TREATMENT OF TUMORS IN PEDIATRIC PATIENTS WITH EPIDERMAL GROWTH FACTOR RECEPTOR ANTAGONISTS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. provisional patent application Ser. No. 60/833,487, filed on July 27, 2006, the contents of which are incorporated by reference herein in its entirety.

FIELD OF THE INVENTION

The present invention relates to treating tumors in pediatric patients by administering a combination of an EGFR antagonist and a chemotherapeutic agent.

BACKGROUND OF THE INVENTION

In the United States, 11,900 children and adolescents under the age of 20 were diagnosed with cancer in 2001 and about 2,200 died of the disease. Solid tumors account for approximately 30% of cases of childhood cancers. Invasive brain and nervous system cancers accounts for 17% of all pediatric cancers, second only to acute lymphocytic leukemia. About half of the diagnosed cases of brain tumors are malignant. Other common childhood cancers include Ewing's sarcoma, leukemia, neuroblastoma, osteosarcoma, rhabdomyosarcoma, soft tissue sarcoma, and Wilms' tumors.

One of the major obstacles in treating brain tumors in children is that the brain is still undergoing rapid development and is vulnerable to toxicities from treatments such as radiation or chemotherapy. Obstacles also arise in treating other types of childhood cancers. Therefore, new therapies for treating tumors in pediatric patients are needed.

The epidermal growth factor receptor (EGFR) family is expressed or over-expressed in various cancers and is generally involved in tumorigenesis. The EGFR family includes the EGF receptor (EGFR, also known as erbB-1/HER1), HER2 (also known as c-neu/erB-2), erbB-3/HER3, and erbB-4/HER4. For example, EGFR and HER2 are thought to play a critical role in processes that regulate tumor cell growth and survival. In particular, EGFR has been implicated in several pathways that affect survival and protection from apoptosis, dedifferentiation, and metastasis (including cell migration and invasion). Among those cancers that express EGFR are some of the most prevalent including head and neck, colorectal, pancreatic, ovarian, renal cell, non-small cell lung,

and gliomas. Prognosis for many of these cancers is poor if not diagnosed at an early stage, and therapy for advanced disease is limited.

There are various EGFR inhibitors currently in clinical trials for the treatment of some of these cancers. One such example is ERBITUX® (cetuximab) (manufactured by ImClone Systems Inc.), which is a chimeric (human/mouse) monoclonal antibody that blocks ligand binding to EGFR, prevents receptor activation, and inhibits growth of cells in culture. Another example is ABX-EGF, which is a fully human monoclonal antibody specific to EGFR that reportedly blocks binding of transforming growth factor alpha (TFG-a) and epidermal growth factor (EGF), two ligands that are known to bind to EGFR. HERCEPTIN® (trastuzumab) is a humanized antibody approved for the treatment of HER2 positive metastatic breast cancer, which is designed to target and block the function of HER2 protein overexpression.

In addition, clinical trials are currently being conducted on various small molecule EGFR inhibitors. An example of a tyrosine kinase inhibitor is IRESSA™, which is a small molecule EGFR tyrosine kinase inhibitor that reportedly inhibits EGFR tyrosine kinase activity, is cytostatic towards a range of human cancer cells that express functional EGFR, and can inhibit tumor cell proliferation via up-regulation of p27.

SUMMARY OF THE INVENTION

In certain embodiments, the present invention provides a method of inhibiting the growth of a tumor in a pediatric patient by treating the pediatric patient with an effective amount of an EGFR antagonist and a chemotherapeutic agent. In a preferred embodiment, the EGFR antagonist is an EGFR antibody that specifically binds to the extracellular domain of EGFR and neutralized activation thereof. In a preferred embodiment, the chemotherapeutic agent is irinotecan.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides methods of treating tumors in pediatric patients by administering an effective amount of an EGFR antagonist and a chemotherapeutic agent. Pediatric patients, according to the present invention, are patients from birth to 18 years of age. Treatment includes: (1) preventing the disease from occurring in a patient who may be predisposed to the disease but does not yet experience or display symptoms of the disease; *e.g.*, prevention of the outbreak of the clinical symptoms; (2) inhibiting the growth of the tumor, *e.g.*, arresting its development; or (3) relieving the tumor, *e.g.*,

causing regression of the symptoms of the tumor. Inhibiting tumor growth includes slowing or stopping growth, as well as causing tumor regression. An effective amount for the treatment of a disease means that amount which, when administered to a patient in need thereof, is sufficient to effect treatment, as defined above of the disease.

