well when initially immunized intraperitoneally (IP) with antigen in complete Freund’s adjuvant, followed by every other week IP immunizations (usually, up to a total of 6) with antigen in incomplete Freund’s adjuvant. Mice can be immunized, first, with cells expressing IGFR1 (e.g., stably transfected HEK293 cells), then with a soluble fragment of IGFR1 (e.g., amino acids 30-902 of SEQ ID NO: 11) and continually receive alternating immunizations with the two antigens. The immune response can be monitored over the course of the immunization protocol with plasma samples being obtained by retroorbital bleeds. The plasma can be screened for the presence of anti-IGFR1 antibodies, for example by ELISA, and mice with sufficient titers of immunoglobulin can be used for fusions. Mice can be boosted intravenously with antigen 3 days before sacrifice and removal of the spleen. It is expected that 2-3 fusions for each antigen may need to be performed. Several mice can be immunized for each antigen. For example, a total of twelve HuMab mice of the HC07 and HC012 strains can be immunized.

Hybridoma cells which produce the monoclonal, fully human anti-IGFR1 antibodies may be produced by methods which are commonly known in the art. These methods include, but are not limited to, the hybridoma technique originally developed by Kohler, et al., (1975) (Nature 256:495-497), as well as the trioma technique (Hering, et al., (1988) Biomed. Biochim. Acta. 47:211-216 and Hagiwara, et al., (1993) Hum. Antibod. Hybridomas 4:15), the human B-cell hybridoma technique (Kozbor, et al., (1983) Immunology Today 4:72 and Cote, et al., (1983) Proc. Natl. Acad. Sci. U.S.A 80:2026-2030), and the EBV-hybridoma technique (Cole, et al., in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96, 1985). Preferably, mouse splenocytes are isolated and fused with PEG to a mouse myeloma cell line based upon standard protocols. The resulting hybridomas may then be screened for the production of antigen-specific antibodies. For example, single cell suspensions of splenic lymphocytes from immunized mice may by fused to one-sixth the number of P3X63-Ag8.653 nonsecreting mouse myeloma cells (ATCC, CRL 1580) with 50% PEG. Cells may be plated at
approximately 2 x 10^6 cells/mL in a flat bottom microtiter plate, followed by a two week incubation in selective medium containing 20% fetal Clone Serum, 18% "653" conditioned media, 5% origen (IGEN), 4 mM L-glutamine, 1 mM L-glutamine, 1 mM sodium pyruvate, 5 mM HEPES, 0.055 mM 2-mercaptoethanol, 50 units/ml penicillin, 50 mg/ml streptomycin, 50 mg/ml gentamycin and 1X HAT (Sigma; the HAT is added 24 hours after the fusion). After two weeks, cells may be cultured in medium in which the HAT is replaced with HT. Individual wells may then be screened by ELISA for human anti-IGFR1 monoclonal IgG antibodies. Once extensive hybridoma growth occurs, medium can be observed usually after 10-14 days. The antibody secreting hybridomas may be replated, screened again, and if still positive for human IgG, anti-IGFR1 monoclonal antibodies, can be subcloned at least twice by limiting dilution. The stable subclones may then be cultured in vitro to generate small amounts of antibody in tissue culture medium for characterization.

The anti-IGFR1 antibodies and antigen-binding fragments thereof of the present invention may also be produced recombinantly (e.g., in an E.coli/T7 expression system as discussed above). In this embodiment, nucleic acids encoding the antibody molecules of the invention (e.g., V_H or V_L) may be inserted into a pET-based plasmid and expressed in the E.coli/T7 system. There are several methods by which to produce recombinant antibodies which are known in the art. One example of a method for recombinant production of antibodies is disclosed in U.S. Patent No. 4,816,567, which is herein incorporated by reference. Transformation can be by any known method for introducing polynucleotides into a host cell. Methods for introduction of heterologous polynucleotides into mammalian cells are well known in the art and include dextran-mediated transfection, calcium phosphate precipitation, polybrene-mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, biolistic injection and direct microinjection of the DNA into nuclei. In addition, nucleic acid molecules may be introduced into mammalian cells by viral vectors. Methods of transforming cells are well known in the art. See, for example, U.S. Patent Nos. 4,399,216; 4,912,040; 4,740,461 and 4,959,455.

Anti-IGFR1 antibodies can also be synthesized by any of the methods set forth in U.S. Patent No. 6,331,415.

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). These include, \textit{inter alia}, Chinese hamster ovary (CHO) cells, NSO,
SP2 cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS),
human hepatocellular carcinoma cells (e.g., Hep G2), A549 cells, 3T3 cells, HEK-293
cells and a number of other cell lines. Mammalian host cells include human, mouse, rat,
dog, monkey, pig, goat, bovine, horse and hamster cells. Cell lines of particular
preference are selected through determining which cell lines have high expression levels.
Other cell lines that may be used are insect cell lines, such as Sf9 cells, amphibian cells,
bacterial cells, plant cells and fungal cells. When recombinant expression vectors
encoding the heavy chain or antigen-binding portion thereof, the light chain and/or
antigen-binding portion thereof are introduced into mammalian host cells, the antibodies
are produced by culturing the host cells for a period of time sufficient to allow for
expression of the antibody in the host cells or, more preferably, secretion of the antibody
into the culture medium in which the host cells are grown.

Antibodies can be recovered from the culture medium using standard protein
purification methods. Further, expression of antibodies of the invention (or other moieties
therefrom) from production cell lines can be enhanced using a number of known
techniques. For example, the glutamine synthetase gene expression system (the GS
system) is a common approach for enhancing expression under certain conditions. The
GS system 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.

It is likely that antibodies expressed by different cell lines or in transgenic animals
will have different glycosylation from each other. However, all antibodies encoded by the
nucleic acid molecules provided herein, or comprising the amino acid sequences provided
herein are part of the instant invention, regardless of the glycosylation of the antibodies.

The term "monoclonal antibody," as used herein, refers to an antibody obtained
from a population of substantially homogeneous antibodies, i.e., the individual antibodies
comprising the population are identical except for possible naturally occurring mutations
that may be present in minor amounts. Monoclonal antibodies are highly specific, being
directed against a single antigenic site. Monoclonal antibodies are advantageous in that
they may be synthesized by a hybridoma culture, essentially uncontaminated by other
immunoglobulins. The modifier "monoclonal" indicates the character of the antibody as
being amongst a substantially homogeneous population of antibodies, and is not to be
construed as requiring production of the antibody by any particular method. As mentioned
above, the monoclonal antibodies to be used in accordance with the present invention
may be made by the hybridoma method first described by Kohler, et al., (1975) Nature 256: 495.

A polyclonal antibody is an antibody which was produced among or in the presence of one or more other, non-identical antibodies. In general, polyclonal antibodies are produced from a B-lymphocyte in the presence of several other B-lymphocytes which produced non-identical antibodies. Usually, polyclonal antibodies are obtained directly from an immunized animal.


The term "fully human antibody" refers to an antibody which comprises human immunoglobulin protein sequences only. A fully human antibody may contain murine carbohydrate chains if produced in a mouse, in a mouse cell or in a hybridoma derived from a mouse cell. Similarly, "mouse antibody" refers to an antibody which comprises mouse immunoglobulin sequences only.

The present invention includes "chimeric antibodies"- an antibody which comprises a variable region of the present invention fused or chimerized with an antibody region (e.g., constant region) from another, non-human species (e.g., mouse, horse, rabbit, dog, cow, chicken). These antibodies may be used to modulate the expression or activity of IGFR1 in the non-human species.

"Single-chain Fv" or "sFv" antibody fragments have the V\textsubscript{H} and V\textsubscript{L} domains of an antibody, wherein these domains are present in a single polypeptide chain. Generally, the sFv polypeptide further comprises a polypeptide linker between the V\textsubscript{H} and V\textsubscript{L} domains which enables the sFv to form the desired structure for antigen binding. Techniques described for the production of single chain antibodies (U.S. Patent Nos. 5,476,786; 5,132,405 and 4,946,778) can be adapted to produce anti-IGFR1-specific single chain antibodies. For a review of sFv see Pluckthun in The Pharmacology of Monoclonal Antibodies, vol. 113, Rosenberg and Moore eds. Springer-Verlag, N.Y., pp. 269-315 (1994).
"Disulfide stabilized Fv fragments" and "dsFv" refer to antibody molecules comprising a variable heavy chain (V_{H}) and a variable light chain (V_{L}) which are linked by a disulfide bridge.

Antibody fragments within the scope of the present invention also include F(ab)_{2} fragments which may be produced by enzymatic cleavage of an IgG by, for example, pepsin. Fab fragments may be produced by, for example, reduction of F(ab)_{2} with dithiothreitol or mercaptoethylamine. A Fab fragment is a V_{L}-C_{L} chain appended to a V_{H}-C_{H} chain by a disulfide bridge. A F(ab)_{2} fragment is two Fab fragments which, in turn, are appended by two disulfide bridges. The Fab portion of an F(ab)_{2} molecule includes a portion of the F_{c} region between which disulfide bridges are located.

An F_{V} fragment is a V_{L} or V_{H} region.

Depending on the amino acid sequences of the constant domain of their heavy chains, immunoglobulins can be assigned to different classes. There are at least five major classes of immunoglobulins: IgA, IgD, IgE, IgG and IgM, and several of these may be further divided into subclasses (isotypes), e.g. IgG-1, IgG-2, IgG-3 and IgG-4; IgA-1 and IgA-2.

The anti-IGFR1 antibody molecules of the invention may also be conjugated to a chemical moiety. The chemical moiety may be, inter alia, a polymer, a radionuclide or a cytotoxic factor. Preferably the chemical moiety is a polymer which increases the half-life of the antibody molecule in the body of a subject. Suitable polymers include, but are not limited to, polyethylene glycol (PEG) (e.g., PEG with a molecular weight of 2kDa, 5 kDa, 10 kDa, 12kDa, 20 kDa, 30kDa or 40kDa), dextran and monomethoxypolyethylene glycol (mPEG). Lee, et al., (1999) (Bioconj. Chem. 10:973-981) discloses PEG conjugated single-chain antibodies. Wen, et al., (2001) (Bioconj. Chem. 12:545-553) disclose conjugating antibodies with PEG which is attached to a radiometal chelator (diethylenetriaminopentaacetic acid (DTPA)).

