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(54) Title: USE OF EGFR BIOMARKERS FOR THE TREATMENT OF GASTRIC CANCER WITH ANTI-EGFR AGENTS

(57) Abstract: The present invention provides methods for treating gastric neoplasias comprising administering to patients effective amounts of lanti-EGFR agents, in particular treating patients who have been previously determined to have an EGFR biomarker.

USE OF EGFR BIOMARKERS FOR THE TREATMENT OF GASTRIC CANCER WITH ANTI-EGFR AGENTS

FIELD OF THE INVENTION

The present invention relates generally to methods for treating gastric neoplasias, in particular treating patients who have been previously determined to have a specific EGFR biomarker.

BACKGROUND OF THE INVENTION

Gastric carcinoma (GC) is one of the most common and deadest cancers with ~ 1 million diagnoses and ~0.7 million deaths each year worldwide [1], with high incidence in Eastern Asia[2]. However, very few effective treatment options are available beyond surgery for the majority of GC patients. Trastuzumab (Herceptin®), a monoclonal antibody targeting HER2, is the only approved target therapy, but is limited to a small fraction of GC patients with higher HER2 (EGFR2) gene expression[3] and amplification. The recently approved drugs that can demonstrate significant benefits in GC could be particularly attractive to meet such an urgent need. EGFR, also referred to as ERBB1/HER1, belongs to the same family of HER2 and is a receptor tyrosine kinase (RTK) expressed in epithelia cancers, including colorectal carcinoma (CRC), GC and non-small cell lung carcinoma (NSCLC), *etc*. Cetuximab is a monoclonal antibody, that binds to EGFR and blocks its ligand induced downstream signaling, thus inhibiting cell proliferation. Cetuximab was approved by Food and Drug Administration (FDA) for treating EGFR-expressing metastatic CRC (mCRC) without activating KRAS mutations at codons 12/13[4], and squamous cell carcinoma of head and neck (SCCHN)[5], but Cetuximab has yet to be approved for treatment for GC. There are several phase II clinical trials on the combination treatments of cetuximab/chemotherapy agents for advanced GC but the studies have yet to demonstrate significant superior clinical benefit over the current standard of care (SOC)[6-8]. A randomized controlled phase III trial sponsored by Merck Serona has been recently reported to fail to meet its primary endpoint (NCT00678535: Erbitux in Combination With Xeloda and Cisplatin in Advanced Esophago-gastric Cancer, or EXPAND).

New effective target therapy is therefore urgently needed. The present invention provides this and other advantages.

SUMMARY OF THE INVENTION

One aspect of the present invention provides a method for treating gastric neoplasia comprising administering to a patient in need of such treatment an effective amount of anti-EGFR agent, wherein the patient has been determined to contain an EGFR biomarker. In one embodiment of the method the gastric neoplasia is gastric adenocarcinoma. In another embodiment of the method the anti-EGFR agent is an anti-EGFR antibody. In another embodiment, the anti-EGFR agent is cetuximab, panitumumab, nimotuzumab, antibody 806, Sym004, or MM-151. In certain embodiments, the anti-EGFR agent is a combination of 2 or more anti-EGFR agents. In another embodiment, the method further comprises administering the anti-EGFR agent in combination with the standard treatment for gastric neoplasia. In one embodiment, the method further comprises administering the anti-EGFR agent in combination with chemotherapy or radiation therapy. In yet a further embodiment, the method comprises administering the anti-EGFR agent in combination with one or more of cisplatin and capecitabine, 5-fluorouracil, oxaliplatin, Irinotecan, docetaxel, paclitaxel, doxorubicin mitomycin C, etoposide, gemcitabine or carboplatin. As further outlined herein, in one embodiment, the EGFR biomarker is EGFR gene amplification or EGFR overexpression. In this regard, the EGFR gene amplification may comprise an EGFR gene copy number that is higher than a predetermined number. The EGFR overexpression may comprise a level of EGFR RNA, protein, or activity that is higher than a predetermined level. In certain embodiments of the method, the patient has been determined to contain an EGFR biomarker and not a HER2 biomarker. Thus, in one embodiment, the patient is administered with an anti-EGFR agent without an anti-HER2 agent.

Another aspect of the present invention provides a method for determining whether a patient is suitable for an anti-EGFR treatment comprising detecting in a sample of the patient the presence or absence of an EGFR biomarker, wherein the presence of an EGFR biomarker is indicative that the patient is suitable for the anti-EGFR treatment.

A further aspect of the present invention provides a method for providing a lab service comprising, receiving a sample of a patient with gastric neoplasia, conducting a test to detect in the sample the presence or absence of an EGFR biomarker, and providing the test result to the healthcare provider of the patient.

Yet another aspect of the present invention provides a kit comprising a reagent suitable for detection of an EGFR biomarker and an instruction for using the EGFR biomarker for the treatment of gastric neoplasia according to the methods described herein.

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BRIEF DESCRIPTION OF THE FIGURES

The invention is more fully appreciated in connection with the following detailed description taken in conjunction with the accompanying drawings, in which:

Figure 1. GC-ADC HuPrime® models responses to cetuximab: Panel A: representative tumor growth inhibition by cetuximab of the responders (top three models) and non-responders (bottom three rows of 8 models). Panel B: Pharmacodynamic analysis of model GA0022. pERK-IHC analysis of model GA0022 tumors post-cetuximab treatment at time points of 0, 6, 24, and 72 hours. Both IHC images and scores are shown.

Figure 2. The correlation of antitumor activities and EGFR gene amplification and over-expression. (Boxed items represent models with EGFR gene amplication). Panel A: Waterfall graph of GC-ADC tumor response to cetuximab; Panel B. EGFR gene copy number analysis of GC models using Affymetrix SNP6 chip analysis. C. Relative mRNA levels as measured Affymetix GeneChip HG-U219. D EGFR mRNA expression as measured by pPCR.

Figure 3. Loboda RAS pathway signature scores were plotted against tumor size over time. The Loboda RAS pathway signature scores were found to have little correlation to the tumor response.

Figure 4. GC-ADC HuPrime® models with EGFR over expression/gene amplification do not have HER2 over-expression/gene amplification. Panel A: EGFR and HER2 expression levels; Panel B: EGFR and HER2 gene copy number.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates generally to methods for treating gastric neoplasias with an anti-EGFR agent in particular treating patients who have been previously determined to have a specific EGFR biomarker. The present invention permits treatment of patients who have a greater likelihood of responding to the treatment by administering therapeutic agents, *i.e.,* anti-EGFR antibodies to patients who are found to have an amplified gene encoding the EGFR protein or overexpression of EGFR (as measured by mRNA expression, protein expression or activity level). The invention is based, in part, on the discovery that EGFR gene amplification, *e.g.*, as detected by fluorescence in situ hybridization (FISH), by microarray analysis, or by other methods known in the art, or EGFR overexpression, provide a basis for selecting patients for treatment because EGFR gene amplification or EGFR overexpression correlates with response to treatment.

In one aspect, the present invention provides a method for determining whether a patient is suitable for an anti-EGFR treatment comprising detecting in a sample of the patient the presence or absence of an EGFR biomarker, wherein the presence of an EGFR biomarker is indicative that the patient is suitable for the anti-EGFR treatment.

As used herein, the term "epidermal growth factor receptor" ("EGFR") refers to a gene that encodes a membrane polypeptide that binds, and is thereby activated by, epidermal growth factor (EGF). EGFR is also known in the literature as ERBB, ERBB1 and HER1. An exemplary EGFR is the human epidermal growth factor receptor (see Ullrich et al. (1984) Nature 309:418-425; Genbank accession number NP-005219.2; complete cds AY588246.1). Binding of an EGF ligand activates the EGFR (e.g. resulting in activation of intracellular mitogenic signaling, autophosphorylation of EGFR). One of skill in the art will appreciate that other ligands, in addition to EGF, can bind to and activate the EGFR. Examples of such ligands include, but are not limited to, amphiregulin, epiregulin, TGF-α., betacellulin, and heparin-binding EGF (HB-EGF). Intracellular domain of, a human, EGFR comprises a polypeptide sequence from amino acid adjacent to the transmembrane domain up to COOH-terminus of the EGFR. Intracellular domain comprises, inter alia, tyrosine kinase domain.

As used herein, an "EGFR gene" refers to a nucleic acid that encodes an EGFR gene product, *e.g.*, an EGFR mRNA, an EGFR polypeptide, and the like.

As used herein, an "anti-EGFR agent" refers to any agent capable of directly or indirectly binding to EGFR and inhibiting activation of an EGFR. Anti-EGFR agents include antibodies that bind to an EGFR and inhibit activation of the EGFR, as well as small molecule tyrosine kinase inhibitors or "kinase inhibitors" that inhibit activation of an EGFR. Antibodies to EGFR include IgG; IgM; IgA; antibody fragments that retain EGFR binding capability, *e.g.,* Fv, Fab, $F(ab)_2$, single-chain antibodies, and the like; chimeric antibodies; etc. Small molecule tyrosine kinase inhibitors of EGFR include EGFR-selective tyrosine kinase inhibitors. Small molecule tyrosine kinase inhibitors of EGFR can have a molecular weight in a range of from about 50 Da to about 10,000 Da.

According to the present invention, an “anti-EGFR agent” or "EGFR inhibitor" is any agent that inhibits (blocks, reduces, antagonizes, decreases, reverses) the expression and/or biological activity of an epidermal growth factor receptor (EGFR), including any EGFR. Therefore, an anti-EGFR agent can include, but is not limited to, a product of drug/compound/peptide design or selection, an antibody or antigen binding fragment thereof, a protein, a peptide, a nucleic acid (including ribozymes, antisense, RNAi and aptamers), or any other agent that inhibits the expression and/or biological activity of an EGFR. For example, known inhibitors of EGFR include the drugs, gefitinib (ZD 1839, Iressa®, AstraZeneca, UK)

and erlotinib (OSI 774, Tarceva®, Genentech, USA), and the monoclonal antibody, Cetuximab (Erbitux®, Imclone, Bristol-Myers Squibb). However, the invention is not limited to these specific agents, and can include an agonist of such agents or agents having substantially similar biological activity as these agents. The biological activity or biological action of a protein, such as an EGFR, refers to any function(s) exhibited or performed by a naturally occurring form of the protein as measured or observed in vivo (i.e., in the natural physiological environment of the protein) or in vitro (i.e., under laboratory conditions). Biological activities of EGFR include, but are not limited to, binding to EGF, receptor homo- or heterodimerization, tyrosine kinase activity, and downstream activities related to cellular homeostasis and development.

Tyrosine kinase inhibitors represent a class of therapeutic agents or drugs that target receptor and/or non-receptor tyrosine kinases in cells such as tumor cells. In certain instances, the tyrosine kinase inhibitor is an antibody-based (e.g., anti-tyrosine kinase monoclonal antibody, etc.) or polynucleotide-based (e.g., tyrosine kinase antisense oligonucleotide, small interfering ribonucleic acid, etc.) form of targeted therapy. Preferably, the tyrosine kinase inhibitor is a small molecule that inhibits target tyrosine kinases by binding to the ATP-binding site of the enzyme. Examples of small molecule tyrosine kinase inhibitors include, but are not limited to, gefitinib (Iressa®), sunitinib (Sutent®; SU11248), erlotinib (Tarceva®; OSI-1774), lapatinib (GW572016; GW2016), canertinib (CI 1033), semaxinib (SU5416), vatalanib (PTK787/ZK222584), sorafenib (BAY 43-9006), imatinib (Gleevec®; ST1571), dasatinib (BMS-354825), leflunomide (SU10), vandetanib (Zactima®; ZD6474), pharmaceutically acceptable salts thereof, derivatives thereof, analogs thereof, and combinations thereof. Additional examples of tyrosine kinase inhibitors suitable for use in the present invention include quinazolines (e.g., PD 153035,4-(3-chloroanilino)quinazoline, etc.), pyridopyrimidines, pyrimidopyrimidines, pyrrolopyrimidines (e.g., CGP 59326, CGP 60261, CGP 62706, etc.), pyrazolopyrimidines, 4-(phenylamino)-7H-pyrrolo[2,3-d]pyrimidines, curcumin (diferuloyl methane), 4,5-bis(4-fluoroanilino)phthalimide, tyrphostines containing nitrothiophene moieties, quinoxalines (see, e.g., U.S. Pat. No. 5,804,396), tryphostins (see, e.g., U.S. Pat. No. 5,804,396), PD0183805, PKI-166, EKB-569, IMC-1C11, Affinitac® (LY900003; ISIS 3521), and the tyrosine kinase inhibitors described in PCT Publication Nos. WO 99/09016, WO 98/43960, WO 97/38983, WO 99/06378, WO 99/06396, WO 96/30347, WO 96/33978, WO 96/33979, and WO 96/33980.

