



- (51) International Patent Classification:
C12Q 1/68 (2006.01)
- (21) International Application Number:
PCT/EP2012/055962
- (22) International Filing Date:
2 April 2012 (02.04.2012)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
11160641.4 31 March 2011 (31.03.2011) EP
11161244.6 5 April 2011 (05.04.2011) EP
- (71) Applicant (for all designated States except US): **SIGNATURE DIAGNOSTICS AG** [DE/DE]; Hermannswerder 20a, 14473 Potsdam (DE).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): **PECHANSKA, Paulina** [PL/DE]; Signature Diagnostics AG, Hermannswerder 20A, 14473 Potsdam (DE). **HINZMANN, Bernd** [DE/DE]; Signature Diagnostics AG, Hermannswerder 20a, 14473 Potsdam (DE). **MAYR, Tobias** [DE/DE]; Signature Diagnostics AG, Hermannswerder 20a, 14473 Potsdam (DE). **ROSENTHAL, André** [DE/DE]; Groebener Dorfstr. 25, 14974 Ludwigsfelde (DE).
- (74) Agent: **HOPPE, Georg, J.**; Beuckestr. 20, 14163 Berlin (DE).
- (81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.
- (84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
- Published:
- without international search report and to be republished upon receipt of that report (Rule 48.2(g))
 - with sequence listing part of description (Rule 5.2(a))

(54) Title: METHOD AND KITS FOR THE PREDICTION OF RESPONSE/NONRESPONSE TO THE TREATMENT WITH AN ANTI-EGFR ANTIBODY IN PATIENTS WITH COLORECTAL CANCER OF ALL UICC STAGES

(57) Abstract: The invention pertains to a method for predicting responsiveness of a human patient in need of treatment with an anti-EGFR antibody to such treatment based on a biological sample. According to the invention, this method comprises: determining the mutation status of KRAS, BRAF and PIK3CA, and concluding based on the mutation status whether the subject will be responsive to treatment with an anti-EGFR antibody.



Method and Kits for the Prediction of Response/Nonresponse to the Treatment with an anti-EGFR Antibody in Patients with Colorectal Cancer of all UICC Stages

The invention relates, among other aspects, to a method for predicting of response/nonresponse to the treatment with an anti-EGFR antibody, including cetuximab and panitumumab, in patients with colorectal cancer of all UICC stages.

Introduction

Colorectal Cancer and Treatment Options

Colorectal cancer affects 73.000 patients in Germany and approximately 145.000 patients in the United States. It is the second most frequent solid tumor after breast and prostate cancer. Treatment of patients with colorectal cancer differs dependent on the location of the tumor, the stage of the disease, various additional risk factors and routine practice in various countries. Standard treatment for patients with colon cancer that is locally defined (stage I and stage II or has spread only to lymph nodes (stage III) always involves surgery to remove the primary tumor. Standard treatment for patients with rectum cancer may differ from country to country and from hospital to hospital as a significant part of these patients will receive neo-adjuvant radio/chemotherapy followed by surgery to remove the tumor tissue.

The five year survival rates of patients with colorectal cancer depend on the clinical stage of the individual patient, the histopathological diagnosis, stage-specific treatment options as well as on routine medical practice that differs from country to country, and often also from hospital to hospital. There are also significant differences in the routine treatment of patient with colorectal cancer in the Western world.

In most countries, patients with UICC stage disease will not receive any additional chemotherapy after surgery, as their five-year survival is approximately 95 %.

Treatment options for patients with UICC stage II colon cancer differ in many Western countries. The five year survival of patients with UICC II disease is approx. 80-82 %, meaning that 18-20 % will experience a progression of disease – often liver or lung metastasis. Once the disease will have spread to distant organs the outcome of the patients is much worse, and the majority of these patient will die relatively quickly despite heavy

treatment of these patients. Therefore guidelines in some Western countries recommend offering adjuvant chemotherapies to patients with UICC II disease including 5-fluorouracil and leucovorine or in combination with oxaliplatin. In other European countries including Germany the guidelines do not recommend to offer patients with UICC II disease adjuvant chemotherapy. There is a controversy if adjuvant chemotherapy should be offered to UICC II patients or not. Randomized clinical data that show a benefit of adjuvant chemotherapy is still missing for these patient cohorts.