The antibodies and antibody fragments of the invention may also be conjugated with labels such as $^{99m}$Tc, $^{90}$Y, $^{111}$In, $^{32}$P, $^{14}$C, $^{125}$I, $^{3}$H, $^{131}$I, $^{11}$C, $^{15}$O, $^{12}$N, $^{18}$F, $^{38}$S, $^{51}$Cr, $^{57}$Te, $^{226}$Ra, $^{60}$Co, $^{59}$Fe, $^{57}$Se, $^{152}$Eu, $^{67}$Cu, $^{217}$Cl, $^{211}$At, $^{212}$Pb, $^{47}$Sc, $^{109}$Pd, $^{234}$Th, and $^{40}$K, $^{157}$Gd, $^{55}$Mn, $^{52}$Tr and $^{56}$Fe.

The antibodies and antibody fragments of the invention may also be conjugated with fluorescent or chemiluminescent labels, including fluorophores such as rare earth chelates, fluorescein and its derivatives, rhodamine and its derivatives, isothiocyanate, phycoerythrin, phycocyanin, allophycocyanin, o-phthalaldehyde, fluorescamine, $^{162}$Eu,
dansyl, umbelliferone, luciferin, luminal label, isoluminal label, an aromatic acridinium ester label, an imidazole label, an acridinium salt label, an oxalate ester label, an aequorin label, 2,3-dihydropthalazinediones, biotin/avidin, spin labels and stable free radicals.

The antibody molecules may also be conjugated to a cytotoxic factor such as diptheria toxin, *Pseudomonas aeruginosa* exotoxin A chain, ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, *Aleurites fordii* proteins and compounds (*e.g.*, fatty acids), dianthin proteins, *Phytoicca americana* proteins PAPI, PAPII, and PAP-S, *Momordica charantia* inhibitor, curcin, crocin, *Saponaria officinalis* inhibitor, mitogellin, restrictocin, phenomycin, and enomycin.


**Chemotherapeutic Agents**

The present invention includes combinations and methods comprising one or more binding compositions, such as an anti-IGFR1 antibody or antigen-binding fragment thereof in association with one or more chemotherapeutic agents. A chemotherapeutic agent provides a therapeutic effect which is helpful in the treatment of any medical condition being treated by administration of a binding composition of the invention (*e.g.*, LCF/HCA). For example, if a binding composition is administered to treat cancer in a subject (*e.g.*, human), the chemotherapeutic agent(s) provide an additional anti-cancer therapeutic effect or some other therapeutic effect which will improve the subject's treatment outcome. The chemotherapeutic agent component of a combination of the invention can operate by any mechanism (*i.e.*, by the same mechanism by which the binding composition acts or by a different mechanism). Chemotherapeutic agents in the combinations and methods of the present invention include, but are, by no means, limited to, signal transduction inhibitors, cell cycle inhibitors, IGF/IGFR1 system modulators (*e.g.*, inhibitors or activators), farnesyl protein transferase (FPT) inhibitors, epidermal growth factor receptor (EGFR) inhibitors, HER2 inhibitors, vascular epidermal growth factor (VEGF) receptor inhibitors, mitogen activated protein (MAP) kinase inhibitors, MEK
inhibitors, AKT inhibitors, mTOR inhibitors, p3 kinase inhibitors, Raf inhibitors, cyclin dependent kinase (CDK) inhibitors, microtubule stabilizers, microtubule inhibitors, SERMs/Antiestrogens, aromatase inhibitors, anthracyclines, proteasome inhibitors and agents which inhibit insulin-like growth factor (IGF) production and anti-sense inhibitors of IGFR1, IGF-1 or IGF2.

FPT inhibitors including tricyclic amide compounds such as those disclosed in U.S. Patent No. 5,719,148 or in U.S. Patent No. 5,874,442 can be combined with an anti-IGFR antibody. For example, any compound represented by formula I, below, may be included in the combinations of the invention:

![Chemical Structure](image)

or a pharmaceutically acceptable salt or solvate thereof, wherein:

- one of a, b, c and d represents N or NR\textsuperscript{9} wherein R\textsuperscript{9} is O\textsuperscript{-}, -CH\textsubscript{3} or -(CH\textsubscript{2})\textsubscript{n}CO\textsubscript{2}H wherein n is 1 to 3, and the remaining a, b, c and d groups represent CR\textsuperscript{1} or CR\textsuperscript{2}; or
- each of a, b, c, and d are independently selected from CR\textsuperscript{1} or CR\textsuperscript{2};
- each R\textsuperscript{1} and each R\textsuperscript{2} is independently selected from H, halo, -CF\textsubscript{3}, -OR\textsuperscript{10} (e.g., -OCH\textsubscript{3}), -COR\textsuperscript{10}, -SR\textsuperscript{10} (e.g., -SCH\textsubscript{3} and -SCH\textsubscript{2}C\textsubscript{6}H\textsubscript{5}), -S(O)\textsuperscript{t}R\textsuperscript{11} (wherein t is 0, 1 or 2, e.g., -SOCH\textsubscript{3} and -SO\textsubscript{2}CH\textsubscript{3}), -SCN, -N(R\textsuperscript{10})\textsubscript{2}, -NR\textsuperscript{10}R\textsuperscript{11}, -NO\textsubscript{2}, -OC(O)R\textsuperscript{10}, -CO\textsubscript{2}R\textsuperscript{10}, -OCO\textsubscript{2}R\textsuperscript{11}, -CN, -NHC(O)R\textsuperscript{10}, -NH\textsubscript{2}SO\textsubscript{2}R\textsuperscript{10}, -CONHR\textsuperscript{10}, -CONHCH\textsubscript{2}CH\textsubscript{2}OH, -NR\textsuperscript{10}COOR\textsuperscript{11},
-SR^{11}C(O)OR^{11} (e.g., -SCH_2CO_2CH_3), -SR^{11}N(R^{75})_2 wherein each R^{75} is independently selected from H and -C(O)OR^{11} (e.g., -S(CH_2)_2NHC(O)O- t-butyl and -S(CH_2)_2NH_2), benzotriazol-1- yloxy, tetrazol-5-ythio, or substituted tetrazol-5-ythio (e.g., alkyl substituted tetrazol5- ythio such as 1-methyl-tetrazol-5-ythio), alkynyl, alkenyl or alkyl, said alkyl or alkenyl group optionally being substituted with halo, -OR^{10} or -CO_2R^{10};

R^3 and R^4 are the same or different and each independently represents H, any of the substituents of R^1 and R^2, or R^3 and R^4 taken together represent a saturated or unsaturated C_5-C_7 fused ring to the benzene ring (Ring III);

R^5, R^6, R^7 and R^8 each independently represents H, -CF_3, -COR^{10}, alkyl or aryl, said alkyl or aryl optionally being substituted with -OR^{10}, -SR^{10}, -S(O)R^{11}, -NR^{10}COOR^{11}, -N(R^{10})_2, -NO_2, -COR^{10}, -OCOR^{10}, -OCO_2R^{11}, -CO_2R^{10}, OPO_3R^{10} or one of R^5, R^6, R^7 and R^8 can be taken in combination with R^{40} as defined below to represent -(CH_2)_r- wherein r is 1 to 4 which can be substituted with lower alkyl, lower alkoxy, -CF_3 or aryl, or R^5 is combined with R^6 to represent =O or =S and/or R^7 is combined with R^8 to represent =O or =S;

R^{10} represents H, alkyl, aryl, or aralkyl (e.g., benzyl);

R^{11} represents alkyl or aryl;

X represents N, CH or C, which C may contain an optional double bond (represented by the dotted line) to carbon atom 11;

the dotted line between carbon atoms 5 and 6 represents an optional double bond, such that when a double bond is present, A and B independently represent -R^{10}, halo, -OR^{11}, -OCO_2R^{11} or -OC(O)R^{10}, and when no double bond is present between carbon atoms 5 and 6, A and B each independently represent H_2, -(OR^{11})_2; H and halo,
dihalo, alkyl and H, (alkyl)2, -H and -OC(O)R10, H and -OR10, =O, aryl and H, =NOR10 or -O-(CH2)p-O- wherein p is 2, 3 or 4;

R represents R40, R42, R44, or R54, as defined below;

R40 represents H, aryl, alkyl, cycloalkyl, alkenyl, alkynyl or -D wherein -D represents

\[
\begin{align*}
\text{N} & \quad \text{R}^3 & \quad \text{R}^4 \\
\text{N} & \quad \text{R}^3 & \quad \text{R}^4 \\
\text{N} & \quad \text{R}^3 & \quad \text{R}^4
\end{align*}
\]

wherein R3 and R4 are as previously defined and W is O, S or NR10 wherein R10 is as defined above; said R40 cycloalkyl, alkenyl and alkynyl groups being optionally substituted with from 1-3 groups selected from halo, -CON(R10)2, aryl, -CO2R10, -OR12, -SR12, -N(R10)2, -N(R10)CO2R11, -COR12, -NO2 or D, wherein -D, R10 and R11 are as defined above and R12 represents R10, -(CH2)mOR10 or -(CH2)qCO2R10 wherein R10 is as previously defined, m is 1 to 4 and q is 0 to 4; said alkenyl and alkynyl R40 groups not containing -OH, -SH or
-N(R^{10})_2 on a carbon containing a double or triple bond respectively; or

R^{40} represents phenyl substituted with a group selected from -SO_2NH_2, -NHSO_2CH_3, -SO_2NHCH_3, -SO_2CH_3, -SOCH_3, -SCH_3, or -NHSO_2CF_3, preferably, said group is located in the para (p-) position of the phenyl ring; or