The tumors that are treated according to the present invention are any of the tumors that express EGFR. Such tumors include blastomas, including hepatoblastomas and neuroblastomas; carcinomas, including adenocarcinomas; gliomas, including ependymomas, astrocytomas, oligodendrogliomas and mixed gliomas; sarcomas, including rhabdomyosarcomas and adenosarcomas; and adenomas. The tumors can occur in virtually all parts of the body, including, for example, the breast, heart, lung, esophagus, small intestine, colon, rectum, stomach, spleen, kidney, bladder, head and neck, larynx, ovary, prostrate, brain, pancreas, skin, bone, bone marrow, blood, thymus, uterus, testicles, cervix, or liver. In a preferred embodiment, the tumors are tumors of the nervous system such as gliomas and neuroblastomas. The tumors to be treated include primary and metastatic tumors, as well as refractory tumors. "Refractory tumors" include tumors that fail to respond or are resistant to treatment with chemotherapeutic agents alone, antibodies alone, radiation alone or combinations thereof. Refractory tumors also encompass tumors that appear to be inhibited by treatment with such agents, but recur up to five years, sometimes up to ten years or longer after treatment is discontinued.

An EGFR antagonist, according to the present invention can be an extracellular antagonist or an intracellular antagonist and more than one antagonist may be employed. Extracellular antagonists include, but are not limited to, proteins or other biological molecules that bind to EGFR. In certain embodiments of the invention, an extracellular antagonist binds to the extracellular domain of EGFR and inhibits binding of EGFR to one or more of its ligands and/or neutralizes ligand-induced activation of EGFR. Ligands for EGFR include EGF, TFG-a, amphiregulin, heparin-binding EGFR (HB-EGF) and betacellulin. Extracellular EGFR antagonists can also include substances that inhibit EGFR dimerization with other EGFR receptor subunits (*i.e.* EGFR homodimers) or heterodimerization with other growth factor receptors (*e.g.*, HER2).

In a preferred embodiment, the EGFR antagonist is an antibody that binds to EGFR and blocks ligand binding. One example of an EGFR antibody is cetuximab (IMC-C225) (GenBank Accession No. 1NQLA), which is a chimeric (human/mouse) IgG monoclonal antibody. *See e.g.*, U.S. Patent No. 4,943,533 (Mendelsohn *et al.*); U.S. Patent No. 6,217,866 (Schlessinger *et al.*); U.S. Application Nos. 08/973,065 (Goldstein *et al.*)

and 09/635,974 (Teufel); WO 99/60023 (Waksal *et al.*) and WO 00/69459, all of which are incorporated by reference herein. Cetuximab specifically binds to EGFR and blocks binding of a ligand, such as EGF. Cetuximab Fab contains the Fab fragment of Cetuximab, *i.e.*, the heavy and light chain variable region sequences of murine antibody M225 (U.S. App. Ser. No. 2004/0006212, incorporated herein by reference) with human IgG1 C_H1 heavy and kappa light chain constant domains. (Cetuximab includes all three IgG1 heavy chain constant domains.) The CDR regions of the heavy chain of cetuximab have the following sequences: a CDR1 region with a sequence of N Y G V H (SEQ ID NO: 1), a CDR2 region with a sequence of V I W S G G N T D Y N T P F T S (SEQ ID NO: 2), and a CDR3 region with a sequence of A L T Y Y D Y E F A Y (SEQ ID NO: 3). The CDR regions of the light chain of cetuximab have the following sequences: a CDR1 region with a sequence of R A S Q S I G T N I H (SEQ ID NO: 4), a CDR2 region with a sequence of Y A S E S I S (SEQ ID NO: 5), and a CDR3 region with a sequence of Q Q N N N W P T T (SEQ ID NO: 6).

Another example of an EGFR antibody is ABX-EGF, which is a fully human IgG_2 monoclonal antibody specific for EGFR. ABX-EGF binds EGFR with high specificity, blocking binding of EGFR to both of its ligands, EGF and TGF-a. *See e.g.*, Figlin *et al.*, Abstract 1102 presented at the 37th Annual Meeting of ASCO, San Francisco, CA, 12-15 May 2001, which is incorporated by reference herein. The sequence and characterization of ABX-EGF, which was formerly known as clone E7.6.3, is disclosed in U.S. Patent No. 6,235,883 (Abgenix, Inc.) at col. 28, line 62 through col. 29, line 36 and in Fig. 29-34, which is incorporated by reference herein. (*See also* Yang *et al.*, *Critical Rev. Oncol./Hematol.*, 38(1): 17-23, 2001, which is incorporated by reference herein.)