Patients with locally advanced colorectal cancer – lymph nodes are infiltrated with cancer cells – have a five year survival rate of 49 %. The treatment guidelines therefore recommend that after surgery all patients should receive adjuvant chemotherapy, either a triple combination of 5-FU, leucovorin and oxaliplatin (FOLFOX4 or FOLFOX6 regimes) or dual combination of capecitabine – an orally available 5-FU derivative – and oxaliplatin (CAPOX). For elderly patients with low ECOG performance scores or known toxicities the dual 5-FU/leucovorin scheme should be used. In the routine practice only 60-80 % of patients with UICC stage III disease will however receive adjuvant chemotherapy. In Germany, only 60 % of UICC III patients will be treated with FOLFOX or 5-FU/leucovorin. There is also a difference in treatment between low densities areas and city populations. In general, approximately 50 % of patients with UICC III disease will experience progression of disease within 1-2 years after surgery. Once distant metastasis is diagnosed, these patients will be offered additional therapies including treatment with targeted antibodies drugs that inhibit the EGFR receptor including cetuximab or panitumumab or antibodies directed against the VEGFA ligand (bevacizumab). Several lines of therapies are offered, but most of these patients with disease progression will die within a five year interval.

The five year survival rate for patients with advanced, metastatic disease is dramatically low. Only 8 % will survive the first five years after surgery. It is these patients for which most of the treatment options with targeted therapies were developed over the last ten years, however, with limited success. The first targeted antibody therapy involved an anti-EGFR antibody cetuximab that was approved in 2004 by the FDA as monotherapy or in combination with Irinotecan, for patients with metastatic CRC (mCRC) that failed prior chemotherapy with irinotecan. In the original BOND study the response rate of the patients for the cetuximab was approximately 11 %. In 2007, a second anti- EGFR antibody, panitumumab, was approved for

the treatment of mCRC patients. However, the FDA approved panitumumab only in combination with KRAS wildtype (wt) mutation status, as it was shown in 2007 that only patients with wt KRAS gene would benefit from panitumumab. However, the data also showed that many patients with mCRC and wt KRAS did not benefit from panitumumab. Also, there were some mCRC patients with mutations in the KRAS gene that showed response to panitumumab. Similar data was also published in 2008-2009 for cetuximab that led to a label change for the approval of cetuximab. At the moment, both cetuximab and panitumumab are only approved for patients with mCRC and wildtype KRAS status.

Companion Diagnostics (CDx)

Accurate prediction of response/nonresponse to therapy is a prerequisite for individualized approaches to treatment. Current clinical practice in the treatment of patients with solid tumors does not offer effective and accurate prediction of response/nonresponse to chemotherapy and hormone therapy.

In prostate cancer no predictive biomarkers are known or established that predict response to radiation, hormone therapy or chemotherapy with taxol. The same is true for advanced non-small cell lung cancer (NSCLC). Approximately 70-80 % of all NSCLC patients are in stage IIIB or stage IV at the time of first diagnosis. For the majority of these patients no predictive markers exist that allow prediction of response to small molecule drugs like erlotinib or iressa that inhibit the kinase function of the EGF receptor. Only in a small cohort of NSCLC patients with EGFR mutations in the kinase domain response to erlotinib was observed. Still 90 % of the NSCLC patients of stage IIIB and IV have a five year survival of less than 8 % despite treatment.

The situation in breast cancer is more complex. For example, most patients with early breast cancer (node negative, ER) will receive radiation, chemotherapy and hormone therapy with tamoxifen after surgical removal of the tumor. The five-year survival of these patient cohorts is between 90-95 %. However, only 4 % of the patients will benefit from the addition of chemotherapy. Current treatment guidelines still recommend the overtreatment of 100 % of these patients with chemotherapy in order to reach the 4 % patients that may benefit. Similarly, a significant portion of the patients do not benefit from tamoxifen although there

are ER positive. Effective methods to predict response to the chemotherapy or hormone therapy are still missing.