R^{40} represents a group selected from

![Chemical structures](attachment:chemical_structures.png)

R^{42} represents

![Chemical structures](attachment:chemical_structures.png)

wherein R^{20}, R^{21} and R^{46} are each independently selected from the group consisting of:

1. H;
2. -(CH_2)_qSC(O)CH_3 wherein q is 1 to 3 (e.g., -CH_2SC(O)CH_3);
3. -(CH_2)_qOSO_2CH_3 wherein q is 1 to 3 (e.g., -CH_2OSO_2CH_3);
4. -OH;
(5) -CS(CH₂)ₓ wherein w is 1 to 3 and the substituents on said substituted phenyl group are the same substituents as described below for said substituted phenyl (e.g., -C-S-CH₂-4-methoxyphenyl);
(6) -NH₂;
(7) -NHC(ơ)OR wherein R is an alkyl group having from 1 to 5 carbon atoms (e.g., R is t-butyl thus forming -NHBOC wherein BOC stands for tert-butyloxycarbonyl—i.e., BOC represents -C(ơ)OC(CH₃)₃), or R represents phenyl substituted with 1 to 3 alkyl groups (e.g., 4-methylphenyl);
(8) alkyl (e.g., ethyl);
(9) -(CH₂)ₓ wherein k is 1 to 6, usually 1 to 4 and preferably 1 (e.g., benzyl);
(10) phenyl;
(11) substituted phenyl (i.e., phenyl substituted with from 1 to 3 substituents, preferably one) wherein the substituents are selected from the group consisting of: halo (e.g., Br, Cl, or I, with Br being preferred); NO₂; -OH; -OCH₃; -NH₂; -NHR; -N(R₂)₂; alkyl (e.g., alkyl having from 1 to 3 carbons with methyl being preferred); -O(CH₂)ᵦ wherein t is from 1 to 3 with 1 being preferred; and -O(CH₂)ᵦ substituted phenyl (wherein t is from 1 to 3 with 1 being preferred); examples of substituted phenyls include, but are not limited to, p-bromophenyl, m-nitrophenyl, o-nitrophenyl, m-hydroxy-phenyl, o-hydroxyphenyl, methoxyphenyl, p-methylphenyl, m-methyl-phenyl, and -OCH₂C₆H₅;
(12) naphthyl;
(13) substituted naphthyl, wherein the substituents are as defined for substituted phenyl above;
(14) bridged polycyclic hydrocarbons having from 5 to 10 carbon atoms (e.g., adamantyl and norbornyl);
(15) cycloalkyl having from 5 to 7 carbon atoms (e.g., cyclopentyl, and cyclohexyl);
(16) heteroaryl (e.g., pyridyl, and pyridyl N-oxide);
(17) hydroxyalkyl (e.g., -(CH₂)ᵥOH wherein v is 1 to 3, such as, for example, -CH₂OH);
(19) substituted pyridyl or substituted pyridyl N-oxide wherein the substituents are selected from methylpyridyl, morpholinyl, imidazolyl, 1-piperidinyl, 1-(4-methylpiperazinyl), -S(O)R^{11}, or any of the substituents given above for said substituted phenyl, and said substituents are bound to a ring carbon by replacement of the hydrogen bound to said carbon;
(20) \[ \text{NHC(O)}-(\text{CH}_2)_k\text{-phenyl} \]
(21) \[ \text{NHC(O)}-(\text{CH}_2)_k\text{-substituted phenyl} \]

(23) \(-\text{NHC(O)}-(\text{CH}_2)_k\text{-phenyl or -NH(C(O)}-(\text{CH}_2)_k\text{-substituted phenyl, wherein said } k \)

is as defined above (i.e., 1-6, usually 1-4 and preferably 1);

(24) \text{piperidine Ring V:}

\[ \text{N-R}^{50} \]

wherein \( R^{50} \) represents H, alkyl (e.g., methyl), alkylcarbonyl (e.g., CH\(_3\)C(O)-),
alkyloxycarbonyl (e.g., -C(O)O-t-C\(_4\)H\(_9\), -C(O)OC\(_2\)H\(_5\), and -C(O)OCH\(_3\)), haloalkyl (e.g.,
trifluoromethyl), or \(-\text{C(O)}\text{NH}(R^{10})\) wherein \( R^{10} \) is H or alkyl; Ring V includes

(25) \(-\text{NHC(O)}\text{CH}_2\text{C}_6\text{H}_5\) or \(-\text{NHC(O)}\text{CH}_2\text{-substituted-C}_6\text{H}_5\), for example \(-\text{NHC(O)}\text{CH}_2\text{-p-hydroxyphenyl, -NHC(O)}\text{CH}_2\text{-m-hydroxyphenyl, and -NHC(O)}\text{CH}_2\text{-o-}
hydroxyphenyl;}

(26) \(-\text{NHC(O)}\text{OC}_6\text{H}_5\);
-OC(O)-heteroaryl, for example

5  (31)  -O-alkyl (e.g., -OCH₃);
(32)  -CF₃;
(33)  -CN;
(34)  a heterocycloalkyl group of the formula

10  (35)  a piperidinyl group of the formula

wherein R²⁶ is H, alkyl, or alkyl substituted by -OH or -SCH₃; or
R²⁰ and R²¹ taken together form a =O group and the remaining R⁴⁶ is as defined above; or

15  Two of R²⁰, R²¹ and R⁴⁶ taken together form piperidine Ring V

wherein R⁵⁰ represents H, alkyl (e.g., methyl), alkylcarbonyl (e.g., CH₃C(O)-),
alkyloxycarbonyl (e.g., -C(O)O-t-C₄H₉, -C(O)OC₂H₅, and -C(O)OCH₃), haloalkyl (e.g.,
trifluoro-methyl), or -C(O)NH(R¹⁰) wherein R¹⁰ is H or alkyl; Ring V includes
examples of Ring V include:

with the proviso $R^{46}$, $R^{20}$, and $R^{21}$ are selected such that the carbon atom to which they are bound does not contain more than one heteroatom (i.e., $R^{46}$, $R^{20}$, and $R^{21}$ are selected such that the carbon atom to which they are bound contains 0 or 1 heteroatom);

$R^{44}$ represents

wherein $R^{25}$ represents heteroaryl (e.g., pyridyl or pyridyl N-oxide), N-methylpiperidinyl or aryl (e.g., phenyl and substituted phenyl); and $R^{48}$ represents H or alkyl (e.g., methyl);

$R^{54}$ represents an N-oxide heterocyclic group of the formula (i), (ii), (iii) or (iv):

(i) 
(ii) 
(iii) 
(iv)
wherein R\textsuperscript{56}, R\textsuperscript{58}, and R\textsuperscript{60} are the same or different and each is independently selected from H, halo, -CF\textsubscript{3}, -OR\textsuperscript{10}, -C(O)R\textsuperscript{10}, -SR\textsuperscript{10}, -S(O)\textsubscript{e}R\textsuperscript{11} (wherein e is 1 or 2), -N(R\textsuperscript{10})\textsubscript{2}, -NO\textsubscript{2}, -CO\textsubscript{2}R\textsuperscript{10}, -OCO\textsubscript{2}R\textsuperscript{11}, -OCOR\textsuperscript{10}, alkyl, aryl, alkenyl or alkynyl, which alkyl may be substituted with -OR\textsuperscript{10}, -SR\textsuperscript{10} or -N(R\textsuperscript{10})\textsubscript{2}

and which alkenyl may be substituted with OR\textsuperscript{11} or SR\textsuperscript{11}; or

R\textsuperscript{54} represents an N-oxide heterocyclic group of the formula (ia), (iia), (iii) or (iva):

\[
\begin{align*}
&\text{(ia)} \quad \text{(iia)} \quad \text{(iii)} \quad \text{or} \quad \text{(iva)} \\
&Y \quad Y \quad Y \quad E
\end{align*}
\]

wherein Y represents N\textsuperscript{\text{+}}-O\textsuperscript{\text{-}} and E represents N; or

R\textsuperscript{54} represents an alkyl group substituted with one of said N-oxide heterocyclic groups (i), (ii), (iii), (iv), (ia), (iia), (iii) or (iva);

Z represents O or S such that R can be taken in combination with R\textsuperscript{5}, R\textsuperscript{6}, R\textsuperscript{7} or R\textsuperscript{8} as defined above, or R represents R\textsuperscript{40}, R\textsuperscript{42}, R\textsuperscript{44} or R\textsuperscript{54}.

Examples of R\textsuperscript{20}, R\textsuperscript{21}, and R\textsuperscript{46} for the above formulas include:
Examples of $R^{25}$ groups include:

wherein $Y$ represents $N$ or $NO$, $R^{28}$ is selected from the group consisting of: $C_1$ to $C_4$ alkyl, halo, hydroxy, $NO_2$, amino (-$NH_2$), -$NHR^{30}$, and -$N(R^{30})_2$ wherein $R^{30}$ represents $C_1$ to $C_6$ alkyl.

In one embodiment, the following tricyclic amide is included with an anti-IGFR antibody:

(Ionafarnib; Sarasar™; Schering-Plough; Kenilworth, NJ). In another embodiment, one of the following FPT inhibitors is included with an anti-IGFR antibody:
FPT inhibitors, which can be included with an anti-IGFR antibody, include BMS-214662 (Hunt et al., J. Med. Chem. 43(20):3587-95 (2000); Dancey et al., Curr. Pharm. Des. 8:2259-2267 (2002); (R)-7-cyano-2,3,4,5-tetrahydro-1-(1H-imidazol-4-ylmethyl)-3-(phenylmethyl)-4-(2-thienylsulfonyl)-1H-1,4-benzodiazepeine)) and R155777 (tipifarnib; Garner et al., Drug Metab. Dispos. 30(7):823-30 (2002); Dancey et al., Curr. Pharm. Des. 8:2259-2267 (2002); (B)-6-[amino(4-chlorophenyl)(1-methyl-1H-imidazol-5-yl)-methyl]-4-(3-chlorophenyl)-1-methyl-2(1H)-quinolinone);
Inhibitors which antagonize the action of the EGF Receptor or HER2, which can be included with an anti-IGFR antibody, include trastuzumab (sold as Herceptin®; Genentech, Inc.; S. San Francisco, CA) ; CP-724714

(TAK-165)

(HKI-272)

(gefitinib (Baselga et al., Drugs 60 Suppl 1:33-40 (2000); ZD-1893; 4-(3-chloro-4-fluoroanilino)-7-methoxy-6-(3-morpholinopropoxy) quinazoline; OSI-774) sold as Iressa™; AstraZeneca; Wilmington, DE;}


Imclone; New York, NY), EKB-569 ( ; Wissner et al., J.