Another examples of an EGFR antibody is HERCEPTIN® (trastuzumab), which is a recombinant DNA-derived humanized monoclonal antibody that selectively binds with high affinity in a cell-based assay (Kd of 5 nM) to the extracellular domain of the human EGFR2 protein, HER2. The antibody is an IgG_1 kappa that contains human framework regions with the complementarity-determining regions of a murine antibody (4D5) that binds to HER2. *See, e.g.*, International Patent Publication No. WO 01/89566 (Mass), which is incorporated by reference herein.

Other EGFR antibodies include EMD 72000 (Merck KGaA), which is a humanized version of the murine anti-EGFR monoclonal antibody EMD 55900; h-R3 (TheraCIM), which is a humanized anti-EGFR monoclonal antibody; Y10, which is a murine monoclonal antibody and was raised against a murine homologue of the human

EGFRvIII mutation; and MDX-447 (Medarex). *See* U.S. Patent Nos. 5,558,864 (Bendig et al.), 5,884,093 (Kettleborough et al.), and 5,891,996 (Mateo de Acosta del Rio et al.), all of which are incorporated by reference herein.

Intracellular EGFR antagonists can be biological molecules, but are usually small molecules, such as synthetic kinase inhibitors that act directly on the cytoplasmic domain of EGFR to inhibit EGFR-mediated signal transduction. One example of a small molecule EGFR antagonist is IRESSA™ (ZD1939), which is a quinozaline derivative that functions as an ATP-mimetic to inhibit EGFR. *See* U.S. Patent No. 5,616,582 (Zeneca Limited); WO 96/33980 (Zeneca Limited) at p. 4; *see also*, Rowinsky *et al.*, Abstract 5 presented at the 37th Annual Meeting of ASCO, San Francisco, CA, 12-15 May 2001; Anido *et al.*, Abstract 1712 presented at the 37th Annual Meeting of ASCO, San Francisco, CA, 12-15 May 2001. Another example of a small molecule EGFR antagonist is TARCEVA® (OSI-774), which is a 4-(substitutedphenylamino)quinozaline derivative [6,7-Bis(2-methoxy-ethoxy)-quinazolin-4-yl]- (3-ethynyl-phenyl)amine hydrochloride] EGFR inhibitor. *See* WO 96/30347 (Pfizer Inc.) at, for example, page 2, line 12 through page 4, line 34 and page 19, lines 14-17. *See also* Moyer *et al.*, *Cancer Res.*, 57: 4838-48 (1997); Pollack *et al.*, *J. Pharmacol.*, 291: 739-48 (1999). TARCEVA® may function by inhibiting phosphorylation of EGFR and its downstream PI3/Akt and MAP (mitogen activated protein) kinase signal transduction pathways resulting in p27-mediated cell-cycle arrest. *See* Hidalgo *et al.*, Abstract 281 presented at the 37th Annual Meeting of ASCO, San Francisco, CA, 12-15 May 2001.

Other small molecules are also reported to inhibit EGFR, many of which are thought to be specific to the tyrosine kinase domain of an EGFR. Some examples of such small molecule EGFR antagonists are described in WO 91/116051, WO 96/30347, WO 96/33980, WO 97/27199 (Zeneca Limited). WO 97/30034 (Zeneca Limited), WO 97/42187 (Zeneca Limited), WO 97/49688 (Pfizer Inc.), WO 98/33798 (Warner Lambert Company), WO 00/18761 (American Cyanamid Company), and WO 00/31048 (Warner Lambert Company). Examples of specific small molecule EGFR antagonists include Cl-1033 (Pfizer), which is a quinozaline (N-[4-(3-chloro-4-fluoro-phenylamino)-7-(3-morpholin-4-yl-propoxy)-quinazolin-6-yl]-acrylamide) inhibitor of tyrosine kinases, particularly EGFR and is described in WO 00/31048 at page 8, lines 22-6; PKI166 (Novartis), which is a pyrrolopyrimidine inhibitor of EGFR and is described in WO 97/27199 at pages 10-12; GW2016 (GlaxoSmithKline), which is an inhibitor of EGFR and HER2; EKB569 (Wyeth), which is reported to inhibit the growth of tumor cells that