Illustrative anti-EGFR agents are anti-EGFR antibodies, including but not limited to the anti-EGFR antibodies: cetuximab (ERBITUX™), panitumumab (VECTIBIX™), matuzumab, nimotuzumab, antibody 806, Sym004, and MM-151 in their murine, chimeric or humanized versions including their immunologically effective fragments (Fab, Fv) and immunoconjugates,

especially immunocytokines. Other antibodies (or other binding molecules) specific for the EGFR extracellular domain are known in the art and are contemplated for use herein (see, *e.g.*, U.S. Pat. Nos. 5,459,061, 5,558,864, 5,891,996, 6,217,866, 6,235,883, 6,699,473, and 7,060,808; European Pat. Nos. EP0359282 and EP0667165).

The term "tumor sample" as used herein means a sample comprising tumor material obtained from a cancerous patient. The term encompasses clinical samples, for example tissue obtained by surgical resection and tissue obtained by biopsy, such as for example a core biopsy or a fine needle biopsy. The term also encompasses samples comprising tumor cells obtained from sites other than the primary tumor, e.g., circulating tumor cells. The term encompasses cells that are the progeny of the patient's tumor cells, e.g. cell culture samples derived from primary tumor cells or circulating tumor cells. The term encompasses samples that may comprise protein or nucleic acid material shed from tumor cells in vivo, e.g. bone marrow, blood, plasma, serum, and the like. The term also encompasses samples that have been enriched for tumor cells or otherwise manipulated after their procurement and samples comprising polynucleotides and/or polypeptides that are obtained from a patient's tumor material.

An “EGFR biomarker” refers to EGFR gene amplification or overexpression of EGFR. Overexpression of EGFR may relate to overexpression as measured by mRNA, protein or protein activity.

In certain embodiments, threshold level(s) of EGFR gene copy number or overexpression of EGFR can be established, and the EGFR gene copy number or the expression level of EGFR mRNA or protein in a patient's tumor sample can be compared to a "predetermined threshold level" (also referred to as "predetermined level" or “predetermined cut-off value”).

A predetermined level, sometimes referred to as a predetermined cut off, of EGFR copy number or expression level may be established using methods known in the art, in particular using Receiver Operator Characteristic curves or “ROC” curves. In practice, ROC curves are typically calculated by plotting the value of a variable versus its relative frequency in "normal" and "disease" populations, and/or by comparison of results from a subject before, during and/or after treatment. In certain embodiments, EGFR expression or gene copy number, or both, in normal samples versus test samples is compared.

To determine the presence or absence of an EGFR biomarker, or other biomarker of interest, such as HER2, the signal detected from the reporter group used in a particular test or assay is generally compared to a signal that corresponds to a predetermined cut-off value, or predetermined threshold level. In one embodiment, the predetermined threshold level for the detection of a biomarker is the average mean level obtained from samples from patients

without the disease, *e.g.*, without gastric cancer. In general, a sample generating a signal that is three standard deviations above the predetermined threshold level is considered positive for the cancer. In an alternate preferred embodiment, the cut-off value is determined using a ROC, according to the method of Sackett et al., Clinical Epidemiology: A Basic Science for Clinical Medicine, Little Brown and Co., 1985, p. 106-7. Briefly, in this embodiment, the cut-off value may be determined from a plot of pairs of true positive rates (i.e., sensitivity) and false positive rates (100%-specificity) that correspond to each possible cut-off value for the diagnostic test result. The cut-off value on the plot that is the closest to the upper left-hand corner (*i.e.*, the value that encloses the largest area) is the most accurate cut-off value, and a sample generating a signal that is higher than the cut-off value determined by this method may be considered positive. Alternatively, the cut-off value may be shifted to the left along the plot, to minimize the false positive rate, or to the right, to minimize the false negative rate. In general, a sample generating a signal that is higher than the cut-off value determined by this method is considered positive for a cancer.

In some embodiments, a ROC curve representing a patient response to a treatment with an anti-EGFR agent may be used to define the objective function. For example, the objective function may reflect the area under the ROC curve. By maximizing the area under the curve in respect to level of EGFR gene copy number (e.g., EGFR gene amplification) or overexpression of EGFR in patients treated with an anti-EGFR agent, one may maximize whether a patient suffering from a cancer will be responsive to the treatment with an anti-EGFR agent. In some other embodiments, the ROC curve may be constrained to provide an area-under-curve of greater than a particular value. ROC curves having an area under the curve of 0.5 indicate complete randomness, while an area under the curve of 1.0 reflects perfect separation of the two sets. Thus, in certain embodiments, a minimum acceptable value, such as 0.75, may be used as a constraint.

In other embodiments, other features such as use of the point at which the slope of the ROC curve is equal to one; the use of the point at which the product of sensitivity and specificity is a maximum; or combinations of two or more of these ROC-curve features may be used to define the objective function.

In certain embodiments, EGFR overexpression (e.g., mRNA or protein expression) is present at a level that is at least about two-fold, three-fold, four-fold, five-fold, six-fold or higher in tumor tissue than in normal tissue of the same type from which the tumor arose.

In some embodiments, increased levels of EGFR gene copy number or overexpression of EGFR, or both, in the tumor sample relative to a predetermined threshold level are indicative that a patient suffering from a cancer will be responsive to the treatment with an anti-EGFR

agent. In some embodiments, decreased levels of EGFR gene copy number in the tumor sample relative to the predetermined threshold level are indicative that a patient suffering from a cancer will be non-responsive to the treatment with an EGFR inhibitor.

The present invention also relates in part to the observation that HuPrime models having an EGFR biomarker (over expression and/or gene amplifications) do not also have a HER2 biomarker (over-expression and/or gene amplification), and vice versa, i.e. no over-expression (gene amplification) of both EGFR and HER2 was observed in a single model (See Examples). Accordingly, in one embodiment, increased levels of EGFR gene copy number or overexpression of EGFR in the tumor sample relative to a predetermined threshold level and the lack of a HER2 biomarker, are used as indicators for selecting a patient for treatment with an anti-EGFR agent. In this regard, the nucleotide sequence of the human tyrosine kinase receptor-type receptor (HER2) gene is also known in the art and can be found, for example, under GenBank Accession Nos. M16789, M16790, M16791, M16792 and M11730 (all incorporated herein by reference). Nucleotide probes and anti-HER2 antibodies are also known in the art and available for use as probes to the HER2 genes and proteins for determining expression levels/activity thereof.

The term "gene copy number" (GCN) is usually defined as the number of genes per genome. The term "EGFR gene copy number" means the ratio of number of EGFR genes per nucleus. In non-tumorigenic or non-neoplastic cells EGFR gene copy number is similar to or less than 2. In tissue sections of non-tumorigenic or non-neoplastic origin, GCN is similar to or less than 2, if detected with in situ hybridization.

The term "increased EGFR gene copy number", "amplified EGFR gene copy number" or "EGFR gene amplification" means that the above-defined ratio in cells of a tumor correlated to a patient is higher or amplified compared to the particular ratio, or threshold level, in cells of a tumor correlated to non-neoplastic cells of the same origin. In one embodiment, the ratio, or threshold level, (number EGFR gene/nucleus) is greater than 2 or 3 or 4 or 5 or 6 or 7. In another embodiment, said ratio or threshold level is similar to or greater than 4. In certain embodiments the term increased or amplified EGFR gene copy number means GCN greater than the EGFR gene copy number in non-tumorigenic or non-neoplastic cells. In certain embodiments, EGFR GCN, or threshold level, may be more than 4, such as 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, 11, 11.5, 12, 12.5, 13, 30.5, 14, 14.5, 15, 50.5, 16 or more. In certain embodiments, EGFR GCN, or threshold level, may be less than 4, such as 3.5, 3, or 2.5.

In some embodiments, EGFR gene copy number similar to or greater than 4 identifies a patient suffering from a cancer who is likely to be responsive to the treatment with –EGFR agent.

According to these afore-mentioned values applicable to an "increased" or "amplified" EGFR gene copy number, the ratio values for a relatively decreased or lower or non-amplified copy number presented by tumor cells of patients, which do not or do not effectively or positively respond, or are non-responsive, to the treatment with EGFR inhibitors or anti-EGFR antibodies are less than 2. In one embodiment, said ratio, or threshold level, is less than 4. In some embodiments, EGFR gene copy number less than 4 identifies a patient suffering from a cancer and who is likely to be non-responsive to the treatment with an anti-EGFR agent.

Methods for determining the presence of an EGFR biomarker, e.g. gene amplification or overexpression of EGFR, or other biomarker of interest (*e.g.*, a HER2 biomarker) include gene expression profiling. Such methods include methods based on hybridization analysis of polynucleotides, methods based on sequencing of polynucleotides, and proteomics-based methods. Exemplary methods known in the art for the quantification of mRNA expression in a sample include northern blotting and in situ hybridization (Parker & Barnes, Methods in Molecular Biology 106:247-283 (1999)); RNAse protection assays (Hod, Biotechniques 13:852-854 (1992)); and PCR-based methods, such as reverse transcription PCT (RT-PCR) (Weis et al., Trends in Genetics 8:263-264 (1992)). Antibodies may be employed that can recognize sequence-specific duplexes, including DNA duplexes, RNA duplexes, and DNA-RNA hybrid duplexes or DNA-protein duplexes. Next generation sequencing, qPCR, qcPCR, and digital PCR may also be used for determining EGFR expression levels.

EGFR expression levels can also be assessed using any of a variety of available microarrays. In this method, polynucleotide sequences of interest (including cDNAs and oligonucleotides) are arrayed on a substrate. The arrayed sequences are then contacted under conditions suitable for specific hybridization with detectably labeled cDNA generated from mRNA of a test sample. The source of mRNA typically is total RNA isolated from a tumor sample, and optionally from normal tissue of the same patient as an internal control or cell lines. mRNA can be extracted, for example, from frozen or archived paraffin-embedded and fixed (e.g. formalin-fixed) tissue samples. Illustrative microarrays for use herein include, but are not limited to Affymetrix HG-U219 GeneChip or Affymetrix SNP6 arrays (Affymetrix, Santa Clara, CA).

In certain embodiments, detection of the EGFR gene copy number or mRNA expression level is accomplished using hybridization assays. Nucleic acid hybridization simply involves contacting a probe (e.g., an oligonucleotide or larger polynucleotide) and target nucleic acid under conditions where the probe and its complementary target can form stable hybrid duplexes through complementary base pairing. As used herein, hybridization conditions refer to standard hybridization conditions under which nucleic acid molecules are used to identify

similar nucleic acid molecules. Such standard conditions are disclosed, for example, in Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Labs Press, 1989. Sambrook et al., ibid., is incorporated by reference herein in its entirety (see specifically, pages 9.31-9.62). In addition, formulae to calculate the appropriate hybridization and wash conditions to achieve hybridization permitting varying degrees of mismatch of nucleotides are known to the skilled artisan.

Under low stringency conditions (e.g., low temperature and/or high salt) hybrid duplexes (e.g., DNA:DNA, RNA:RNA, or RNA:DNA) will form even where the annealed sequences are not perfectly complementary. Thus specificity of hybridization is reduced at lower stringency. Conversely, at higher stringency (e.g., higher temperature or lower salt) successful hybridization requires fewer mismatches.

The hybridized nucleic acids are detected by detecting one or more labels attached to the sample nucleic acids. The labels may be incorporated by any of a number of means well known to those of skill in the art.

In one embodiment, EGFR gene copy number is detected using CFISH analysis. FISH (dual-color) procedures can be performed using commercially available reagents and methods, such as those described in the examples herein. (see, *e.g.*, Abbott PathVysion EGFR DNA Probe Kit (Abbott, Downers Grove, IL). In certain embodiments, the labeled probe used for such FISH analysis comprises Spectrum Orange fluorophore-labeled EGFR (303 kb) specific for the EGFR gene locus on chromosome 7p12, and/or the Spectrum Green fluorophore-labeled chromosome enumerator probe (5.4 kb) targeted to the α-satellite DNA sequence located at the centromeric region of chromosome 7 (CEP7; 7p11.1–q11.1).