Numerous other small molecules which have been described as being useful to inhibit EGFR can be combined with an anti-IGFR antibody. For example, U.S. Patent 5,656,655, discloses styryl substituted heteroaryl compounds that inhibit EGFR. U.S. Patent 5,646,153 discloses bis mono and/or bicyclic aryl heteroaryl carbocyclic and
heterocarbo cyclic compounds that inhibit EGFR and/or PDGFR. U.S. Patent 5,679,683
discloses tricyclic pyridine compounds that inhibit the EGFR. U.S. Patent 5,616,582
discloses quinazoline derivatives that have receptor tyrosine kinase inhibitory activity. Fry et al., Science 265 1093-1095 (1994) discloses a compound having a structure that
inhibits EGFR (see Figure 1 of Fry et al.). U.S. Patent 5,196,446, discloses
heteroarylethenediyl or heteroarylethenediylaryl compounds that inhibit EGFR. Panek, et al., Journal of Pharmacology and Experimental Therapeutics 283, 1433-1444 (1997)
disclose a compound identified as PD166285 that inhibits the EGFR, PDGFR, and FGFR families of receptors. PD166285 is identified as 6- (2,6- dichlorophenyl)-2-(4-(2-
diethylaminoethoxy)phenylamino)-8-methyl-8H- pyrido(2,3- d)pyrimidin-7-one.

VEGF receptor inhibitors, which can be combined with an anti-IGF antibody, include PT K787/ZK 222584 (Thomas et al., Semin Oncol. 30(3 Suppl 6):32-8 (2003)) and the humanized anti-VEGF antibody Bevacizumab (sold under the brand name Avastin™; Genentech, Inc.; South San Francisco, CA).

MAP kinase inhibitors, which can be combined with an anti-IGF antibody, include VX-745 (Haddad, Curr Opin. Investig. Drugs 2(8):1070-6 (2001)).

MAP kinase kinase (MEK) inhibitors, which can be combined with an anti-IGF antibody, include PD 184352 (Sebolt-Leopold, et al. Nature Med. 5: 810-816 (1999)).


p13 kinase inhibitors, which can be combined with an anti-IGF antibody, include LY294002, LY292232, LY292696, LY293684, LY293646 (Vlahos et al., J. Biol. Chem. 269(7): 5241-5248 (1994)) and wortmannin.


Cyclin dependent kinase inhibitors, which can be combined with an anti-IGF antibody, include flavopiridol (L86-8275/HMR 1275; Senderowicz, Oncogene 19(56): 6600-6606 (2000)) and UCN-01 (7-hydroxy staurosporine; Senderowicz, Oncogene 19(56): 6600-6606 (2000)).

IGF/IGFR inhibitors, which can be combined with an anti-IGF antibody, include IGF inhibitory peptides (U.S. Published Patent Application No. 20030092631 A1; PCT Application Publication NOs. WO 03/27246 A2; WO 02/72780), 4-amino-5-phenyl-7-
cyclobutyl-pyrrole[2,3-d] pyrimidine derivatives such as those disclosed in PCT

Application Publication No. WO 02/92599 (e.g., ), flavonoid glycones such as quercetin (PCT Application Publication No. WO 03/39538)

and anti-IGFR1 antibodies other than those of the present invention.

Other Anti-IGFR1 antibodies, which can be combined with an anti-IGFR antibody of the invention, are disclosed, for example, in Burtrum et al Cancer Research 63:8912-8921 (2003); in French Patent Applications FR2834990, FR2834991 and FR2834900 and in PCT Application Publication Nos. WO 03/59951; WO 04/71529; WO 03/106621; WO

04/83248; WO 04/87756 and WO 02/53596.

Agents which inhibit IGF production, which can be combined with an anti-IGFR antibody, include octreotide (L-Cysteinamide, D-phenylalanyl-L-cysteiny1-L-phenylalanyl-D-tryptophyl-L-lysyl-L-threony1-N-[2-hydroxy-1-(hydroxymethyl) propyl]-, cyclic (2,7)-disulfide; [R

R*,R*]);

Katz et al., Clin Pharm. 8(4):255-73 (1989); sold as Sandostatin LAR® Depot; Novartis Pharm. Corp; E. Hanover, NJ).

Proteasome inhibitors, which can be combined with an anti-IGFR antibody, include bortezomib (
[(1R)-3-methyl-1-[[2S]-1-oxo-3-phenyl-2-[(pyrazinylcarbonyl)amino]propyl]amino]butyl] boronic acid; sold as Velcade™; Millennium Pharm., Inc.; Cambridge, MA).

Microtubule stabilizers and microtubule depolymerizers/inhibitors, which can be combined with an anti-IGFR antibody, include paclitaxel (sold as Taxol®; Bristol-Myers Squibb; New York, NY) and docetaxel (sold as Taxotere®; Aventis Pharm, Inc.; Bridgewater, NJ); vincristine (epothilone B and BMS-247550).
podophyllotoxins and derivatives thereof including Etoposide (VP-16; Lee et al., Clin. Cancer Res. 7(5):1429-37 (2001)),

) and BMS-310705

( Temozolomide (CONH₂)

; sold by Schering Corp.; Kenilworth, NJ as Temodar®) may also be combined with an anti-IGFR antibody of the invention.

Anthracyclines which may be combined with an anti-IGFR antibody include doxorubicin (}
48.

; sold as Doxil®; Ortho Biotech Products L.P.; Raritan, NJ); daunorubicin

; sold as Cerubidine®; Ben Venue Laboratories, Inc.; Bedford, OH) and epirubicin ( ; sold as Ellence®; Pharmacia & Upjohn Co; Kalamazoo, MI).

Anti-estrogens and selective estrogen receptor modulators (SERMs), which can be combined with the anti-IGFR antibodies of the invention include droloxifene (3- hydroxytamoxifen), 4-hydroxytamoxifen ( ), tamoxifen

( ; sold as Nolvadex®; Astra Zeneca; Wilmington, DE); pipendoxifene

( ; ERA-923; Greenberger et al., Clin. Cancer Res. 7(10):3166-77
(2001)); arzoxifene (LY353381; Sato et al., J. Pharmacol. Exp. Ther. 287(1):1-7 (1998)); raloxifene (sold as Evista®; Eli Lilly & Co.; Indianapolis, IN); fulvestrant (ICI-182780; sold as Faslodex; Astra Zeneca; Wilmington, DE); acolbifene (EM-652);

toremifene (CP-336,156; Ke et al., Endocrinology 139(4):2068-76 (1998)); lasoxifene (CP-
idoxifene (pyrrolidino-4-iodotamoxifen; ; Nuttall et al., Endocrinology 139(12):5224-34 (1998)); TSE-424

( ); Bazedoxifene; WAY-140424); HMR-3339 and ZK-186619.

Aromatase inhibitors, which can be included with an anti-IGFR antibody, include

anastrazole ( ); Dukes et al., J. Steroid. Biochem. Mol. Biol. 58(4):439-45 (1996)), letrozole (NC; sold as Femara®; Novartis Pharmaceuticals Corp.; E. Hanover, NJ) and exemestane

( ; sold as Aromasin®; Pharmacia Corp.; Kalamazoo, MI).

Oxaliplatin (}
An anti-IGFR antibody can also be combined with gemcitabine HCl

(with retinoic acid or with any IGFR inhibitor set forth in any of Mitsiades et al., Cancer Cell 5:221-230 (2004); Garcia-Echeverria et al., Cancer Cell 5:231-239, 2004; WO 2004/036627 or WO 2004/030625.

Antisense oligonucleotides can be produced that are complementary to the mRNA of the IGFR1, IGF-1 or IGF-2 gene and can be used to inhibit transcription or translation of the genes. Production of antisense oligonucleotides effective for therapeutic uses is well known in the art. Antisense oligonucleotides are often produced using derivatized or modified nucleotides in order to increase half-life or bioavailability. The primary sequence of the IGFR1, IGF-1 or IGF-2 gene can also be used to design ribozymes. Most synthetic ribozymes are generally hammerhead, tetrahymena and harpin ribozymes. Methods of designing and using ribozymes to cleave specific RNA species are well known in the art.
The chemical structures and other useful information regarding many of the foregoing agents can be found in the *Physicians’ Desk Reference*, 57th ed., 2003; Thompson PDR; Montvale, NJ.

Categorization of a particular agent into a particular class (e.g., FPT inhibitor or microtubule stabilizer) is only done for descriptive purposes and is not meant to limit the invention in any way.

The scope of present invention includes compositions and methods comprising an anti-IGFR antibody along with one or more of the foregoing chemotherapeutic agents or any salt, hydrate, isomer, formulation, solvate or prodrug thereof.

**Pharmaceutical Compositions**

A combination, or any component thereof, of the invention can be incorporated into a pharmaceutical composition, along with a pharmaceutically acceptable carrier, suitable for administration to a subject *in vivo*. The scope of the present invention includes pharmaceutical compositions which may be administered to a subject by any route, such as a non-parenteral (e.g., oral, ocular, topical or pulmonary (inhalation)) or a parenteral route (e.g., intratumoral injection, intravenous injection, intraarterial injection, subcutaneous injection or intramuscular injection). In one embodiment, the pharmaceutical compositions of the invention comprise an antibody comprising 15H12/19D12 LCF and 15H12/19D12 HCA in association with one or more chemotherapeutic agents and a pharmaceutically acceptable carrier.