There is one FDA approved CDx in breast cancer. Determination of the HERII status is predictive of response to trastuzumab, an anti HERII antibody. Thus patients with HERII positive breast cancer will receive trastuzumab at some point of their treatment history. However, only 25 % of all breast cancer patients are HERII positive and of those only 20-25 % of the patients benefit from trastuzumab, meaning that 75-80 % of HERII positive breast cancer patients are over treated and have no benefit from this expensive treatment.

In colorectal cancer, no predictive biomarkers are established in the adjuvant treatment of UICC II or UICC III patients.

Clinical Need for CDx in CRC UICC stage II and stage III

At time of first diagnosis, 70 % of the CRC patients are in UICC stage II and UICC stage III. 20 % of the UICC stage II and 49 % of the UICC stage III patients will suffer from progression of disease within 1-2 years after surgery. The majority of the relapsed patients will receive metastasis in the liver followed by metastasis in the lung. Hence, anti-EGFR antibody drugs like cetuximab and panitumumab would be ideal drugs to treat these patients before metastasis will occur if responders to these drugs could be identified and separated from non-responders. Recently, two randomized phase III trials, one in the US and one in Europe, evaluating cetuximab vs. cetuximab plus FOLFOX in UICC stage III patients did not meet their endpoints. Secondary endpoint analysis showed that patients with wild type KRAS did not benefit in the Cetuximab/FOLFOX arm in comparison to patients in the FOLFOX arm (ASCO 2010).

Therefore, there is a large clinical need in the art to predict whether a patient with colorectal cancer will respond to a treatment with an anti-EGFR antibody or not.

Description of the invention

The inventors discovered combinations of genes that can be used to predict whether a human subject (patient) in need of treatment with an anti-EGFR antibody will respond to such treatment.

Examples for anti-EGFR antibodies that can be used according to the invention are cetuximab, panitumumab, and IMC-11F8. Commercially available anti-EGFR antibodies are sold and developed, e.g. by Merck-Serono, Bristol-Myer Squibbs (BMS), and Amgen. Anti-EGFR antibodies are developed by other companies, e.g. by Eli Lilly.

Cetuximab (IMC-C225, Erbitux[®]) is a chimeric (mouse/human) monoclonal antibody, an epidermal growth factor receptor (EGFR) inhibitor, usually given by intravenous infusion. Cetuximab is administered for the treatment of cancer, in particular for treatment of metastatic colorectal cancer (stage Dukes D) and head and neck cancer.

Cetuximab binds specifically to the extracellular domain of the human epidermal growth factor receptor. It is composed of the Fv regions of a murine anti-EGFR antibody with human IgG1 heavy and kappa light chain constant regions and has an approximate molecular weight of 152 kDa. Cetuximab is produced in mammalian (murine myeloma) cell culture.

Panitumumab, also known as ABX-EGF, is a fully human monoclonal antibody specific to the epidermal growth factor receptor (EGFR). Panitumumab is manufactured by Amgen and sold as Vectibix[®].

IMC-11F8 is a potent, fully human monoclonal antibody that targets the epidermal growth factor receptor (EGFR). It is currently in Phase II studies for metastatic colorectal cancer with one or more Phase III trials planned in 2009. IMC-11F8 is in development by Eli Lilly.

Specifically, the inventors found that the absence or presence of certain mutations in the sequence of the genes PIK3CA, KRAS, and BRAF allows for the prediction whether a subject in need of anti-EGFR antibody treatment will respond to such an anti-EGFR antibody treatment or not. Accordingly, the mutation status of these genes is determined. The mutation

status can either be wild type (unaltered) or mutated. Any change of nucleotide in a nucleic acid or of an amino acid in a protein is referred to as a mutation.