overexpress EGFR or HER2 *in vitro* and *in vivo*; AG-1478 (Tryphostin), which is a quinazoline small molecule that inhibits signaling from both EGFR and erbB-2; AG-1478 (Sugen), which is a bisubstrate inhibitor that also inhibits protein kinase CK2; PD 153035 (Parke-Davis) which is reported to inhibit EGFR kinase activity and tumor growth, induce apoptosis in cells in culture, and enhance the cytotoxicity of cytotoxic chemotherapeutic agents; SPM-924 (Schwarz Pharma), which is a tyrosine kinase inhibitor targeted for treatment of prostrate cancer; CP-546,989 (OSI Pharmaceuticals), which is reportedly an inhibitor of angiogenesis for treatment of solid tumors; ADL-681, which is a EGFR kinase inhibitor targeted for treatment of cancer; PD 158780, which is a pyridopyrimidine that is reported to inhibit the tumor growth rate of A4431 xenografts in mice; CP-358,774, which is a quinzoline that is reported to inhibit autophosphorylation in HN5 xenografts in mice; ZD1839, which is a quinzoline that is reported to have antitumor activity in mouse xenograft models including vulvar, NSCLC, prostrate, ovarian, and colorectal cancers; CGP 59326A, which is a pyrrolopyrimidine that is reported to inhibit growth of EGFR-positive xenografts in mice; PD 165557 (Pfizer); CGP54211 and CGP53353 (Novartis), which are dianilnophthalimides. Naturally derived EGFR tyrosine kinase inhibitors include genistein, herbimycin A, quercetin, and erbstatin.

Further small molecules reported to inhibit EGFR and that are therefore within the scope of the present invention are tricyclic compounds such as the compounds described in U.S. Patent No. 5,679,683; quinazoline derivatives such as the derivatives described in U.S. Patent No. 5,616,582; and indole compounds such as the compounds described in U.S. Patent No. 5,196,446.

It will be appreciated that useful small molecule to be used in the invention are inhibitors of EGFR, but need not be completely specific for EGFR.

In a preferred embodiment, the EGFR antagonist is an anti-EGFR antibody that exhibits one or more of following properties:

1) The antibody binds to the external domain of EGFR and inhibits ligand binding. Inhibition can be determined, for example, by a direct binding assay using purified or membrane bound receptor. In this embodiment, the antibodies of the present invention or fragments thereof, preferably bind EGFR at least as strongly as the natural ligands of EGFR (EGF, TFG-a).

2) The antibodies neutralize EGFR. Binding of a ligand to an external, extracellular domain of EGFR stimulates tyrosine kinase activity and receptor phosphorylation and/or the phosphorylation of other proteins involved in the various

signaling pathways. Neutralization of EGFR includes inhibition, diminution, inactivation and/or disruption of one or more of the activities normally associated with signal transduction. Neutralization can be determined *in vivo*, *ex vivo*, or *in vitro* using, for example, tissues, cultured cell, or purified cellular components.

One measure of EGFR neutralization is inhibition of the tyrosine kinase activity of the receptor. Tyrosine kinase inhibition can be determined using well-known methods; for example, by measuring the autophosphorylation level of recombinant kinase receptor, and/or phosphorylation of natural or synthetic substrates. Thus, phosphorylation assays are useful in determining neutralizing antibodies in the context of the present invention. Phosphorylation can be detected, for example, using an antibody specific for phosphotyrosine in an ELISA assay or on a western blot. Some assays for tyrosine kinase activity are described in Panek et al., *J. Pharmacol. Exp. Thera.* 283: 1433-44 (1997) and Batley et al., *Life Sci.* 62:143-50 (1998). Antibodies of the invention cause a decrease in tyrosine phosphorylation of EGFR of at least about 75%, preferably at least about 85%, and more preferably at least about 90% in cells that respond to ligand.

Another measure of EGFR neutralization is inhibition of phosphorylation of downstream substrates of EGFR. Accordingly, the level of phosphorylation of MEK and ERK can be measured. The decrease in phosphorylation is at least about 40%, and can be at least about 60%, or at least about 80%.