As stated above, the combinations of the invention include the binding composition component and chemotherapeutic agent component “in association” with one another. The term “in association” indicates that the components of the combinations of the invention can be formulated into a single composition for simultaneous delivery or formulated separately into two or more compositions (e.g., a kit). For example, the scope of the present invention includes combinations comprising an anti-IGFR1 antibody formulated for parenteral administration (e.g., intravenous) to a subject and a chemotherapeutic agent formulated for oral delivery (e.g., pill, tablet, capsule).

Alternatively, both components of the combination can be formulated, separately or together, for parenteral delivery or non-parenteral delivery (e.g., oral).


Pharmaceutically acceptable carriers are conventional and very well known in the art. Examples include aqueous and nonaqueous carriers, stabilizers, antioxidants, solvents, dispersion media, coatings, antimicrobial agents, buffers, serum proteins, isotonic and absorption delaying agents, and the like that are physiologically compatible. Preferably, the carrier is suitable for injection into a subject’s body.

Examples of suitable aqueous and nonaqueous carriers which may be employed in the pharmaceutical compositions of the invention include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

Stabilizers, such as α, α-trehalose dihydrate may be included for stabilizing the antibody molecules of the invention from degrading effects of dessication or freeze-drying.

Examples of pharmaceutically-acceptable antioxidants include: water soluble antioxidants such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; and oil-soluble antioxidants such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like; and metal chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

Prevention of the presence of microorganisms may be ensured both by sterilization procedures, and by the inclusion of various antimicrobial agents such as EDTA, EGTA, paraben, chlorobutanol, phenol sorbic acid, and the like.

Suitable buffers which may be included in the pharmaceutical compositions of the invention include L-histidine based buffers, phosphate based buffers (e.g., phosphate buffered saline, pH ≈ 7), sorbate based buffers or glycine-based buffers.
Serum proteins which may be included in the pharmaceutical compositions of the invention may include human serum albumin.

Isotonic agents, such as sugars, ethanol, polyalcohols (e.g., glycerol, propylene glycol, liquid polyethylene glycol, mannitol or sorbitol), sodium citrate or sodium chloride (e.g., buffered saline) may also be included in the pharmaceutical compositions of the invention.

Prolonged absorption of an injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption such as aluminum monostearate and/or gelatin.

Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils.

Pharmaceutically acceptable carriers include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. The use of such media and agents for pharmaceutically active substances is well known in the art.

Sterile injectable solutions can be prepared by incorporating a combination of the invention or any component thereof (e.g., binding composition and/or chemotherapeutic agent), in the required amount, in an appropriate solvent, optionally with one or a combination of ingredients enumerated above, as required, followed by sterilization microfiltration. Generally, dispersions are prepared by incorporating the active ingredient (e.g., binding composition and/or chemotherapeutic agent) 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 (lyophilization) that yield a powder of the active ingredient plus any additional, desired ingredient from a previously sterile-filtered solution thereof.

A combination or the invention or any component thereof (e.g., binding composition and/or chemotherapeutic agent) may also be orally administered.

Pharmaceutical compositions for oral administration may include additives and carriers such as starch (e.g., potato, maize or wheat starch or cellulose), starch derivatives (e.g., microcrystalline cellulose or silica), sugars (e.g., lactose), talc, lactose, stearate, magnesium carbonate or calcium phosphate. In order to ensure that oral compositions are well tolerated by the patient's digestive system, mucus formers or resins may be included. It may also be desirable to improve tolerance by formulating in a capsule which
is insoluble in the gastric juices. An exemplary pharmaceutical composition of this
invention in the form of a capsule is prepared by filling a standard two-piece hard gelatin
capsule with the combination of the invention or any component thereof in powdered form,
lactose, talc and magnesium stearate. Oral administration of immunoglobulins has been
described (Foster, et al., (2001) Cochrane Database System rev. 3:CD001816)

A combination of the invention or any component thereof (e.g., binding composition
and/or chemotherapeutic agent) may also be included in a pharmaceutical composition for
topical administration. Formulations suitable for topical administration include liquid or
semi-liquid preparations suitable for penetration through the skin to the site where
treatment is required, such as liniments, lotions, creams, ointments or pastes, and drops
suitable for administration to the eye, ear or nose.

Drops according to the present invention may comprise sterile aqueous or oily
solutions or suspensions and may be prepared by dissolving the combination of the
invention or any component thereof (e.g., binding composition and/or chemotherapeutic
agent) in a suitable aqueous solution of a bactericidal and/or fungicidal agent and/or any
other suitable preservative, and preferably including a surface active agent. The resulting
solution may then be clarified by filtration.

Lotions according to the present invention include those suitable for application to
the skin or eye. An eye lotion may comprise a sterile, aqueous solution optionally
containing a bactericide and may be prepared by methods similar to those for the
preparation of drops. Lotions or liniments for application to the skin may also include an
agent to hasten drying and to cool the skin, such as an alcohol or acetone, and/or a
moisturizer such as glycerol or an oil such as castor oil or arachis oil.

Creams, ointments or pastes according to the present invention are semi-solid
formulations of the active ingredient for external application. They may be made by
mixing the combination of the invention or any component thereof in finely-divided or
powdered form, alone or in solution or suspension in an aqueous or non-aqueous fluid,
with the aid of suitable machinery, with a greasy or non-greasy basis. The basis may
comprise hydrocarbons such as hard, soft or liquid paraffin, glycerol, beeswax, a metallic
soap; a mucilage; an oil of natural origin such as almond, corn, arachis, castor or olive oil;
wool fat or its derivatives, or a fatty acid such as stearic or oleic acid together with an
alcohol such as propylene glycol or macrogels. The formulation may incorporate any
suitable surface active agent such as an anionic, cationic or non-ionic surface active such
as sorbitan esters or polyoxyethylene derivatives thereof. Suspending agents such as
natural gums, cellulose derivatives or inorganic materials such as siliceous silicas, and other ingredients such as lanolin, may also be included.

A combination of the invention or any component thereof (e.g., binding composition and/or chemotherapeutic agent) may also be administered by inhalation. A suitable pharmaceutical composition for inhalation may be an aerosol. An exemplary pharmaceutical composition for inhalation of a combination of the invention or any component thereof may include: an aerosol container with a capacity of 15-20 ml comprising the active ingredient (e.g., binding composition and/or chemotherapeutic agent), a lubricating agent, such as polysorbate 85 or oleic acid, dispersed in a propellant, such as freon, preferably in a combination of 1,2-dichlorotetrafluoroethane and difluorochloromethane. Preferably, the composition is in an appropriate aerosol container adapted for either intranasal or oral inhalation administration.

**Dosage**

Preferably, a combination of the invention is administered to a subject at a "therapeutically effective dosage" or "therapeutically effective amount" which preferably inhibits a disease or condition (e.g., tumor growth) to any extent-preferably by at least about 20%, more preferably by at least about 40%, even more preferably by at least about 60%, and still more preferably by at least about 80%-100% relative to untreated subjects. The ability of a combination of the invention or any component thereof to inhibit cancer can be evaluated in an animal model system predictive of efficacy in human tumors. Alternatively, this property can be evaluated by examining the ability of a combination of the invention or any component thereof to inhibit tumor cell growth in vitro by assays well-known to the skilled practitioner. One of ordinary skill in the art would be able to determine such amounts based on such factors as the subject's size, the severity of the subject's symptoms, and the particular composition or route of administration selected.

Dosage regimens may be adjusted to provide the optimum desired response (e.g., a therapeutic response). For example, a dose may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage.

A physician or veterinarian having ordinary skill in the art can readily determine and prescribe the effective amount of the pharmaceutical composition required. For
example, the physician or veterinarian could start doses of the antibody or antigen-binding fragment of the invention employed in the pharmaceutical composition at levels lower than that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved. The effectiveness of a given dose or treatment regimen of an antibody or combination of the invention can be determined, for example, by determining whether a tumor being treated in the subject shrinks or ceases to grow. The size of tumor can be easily determined, for example, by X-ray, magnetic resonance imaging (MRI) or visually in a surgical procedure.

In general, a suitable daily dose of a combination of the invention or any component thereof may be that amount which is the lowest dose effective to produce a therapeutic effect. Such an effective dose will generally depend upon the factors described above. It is preferred that administration be by injection, preferably proximal to the site of the target (e.g., tumor). If desired, a therapeutically effective daily dose of an antibody or antibody/chemotherapeutic agent combination of the invention or pharmaceutical composition thereof may be administered as two, three, four, five, six or more sub-doses administered separately at appropriate intervals throughout the day. In an embodiment, a “therapeutically effective” dosage of any anti-IGFR antibody of the present invention is in the range of about 3 mg/kg (body weight) to about 10 mg/kg (e.g., 3, 4, 5, 6, 7, 8, 9 or 10 mg/kg) per day. In an embodiment, a “therapeutically effective dosage” of a chemotherapeutic agent is as set forth in the Physicians’ Desk Reference 2003 (Thomson Healthcare; 57th edition (November 1, 2002)) which is herein incorporated by reference. For example, in an embodiment, the daily dose of gefitinib is 250 mg/day or the daily dose of paclitaxel is about 135 mg/m² to about 175 mg/m².

Therapeutic Methods and Administration

A combination of the invention or an anti-IGFR antibody or antigen-binding fragment thereof of the invention, alone, can be used to inhibit or reduce the growth or proliferation of any cell, such as a malignant cell, either in vitro (e.g., in cell culture) or in vivo (e.g., within the body of a subject suffering from a disease mediated by elevated expression or activity of IGFR1 or by elevated expression of its ligand (e.g., IGF-I or IGF-II)). Such inhibition or reduction of growth or proliferation of a cell can be achieved by contacting the cell with the combination.