The mutation status can be determined, e.g., from genomic DNA, cDNA, mRNA, or cRNA, or from fragments of the before-mentioned molecules, e.g. by using sequencing, real-time polymerase chain reaction, ligation chain reaction, Northern blotting, or microarray technology and the like.

The sequence of the mRNAs transcribed from the genes PIK3CA, KRAS, and BRAF is described herein as SEQ ID NOs 1, 2, and 3, respectively.

KRAS and its principal effector BRAF are important proteins in the EGFR signaling pathway and are involved in promotion of tumor growth, spread and differentiation. The PIK3CA gene encodes for the p110 α protein, the class I PI-3-kinase catalytic subunit.

The mutations in the genes PIK3CA, KRAS, BRAF, CTNNB1, and NRAS that can be determined according to the invention are as follows: Table A

Gene	Mutation nt	Mutation aa
PIK3CA	1624G>A	E542K (Glu → Lys)
PIK3CA	1633G>A	E545K (Glu → Lys)
PIK3CA	3140A>G	H1047R (His – Arg)
KRAS	34G>A	G12S (Gly → Ser)
KRAS	34G>T	G12C (Gly → Cys)
KRAS	34G>C	G12R (Gly → Arg)
KRAS	35G>A	G12D (Gly → Asp)
KRAS	35G>T	G12V (Gly → Val)
KRAS	35G>C	G12A (Gly → Ala)
KRAS	37G>T	G13C (Gly → Cys)
KRAS	38G>A	G13D (Gly → Asp)
KRAS	436G>A	A146T (Ala → Thr)
KRAS	181C>A	Q61K (Gln → Lys)
KRAS	181C>G	Q61E (Gln → Glu)
KRAS	182A>C	Q61P (Gln → Pro)
KRAS	182A>G	Q61R (Gln → Arg)
KRAS	182A>T	Q61L (Gln → Leu)
KRAS	183A>C	Q61H (Gln → His)

KRAS	183A>T	Q61H (Gln -> His)
BRAF	1799T>A	V600E (Val -> Glu)
CTNNB1	121A>G	T41A (Thr -> Ala)
CTNNB1	134C>T	S45F (Ser -> Phe)
NRAS	34G>A	G12S (Gly -> Ser)
NRAS	34G>T	G12C (Gly -> Cys)
NRAS	35G>A	G12D (Gly -> Asp)
NRAS	35G>t	G12V (Gly -> Val)
NRAS	37G>C	G13R (Gly -> Arg)
NRAS	38G>A	G13D (Gly -> Asp)
NRAS	181C>A	Q61K (Gln -> Lys)
NRAS	182A>G	Q61R (Gln -> Arg)
NRAS	182A>T	Q61L (Gln -> Leu)

In another aspect of the invention, the mutation status of a gene can also be deduced from the protein sequence of the protein encoded by the particular gene (given in table A). The protein sequence of the proteins of the invention are known in the art and can also be deduced from the nucleic acid sequence provided herein.

In a preferred embodiment, the human patient is clinically “responding” to anti-EGFR antibody treatment as used herein when the T/C value is < 20. The T/C value represents the treated-to-control ratio of relative median tumor volumes.

The invention refers, in a first aspect, to a method for predicting the responsiveness of a human patient in need of anti-EGFR antibody treatment to such treatment based on a biological sample, i.e. whether the patient will respond or will not respond. Such a method comprises the following two steps. Firstly, the mutation status of at least PIK3CA, KRAS, and BRAF is determined in the biological sample, and secondly, it is concluded based on the mutation status of PIK3CA, KRAS, and BRAF, whether the subject is responsive or non-responsive to anti-EGFR antibody treatment. The term responsiveness relates to whether a subject will be respond or not respond to a treatment with anti-EGFR antibody.

When determining the mutation status of PIK3CA, the method comprises determining whether at least one mutation chosen from the group consisting of 1624G>A, 1633G>A, and 3140A>G is present in the biological sample.