Many conventional detection methods utilize enzymes. The types of enzyme substrates popularly used for sensitive detection are typically colorimetric, radioactive, or fluorescent. Conventional colorimetric substrates produce a new color (or change in spectral absorption) upon enzyme action on a chromogenic substrate. This type of detection is advantageous in that the chromogens produced are easily detected by light-based microscopy or with spectral equipment. The cost of equipment for detection is also generally less than with other methods; for example in pathology, the brown color produced by the enzyme horseradish peroxidase acting on the substrate 3,3'-diaminobenzidine (DAB), requires only a simple bright field light microscope for observation of biopsied sections. Other chromogens which can be used in conjunction with horseradish peroxidase include, but are not limited to, 3-Amino-9-ethylcarbazole (AEC) and Bajoran Purple. Other chromogens which can be used in conjunction with alkaline phosphatase include, but are not limited to, Fast Red and Ferangi Blue. Numerous

chromogens are available to a person having ordinary skill in the art, and are commercially available through catalogs provided by companies such as Thermo Fisher Scientific.

Various labels used in detection methods include fluorescent dyes (e.g., fluorescein isothiocyanate, Texas red, rhodamine, and the like), and enzymes (e.g., LacZ, CAT, horseradish peroxidase, alkaline phosphatase, .beta.-galactosidase, and glucose oxidase, acetylcholinesterase and others, commonly used as detectable enzymes), or members of a binding pair that are capable of forming complexes such as streptavidin/biotin, avidin/biotin or an antigen/antibody complex including, for example, rabbit IgG and anti-rabbit IgG; fluorophores; light scattering or plasmon resonant materials such as gold or silver particles or quantum dots; or radiolabels; and probes labeled with any other signal generating label known to those of skill in the art, as described, for example, in the 6.sup.th Edition of the Molecular Probes Handbook by Richard P. Hoagland. In certain embodiments, the labeled probe comprises Spectrum Orange fluorophore-labeled EGFR (303 kb) specific for the EGFR gene locus on chromosome 7p12, and/or the Spectrum Green fluorophore-labeled chromosome enumerator probe (5.4 kb) targeted to the α-satellite DNA sequence located at the centromeric region of chromosome 7 (CEP7; 7p11.1–q11.1).

In certain embodiments, the hybridizing nucleic acids, such as the EGFR gene or fragment thereof, are detected by metal labels or "enzymatic metallography" and most preferably, in the context of a silver in situ hybridization (SISH) assay (see e.g. patent publication US20080299555 A1). In particular, the enzymatic metallography allows detection of a single copy of a target gene in a chromosome by a conventional bright field microscope without requiring oil immersion. SISH also enables detection of gene copies with a resolution that allows for individual enumeration of signals, such as discrete metal deposit dots for individual gene copies. In one embodiment, the invention allows for detection of at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or more copies of EGFR gene in human chromosome 7 in a nucleus, as discrete metal deposit dots.

The copy number of genes and chromosomes in tumor cells according to the invention can be measured, for example using FISH or SISH assays, in nuclei, and the protein expression can be evaluated, for example in immunohistochemistry assays, in tumor cell nuclei, cytoplasm and/or membranes. These tests, *e.g.,* FISH, SISH and immunohistochemistry, as well as other detection methods, can be performed in primary tumors, metastatic tumors, locally recurring tumors, or other tumoral settings. The tumor specimens can be fresh, frozen, fixed or otherwise preserved.

The nucleotide sequence of the human epidermal growth factor receptor (EGFR) gene is known in the art and can be found under GenBank Accession No. AY588246 (incorporated

herein by reference), for example. Nucleotide probes are also known in the art and available for use as probes to detect EGFR genes. For example, such a probe for detecting EGFR and chromosome 7 centromere sequences is available (*e.g.*, LSI EGFR SpectrumOrange/CEP 7 SpectrumGreen probe (Vysis, Abbott Laboratories).

Protein expression can be detected in suitable tissues, such as tumor tissue and cell material obtained by biopsy. For example, the patient tumor biopsy sample, which can be immobilized, can be contacted with an antibody, an antibody fragment, or an aptamer, that selectively binds to the protein to be detected, and determining whether the antibody, fragment thereof or aptamer has bound to the protein. Protein expression can be measured using a variety of methods standard in the art, including, but not limited to: Western blot, immunoblot, enzyme-linked immunosorbant assay (ELISA), radioimmunoassay (RIA), immunoprecipitation, surface plasmon resonance, chemiluminescence, fluorescent polarization, phosphorescence, immunohistochemical analysis, matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry, microcytometry, microarray, microscopy, fluorescence activated cell sorting (FACS), and flow cytometry. In one embodiment, immunohistochemical (IHC) analysis is used to detect protein expression. IHC methods and assessment criteria for detection of protein expression are described in detail, for example, in Hirsch et al., J. Clin. Oncol. 2003, 21:3798-3807.

EGFR gene amplification or overexpression can result in increased EGFR activity. Abnormally high EGFR activation results in phosphorylation of several intracellular substrates, which in turns gives rise to mitogenic signaling as well as other tumor-inducing activities. Accordingly, in certain embodiments, determining the presence of an EGFR biomarker may involve measuring one or more indicators of EGFR biological activity as determined using either a cell proliferation assay, an apoptosis assay, a receptor binding assay, a receptor phosphorylation assay, and the like.

Another aspect of the present invention provides a method for providing a lab service comprising receiving a sample of a patient with gastric neoplasia, conducting a test as described herein to detect in the sample the presence or absence of an EGFR biomarker, and providing the test result to the healthcare provider of the patient.

The present invention also provides kits comprising a reagent suitable for detection of an EGFR biomarker and an instruction for using the EGFR biomarker for determining treatment options for patients with gastric neoplasia according to the methods described herein. Such kits typically comprise two or more components necessary for performing a diagnostic assay. Components may be compounds, reagents, containers and/or equipment. For example, one container within a kit may contain a monoclonal antibody or fragment thereof that specifically

binds to EGFR (and/or in certain embodiments, HER2 protein). Such antibodies or fragments may be provided attached to a support material, as described above. One or more additional containers may enclose elements, such as reagents or buffers, to be used in the assay. Such kits may also, or alternatively, contain a detection reagent as described above that contains a reporter group suitable for direct or indirect detection of antibody binding.

Alternatively, a kit may be designed to detect the level of mRNA encoding EGFR (and/or HER2) protein in a biological sample, or for the detection of EGFR gene amplification. Such kits generally comprise at least one oligonucleotide probe or primer, as described above, that hybridizes to the EGFR DNA or to a polynucleotide encoding the EGFR protein. Such an oligonucleotide may be used, for example, within a PCR or hybridization assay. Additional components that may be present within such kits include a second oligonucleotide and/or a diagnostic reagent or container to facilitate the detection of a polynucleotide encoding EGFR or HER2 protein.

Pharmaceutical Compositions and Methods of Treatment

The present invention provides methods for treating gastric neoplasia comprising administering to a patient in need of such treatment a therapeutically effective amount of anti-EGFR agent as described herein, wherein the patient has been determined to contain an EGFR biomarker. In one embodiment, the gastric neoplasia is gastric adenocarcinoma. The methods described herein include methods for treating any type of gastric cancer, including intestinal and diffuse gastric adenocarcinoma, gastrointestinal stromal tumor (GIST), gastrointestinal leiomyosarcoma, gastrointestinal carcinoid and gastrointestinal lymphoma, where the patient has been determined to contain an EGFR biomarker as defined herein. In certain embodiments, the methods are for treating gastric cancer. In particular embodiments the methods comprise methods for treating EGFR-expressing cancer, such as gastric cancer, wherein the cancer is not esophagogastric adenocarcinoma (OGA) or metastatic colorectal carcinoma. In one embodiment, the present invention provides methods for treating gastric neoplasia comprising administering to a patient in need of such treatment a therapeutically effective amount of anti-EGFR agent as described herein, wherein the patient has been determined to contain an EGFR biomarker and not a HER2 biomarker.

Another embodiment provides a method for preventing metastasis of a gastric cancer including, but not limited to, intestinal and diffuse gastric adenocarcinoma, gastrointestinal stromal tumor (GIST), gastrointestinal leiomyosarcoma, gastrointestinal carcinoid and gastrointestinal lymphoma, where the patient has been determined to contain an EGFR

biomarker as defined herein, by administering to a cancer patient a therapeutically effective amount of a herein disclosed anti-EGFR agent (such as an EGFR-specific antibody). In this regard, the method comprises administering an amount of an anti-EGFR agent that, following administration, inhibits, prevents or delays metastasis of a gastric cancer in a statistically significant manner, *i.e.,* relative to an appropriate control as will be known to those skilled in the art. In certain embodiments, the patient has been determined to contain an EGFR biomarker and not a HER2 biomarker. Thus, in one embodiment, the present invention provides methods for preventing metastasis of a gastric cancer where the patient in need thereof is administered with an anti-EGFR agent without an anti-HER2 agent.

As used herein, the terms "treatment," "treating," and the like, refer to administering an agent, or carrying out a procedure (e.g., radiation, a surgical procedure, etc.), for the purposes of obtaining an effect. The effect may be prophylactic in terms of completely or partially preventing a disease, such as cancer, or symptom thereof and/or may be therapeutic in terms of effecting a partial or complete cure for a disease and/or symptoms of the disease. "Treatment," as used herein, covers any treatment of a disease, such as cancer, in a mammal, particularly in a human, and includes: (a) preventing the disease or a symptom of a disease from occurring in a subject which may be predisposed to the disease but has not yet been diagnosed as having it (e.g., including diseases that may be associated with or caused by a primary disease; (b) inhibiting the disease, i.e., arresting its development; and (c) relieving the disease, i.e., causing regression or halting progression of the disease.

As used herein in the context of patient response to an anti-EGFR agent treatment, the terms "responsive", "beneficial response," "beneficial patient response," and "clinically beneficial response," "clinical benefit," and the like, are used interchangeably and refer to favorable patient response to a drug as opposed to unfavorable responses, i.e. nonresponsive to a treatment and/or having adverse events. In individual patients, beneficial response can be expressed in terms of a number of clinical parameters, including loss of detectable tumor (complete response, CR), decrease in tumor size and/or cancer cell number (partial response, PR), tumor growth arrest (stable disease, SD), enhancement of anti-tumor immune response, possibly resulting in regression or rejection of the tumor; relief, to some extent, of one or more symptoms associated with the tumor; increase in the length of survival following treatment; and/or decreased mortality at a given point of time following treatment. Continued increase in tumor size and/or cancer cell number and/or tumor metastasis is indicative of lack of beneficial response to treatment.

In a population the clinical benefit of a drug, i.e. its efficacy can be evaluated on the basis of one or more endpoints. For example, in one embodiment, analysis of overall response rate

(ORR) classifies as responders those patients who experience CR or PR after treatment with drug. Analysis of disease control (DC) classifies as responders those patients who experience CR, PR or SD after treatment with drug.

As is used herein, the term "progression free survival" refers to the time interval from treatment of the patient until the progression of cancer or death of the patient, whichever occurs first.

As used herein, the term "responder" or "responsive" refers to a patient who has been determined to have an EGFR biomarker, and who exhibits a beneficial clinical response following treatment with an anti-EGFR agent.

As used herein, the term "non-responder" or "non-responsive" refers to a patient who does not exhibit a beneficial clinical response following treatment with an EGFR inhibitor. In certain embodiments, a nonresponder may have been determined to have an EGFR biomarker. In other embodiments, a nonresponder may have been determined not to have an EGFR biomarker.

Administration of the anti-EGFR agents described herein, such as anti-EGFR antibodies, in pure form or in an appropriate pharmaceutical composition, can be carried out via any of the accepted modes of administration of agents for serving similar utilities. The pharmaceutical compositions can be prepared by combining an anti-EGFR agent (e.g., antibody) or an anti-EGFR agent-containing composition with an appropriate physiologically acceptable carrier, diluent or excipient, and may be formulated into preparations in solid, semi-solid, liquid or gaseous forms, such as tablets, capsules, powders, granules, ointments, solutions, suppositories, injections, inhalants, gels, microspheres, and aerosols. In addition, other pharmaceutically active ingredients (including other anti-cancer agents as described elsewhere herein) and/or suitable excipients such as salts, buffers and stabilizers may, but need not, be present within the composition. Administration may be achieved by a variety of different routes, including oral, parenteral, nasal, intravenous, intradermal, subcutaneous or topical. Preferred modes of administration depend upon the nature of the condition to be treated or prevented. An amount that, following administration, reduces, inhibits, prevents or delays the progression and/or metastasis of a cancer is considered effective.