In addition, methods for detection of protein expression can be utilized to determine EGFR neutralization, wherein the proteins being measured are regulated by EGFR tyrosine kinase activity. These methods include immunohistochemistry (IHC) for detection of protein expression, fluorescence in situ hybridization (FISH) for detection of gene amplification, competitive radioligand binding assays, solid matrix blotting techniques, such as Northern and Southern blots, reverse transcriptase polymerase chain reaction (RT-PCR) and ELISA. See, e.g., Grandis et al., *Cancer*, 78:1284-92 (1996); Shimizu et al., *Japan J. Cancer Res.*, 85:567-71 (1994); Sauter et al., *Am. J. Path.*, 148:1047-53 (1996); Collins, *Glia* 15:289-96 (1995); Radinsky et al., *Clin. Cancer Res.* 1:19-31 (1995); Petrides et al., *Cancer Res.* 50:3934-39 (1990); Hoffmann et al., *Anticancer Res.* 17:4419-26 (1997); Wikstrand et al., *Cancer Res.* 55:3140-48 (1995).

Ex vivo assays can also be utilized to determine EGFR neutralization. For example, receptor tyrosine kinase inhibition can be observed by mitogenic assays using cell lines stimulated with receptor ligand in the presence and absence of inhibitor. An example of such a mitogenic assay is a 3T3 cells mitogenic assay (3T3 (clone A31-714)

cells from American Type Culture Collection (Manassas, VA)). Another method involves testing for inhibition of growth of EGFR-expressing tumor cells or cells transfected to express EGFR. Inhibition can also be observed using tumor models, for example, human tumor cells injected into a mouse.

The antibodies of the present invention are not limited by any particular mechanism of EGFR neutralization. The anti-EGFR antibodies of the present invention can bind externally to EGFR cell surface receptor, block binding of ligand and subsequent signal transduction mediated via the receptor-associated tyrosine kinase, and prevent phosphorylation of the EGFR and other downstream proteins in the signal transduction cascade.

3) The antibodies down modulate EGFR. The amount of EGFR present on the surface of a cell depends on receptor protein production, internalization, and degradation. The amount of EGFR present on the surface of a cell can be measured indirectly, by detecting internalization of the receptor or a molecule bound to the receptor. For example, receptor internalization can be measured by contacting cells that express EGFR with a labeled antibody. Membrane-bound antibody is then stripped, collected and counted. Internalized antibody is determined by lysing the cells and detecting label in the lysates.

Another way is to directly measure the amount of the receptor present on the cell following treatment with an anti-EGFR antibody or other substance, for example, by fluorescence-activated cell-sorting analysis of cells stained for surface expression of EGFR. Stained cells are incubated at 37°C and fluorescence intensity measured over time. As a control, part of the stained population can be incubated at 4°C (conditions under which receptor internalization is halted).

Another measure of down-modulation is reduction of the total receptor protein present in a cell, and reflects degradation of internal receptors. Accordingly, treatment of cells (particularly cancer cells) with antibodies of the invention results in a reduction in total cellular EGFR. In a preferred embodiment, the reduction is at least about 70%, more preferably at least about 80%, and even more preferably at least about 90%.

For treatment of human subjects, antibodies according to the invention are preferably human. Alternatively, the antibodies can be from non-human primates or other mammals, or be humanized or chimeric antibodies.

Antibody fragments according to the invention can be produced by cleaving a whole antibody, or by expressing DNA that encodes the fragment. Fragments of antibodies may be prepared by methods described by Lamoyi et al., *J. Immunol. Methods*,

56: 235-243 (1983) and by Parham, *J. Immunol.* 131: 2895-2902 (1983). Such fragments may contain one or both Fab fragments or the $F(ab')_2$ fragment. Such fragments may also contain single-chain fragment variable region antibodies, i.e. scFv, dibodies, or other antibody fragments. Methods of producing such functional equivalents are disclosed in PCT Application WO 93/21319, European Patent Application No. EP 239400; PCT Application WO 89/09622; European Patent Application EP 338745; and European Patent Application EP 332424.

Preferred host cells for transformation of vectors and expression of the antibodies of the present invention are mammalian cells, e.g., COS-7 cells, Chinese hamster ovary (CHO) cells, and cell lines of lymphoid origin such as lymphoma, myeloma (e.g. NS0), or hybridoma cells. Other eukaryotic hosts, such as yeasts, can be alternatively used.

The transformed host cells are cultured by methods known in the art in a liquid medium containing assimilable sources of carbon (carbohydrates such as glucose or lactose), nitrogen (amino acids, peptides, proteins or their degradation products such as peptones, ammonium salts or the like), and inorganic salts (sulfates, phosphates and/or carbonates of sodium, potassium, magnesium and calcium). The medium furthermore contains, for example, growth-promoting substances, such as trace elements, for example iron, zinc, manganese and the like.