When determining the mutation status of KRAS the method comprises determining whether at least one mutations chosen from the group consisting of 34G>A, 34G>T, 34G>C, 35G>A, 35G>T, 35G>C, 37G>T, 38 G>A, 181C>A, 181C>G, 182A>C, 182A>G, 182A>T, 183A>C, 183A>T, and 436 G>A is present in the biological sample.

When determining the mutation status of BRAF the method comprises determining whether a T>A mutation is present at position 1799 of the BRAF gene.

A wild type (wt) mutation status of PIK3CA, KRAS, and BRAF allows concluding that the subject is likely to respond to anti-EGFR antibody treatment.

Specifically, in a preferred embodiment, a subject that is found to have a mutation status = wild type in:

- the gene PIK3CA at positions 1624G, 1633G, and 3140A,
- the gene KRAS at positions 34G, 35G, 38G and 436G, and
- the gene BRAF at position 1799T

is a responder to anti-EGFR antibody treatment with a sensitivity of 83 % and a specificity of 76 %.

In an alternative, preferred embodiment, a subject that is found to have a mutation status = wild type in:

- the gene PIK3CA at positions 1624G, 1633G, and 3140A, and
- the gene KRAS at positions 34G, 35G, 38G and 436G, and
- the gene BRAF at position 1799T, and
- a mutation status = 35G>A in the gene KRAS

is a responder to anti-EGFR antibody treatment with a sensitivity of 94 % and a specificity of 67 %.

If a mutation status of one ore more of the mutations

PIK3CA 1624G>A, 1633G>A, 3140A>G

KRAS 34G>A, 34G>T, 34G>C, 35G>T, 35G>C, 38 G>A, 436 G>A

BRAF 1799T>A

is determined based on the tumor sample from the subject in the method, then it is concluded that the subject is a non-responder to anti-EGFR antibody treatment, wherein any single mutation of the ones listed above allows for this conclusion.

In this context, reference is made to tables 3A, 3B and 4.

Colorectal cancer (CRC) refers to both colorectal adenoma and colorectal carcinoma. A colorectal adenoma is characterized by atypical growth of epithelial cells in mucosal tissue, i.e. neoplasia. Hypercellularity with enlarged, hyperchromatic nuclei, varying degrees of nuclear stratification, nuclear polymorphisms, and loss of polarity are the histologically defining features. In colorectal adenoma, this abnormal growth is confined to the mucosa; a synonym of adenoma is intraepithelial neoplasia (IEN). If this atypical growth of epithelial cells extends/invades through the muscularis mucosae, the muscle layer under the mucosa, with destruction of the usual anatomical wall, the pathologist terms this atypical growth a colorectal carcinoma.

The colorectal cancer can be of any of stage according to the “Union International Contre le Cancer” (UICC). This allows for the testing of cetuximab responsiveness even in cancer stages that cetuximab is not approved for (e.g. cetuximab is approved only for the treatment colorectal cancer of UICC-stage IV). It is preferred that the subject is in either UICC stage II, stage III or stage IV.

UICC-stage 0 includes CIS only. UICC-stages I and II are comprised of the localized stages, whereas UICC-stage III describes CRC where tumor cells have metastasized into regional lymph nodes. The worst case is UICC-stage IV; it describes CRC which has metastasized into other organ(s), usually liver (~75 %), peritoneum (~22 %), and/or lung (~21 %).

The colorectal cancer can be of any of Dukes stage A to D. This allows for the testing of anti EGFR antibody responsiveness even in cancer stages that the anti EGFR antibody is not approved for (e.g., cetuximab is approved only for the treatment of colorectal cancer of the stage Dukes D).

Dukes A describes a colorectal tumor that has not penetrated the wall and has no nodal involvement. Dukes B involves a penetrating cancer through the rectal wall but with no nodal involvement. Dukes C indicates lymph node involvement. Dukes D includes metastases to distant organs.

Further, it is preferred that the subject is chemo-naïve at the time the tumor tissue sample is obtained for using the method.