In certain embodiments, the amount administered is sufficient to result in tumor regression, as indicated by a statistically significant decrease in the amount of viable tumor, for example, at least a 50% decrease in tumor mass, or by altered (*e.g.*, decreased with statistical significance) scan dimensions. In other embodiments, the amount administered is sufficient to result in clinically relevant reduction in gastric cancer.

The precise dosage and duration of treatment is a function of the disease being treated and may be determined empirically using known testing protocols or by testing the compositions in model systems known in the art and extrapolating therefrom. Controlled clinical trials may also be performed. Dosages may also vary with the severity of the condition to be alleviated. A pharmaceutical composition is generally formulated and administered to exert a therapeutically useful effect while minimizing undesirable side effects. The composition may be administered one time, or may be divided into a number of smaller doses to be administered at intervals of time. For any particular subject, specific dosage regimens may be adjusted over time according to the individual need.

The compositions comprising the anti-EGFR agents as described herein may be administered alone or in combination with other known cancer treatments, such as radiation therapy, chemotherapy, transplantation, immunotherapy, hormone therapy, photodynamic therapy, *etc*. The compositions may also be administered in combination with antibiotics.

Typical routes of administering these and related pharmaceutical compositions thus include, without limitation, oral, topical, transdermal, inhalation, parenteral, sublingual, buccal, rectal, vaginal, and intranasal. The term parenteral as used herein includes subcutaneous injections, intravenous, intramuscular, intrasternal injection or infusion techniques. Pharmaceutical compositions according to certain embodiments of the present invention are formulated so as to allow the active ingredients contained therein to be bioavailable upon administration of the composition to a patient. Compositions that will be administered to a subject or patient may take the form of one or more dosage units, where for example, a tablet may be a single dosage unit, and a container of a herein described anti-EGFR agent in aerosol form may hold a plurality of dosage units. Actual methods of preparing such dosage forms are known, or will be apparent, to those skilled in this art; for example, see *Remington: The Science and Practice of Pharmacy*, 20th Edition (Philadelphia College of Pharmacy and Science, 2000). The composition to be administered will, in any event, contain a therapeutically effective amount of an anti-EGFR agent, e.g., an anti-EGFR antibody as described herein, for treatment of a disease or condition of interest in accordance with teachings herein.

A pharmaceutical composition may be in the form of a solid or liquid. In one embodiment, the carrier(s) are particulate, so that the compositions are, for example, in tablet or powder form. The carrier(s) may be liquid, with the compositions being, for example, an oral oil, injectable liquid or an aerosol, which is useful in, for example, inhalatory administration. When intended for oral administration, the pharmaceutical composition is

preferably in either solid or liquid form, where semi-solid, semi-liquid, suspension and gel forms are included within the forms considered herein as either solid or liquid.

As a solid composition for oral administration, the pharmaceutical composition may be formulated into a powder, granule, compressed tablet, pill, capsule, chewing gum, wafer or the like. Such a solid composition will typically contain one or more inert diluents or edible carriers. In addition, one or more of the following may be present: binders such as carboxymethylcellulose, ethyl cellulose, microcrystalline cellulose, gum tragacanth or gelatin; excipients such as starch, lactose or dextrins, disintegrating agents such as alginic acid, sodium alginate, Primogel, corn starch and the like; lubricants such as magnesium stearate or Sterotex; glidants such as colloidal silicon dioxide; sweetening agents such as sucrose or saccharin; a flavoring agent such as peppermint, methyl salicylate or orange flavoring; and a coloring agent. When the pharmaceutical composition is in the form of a capsule, for example, a gelatin capsule, it may contain, in addition to materials of the above type, a liquid carrier such as polyethylene glycol or oil.

The pharmaceutical composition may be in the form of a liquid, for example, an elixir, syrup, solution, emulsion or suspension. The liquid may be for oral administration or for delivery by injection, as two examples. When intended for oral administration, preferred composition contain, in addition to the present compounds, one or more of a sweetening agent, preservatives, dye/colorant and flavor enhancer. In a composition intended to be administered by injection, one or more of a surfactant, preservative, wetting agent, dispersing agent, suspending agent, buffer, stabilizer and isotonic agent may be included.

The liquid pharmaceutical compositions, whether they be solutions, suspensions or other like form, may include one or more of the following: sterile diluents such as water for injection, saline solution, preferably physiological saline, Ringer's solution, isotonic sodium chloride, fixed oils such as synthetic mono or diglycerides which may serve as the solvent or suspending medium, polyethylene glycols, glycerin, propylene glycol or other solvents; antimicrobial agents such as benzyl alcohol or methyl paraben; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic. In certain embodiments, physiological saline is a preferred composition. An injectable pharmaceutical composition is preferably sterile.

A liquid pharmaceutical composition intended for either parenteral or oral administration should contain an amount of an anti-EGFR agent, such as an EGFR-specific antibody as herein

disclosed such that a suitable dosage will be obtained. Typically, this amount is at least 0.01% of the antibody in the composition. When intended for oral administration, this amount may be varied to be between 0.1 and about 70% of the weight of the composition. Certain oral pharmaceutical compositions contain between about 4% and about 75% of the antibody. In certain embodiments, pharmaceutical compositions and preparations according to the present invention are prepared so that a parenteral dosage unit contains between 0.01 to 10% by weight of the antibody prior to dilution.

The pharmaceutical composition may be intended for topical administration, in which case the carrier may suitably comprise a solution, emulsion, ointment or gel base. The base, for example, may comprise one or more of the following: petrolatum, lanolin, polyethylene glycols, bee wax, mineral oil, diluents such as water and alcohol, and emulsifiers and stabilizers. Thickening agents may be present in a pharmaceutical composition for topical administration. If intended for transdermal administration, the composition may include a transdermal patch or iontophoresis device. The pharmaceutical composition may be intended for rectal administration, in the form, for example, of a suppository, which will melt in the rectum and release the drug. The composition for rectal administration may contain an oleaginous base as a suitable nonirritating excipient. Such bases include, without limitation, lanolin, cocoa butter and polyethylene glycol.

The pharmaceutical composition may include various materials, which modify the physical form of a solid or liquid dosage unit. For example, the composition may include materials that form a coating shell around the active ingredients. The materials that form the coating shell are typically inert, and may be selected from, for example, sugar, shellac, and other enteric coating agents. Alternatively, the active ingredients may be encased in a gelatin capsule. The pharmaceutical composition in solid or liquid form may include an agent that binds to the antibody of the invention and thereby assists in the delivery of the compound. Suitable agents that may act in this capacity include other monoclonal or polyclonal antibodies, one or more proteins or a liposome. The pharmaceutical composition may consist essentially of dosage units that can be administered as an aerosol. The term aerosol is used to denote a variety of systems ranging from those of colloidal nature to systems consisting of pressurized packages. Delivery may be by a liquefied or compressed gas or by a suitable pump system that dispenses the active ingredients. Aerosols may be delivered in single phase, bi-phasic, or tri-phasic systems in order to deliver the active ingredient(s). Delivery of the aerosol includes the necessary container, activators, valves, subcontainers, and the like, which together may form a kit. One of ordinary skill in the art, without undue experimentation may determine preferred aerosols.

The pharmaceutical compositions may be prepared by methodology well known in the pharmaceutical art. For example, a pharmaceutical composition intended to be administered by injection can be prepared by combining a composition that comprises an anti-EGFR agent, such an EGFR-specific antibody as described herein and optionally, one or more of salts, buffers and/or stabilizers, with sterile, distilled water so as to form a solution. A surfactant may be added to facilitate the formation of a homogeneous solution or suspension. Surfactants are compounds that non-covalently interact with an antibody composition so as to facilitate dissolution or homogeneous suspension of the antibody in the aqueous delivery system.

The compositions may be administered in a therapeutically effective amount, which will vary depending upon a variety of factors including the activity of the specific compound (*e.g.*, an EGFR-specific antibody) employed; the metabolic stability and length of action of the compound; the age, body weight, general health, sex, and diet of the patient; the mode and time of administration; the rate of excretion; the drug combination; the severity of the particular disorder or condition; and the subject undergoing therapy.

Compositions comprising an anti-EGFR agent, such as an EGFR-specific antibody, may also be administered simultaneously with, prior to, or after administration of one or more other therapeutic agents. In one embodiment, the present invention provides methods for treating gastric neoplasia comprising administering to a patient in need of such treatment an effective amount of a combination of two or more anti-EGFR agents, wherein the patient has been determined to contain an EGFR biomarker. In this regard, the treatment may comprise an effective amount of a combination of two or more anti-EGFR agents selected from cetuximab, panitumumab, nimotuzumab, antibody 806, Sym004, MM-151, and other anti-EGFR agents described herein.

In one embodiment, the present invention provides methods for treating gastric neoplasia comprising administering to a patient in need of such treatment an effective amount of an anti-EGFR agents, wherein the patient has been determined to contain an EGFR biomarker, in combination with a standard of care treatment for gastric cancer. Standard treatments for gastric cancer include: surgery, radiation therapy, or chemotherapy, or a combination of these treatments. Surgery may comprise surgery to remove the affected part of the stomach and nearby lymph nodes or in certain cases can include gastrectomy. Currently, there is no single standard chemotherapy treatment plan used worldwide for the treatment of gastric cancer. However, chemotherapy treatments may include the combination of at least two drugs, fluorouracil (5-FU, Adrucil) and cisplatin (Platinol). Other drugs similar to 5-FU (such as capecitabine or Xeloda) and similar to cisplatin (such as oxaliplatin or Eloxatin) appear to be

equivalent. Other drugs commonly used include docetaxel (Taxotere), paclitaxel (Taxol), irinotecan (Camptosar), and epirubicin (Ellence).

As would be understood by the skilled person, combination therapy may include administration of a single pharmaceutical dosage formulation which contains an anti-EGFR agent and one or more additional active agents, as well as administration of compositions comprising anti-EGFR agents and each active agent in its own separate pharmaceutical dosage formulation. For example, an anti-EGFR antibody as described herein and the other active agent can be administered to the patient together in a single oral dosage composition such as a tablet or capsule, or each agent administered in separate oral dosage formulations. Similarly, an anti-EGFR antibody as described herein and the other active agent can be administered to the patient together in a single parenteral dosage composition such as in a saline solution or other physiologically acceptable solution, or each agent administered in separate parenteral dosage formulations. Where separate dosage formulations are used, the compositions comprising anti-EGFR agents, such as antibodies, and one or more additional active agents can be administered at essentially the same time, *i.e.,* concurrently, or at separately staggered times, *i.e.,* sequentially and in any order; combination therapy is understood to include all these regimens.

Thus, in certain embodiments, also contemplated is the administration of a composition comprising an anti-EGFR agent, such as an EGFR-specific antibody, in combination with one or more other therapeutic agents. Such therapeutic agents may be accepted in the art as a standard treatment for a particular disease state as described herein, such as a cancer, in particular gastric cancer. Exemplary therapeutic agents contemplated include cytokines, growth factors, steroids, NSAIDs, DMARDs, anti-inflammatories, chemotherapeutics, radiotherapeutics, or other active and ancillary agents.

In certain embodiments, an anti-EGFR agent, such as an anti-EGFR antibody, may be administered to a patient identified as having an EGFR biomarker, in conjunction with any number of chemotherapeutic agents. Examples of chemotherapeutic agents include alkylating agents such as thiotepa and cyclophosphamide (CYTOXAN^{TM}); alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, trietylenephosphoramide, triethylenethiophosphaoramide and trimethylolomelamine; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, ranimustine; antibiotics such as

aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, calicheamicin, carabicin, carminomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin, epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine, 5-FU; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elformithine; elliptinium acetate; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidamine; mitoguazone; mitoxantrone; mopidamol; nitracrine; pentostatin; phenamet; pirarubicin; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK.RTM.; razoxane; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2, 2',2"-trichlorotriethylamine; urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxoids, e.g. paclitaxel (TAXOL®, Bristol-Myers Squibb Oncology, Princeton, N.J.) and docetaxel (TAXOTERE®., Rhne-Poulenc Rorer, Antony, France); chlorambucil; gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; oxaliplatin; Irinotecan; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitomycin C; mitoxantrone; vincristine; vinorelbine; navelbine; novantrone; teniposide; daunomycin; aminopterin; xeloda; ibandronate; CPT-11; topoisomerase inhibitor RFS 2000; difluoromethylomithine (DMFO); retinoic acid derivatives such as Targretin™ (bexarotene), Panretin™ (alitretinoin) ; ONTAK™ (denileukin diftitox) ; esperamicins; capecitabine; and pharmaceutically acceptable salts, acids or derivatives of any of the above. Also included in this definition are anti-hormonal agents that act to regulate or inhibit hormone action on tumors such as anti-estrogens including for example tamoxifen, raloxifene, aromatase inhibiting 4(5)-imidazoles, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and toremifene (Fareston); and anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; and pharmaceutically acceptable salts, acids or derivatives of any of the above.