High affinity anti-EGFR antibodies according to the present invention can be isolated from a phage display library constructed from human heavy chain and light chain variable region genes. For example, a variable domain of the invention can be obtained from a peripheral blood lymphocyte that contains a rearranged variable region gene. Alternatively, variable domain portions, such as CDR and FW regions, can be obtained from different sources and recombined. Further, portions of the variable domains (*e.g.*, FW regions) can be synthetic consensus sequences.

Antibodies and antibody fragments of the present invention can be obtained, for example, from naturally occurring antibodies, or Fab or scFv phage display libraries. It is understood that, to make a single domain antibody from an antibody comprising a V_H and a V_L domain, certain amino acid substitutions outside the CDRs can be desired to enhance binding, expression or solubility. For example, it can be desirable to modify amino acid residues that would otherwise be buried in the V_H-V_L interface.

Further, antibodies and antibody fragments of the invention can be obtained by standard hybridoma technology (Harlow & Lane, ed., Antibodies: A Laboratory Manual, Cold Spring Harbor, 211-213 (1998), which is incorporated by reference herein) using

transgenic mice (*e.g.*, KM mice from Medarex, San Jose, Calif.) that produce human immunoglobulin gamma heavy and kappa light chains. In a preferred embodiment, a substantial portion of the human antibody producing genome is inserted into the genome of the mouse, and is rendered deficient in the production of endogenous murine antibodies. Such mice may be immunized subcutaneously (s.c.) with PDGFRa (usually in complete Freund's adjuvant) with boosts as needed. Immunization methods are well known in the art.

Antibodies that can be used according to the invention include complete immunoglobulins, antigen binding fragments of immunoglobulins, as well as antigen binding proteins that comprise antigen binding domains of immunoglobulins. Antigen binding fragments of immunoglobulins include, for example, Fab, Fab', and $F(ab')_2$. Other antibody formats have been developed which retain binding specificity, but have other characteristics that may be desirable, including for example, bispecificity, multivalence (more than two binding sites), compact size (e.g., binding domains alone).

Single chain antibodies lack some or all of the constant domains of the whole antibodies from which they are derived. Therefore, they can overcome some of the problems associated with the use of whole antibodies. For example, single-chain antibodies tend to be free of certain undesired interactions between heavy-chain constant regions and other biological molecules. Additionally, single-chain antibodies are considerably smaller than whole antibodies and can have greater permeability than whole antibodies, allowing single-chain antibodies to localize and bind to target antigen-binding sites more efficiently. Furthermore, the relatively small size of single-chain antibodies makes them less likely to provoke an unwanted immune response in a recipient than whole antibodies.

Multiple single chain antibodies, each single chain having one V_H and one V_L domain covalently linked by a first peptide linker, can be covalently linked by at least one or more peptide linker to form a multivalent single chain antibodies, which can be monospecific or multispecific. Each chain of a multivalent single chain antibody includes a variable light chain fragment and a variable heavy chain fragment, and is linked by a peptide linker to at least one other chain. The peptide linker is composed of at least fifteen amino acid residues. The maximum number of amino acid residues is about one hundred.

Two single chain antibodies can be combined to form a diabody, also known as a bivalent dimer. Diabodies have two chains and two binding sites, and can be monospecific or bispecific. Each chain of the diabody includes a V_H domain connected to

a V_L domain. The domains are connected with linkers that are short enough to prevent pairing between domains on the same chain, thus driving the pairing between complementary domains on different chains to recreate the two antigen-binding sites.

Three single chain antibodies can be combined to form triabodies, also known as trivalent trimers. Triabodies are constructed with the amino acid terminus of a V_L or V_H domain directly fused to the carboxyl terminus of a V_L or V_H domain, i.e., without any linker sequence. The triabody has three Fv heads with the polypeptides arranged in a cyclic, head-to-tail fashion. A possible conformation of the triabody is planar with the three binding sites located in a plane at an angle of 120 degrees from one another. Triabodies can be monospecific, bispecific or trispecific.

Thus, antibodies of the invention and fragments thereof include, but are not limited to, naturally occurring antibodies, bivalent fragments such as $(Fab')_2$, monovalent fragments such as Fab, single chain antibodies, single chain Fv (scFv), single domain antibodies, multivalent single chain antibodies, diabodies, triabodies, and the like that bind specifically with antigens.