A combination of the invention or an anti-IGFR antibody or antigen-binding fragment thereof, alone, of the invention can be used for treating or preventing any
disease or condition in a subject in need of such treatment or prevention which is mediated, for example, by elevated expression or activity of IGFR1 or by elevated expression of its ligand (e.g., IGF-I or IGF-II) and which may be treated or prevented by modulation of IGFR1 ligand binding, activity or expression. Preferably, the disease or condition is mediated by an increased level of IGFR1, IGF-I or IGF-II and is treated or prevented by decreasing IGFR1 ligand binding, activity (e.g., autophosphorylation activity) or expression. Preferably, the disease or condition is malignancy, more preferably a malignancy characterized by a tumor which expresses IGFR1, such as, but not limited to, bladder cancer, Wilms' cancer, bone cancer, prostate cancer, lung cancer, colorectal cancer, breast cancer, cervical cancer, synovial sarcoma, ovarian cancer, pancreatic cancer, benign prostatic hyperplasia (BPH), diarrhea associated with metastatic carcinoid and vasoactive intestinal peptide secreting tumors (e.g., VIPoma or Werner-Morrison syndrome). Acromegaly may also be treated with a combination of the invention. Antagonism of IGF-I has been reported for treatment of acromegaly (Drake, et al., (2001) Trends Endocrin. Metab. 12: 408-413). Other non-malignant medical conditions which may also be treated, in a subject, by administering a combination of the invention, include gigantism, psoriasis, atherosclerosis, smooth muscle restenosis of blood vessels or inappropriate microvascular proliferation, such as that found as a complication of diabetes, especially of the eye rheumatoid arthritis, Grave's disease, multiple sclerosis, systemic lupus erythematosus, Hashimoto's Thyroiditis, Myasthenia Gravis, auto-immune thyroiditis and Bechet's disease.

The term “subject” may refer to any organism, preferably an animal, more preferably a mammal (e.g., rat, mouse, dog, cat, rabbit) and most preferably a human.

In an embodiment of the invention, where possible, a composition of the invention is administered to a subject in accordance with the Physicians' Desk Reference 2003 (Thomson Healthcare; 57th edition (November 1, 2002)).

A combination of the invention or any component thereof can be administered by an invasive route such as by injection (see above). Administration by a non-invasive route (e.g., orally; for example, in a pill, capsule or tablet) is also within the scope of the present invention. In an embodiment of the invention, an anti-IGFR antibody of the invention, or pharmaceutical composition thereof, is administered intravenously, subcutaneously, intramuscularly, intraarterially or intratumorally while a chemotherapeutic agent of the invention (e.g., gefitinib (e.g., Iressa™)) is administered orally in tablet form.
In another embodiment, the chemotherapeutic agent is paclitaxel (e.g., Taxol®) which is administered intravenously.

Compositions can be administered with medical devices known in the art. For example, a pharmaceutical composition of the invention can be administered by injection with a hypodermic needle.

The pharmaceutical compositions of the invention may also be administered with a needleless hypodermic injection device; such as the devices disclosed in U.S. Patent Nos. 6,620,135; 6,096,002; 5,399,163; 5,383,851; 5,312,335; 5,064,413; 4,941,880; 4,790,824 or 4,596,556.

Examples of well-known implants and modules form administering pharmaceutical compositions include: U.S. Patent No. 4,487,603, which discloses an implantable micro-infusion pump for dispensing medication at a controlled rate; U.S. Patent No. 4,447,233, which discloses a medication infusion pump for delivering medication at a precise infusion rate; U.S. Patent No. 4,447,224, which discloses a variable flow implantable infusion apparatus for continuous drug delivery; U.S. Patent. No. 4,439,196, which discloses an osmotic drug delivery system having multi-chamber compartments. Many other such implants, delivery systems, and modules are well known to those skilled in the art.

**Kits**

The present invention also provides kits comprising the components of the combinations of the invention in kit form. A kit of the present invention includes one or more components including, but not limited to, a binding composition, as discussed herein, which specifically binds IGFR1 (e.g., 19D12/15H12 LCF/HCA) in association with one or more additional components including, but not limited to, a chemotherapeutic agent, as discussed herein. The binding composition and/or the chemotherapeutic agent can be formulated as a pure composition or in combination with a pharmaceutically acceptable carrier, in a pharmaceutical composition.

In one embodiment, a kit includes a binding composition of the invention (e.g., 19D12/15H12 LCF/HCA) or a pharmaceutical composition thereof in one container (e.g., in a sterile glass or plastic vial) and a chemotherapeutic agent or a pharmaceutical composition thereof in another container (e.g., in a sterile glass or plastic vial).

In another embodiment of the invention, the kit comprises a combination of the invention, including a binding composition component (e.g., 19D12/15H12 LCF/HCA) along with a chemotherapeutic agent component formulated together, optionally, along
with a pharmaceutically acceptable carrier, in a pharmaceutical composition, in a single, common container.

If the kit includes a pharmaceutical composition for parenteral administration to a subject, the kit can include a device for performing such administration. For example, the kit can include one or more hypodermic needles or other injection devices as discussed above.

The kit can include a package insert including information concerning the pharmaceutical compositions and dosage forms in the kit. Generally, such information aids patients and physicians in using the enclosed pharmaceutical compositions and dosage forms effectively and safely. For example, the following information regarding a combination of the invention may be supplied in the insert: pharmacokinetics, pharmacodynamics, clinical studies, efficacy parameters, indications and usage, contraindications, warnings, precautions, adverse reactions, overdosage, proper dosage and administration, how supplied, proper storage conditions, references, manufacturer/distributor information and patent information.

EXAMPLES

The following examples are provided to further describe the present invention and should not be construed to limit the scope of the invention in any way.

Example 1: Proliferation Assay Using an Anti-IGFR1 Antibody and a Chemotherapeutic Agent.

The ability of cells in culture to proliferate when exposed to varying concentrations of the 19D12/15H12 wild-type or 19D12/15H12 LCF/HCA anti-IGFR1 antibody and either paclitaxel, gefitinib, lonafarnib 4-hydroxy tamoxifen or doxorubicin was evaluated in this example.

Cell Preparation. H322 NSCLC cells or MCF7 cells were cultured for several passages no greater than 80% confluency in T-75 TC treated filtered flasks. The cells were trypsinized, counted and resuspended at a concentration of 25000 cells/ml in 10% HI-FBS (heat-inactivated fetal bovine serum) RPMI medium containing NEAA (non-essential amino acids), L-Glu, MEM Vitamins and PS. 100ul of cell suspension (2500 cells) was added to each well of a BD Falcon 96 well black, clear bottom TC treated plate. The cells were allowed to attach and spread overnight at 37°C. The 10% RPMI solution
was replaced with 100μl RPMI containing 2% HI-FBS containing NEAA, L-Glu, MEM Vitamins and PS.

**Solution Preparation.** All assay reagents were prepared in RPMI containing 2% HI-FBS at 20X concentration and serial diluted for a total of 10 test concentrations per treatment. Every test point was prepared in triplicate on separate assay plates. Each plate included experimental wells containing either (i) antibody 19D12/15H12 and paclitaxel, (ii) antibody 19D12/15H12 and gefitinib; (iii) antibody 19D12/15H12 LCF/HCA and lonafarnib; (iv) antibody 19D12/15H12 and 4-hydroxy tamoxifen; or (v) antibody 19D12/15H12 and doxorubicin along with internal controls of containing either (a) no treatment, (b) reagent1 (paclitaxel, gefitinib, lonafarnib, 4-hydroxy tamoxifen or doxorubicin) alone, and (c) antibody 19D12/15H12 or 19D12/15H12 LCF/HCA alone.

Reagent 1 and 19D12/15H12 or 19D12/15H12 LCF/HCA were set up individually as dose responses as well as in combination with each other. Cell proliferation was measured on Day4.

**Assay.** Cell proliferation was measured using the Promega Cell Titer-Glo Luminescent Cell Viability Assay (Promega Corp.; Madison, WI). This assay provided a method for determining the number of viable cells in culture based on quantitation of ATP in the culture, which indicates the presence of metabolically active cells.

The assay reagents and assay plates were equilibrated to room temperature and prepared immediately before addition to the assay plates. One volume of assay reagent was added to each well of the assay plate and shaken on an orbital platform for at least ten minutes to allow for equilibration of the ATP reaction and to ensure total lysis of all cells in the assay plate. The reaction had a half-life of five hours but in no case was reading done later than 30 minutes after addition of reagent. Luminescence was detected on Wallac 420 Plate Reader with stacker.

The results from these experiments are shown below in Tables 2-6. The units in the tables (proliferation index) are arbitrary and are proportional to the number of viable cells observed in the culture under each respective condition. The data from the "no treatment" experiments indicate the proliferation index observed in the absence of any drug (i.e., antibody or chemotherapeutic composition).

In Tables 2-6, "μg" indicates micrograms and "μM" indicates micromolar.
Table 2. Proliferation of H322 NSCLC cells in the presence of anti-IGFR1 antibody 19D12/15H12 and paclitaxel (“Taxol”).

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No treatment: 71974; 81788; 75410; 75124; 75558; 79618; 77860; 83468; 78992; 79840; 85414; 87962; 84304; 88926; 77074; 86696; 74354; 77454.
Table 3. Proliferation of H322 NSCLC cells in the presence of anti-IGFR1 antibody 19D12/15H12 and gefitinib (“Iressa”).

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Table 3. Proliferation of H322 NSCLC cells in the presence of anti-IGFR1 antibody 19D12/15H12 and gefitinib (“Iressa”).