A variety of other therapeutic agents may be used in conjunction with the anti-EGFR agents described herein. In one embodiment, a patient identified as having an EGFR biomarker is administered an anti-EGFR agent with an anti-inflammatory agent. Anti-inflammatory agents or drugs include, but are not limited to, steroids and glucocorticoids (including betamethasone, budesonide, dexamethasone, hydrocortisone acetate, hydrocortisone, hydrocortisone, methylprednisolone, prednisolone, prednisone, triamcinolone), nonsteroidal anti-inflammatory drugs (NSAIDS) including aspirin, ibuprofen, naproxen, methotrexate, sulfasalazine, leflunomide, anti-TNF medications, cyclophosphamide and mycophenolate.

Exemplary NSAIDs are chosen from the group consisting of ibuprofen, naproxen, naproxen sodium, Cox-2 inhibitors such as VIOXX® (rofecoxib) and CELEBREX® (celecoxib), and sialylates. Exemplary analgesics are chosen from the group consisting of acetaminophen, oxycodone, tramadol of proporxyphene hydrochloride. Exemplary glucocorticoids are chosen from the group consisting of cortisone, dexamethasone, hydrocortisone, methylprednisolone, prednisolone, or prednisone. Exemplary biological response modifiers include molecules directed against cell surface markers (*e.g.*, CD4, CD5, etc.), cytokine inhibitors, such as the TNF antagonists (*e.g.*, etanercept (ENBREL®), adalimumab (HUMIRA®) and infliximab (REMICADE®)), chemokine inhibitors and adhesion molecule inhibitors. The biological response modifiers include monoclonal antibodies as well as recombinant forms of molecules. Exemplary DMARDs include azathioprine, cyclophosphamide, cyclosporine, methotrexate, penicillamine, leflunomide, sulfasalazine, hydroxychloroquine, Gold (oral (auranofin) and intramuscular) and minocycline.

The compositions comprising anti-EGFR agents described herein, such as EGFR-specific antibodies, may be prepared with carriers that protect the antibody against rapid elimination from the body, such as time release formulations or coatings. Such carriers include controlled release formulations, such as, but not limited to, implants and microencapsulated delivery systems, and biodegradable, biocompatible polymers, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, polyorthoesters, polylactic acid and others known to those of ordinary skill in the art.

EXAMPLE 1

EGFR GENE AMPLIFICATION AND OVEREXPRESSION ARE PREDICTIVE BIOMARKERS FOR RESPONSE TO CETUXIMAB TREATMENT IN GASTRIC ADENOCARCINOMA

This example describes the results of a random cetuximab trial that was conducted in a cohort of fully molecularly annotated (expression and mutation profiling) naïve Asian gastric adenocarcinoma (GC-ADC) patient derived xenografts (PDX) to identify responders and non-responders, followed by discovery of predictive biomarker.

INTRODUCTION AND SUMMARY

Patient derived xenograft (PDX), without any *in vitro* manipulation, mirrors patients' histopathological and genetic profiles[9-14]. It has improved predictive power as preclinical cancer models, and enables the true individualized therapy and discovery of predictive biomarkers. The models are also called as "Avatar mice" or "xenopatients", and the large collection of them can potentially reflect the diversity of tumors in patients. Due to the extensive diversity of cancer patient populations, successfulness of the clinical trial largely relies on the inclusion of the likely responders who express the intended target and have the correct genetic profiles, and exclusion of non-responders. These models thus can be used to test investigational targeted drugs by modeling clinical trial format.

A large collection of gastric adenocarcinoma (GC-ADC) PDX called GC-ADC HuPrime® models (similar NSCLC-HuPrime was previously described)[15] were established. A cohort of 19 GC HuPrime® were tested to evaluate the tumor response to cetuximab and it was found that a subset of the GC-ADC with EGFR gene amplification and over-expression responded well to cetuximab. This observation suggested that EGFR gene copy and/or over expression could serve as a potential practical single biomarker to predict patient response to cetuximab, a situation similar to that of HER2/Herceptin® scenario in GC-ADC. A prospective clinical study using EGFR gene copy number as a patient selection criterion is warranted to further confirm the observation and could lead to ultimate regulatory approval of cetuximab as GC-ADC treatment.

Findings: Among the 19 PDX GC models tested, 4 models (21%) responded to cetuximab (defined by $^{\Delta T}/_{\Delta C} > 20\%$). The expression profiling and copy number variation analysis revealed that all the 4 responders have amplified EGFR gene and/or corresponding

high EGFR expression, in contrast to the 15 non-responders ($^{\Delta T}/_{\Delta C}$ > 20%). This result is consistent with an observation made in EXTRA, a phase II trial where all 4 patients with EGFR amplification are responders. These results suggested that EGFR gene amplification and/or high expression are the key oncogenic driver for this subset of GC-ADC, which is not typical for NSCLC and CRC.

Interpretation: EGFR gene amplification and over-expression can serve as a single predictive biomarker for cetuximab response in GC-ADC.

MATERIALS AND METHODS

Patient samples, engraftment, cetuximab treatment experiments and model cahracterizations. Surgically removed fresh GC tumor tissues were used to subcutaneously engraft into 6-8 week old, female Balb/c nude mice (Beijing HFK Bioscience Co. Ltd., Beijing, China) immediately after surgery per procedures described previously[10]. The established tumor models were serially re-engrafted for passage and conducting studies. Access and use of the patient samples were approved by the Ethic Committee of Beijing Cancer Hospital along with the informed consents from the patients. All procedures were performed under a sterilization conditions in Crown Bioscience SFP facility. All the experimental animals that involved in our studies were conducted in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Committee on the Ethics of Animal Experiments of Crown Bioscience, Inc. (Crown Bioscience IACUC Committee).

The procedure for evaluating tumor response to cetuximab in PDX models was detailed described previously [15, 17]. The tumor growth was monitored twice weekly, and $\%^{\Delta T}/_{\Delta C}$ value were calculated for assessing tumor response to cetuximab (Δ T = tumor volume change in the treatment group and Δ C = tumor volume change in control group).

Model characterizations, including expression profiling using Affy U219, SNP6, IHC, qPCR, oncogene mutation analysis, have all been detailed described previously [15, 17].

CFISH analysis. FISH (dual-color) procedures were performed using Abbott PathVysion EGFR DNA Probe Kit per the manufacturer's protocol (Abbott, Downers Grove, IL). The Spectrum Orange fluorophore-labeled EGFR (303 kb) are specific for the EGFR gene locus on chromosome 7p12, and the Spectrum Green fluorophore-labeled chromosome enumerator probe (5.4 kb) targeted to the α-satellite DNA sequence located at the centromeric region of chromosome 7 (CEP7; 7p11.1–q11.1). Briefly, the FFPE sections were deparaffinized followed

by digestion with pepsin and hybridization. The treated slide were denatured and hybridized with probes, followed by counterstaining with 15 μL DAPI/anti-fade solution and scanning using OLYMPUS BX51 fluorescent microscope (OLYMPUS BX51, Japan) equipped with single band pass filter set to detect DAPI, Rhodamine (7p12) and FITC (chromosome 7) at 1000×.

Statistical Analysis. The data of tumor volume were evaluated using Student's t- test for two comparisons, and one-way ANOVA test for multiple comparisons. All data were analyzed using SPSS 16.0. $P < 0.05$ was considered to be statistically significant. Fisher's Exact Test was used to access the response difference between the EGFR amplified models and non-amplified models (see quantitativeskills.com/sisa/statistics/fisher.htm).

RESULTS

A subset of GC HuPrime® models responds to cetuximab. We set out to test a randomly selected cohort of GC-ADC HuPrime® models by conducting a clinical trial-like study for assessing potential cetuximab activities. These models were first established by transplanting surgically removed tumor tissues from GC-ADC patients into immunocompromised Balb/c nude mice via subcutaneous inoculation. The original patient diagnosis and description are summarized in Table 2. Secondly, 19 randomly selected models were subjected to once-weekly cetuximab treatment for 2 weeks (1 mg/mouse or 50mg/kg). The models can be divided into two categories according to the activities: 4/19 or 21% of GC-ADC HuPrime® responded (nearly complete responsive with $^{\Delta T}/_{\Delta C} < 0\%$) to cetuximab treatment; 15/19 or 79% did not (partial or complete resistant with $^{\Delta T}/_{\Delta C} > 30\%$). The representative tumor growth inhibition curves of these two categories are shown in Figure 1A. The quantification of tumor response as measured by $^{\Delta T}/_{\Delta C}$ values are summarized in Table 1. These rather distinct responses seen in GC-ADC models are somewhat in contrast to the responses that were observed in CRC[17] and NSCLC[15] HuPrime® models. Nevertheless, our data suggested that a subset of GC can potentially benefit from cetuximab. It was also worth noting that the 21% responders observed in this GC-ADC study may not necessarily reflect true percentage of potential responders in the GC-ADC patient population due to the less than 100% take-rate of the engraftment (usually between 30 to 50% in our hand) and the possibility of the biased take-rate among the responders or non-responders.

Next it was investigated whether EGFR signaling was indeed inactivated in these tumors by cetuximab by performing a single dose pharmacodynamic analysis. The tumor bearing animals were first treated with cetuximab and the tumors were harvested at time points of 6, 24

and 72 hours post treatment. As an example, GA0022 tumor tissues were analyzed post-treatment for pERK (a downstream effector of EGFR signaling) by immunochemistry analysis (IHC). The results clearly demonstrated the reduction of pERK in tumors (Figure 1B), correlating to the observed antitumor activity in the same model.

The factors governing the response to cetuximab in CRC seem to contribute significantly less to the response in GC-ADC. In order to discover the predictive biomarker(s) for the observed response GC-ADC, this cohort of models was systemically profiled for expression and gene copy number, as well as genetic mutations, of some common oncogenes. Activating mutations, including those of KRAS, BRAF (V600E), c-MET, EGFR, AKT and PI3KC have been associated with resistance to cetuximab in CRC patients[15, 17-21]. Thus, these models were first analyzed by hot-spot mutation sequencing of these oncogenes. Interestingly, few of the tested models, regardless responders or non-responders, showed any mutations with exception of GA0139 containing G13D and GA044 containing 327-329 deletion in PIK3CA (Table 1). Therefore, the non-response of GC HuPrime® to cetuximab apparently cannot be simply attributed to these oncogene mutations.

In a separate study earlier, it was observed that CRC HuPrime® response to cetuximab is dependent on RAS pathway signaling, or associated with the low Loboda-RAS pathway signature scores [17, 22]. It would therefore be interesting to know whether RAS signaling pathway or Loboda scores have similarly impacts on the GC HuPrime® response. To our surprise, Loboda RAS pathway signature scores were found to have little correlation to the tumor response (see Figure 3), suggesting that the activation status of RAS pathway contribute significantly less to the response or resistance to cetuximab in GC-ADC than those seen in CRC.

EGFR gene amplification seems to be an oncogenic driver in a subset of GC-ADC HuPrime® that respond to cetuximab. However, on the other hand, Affymetix HG-U219 GeneChip analysis revealed that all of the 4 responders expressed high levels of EGFR (mRNA levels), and that all the 15 poor responders are associated with lower levels of expression (Figure 2A and 2C, Table 1). This observation seems plausible since the higher activity of EGFR via higher expression could drive the oncogenic transformation in these tumors and the inactivation by cetuximab could thus inhibit tumor growth.

Furthermore, it was investigated what genetic defects behind the higher EGFR expression by examining gene copy using Affymetrix SNP6 analysis (Figure 2B). Interestingly, all the responders have the corresponding EGFR gene amplification (Figure 2A and 2B) (Table 1,

Table 3) (P-value < 0.00026 per Fisher's Exact Test). This near-perfect correlation suggested that EGFR gene amplification is likely the key oncogenic driver in the responders and a potential practical single biomarker for predicting response to cetuximab in GC-ADC. To further confirm the gene amplification, we also performed EGFR-fluorescence in situ hybridization, or FISH, a clinically practical assay, to assess EGFR gene amplification status of all these models. The FISH data indeed confirm the observations seen by SNP6. The GA152 FISH analysis as an example, clearly indicated EGFR amplification. The clinically accepted FISH procedure enables the development of companion diagnosis for cetuximab treatment in the clinic for cetuximab GC-ADC treatment.