According to the present invention, an EGFR antagonist is administered in combination with one or more anti-neoplastic agents. As employed herein, the term, "anti-neoplastic" agent excludes an EGFR antagonist, unless otherwise specified. Any suitable anti-neoplastic agent can be used, such as a chemotherapeutic agent, radiation, or combinations thereof. The anti-neoplastic agent can be an alkylating agent or an anti-metabolite. Examples of alkylating agents include, but are not limited to, cisplastin, cyclophosphamide, melphalan, and dacarbazine. Examples of anti-metabolites include, but are not limited to doxorubicin, dunorubicin, paclitaxel, and gemcitabine.

In a preferred embodiment, the anti-neoplastic agent is a chemotherapeutic agent. Preferred chemotherapeutic agents include amifostine (ethyol), cisplatin, dacarbazine (DTIC), dactinomycin, mechlorethamine (nitrogen mustard), streptozocin, cyclophosphamide, carmustine (BCNU), lomustine (CCNU), doxorubicin (adriamycin), doxorubicin lipo (doxil), gemcitabine (gemzar), daunorubicin, daunorubicin lipo (daunoxome), procarbazine, mitomycin, cytarabine, etoposide, methotrexate, 5-fluorouracil, vinblastine, vincristine, bleomycin, paclitaxel (taxol), docetaxel (taxotere), aldesleukin, asparaginase, busulfan, carboplatin, cladribine, camptothecin, CPT-11,10-hydroxy-7-ethyl-camptothecin (SN38), dacarbazine, floxuridine, fludarabine, hydroxyurea, ifosfamide, idarubicin, mesna, interferon alpha, interferon beta, irinotecan, mitoxantrone, topotecan, leuprolide, megestrol, melphalan, mercaptopurine, plicamycin,

mitotane, pegaspargase, pentostatin, pipobroman, plicamycin, streptozocin, tamoxifen, teniposide, testolactone, thioguanine, thiotepa, uracil mustard, vinorelbine, chlorambucil and combinations thereof. In a more preferred embodiment, the chemotherapeutic agent is irinotecan.

An anti-neoplastic agent, including a chemotherapeutic agent and/or an anti-metabolite, is administered in a dose and on a schedule according to the package directions and/or the clinical response of the patient. Such doses will be apparent to one of ordinary skill and do not require undue experimentation. For example, Ironotecan is preferably administered over the recommended dose range of from about 50 to about 350 mg/m^2, although the dose may be higher or lower, depending on the clinical response of the patient. Other agents have dosage ranges of from about 1 to about 1,000 mg/m^2 per administration and/or treatment regimen. Radiation treatment is administered based on the appropriate clinical treatment protocols and/or clinical experience of one of skill in the art.

In a combination therapy, the EGFR antagonist is administered before, during, or after commencing therapy with another agent, as well as any combination thereof, i.e., before and during, before and after, during and after, or before, during and after commencing the anti-neoplastic agent therapy. For example, an EGFR antibody can be administered between 1 and 30 days, preferably 3 and 20 days, more preferably between 5 and 12 days before commencing radiation therapy. In a preferred embodiment of the invention, chemotherapy is administered concurrently with or, more preferably, subsequent to antibody therapy.

In the present invention, any suitable method or route can be used to administer the antagonists of the invention, and optionally, to co-administer anti-neoplastic agents and/or antagonists of other receptors. The anti-neoplastic agent regimens utilized according to the invention, include any regimen believed to be optimally suitable for the treatment of the patient's tumor. Different tumors can require use of specific anti-tumor antibodies and specific anti-neoplastic agents, which will be determined on a patient to patient basis. Routes of administration include, for example, oral, intravenous, intraperitoneal, subcutaneous, or intramuscular administration. The dose of antagonist administered depends on numerous factors, including, for example, the type of antagonists, the type and severity of the tumor being treated and the route of administration of the antagonists. It should be emphasized, however, that the present invention is not limited to any particular method or route of administration.

One of skill in the art would understand that dosages and frequency of treatment depend on the tolerance of the individual patient and on the pharmacological and pharmacokinetic porperties of blocking or inhibitory agent used. Ideally, one wishes to achieve saturable pharmacokinetics for the agent used. A loading dose for an anti-EGFR antibody can range, for example, from about 10 to about 1000 mg/m^2, preferably from about 200 to about 400 mg/m^2. This can be followed by several additional daily or weekly dosages ranging, for example, from about 200 to about 400 mg/m^2. The patient is monitored for side effects and the treatment is stopped when such side effects are severe.