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| Table 4. Proliferation of H322 NSCLC cells in the presence of anti-IGFR1 antibody |
|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| Ionafarnib (uM)          | Ionafarnib (uM)          | Ionafarnib (uM)          | Ionafarnib (uM)          | Ionafarnib (uM)          |
| 10                       | 2                        | 0.4                      | 0.08                     | 0.016                    | 0.0032                   |
| 20                       | 2.28676                  | 6.5425                   | 8.5858                   | 8.3633                   | 9.0651                   |
| 4                        | 10249                    | 64299                    | 74003                    | 87732                    | 90544                    |
| 0.8                      | 16422                    | 96038                    | 81121                    | 88590                    | 93368                    |
| 0.16                     | 19691                    | 74212                    | 79370                    | 95207                    | 94692                    |
| 0.032                    | 32604                    | 95626                    | 109768                   | 119933                   | 124480                   |
| 0.0054                   | 39216                    | 117821                   | 125523                   | 139608                   | 143282                   |
| 0.002128                 | 18089                    | 117945                   | 126282                   | 149075                   | 154670                   |
| 0.000256                 | 47214                    | 122431                   | 125867                   | 143050                   | 147594                   |
| 5.12E-05                 | 37457                    | 126574                   | 123493                   | 141257                   | 144190                   |
| 1.02E-05                 | 33216                    | 127909                   | 123379                   | 140804                   | 142810                   |
| Ionafarnib (uM)          | Ionafarnib (uM)          | Ionafarnib (uM)          | Ionafarnib (uM)          | Ionafarnib (uM)          |
| 10                       | 2                        | 0.4                      | 0.08                     | 0.016                    | 0.0032                   |
| 20                       | 2.5599                   | 8.5353                   | 9.4441                   | 10.2269                  | 10.5279                  |
| 4                        | 20764                    | 77990                    | 9.0928                   | 10.0880                  | 10.7228                  |
| 0.8                      | 20754                    | 8.2864                   | 9.4054                   | 9.9707                   | 10.0385                  |
| 0.16                     | 31766                    | 8.3865                   | 9.5944                   | 10.2713                   | 11.1415                   |
| 0.032                    | 21376                    | 9.9495                   | 11.1094                   | 12.1189                   | 12.5248                   |
| 0.0064                   | 39998                    | 11.9937                  | 13.6846                  | 14.3037                  | 15.9477                  |
| 0.002128                 | 121024                   | 123895                   | 131212                   | 143031                   | 145420                   |
| 0.000256                 | 48311                    | 126873                   | 132897                   | 145890                   | 151220                   |
| 5.12E-05                 | 46956                    | 114648                   | 125321                   | 133849                   | 138326                   |
| 1.02E-05                 | 43592                    | 118218                   | 121167                   | 135102                   | 140693                   |
| Ionafarnib (uM)          | Ionafarnib (uM)          | Ionafarnib (uM)          | Ionafarnib (uM)          | Ionafarnib (uM)          |
| 10                       | 2                        | 0.4                      | 0.08                     | 0.016                    |
| 20                       | 2.5332                   | 7.9142                   | 8.7710                   | 9.6999                   |
| 4                        | 18000                    | 7.9598                   | 9.9074                   | 9.2891                   |
| 0.8                      | 19709                    | 8.2241                   | 9.8781                   | 9.5006                   |
| 0.16                     | 23109                    | 8.0702                   | 9.1006                   | 10.0372                   | 10.2768                   |
| 0.032                    | 31689                    | 10.1343                  | 10.3480                  | 11.6640                  |
| 0.0064                   | 26899                    | 11.7922                  | 11.9493                  | 13.1017                  |
| 0.002128                 | 23945                    | 12.2721                  | 12.2778                  | 13.3720                  |
| 0.000256                 | 35691                    | 13.5030                  | 13.9571                  |
| 5.12E-05                 | 7.6140                   | 11.6078                  | 12.0058                  |
| 1.02E-05                 | 2.3393                   | 12.1269                  |
| Ionafarnib (uM)          | Ionafarnib (uM)          | Ionafarnib (uM)          | Ionafarnib (uM)          |
| 10                       | 2                        | 0.4                      |
| 20                       | 18478                    | 10.458                    | 23.209                    | 21.356                   |
| 4                        | 90009                    | 11.4766                   | 10.3595                   | 12.6305                   |
| 0.8                      | 96560                    | 11.0650                   | 11.0859                   | 12.6559                   |
| 0.16                     | 115359                   | 12.2519                   | 12.1571                   |
| 0.032                    | 111904                   | 12.9612                   | 13.2994                   | 13.8400                   |
| 0.016                    | 111655                   | 12.9204                   | 12.3992                   | 14.1908                   |
| 0.0054                   | 107175                   | 12.7792                   | 11.8530                   |
| 0.00128                   | 111619                   | 11.9944                   | 12.8736                   |
| 2.56E-05                 | 106451                   | 12.0521                   | 12.1819                   |
| 19D12 (LCF/HCA) and lonafarnib. |
| No treatment: 114280; 118325; 135058; 129246; 125513; 119709; 134363; 129286; 138048; 132272; 138562; 140206; 135510; 138660; 132918; 131451; 140071; 135689. |
Table 5. Proliferation of MCF7 cells in the presence of anti-IGFR1 antibody

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<th>Antibody 10/12/15H12 (H)</th>
<th>4-hydroxy tamoxifen (ng/mL)</th>
<th>20000</th>
<th>4000</th>
<th>800</th>
<th>160</th>
<th>32</th>
<th>6.4</th>
<th>1.28</th>
<th>0.256</th>
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<th>4-hydroxy tamoxifen (ng/mL)</th>
<th>20000</th>
<th>4000</th>
<th>800</th>
<th>160</th>
<th>32</th>
<th>6.4</th>
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19D12/15H12 and 4-hydroxy tamoxifen.

No treatment: 38094; 32799; 43225; 30131; 35545; 28400; 35256; 18841; 34641; 24138; 28849; 21562; 36446; 25365; 34561; 21852; 40120; 23587.
Table 6. Proliferation of MCF7 cells in the presence of anti-IGFR1 antibody 19D12/15H12 and doxorubicin.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Doxorubicin (µg/mL)</th>
<th>20</th>
<th>4</th>
<th>0.16</th>
<th>0.08</th>
<th>0.04</th>
<th>0.02</th>
<th>0.0125</th>
<th>0.00512</th>
<th>0.000512</th>
<th>0.0000512</th>
<th>0.00000512</th>
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<td></td>
<td>20</td>
<td>4</td>
<td>0.16</td>
<td>0.08</td>
<td>0.04</td>
<td>0.02</td>
<td>0.0125</td>
<td>0.00512</td>
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<td>No treatment</td>
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<td>116244</td>
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<td>117403</td>
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5

No treatment: 126997; 128567; 116244; 117342; 112806; 114636; 122023; 117403;
Example 2: *In vivo* tumor inhibition assay of anti-IGFR and paclitaxel using a NSCLC xenograft model H322.

In this example, the effectiveness of an anti-IGFR/paclitaxel combination of the invention for tumor growth inhibition was demonstrated *in vivo*.

Five million H322 human NSCLC cells in Matrigel were inoculated subcutaneously into nude mice. Anti-IGFR antibody 19D12 and/or paclitaxel treatment was initiated when the tumor size reached ~105-115 mm$^3$ at day 0. Both 19D12 and paclitaxel were dosed twice per week. Anti-IGFR antibody 19D12 was dosed at 0.5 mg per mouse. Paclitaxel was at 15 mpk. Ten animals per group. Tumor volumes were measured by Labcat.

**Table 7. Tumor growth inhibition in mice.**

<table>
<thead>
<tr>
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<th>Day 0</th>
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<tr>
<td><strong>Vehicle control</strong></td>
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<tr>
<td>Volume (mm$^3$)</td>
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<tr>
<td>SEM</td>
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<tr>
<td>SD</td>
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<td>218.25</td>
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<tr>
<td>Growth</td>
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<td>271.02</td>
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<tr>
<td>% inhibition</td>
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<tr>
<td><strong>0.5 mg 19D12</strong></td>
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<tr>
<td>Volume (mm$^3$)</td>
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<tr>
<td>Growth</td>
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<tr>
<td>% inhibition</td>
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<td>75.50%</td>
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<tr>
<td><strong>15 mpk Taxol</strong></td>
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<tr>
<td>Volume (mm$^3$)</td>
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<td><strong>0.5 mg 19D12+15 mpk Taxol</strong></td>
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The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and the accompanying figures. Such modifications are intended to fall within the scope of the appended claims.
Patents, patent applications, Genbank Accession Numbers and publications are cited throughout this application, the disclosures of which, particularly, including all disclosed chemical structures and antibody amino acid sequences therein, are incorporated herein by reference.
We Claim:

1. A combination comprising:
   (a) one or more binding compositions comprising a member selected from the group consisting of: a light chain amino acid sequence which comprises CDR-L1 defined by SEQ ID NO: 5, CDR-L2 defined by SEQ ID NO: 6 and CDR-L3 defined by SEQ ID NO: 7; and a heavy chain amino acid sequence which comprises CDR-H1 defined by SEQ ID NO: 8 or 12, CDR-H2 defined by SEQ ID NO: 9 and CDR-H3 defined by SEQ ID NO: 10; in association with
   (b) one or more chemotherapeutic agents.

2. The combination of claim 1 wherein the binding composition comprises an isolated light chain amino acid sequence which comprises CDR-L1 defined by SEQ ID NO: 5, CDR-L2 defined by SEQ ID NO: 6 and CDR-L3 defined by SEQ ID NO: 7; and an isolated heavy chain amino acid sequence which comprises CDR-H1 defined by SEQ ID NO: 8 or 12, CDR-H2 defined by SEQ ID NO: 9 and CDR-H3 defined by SEQ ID NO: 10.

3. The combination of claim 2 wherein a binding composition comprises an isolated light chain immunoglobulin comprising amino acids 20-128 of SEQ ID NO: 2 and an isolated heavy chain immunoglobulin comprising amino acids of 20-137 of SEQ ID NO: 4.