Table 1. Profiles of GC HuPrimer® Model Panels

model ID	T/C	P value	EGFR | Exon 18;19;20;21	k-RAS | Exon 2;3;4	BRAF | Exon 15	c-MET Exon 14; 16;17;18;19;21	PIK3CA | Exon1;9;20	EGFR SNP6 PICNIC	FGFR2 SNP6	HER2 SNP6	EGFR U219 intensity
GA0114	1.62		WT	WT	WT	WT	WT	6	15	5	2.9
GA2140	1.32										
GA0119	0.93	0.69	WT	WT	WT	WT	WT	5	3	5	3.6
GA0139	0.91	0.91	WT	G13D	WT	WT	WT	5	5	15	2.9
GA0138	0.90	0.62	WT	WT	WT	WT	WT	5	5	5	2.3
GA0037	0.88	0.82	WT	WT	WT	WT	WT	5	5	6	2.5
GA0033	0.81	0.13	WT	WT	WT	WT	WT	5	15	4	2.4
GA0023	0.78	0.26	WT	WT	WT	WT	WT	4	4	5	2.5
GA0080	0.75	0.43	WT	WT	WT	WT	WT	6	6	4	2.8
GA0151	0.7		WT	WT	WT	WT	WT				4.0
GA0044	0.69	0.23	WT	WT	WT	WT	deletion 327-329	5	6	4	3.1
GA0098	0.55		WT	WT	WT	WT	G545Y	5	4	5	4.2
GA0060	0.45		WT	WT	WT	WT	WT	5	4	15	4.2
GA0025	0.31	0.014	WT	WT	WT	WT	WT				3.8
GA0022	-0.071	0.001	WT	WT	WT	WT	WT	7	5	4	6.5
GA046	-0.072		WT	WT	WT	WT	WT	7	4	3	6.9
GA0075	-0.098	0.000	WT	WT	WT	WT	WT	8	4	5	5.8
GA0152	-0.121	0.013	WT	WT	WT	WT	WT	15	3	5	10.5

Shading denotes responder

Table 2. GC Patient Diagnosis and Pathology, and The Corresponding Model Pathology Confirmation

ID	Gender	Original hospital pathology report
GA0114	F	Gastric adenocarcinoma.
GA2140	M	Moderately to poorly differentiated adenocarcinoma of gastric body to antrum with necrosis, tuomor mass: 12×11cm. The tumor invades through gastric wall. Regional LN: NO.1 LN (0/6), NO.2 LN (0/1), NO.3 LN (0/6), NO.5 LN (0/2), NO.6 LN (0/2), NO.7, 8, 9 LN (0/5), NO.4d LN (0/4).

ID	Gender	Original hospital pathology report
		IHC results: CD44(+), cMet(+), EGFR(+), HER2(1+), Ki-67(+＞75%), MMP7(+), P170(+), P27(+25～50%), P53(+＜25%), TOPOII(+50～75%).
GA0119	M	Adenocarcinoma of gastric cardia with mucinous adenocarcinoma and some signet-ring cell carcinoma, ulcerative type,poorly differentiated, tumor mass: 6.5cm×7cm×2cm, invaded serosa and nerve plexus. Malignant cells adjacent to inferior stump. Regional LN: LN of lesser curvature (6/6), left gastric LN (5/6), inferior pyloric LN (1/1).
GA0139	M	Adenocarcinoma of gastric cardia with papillary adenocarcinoma, ulcerative type, moderately differentiated, tumor mass: 6.5cm×7cm×1cm, invaded adipose tissue external serosa. No malignant cells adjacent to both stump. Regional LN: LN of lesser curvature (0/11), paraesophageal LN (0/4).
GA0138	M	Adenocarcinoma of stomach, invasive, ulcerative type, poorly differentiated, tumor mass: 11cm×9cm×3cm, invaded deep muscular layer, no visible malignant cells adjacent to both stump. Regional LN: LN of lesser curvature (1/5).
GA0037	M	Adenocarcinoma of gastric cardia, ulcerative type,poorly differentiated, tumor mass: 4cm×4cm×2.5cm, invaded serosa. No visible malignant cells adjacent to both stump. Regional LN: LN of lesser curvature (0/9).
GA0033	M	Adenocarcinoma of gastric cardia, ulcerative type, poorly differentiated, tumor mass: 9cm×6cm×1.5cm, invaded serosa, and accompanied with necrosis and a few lymphocytes deposition. No visible malignant cells adjacent to both stump. Regional LN: cardial LN (0/5), left gastric LN (0/7).
GA0023	F	Adenocarcinoma, ulcerative type, poorly differenciated.
GA0080	M	1. Adenocarcinoma of gastric cardia, invasive type, moderately-poorly differentiated, tumor mass: 8cm×6cm×1.5cm, invaded serosa. No visible malignant cells adjacent to both stump. Regional LN: Regional LN: cardial LN (1/5), left gastric LN (2/2). 2. Hepatitis, type B
GA0044	M	Metastatic adenocarcinoma derived from liver, moderately-poorly differentiated. The patient had been done gastric cancer surgery 2 years ago.
GA0025	F	Signet-ring cell carcinoma of gastric greater curvature, parts are mucinous adenocarcinoma, protruded type, tumor mass: 3.5cm×3.5cm×2cm, invaded through gastric wall. Regional LN: LN surround celiac trunk (1/1). IHC results: HER-1(-)，HER-2(-)，p53(-)，p170(-)，Ki-67(+ 25～50%)，VEGF(+)，Top-Ⅱα(+<25%)，p16(-).
GA0022	M	Adenocarcinoma of gastic cardia, ulcerative type, moderately-poorly differentiated, tumor mass: 5cm×4cm×2.5cm, invaded through gastric wall to serosa. No visible malignant cells adjacent to both stump. Regional LN: left gastric LN (5/9), LN of inferior parapulmonary vein (0/6).
GA0046	M	Adenocarcinoma derived from juncture of stomach and esophagus in lesser gastric curvature, protruded ulcerative type, poorly differentiated, tumor mass: 6cm×5cm×1.5cm. Malignant cells invade through gastric wall and esophagus. Regional LN: LN of lesser curvature (3/14). IHC results: HER-1(+), HER-2(-), P53(+ 25～50%), P170(-), Ki-67(+ 25～50, VEGF(++). Top-Ⅱα(+ about 10%). p16 (-).
GA0075	F	Adenocarcinoma of cardia and fundus of stomach, infiltrating ulcerative type, moderately-poorly differentiated, tumor mass: 6.5cm×5cm×1.5cm, invaded serosa. No malignant cells adjacent to both stump. Regional LN: LN of lesser curvature (1/6), cardial LN (0/4), LN of greater curvature (0/4), LN of para-inferior pulmonary vein (0/3).
GAF152	F	Poorly differentiated adenocarcinoma (P0, P5).

Shading denotes responder

Table 3. EGFR copy number determined by different methods.

model ID	EGFR SNP6 PICNIC	EGFR qPCR	EGFR FISH
GA0114	6		N/A
GA2140			N/A
GA0119	5		0.99
GA0139	5	1.06	
GA0138	5	0.96	
GA0037	5	1.50	
GA0033	5	+0.64	
GA0023	4	0.00	
GA0080	6	0.94	
GA0151		N/A	
GA0044	5	N/A	
GA0098	5	N/A	
GA0060	5	N/A	+
GA0025	N/A	0.32	
GA0022	7	1.21	
GA046	7	N/A	
GA0075	8	2.29	
GA0152	15	8.22	

Shading denotes responder

DISCUSSION

This study clearly demonstrated that a subset of GC-ADC HuPrime® models with EGFR gene amplification and over expression responded to cetuximab (4/4). Consistent with this, 4 out of 4 EGFR amplified patients in EXTRA phase II trial were responders to the combination treatment of cetuximab and cisplatin/capecitabine (NCT00477711)[16], supporting the assumed predicative power of PDX models and our hypothesis of EGFR-predictive biomarker for cetuximab GC-ADC treatment. This assumption can also be further confirmed by retrospectively examining recently completed phase III trial in gastro-esophageal carcinoma, EXPAND, when the data becomes available. FISH for EGFR gene amplification can routinely be performed in the clinic setting, thus enabling this to be used as a companion diagnostic. Therefore, such a prospective trial can readily be implemented. The ultimate approval would offer one more targeted therapy option for GC-ADC patients, in addition to trastuzumab (Herceptin®) for patients with HER2 gene amplification[3].

The heavy dependency on EGFR gene amplification seen in GC HuPrime® response to cetuximab was however not observed in NSCLC (Yang et al., in submission) and CRC [17] HuPrime®, suggesting drastic difference among different cancer types. Very interestingly,

this EGFR dependency in GC-ADC somehow mirrors the Her2 dependency for trastuzumab response in GC-ADC. This similarity provides a clear feasible development path for a companion diagnostic for cetuximab treatment of GC, just as the companion diagnostic for trastuzumab.

Another interesting observation is that the models with EGFR over expression (also gene amplifications) do not have HER2 over-expression (or gene amplification), and vice versa, i.e. no over-expression (gene amplification) of both of these genes was observed in a single model (see Figure 4A and 4B). This observation indicates that amplification/overexpression of both genes does not drive oncogenesis. Thus, it is reasonable that there would be no reason to use the combination of cetuximab and trastuzumab for GC patient treatment.

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CLAIMS

What is claimed is:

1. A method for treating gastric neoplasia comprising administering to a patient in need of such treatment an effective amount of anti-EGFR agent, wherein the patient has been determined to contain an EGFR biomarker.

2. The method of claim 1, wherein the gastric neoplasia is gastric adenocarcinoma.

3. The method of claim 1, wherein the anti-EGFR agent is an anti-EGFR antibody.

4. The method of claim 1, wherein the anti-EGFR agent is cetuximab, panitumumab, nimotuzumab, antibody 806, Sym004, or MM-151.

5. The method of claim 1, wherein the anti-EGFR agent is a combination of two or more anti-EGFR agents.

6. The method of claim 1 further comprising administering the anti-EGFR agent in combination with the standard treatment for gastric neoplasia.

7. The method of claim 1 further comprising administering the anti-EGFR agent in combination with chemotherapy or radiation therapy.

8. The method of claim 1 further comprising administering the anti-EGFR agent in combination with cisplatin and capecitabine, or 5-fluorouracil, oxaliplatin, Irinotecan, docetaxel, paclitaxel, doxorubicin mitomycin C, etoposide, gemcitabine, carboplatin.

9. The method of claim 1, wherein the EGFR biomarker is EGFR gene amplification or EGFR overexpression.

10. The method of claim 9, wherein the EGFR gene amplification comprises an EGFR gene copy number that is higher than a predetermined number.

11. The method of claim 9, wherein the EGFR overexpression comprises a level of EGFR RNA, protein, or activity that is higher than a predetermined level.