One of skill in the art would also know how to monitor the progress of the treatment in order to determine an effective dose. For example, one such way is to monitor MRI, CT, or other brain scans.

EXAMPLES

Example 1: The following example discloses a method of treating refractory solid tumors by administering a combination of irinotecan and an EGFR antibody.

From August 2005 to March 2006, 20 pediatric patients with refractory solid tumors and life expectancy of at least 8 weeks were administered irinotecan and different dose levels of cetuximab. The patients were divided into two groups (ages 1-12 = Arm A, and 13-18 = Arm B) with the following tumor types: brainstem glioma/ astrocytoma (10), hepatoblastoma, osteosarcoma (1), ependymoma (1), neuroblastoma (1), rhabdomyosarcoma (1) and other (5). Irinotecan was administered at a dose of 20 mg/m^2/day as a 60 minute infusion for 5 days x 2 weeks, every 21 days. The dose schedules and number of subjects with dose-limiting toxicities (DLTs) are provided in Table 1.

Table 1

Arm	Dose level	Cetuximab (mg/m^2/day days 1, 8, 15)	Irinotecan (mg/m^2/day days 1-5 and 8-12)	No. of subjects	No. of subjects with DLT (dose-limiting toxicity)
A	1	75	20	6	1 (Gr 3 diarrhea)
A	2	150	20	6	2 (Gr 4 neutropenia, Gr 3 diarrhea)
B	1	75	20	8	1 (Gr 3 diarrhea)

Due to the DLTs experienced by two out of the six patients in Arm A, dose level 2, which were deemed to be related to irinotecan, irinotecan was de-escalated to 16 mg/m^2.

One subject in Arm B experienced a Grade 3 infusion reaction and treatment was discontinued. Other observed toxicities included grade 1 rash.

One subject with an EGFR-negative high grade glioma (group A dose level 1) achieved a partial response and is currently in cycle 13. One subject with Neuroblastoma (group A dose level 1) has experienced a minor response and is currently receiving cycle 9. Seven subjects had a best response of stable disease for an average of 3.4 months (ranging from 2- 7+ mo).

In conclusion, the combination of cetuximab and irinotecan shows promising anti-cancer activity in pediatric solid tumors.

The foregoing description and example have been set forth merely to illustrate the invention and are not intended as being limiting. Each of the disclosed aspects and embodiments of the present invention may be considered individually or in combination with other aspects, embodiments, and variations of the invention. Further, while certain features of embodiments of the present invention may be shown in only certain figures, such features can be incorporated into other embodiments shown in other figures while remaining within the scope of the present invention. In addition, unless otherwise specified, none of the steps of the methods of the present invention are confined to any particular order of performance. Modifications of the disclosed embodiments incorporating the spirit and substance of the invention may occur to persons skilled in the art and such modifications are within the scope of the present invention. Furthermore, all references cited herein are incorporated by reference in their entirety.

What is claimed is:

1. A method of inhibiting the growth of a tumor in a pediatric patient comprising treating the pediatric patient with an effective amount of an epidermal growth factor receptor (EGFR) antagonist and a chemotherapeutic agent.

2. The method of claim 1, wherein the tumor is a refractory tumor.

3. The method of claim 1, wherein the tumor is a glioma.

4. The method of claim 1, wherein the tumor is a neuroblastoma.

5. The method of claim 1, wherein the antagonist is an antibody or fragment thereof.

6. The method of claim 5, wherein the antibody or fragment thereof specifically binds to the extracellular domain of EGFR.

7. The method of claim 5, wherein the antibody or fragment thereof inhibits binding of an EGFR ligand to an EGFR and neutralizes activation thereof.

8. The method of claim 5, wherein the antibody is cetuximab.

9. The method of claim 5, wherein the antibody or fragment thereof is monoclonal.

10. The method of claim 5, wherein the antibody or fragment thereof is chimeric.

11. The method of claim 5, wherein the antibody or fragment thereof is humanized.

12. The method of claim 5, wherein the antibody or fragment thereof is human.

13. The method of claim 1, wherein the chemotherapeutic agent is irinotecan.

14. The method of claim 1, further comprising administering radiation to the pediatric patient.