4. The combination of claim 1 wherein a chemotherapeutic agent is one or more members selected from the group consisting of a taxane, a topoisomerase inhibitor, a signal transduction inhibitor, a cell cycle inhibitor, an IGFR/IGFR1 system modulator, a farnesyl protein transferase (FPT) inhibitor, an epidermal growth factor receptor (EGFR) inhibitor, a HER2 inhibitor, a vascular epidermal growth factor (VEGF) receptor inhibitor, a mitogen activated protein (MAP) kinase inhibitor, a MEK inhibitor, an AKT inhibitor, a mTOR inhibitor, a pI3 kinase inhibitor, a Raf inhibitor, a cyclin dependent kinase (CDK) inhibitor, a microtubule stabilizer, a microtubule inhibitor, a SERM/Antiestrogen, an aromatase inhibitor, an anthracycline, a proteasome inhibitor, an agent which inhibits insulin-like growth factor (IGF) production and an anti-sense inhibitor of IGFR1, IGF-1 or IGF2.

5. The combination of claim 4 wherein a chemotherapeutic agent is a taxane selected from: paclitaxel and docetaxel.
6. The combination of claim 4 wherein a chemotherapeutic agent is a microtubule inhibitor selected from: vincristine, vinblastine, a podophyllotoxin, epothilone B, BMS-247550 and BMS-310705.

7. The combination of claim 4 wherein a chemotherapeutic agent is an epidermal growth factor receptor (EGFR) inhibitor selected from: gefitinib, erlotinib, cetuximab, ABX-EGF, lapatanib, canertinib, EKB-569 and PKI-166.

8. The combination of claim 4 wherein a chemotherapeutic agent is a farnesyl protein transferase inhibitor selected from: lonafarnib and tipifarnib (R155777).

9. The combination of claim 4 wherein a chemotherapeutic agent is a selective estrogen receptor modulator (SERM)/antiestrogen selected from: tamoxifen, raloxifene, fulvestrant, acolbifene, pipendoxifene, arzoxifene, toremifene, lasofoxifene, bazedoxifene (TSE-424), idoxifene, HMR-3339 and ZK-186619.

10. The combination of claim 4 wherein a chemotherapeutic agent is an anthracycline selected from: doxorubicin, daunorubicin and epirubicin.

11. The combination of claim 4 wherein a chemotherapeutic agent is a HER2 inhibitor selected from: trastuzumab, HKI-272, CP-724714 and TAK-165.

12. The combination of claim 4 wherein a chemotherapeutic agent is a topoisomerase inhibitor selected from: etoposide, topotecan, camptothecin and irinotecan.

13. A pharmaceutical composition comprising a combination of claim 1 along with a pharmaceutically acceptable carrier.

14. A combination comprising:

(a) one or more fully-human, monoclonal antibodies comprising a light chain immunoglobulin comprising amino acids 20-128 of SEQ ID NO: 2 and a heavy chain immunoglobulin comprising amino acids 20-137 of SEQ ID NO: 4; in association with

(b) one or more chemotherapeutic agents selected from
; and
15. A method for treating or preventing a medical condition in a subject in need of such treatment or prevention, which medical condition is mediated by elevated expression or activity of Insulin-like Growth Factor Receptor-I (IGFR1), comprising administering a therapeutically effective amount of a combination of claim 1 to the subject.


17. The method of claim 15 wherein a chemotherapeutic agent is one or more members selected from the group consisting of:
18. The method of claim 15 wherein the medical condition is selected from the group consisting of acromegaly, bladder cancer, Wilm's cancer, ovarian cancer, pancreatic cancer, benign prostatic hyperplasia, breast cancer, prostate cancer, bone cancer, lung cancer, colorectal cancer, cervical cancer, synovial sarcoma, diarrhea associated with metastatic carcinoid, vasoactive intestinal peptide secreting tumors, gigantism, psoriasis, atherosclerosis, smooth muscle restenosis of blood vessels, inappropriate microvascular proliferation, rheumatoid arthritis, Grave's disease, multiple sclerosis, systemic lupus erythematosus, Hashimoto's Thyroiditis, Myasthenia Gravis, auto-immune thyroiditis and Bechet's disease.

19. The method of claim 15 wherein the combination is administered to the subject by a parenteral route.

20. A method for treating or preventing a medical condition in a subject in need of such treatment or prevention comprising administering a combination comprising:

(a) a therapeutically effective amount of one or more fully human, monoclonal antibodies comprising a light chain immunoglobulin comprising amino acids 20-128 of SEQ ID NO: 2
and a heavy chain immunoglobulin comprising amino acids 20-137 of SEQ ID NO: 4; in association with

(b) a therapeutically effective amount of one or more chemotherapeutic agents selected from:

22. A method for inhibiting the growth or proliferation of a malignant cell comprising contacting the cell with a combination of claim 1.

23. The method of claim 22 wherein the cell is in vitro.


25. The method of claim 22 wherein a chemotherapeutic agent is one or more members selected from the group consisting of:
; and

[Chemical structure image]
26. The method of claim 22 wherein a cell is selected from a non-small cell lung
carcinoma, a breast cancer cell, an ovarian cancer cell, a colorectal cancer cell, a prostate
cancer cell, a pediatric cancer cell and a pancreatic cancer cell.

27. The method of claim 26 wherein a cell is an NCI-H322 cell, an A2780 cell or an MCF7
cell.

28. A kit comprising:
   (a) one or more binding compositions comprising a member selected from the group
   consisting of: an isolated light chain amino acid sequence which comprises CDR-L1
defined by SEQ ID NO: 5, CDR-L2 defined by SEQ ID NO: 6 and CDR-L3 defined by
SEQ ID NO: 7; and an isolated heavy chain amino acid sequence which comprises CDR-
H1 defined by SEQ ID NO: 8 or 12, CDR-H2 defined by SEQ ID NO: 9 and CDR-H3
defined by SEQ ID NO: 10; in association with
   (b) one or more chemotherapeutic agents.

29. The kit of claim 28 wherein said binding compositions and said chemotherapeutic
agents are in separate containers.
SEQUENCE LISTING

Schering Corporation

NEUTRALIZING HUMAN ANTI-IGFR ANTIBODY

OC06100K

60/524,732
2003-21-11
14

PatentIn version 3.1
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DNA
Homo sapiens

CDS
(1)..(384)

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Ser Arg Gly Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Val
20 25 30

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Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Ile
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Gly Ser Ser Leu His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg
50 55 60

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Leu Leu Ile Lys Tyr Ala Ser Gln Ser Leu Ser Gly Ile Pro Asp Arg
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Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Ile
35 40 45

Gly Ser Ser Leu His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg
50 55 60

Leu Leu Ile Lys Tyr Ala Ser Gln Ser Leu Ser Gly Ile Pro Asp Arg
65 70 75 80

Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg
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411
Homo sapiens

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Cys Gly Pro Gly Ile Asp Ile Arg Asn Asp Tyr Glu Glu Ile Lys Arg
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Ser Ser Phe Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
50 55 60

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

Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asn Ala Lys Asn Ser
85 90 95

Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Met Ala Val Tyr
100 105 110

Tyr Cys Ala Arg Leu Gly Asn Phe Tyr Tyr Gly Met Asp Val Trp Gly
115 120 125

Gln Gly Thr Thr Val Thr Val Ser Ser
130 135
**INTERNATIONAL SEARCH REPORT**

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC 7 C07K16/28 A61K39/395
//A61K39/395,31:000

According to International Patent Classification (IPC) or to both national classification and IPC.

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, EMBASE, BIOSIS

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

<table>
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<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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<td>WO 03/100008 A (SCHERING CORPORATION; WANG, YAN; GREENBERG, ROBERT; PRESTA, LEONARD; P) 4 December 2003 (2003-12-04) cited in the application See page 28, lines 26-33 for specific claim 27 page 47, line 19 - page 49, line 18; claims; sequences 45,8,2,17,15,16,78,74,76,72,41,43</td>
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<td>WO 02/053596 A (PFIZER INC; ABGENIX, INC; COHEN, BRUCE, D; BEEBE, JEAN; MILLER, PENEL0) 11 July 2002 (2002-07-11) page 61, line 11 - page 64, line 32</td>
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Further documents are listed in the continuation of box C

Patent family members are listed in annex

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* Special categories of cited documents

- **A** document defining the general state of the art which is not considered to be of particular relevance
- **E** earlier document but published on or after the international filing date
- **L** document which may throw doubts on priority claim(s) or which is cited to establish the publication date of an earlier citation or other special reason (as specified)
- **O** document relating to an oral disclosure, use, exhibition or other means
- **P** document published prior to the international filing date but later than the priority date claimed

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**5 April 2005**

**17/05/2005**

**Date of the actual completion of the international search**

**Date of mailing of the international search report**

**Name and mailing address of the ISA**

European Patent Office, P B 5816 Patentlaan 2
NL – 2280 HV Rijswijk
Tel. (+31-37) 340-2040, Tx 31 651 eipo nl,
Fax. (+31-37) 345-3016

**Authorized officer**

Vadot, P

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Form PCT/ISA/10 (second sheet) (January 2004)
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<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
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INTernational search report

Box No. I  Nucleotide and/or amino acid sequence(s) (Continuation of item 1.b of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:

   a. type of material
      - [X] a sequence listing
      - [ ] table(s) related to the sequence listing

   b. format of material
      - [X] in written format
      - [X] in computer readable form

   c. time of filing/furnishing
      - [X] contained in the international application as filed
      - [X] filed together with the international application in computer readable form
      - [ ] furnished subsequently to this Authority for the purpose of search

2. [ ] In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:
**INTERNATIONAL SEARCH REPORT**

**Box II** Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [x] Claims Nos.:
   - because they relate to subject matter not required to be searched by this Authority, namely:
     
     Although claims 15-27 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
   - because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
   - because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box III** Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

- [ ] The additional search fees were accompanied by the applicant’s protest.

- [ ] No protest accompanied the payment of additional search fees.
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US 2004047835 A1 11-03-2004 NONE