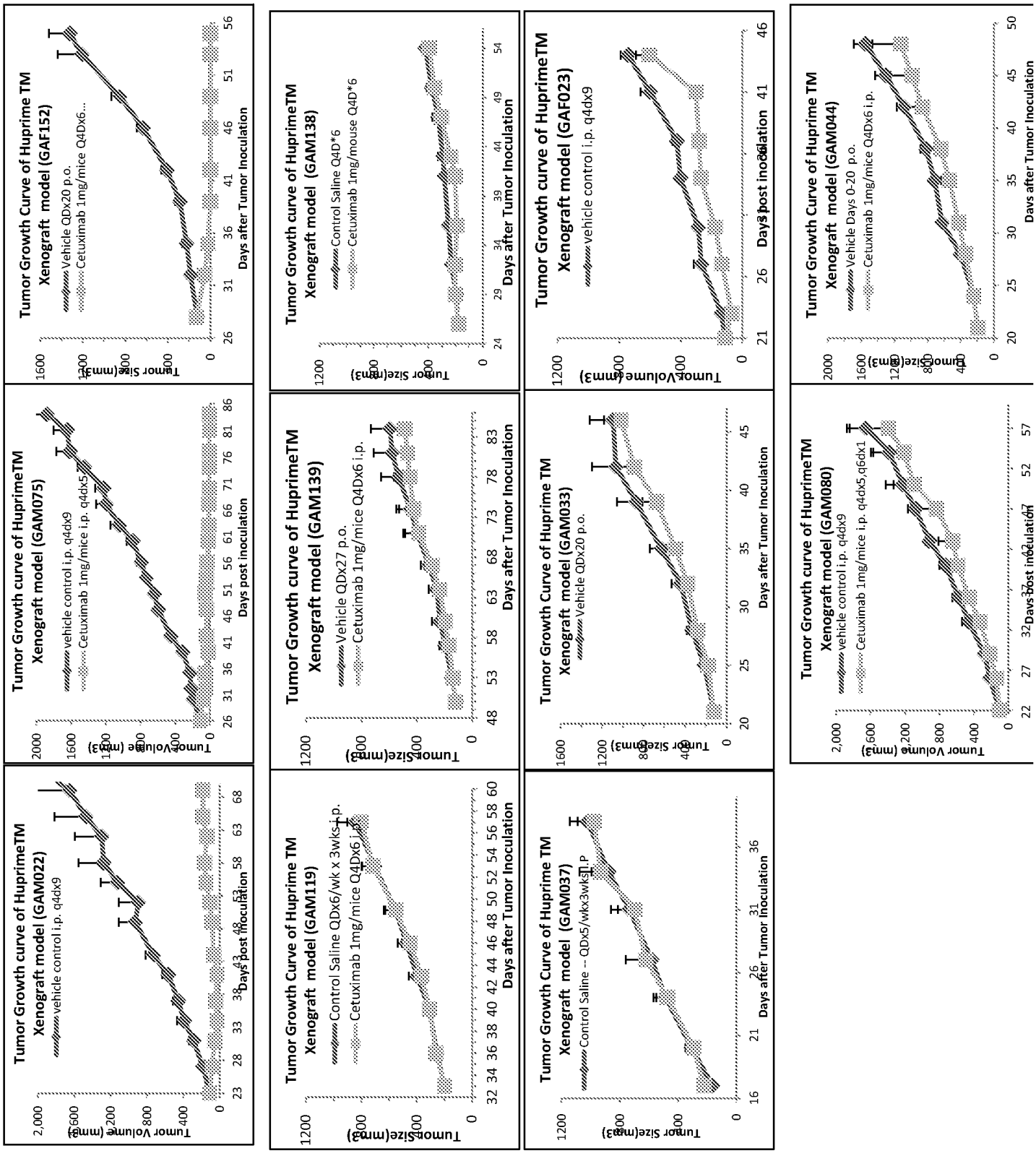
12. The method of claim 1, wherein the patient has been determined to contain an EGFR biomarker and not a HER2 biomarker.

13. The method of claim 12, wherein the patient is administered with an anti-EGFR agent without an anti-HER2 agent.

14. A method for determining whether a patient is suitable for an anti-EGFR treatment comprising detecting in a sample of the patient the presence or absence of an EGFR biomarker, wherein the presence of an EGFR biomarker is indicative that the patient is suitable for the anti-EGFR treatment.

15. A method for providing a lab service comprising, receiving a sample of a patient with gastric neoplasia, conducting a test to detect in the sample the presence or absence of an EGFR biomarker, and providing the test result to the healthcare provider of the patient.

16. A kit comprising a reagent suitable for detection of an EGFR biomarker and an instruction for using the EGFR biomarker for the treatment of gastric neoplasia according to the method of claim 1.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Days post inoculation
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Tumor Volume (mm3)
Days post inoculation
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Days after Tumor Inoculation
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Days after Tumor Inoculation
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Days post inoculation
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Tumor Volume (mm3)
Days post inoculation
Tumor Growth Curve of Huprime TM Xenograft model (GAM044)
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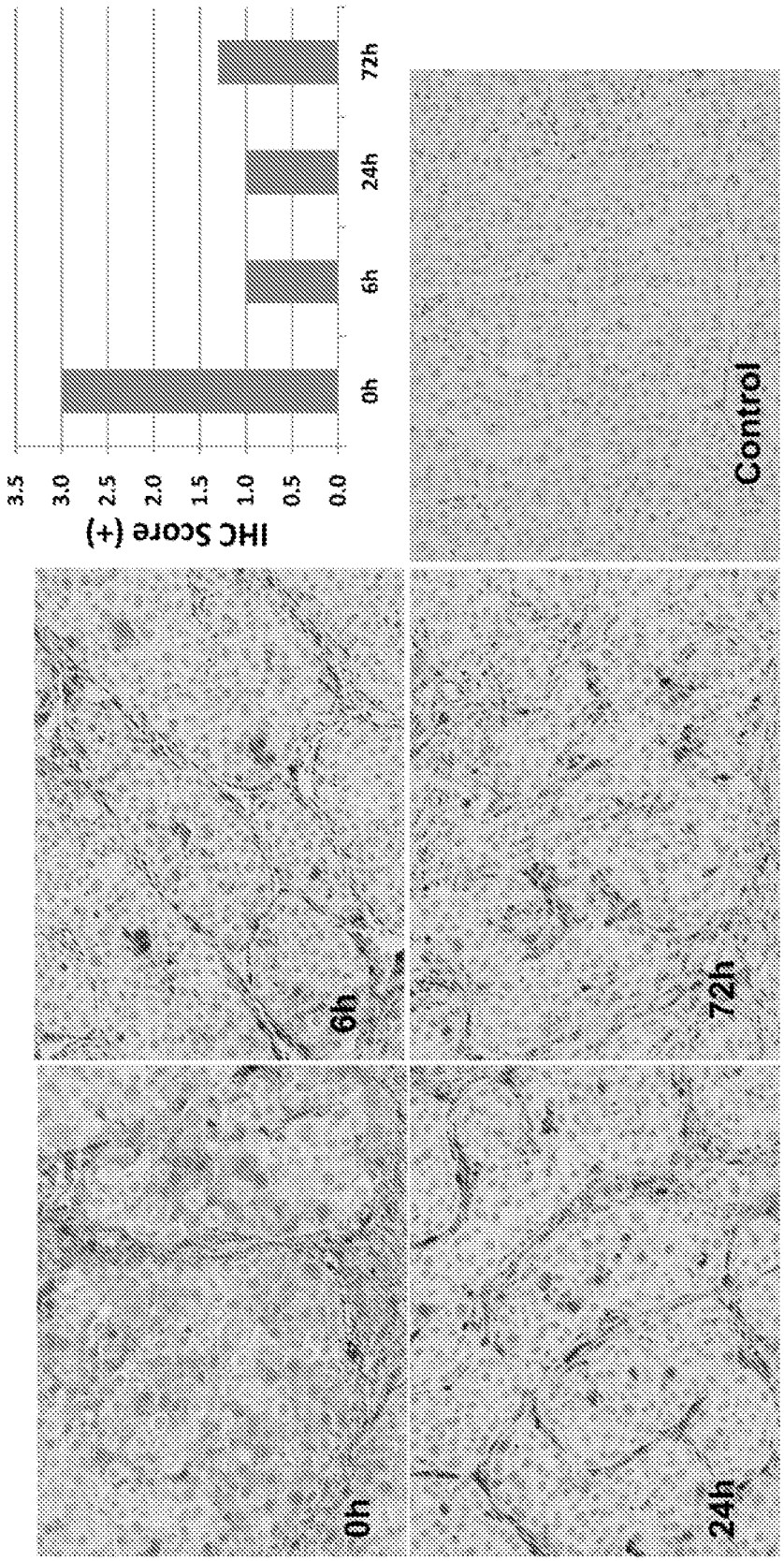
Fig. 1A.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0h
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24h
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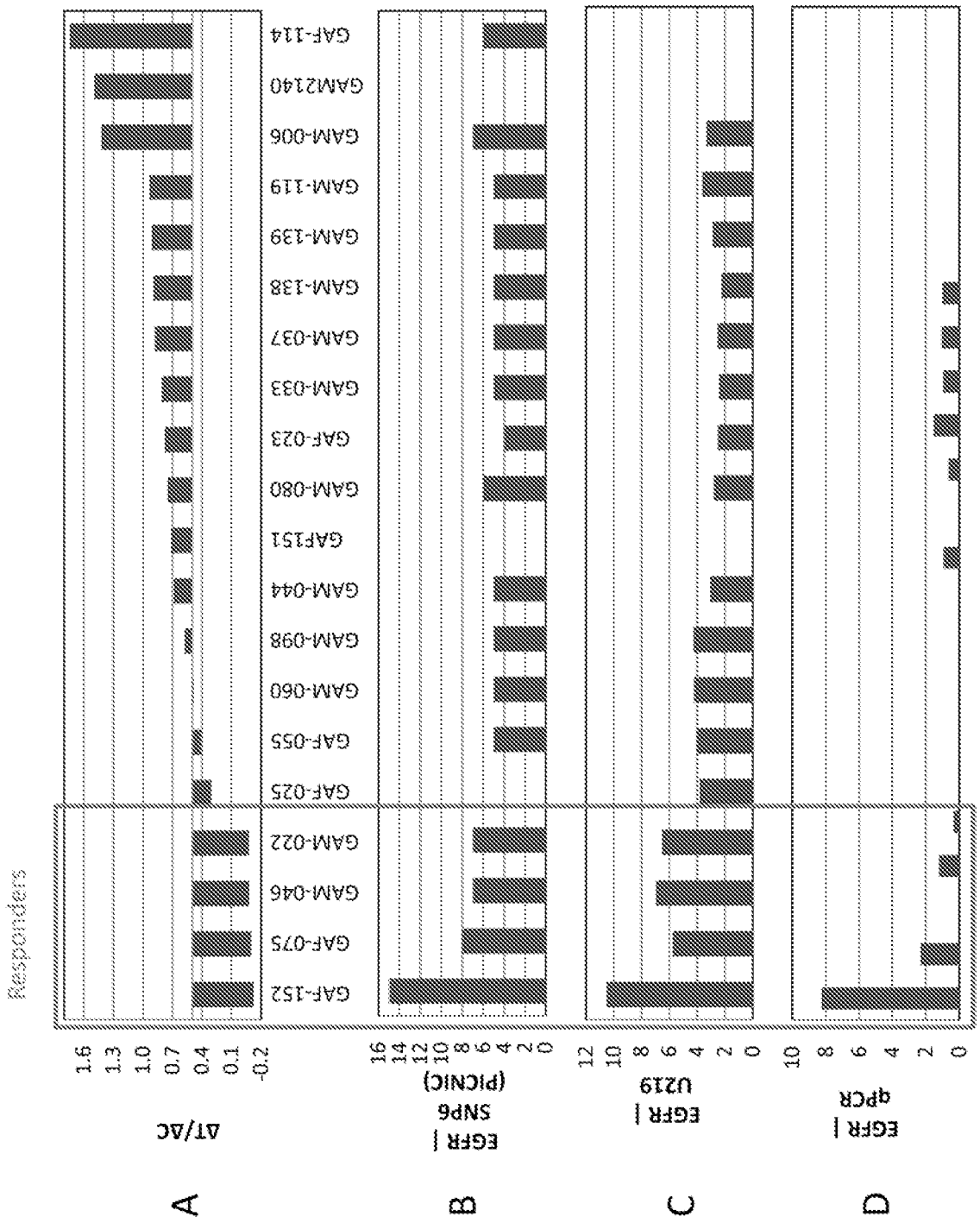
Fig. 1B.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B
EGFR | SNP6 (pICNIC)
16
14
12
10
8
6
4
2
0
C
EGFR | U219
12
10
8
6
4
2
0
D
EGFR | qPCR
10
8
6
4
2
0
Responders
GAF-152
GAF-075
GAM-046
GAM-022
GAF-025
GAF-055
GAM-060
GAM-098
GAM-044
GAF151
GAM-080
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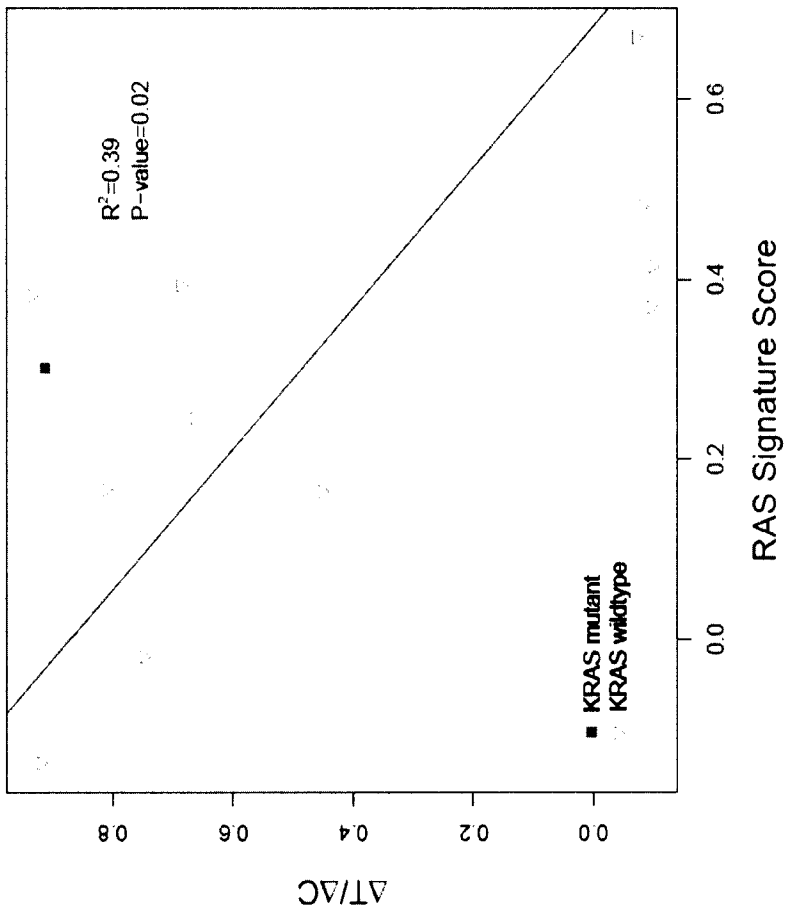
Fig. 2.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KRAS wildtype
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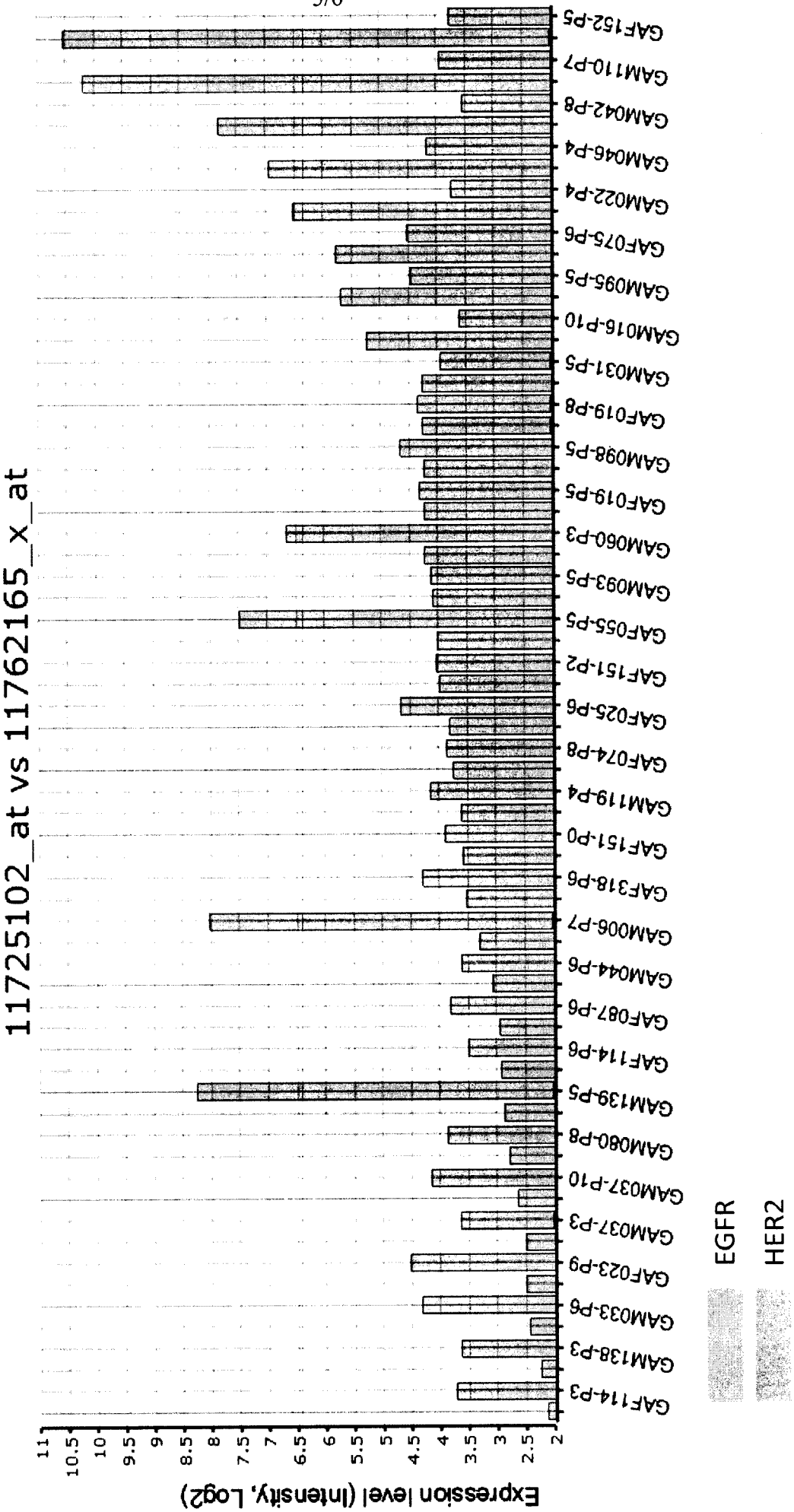
0.4
0.6
0.8

Fig. 3.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8.5
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7
6.5
6
5.5
5
4.5
4
3.5
3
2.5
2
GAF152-P5
GAM110-P7
GAM042-P8
GAM046-P4
GAM022-P4
GAF075-P6
GAM095-P5
GAM016-P10
GAM031-P5
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GAF151-P0
GAF318-P6
GAM006-P7
GAM044-P6
GAF087-P6
GAF114-P6
GAM139-P5
GAM080-P8
GAM037-P10
GAM037-P3
GAF023-P9
GAM033-P6
GAM138-P3
GAF114-P3
EGFR
HER2

Figure 4A

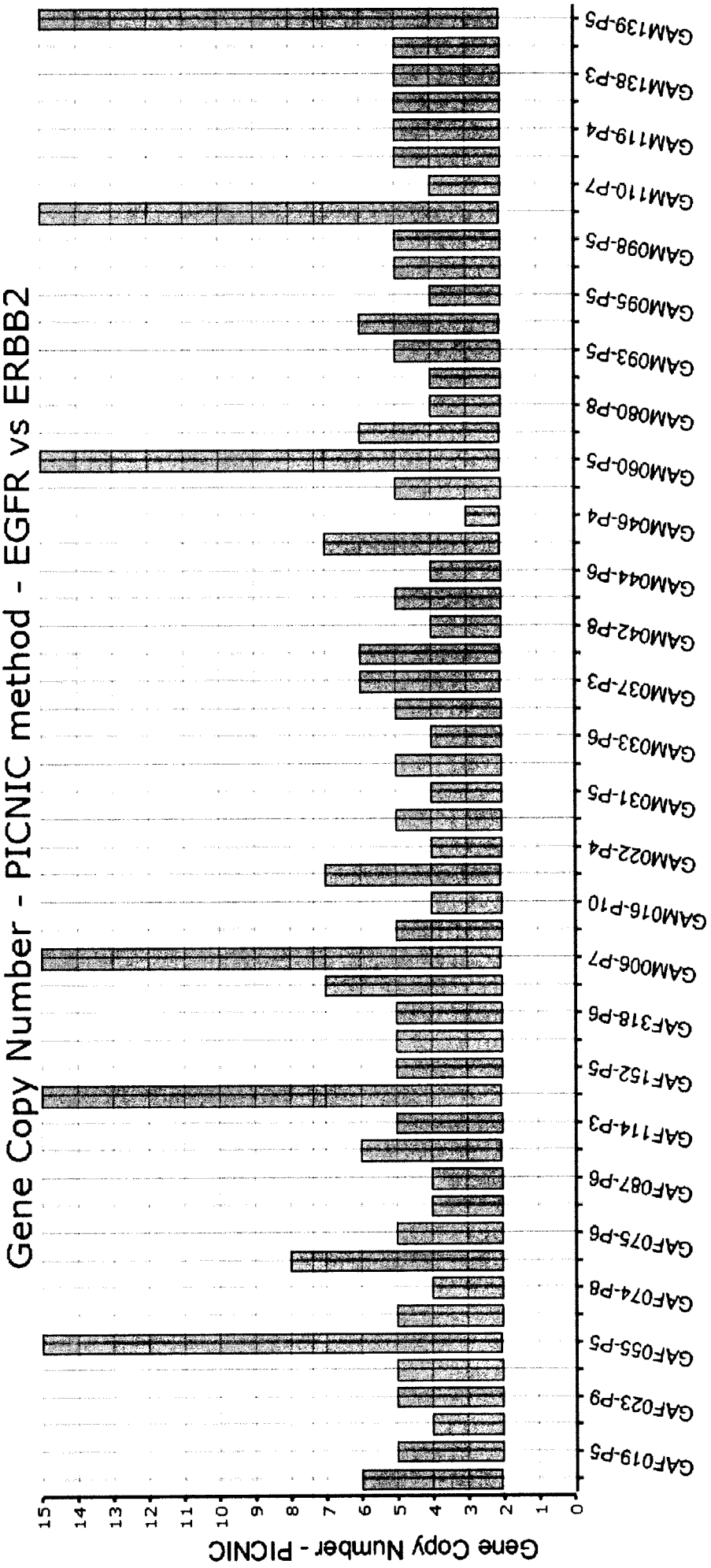
Gene Copy Number - PICNIC method - EGFR vs ERBB2
GAM139-P5
GAM138-P3
GAM119-P4
GAM110-P7
GAM098-P5
GAM095-P5
GAM093-P5
GAM080-P8
GAM060-P5
GAM046-P4
GAM044-P6
GAM042-P8
GAM037-P3
GAM033-P6
GAM031-P5
GAM022-P4
GAM016-P10
GAM006-P7
GAF318-P6
GAF152-P5
GAF114-P3
GAF087-P6
GAF075-P6
GAF074-P8
GAF055-P5
GAF023-P9
GAF019-P5
15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0
Gene Copy Number - PICNIC
EGFR
HER2

Figure 4B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2013/072638

A. CLASSIFICATION OF SUBJECT MATTER

See the extra sheet

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: A61K, A61P

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

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

CNPAT, CNKI, DWPI, EPOABS, CPRSABS, CNABS, CNTXT, VEN, CJFD, ISI Web of Knowledge, PUBMED: EGFR, ERBB, cetuximab, gastric carcinoma, amplification, adenocarcinoma, neoplasia

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ZHAO Ya-juan et al., Expression of EGFR and its clinic signigicance in gastric adenocarcinoma tissues, CHIN J CANCER PREV TREAT, February 2010, Vol.17, No.4, pages 273-274, see page 274.	1-6, 9-10, 12-16
Y		7, 8
X	M A Kim et al., EGFR in gastric carcinomas: prognostic significance of protein overexpression and high gene copy number, Histopathology 2008, 52: 738-746, see page 739, 743, 745.	1-6, 9, 11-16
Y		7, 8
Y	C Pinto et al., Phase II study of cetuximab in combination with cisplatin and docetaxel in patients with untreated advanced gastric or gastro-oesophageal junction adenocarcinoma (DOCETUX study), British Journal of Cancer, 22 September 2009, 101: 1261-1268, see the abstract.	7, 8

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim (S) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 December 2013 (12.12.2013)	**19 Dec. 2013 (19.12.2013)**
Name and mailing address of the ISA/CN The State Intellectual Property Office, the P.R.China 6 Xitucheng Rd., Jimen Bridge, Haidian District, Beijing, China 100088 Facsimile No. 86-10-62019451	Authorized officer ZHANG, Yanxia Telephone No. (86-10)62411049

Form PCT/ISA /210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2013/072638

Box No. 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. ☒ Claims Nos.: 1-13
because they relate to subject matter not required to be searched by this Authority, namely:
Claims 1-13 relate to subject matters of methods for treating gastric neoplasia, belonging to methods of treatment for living human or animal bodies which are not required to be searched by PCT Rule 39.1(iv).
Since the subject matter of claims 1-13 could be drafted as the use of an effective amount of anti-EFGR agent for the manufacturing of a medicament for the treatment of gastric neoplasia, so claims 1-13 are searched based on the anticipated subject matters.

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 No. 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 fees, this Authority did not invite payment of 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 and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2013/072638

A. CLASSIFICATION OF SUBJECT MATTER

A61K 39/395 (2006.01) i

A61P 35/00 (2006.01) i