Preferably, the determination of the mutation status of the subject's tumor sample is performed using any molecule from which the mutation status of a gene can be deduced, such as genomic DNA, cDNA, mRNA, cRNA, or its fragments.

For the determination of the mutation status, any method known in the art suitable for finding mutations can be used, such as sequencing, real-time polymerase chain reaction (real-time PCR), Scorpions PCR, High Resolution Melt (HRM) analysis, COLD-PCR, Allel Specific PCR, Single-Stranded Conformation Polymorphism Analysis, Denaturing High Performance Liquid Chromatography, ligation chain reaction (LCR), Northern blotting, or microarray technology. Molecules that can be used for real-time PCR can be found in the examples.

In one embodiment of the invention, any two, more preferred any three or more genes of the group consisting of PIK3CA, KRAS, BRAF, CTNNB1, and/or NRAS are at least used to predict the responsiveness of a human cancer patient to anti-EGFR antibody treatment.

In another embodiment of the invention, human cancer patients may involve patients with colorectal cancer of Dukes A stage (UICC I), Dukes B stage (UICC II), Dukes C stage (UICC III), and Dukes D stage (UICC IV).

In another preferred embodiment of the invention human cancer patients may involve patients with colorectal cancer of Dukes B stage (UICC II) and Dukes C stage (UICC III).

In another aspect, the invention refers to a microarray for predicting the responsiveness of a human patient in need of anti-EGFR antibody treatment to such anti-EGFR antibody

treatment. Such a microarray comprises or consists of probes for determining the mutation status of PIK3CA, KRAS, and BRAF in a biological sample, optionally also for determining the mutation status of CTNNB1, and/or NRAS.

A "microarray" includes a specific set of probes, such as oligonucleotides and/or cDNAs (*e.g.*, expressed sequence tags, "ESTs") corresponding in whole or in part, and/or continuously or discontinuously, to regions of genomic DNA or RNAs that can be extracted from a biological sample of a human patient. The probes are bound to a solid support. The support may be selected from beads (magnetic, paramagnetic, etc.), glass slides, and silicon wafers. The probes can correspond in sequence to the genes of the invention such that hybridization between the gene from the subject sample (genomic DNA or cDNA derived therefrom, etc.) and the probe occurs. In the microarray embodiments, the sample genomic DNA, mRNA etc. can optionally be amplified before hybridization to the microarray. Prior to hybridization, the sample genomic DNA, mRNA etc. is fluorescently labeled. Upon hybridization to the array and excitation at the appropriate wavelength, fluorescence emission is quantified. Fluorescence emission for each particular genomic DNA, mRNA etc. is directly correlated with the amount of the particular genomic DNA, mRNA etc. in the sample. The signal can be detected and together with its location on the support can be used to determine which probe hybridized with genomic DNA, mRNA etc. from the subject's biological sample.

Accordingly, in certain aspects, the invention is directed to a kit or microarray for detecting the mutation status in the subject's tumor sample, where this "status" allows for the conclusion of whether the subject will be responding or is likely to respond to anti-EGFR antibody treatment or not. Probes are designed to minimize cross reactivity and false positives and allow under appropriate conditions for the detection of single mutation. Thus, the invention in certain aspects provides a microarray, which generally comprises a solid support and a set of oligonucleotide probes.

In another aspect, the invention relates to a kit for predicting the responsiveness of a human patient in need of anti-EGFR antibody treatment to such anti-EGFR antibody treatment. Such a kit comprises means for determining the mutation status of at least PIK3CA, KRAS, and BRAF in a biological sample.

The means for determining the mutation status of PIK3CA, KRAS, and/or BRAF, as well as optionally CTNNB1, and/or NRAS are chosen from the group consisting of primers, probes, and blocker oligonucleotides (examples given in Tables 5A and 5B). These means are suitable in particular for use in sequencing, real-time polymerase chain reaction, ligation chain reaction, Northern blotting, or microarray technology. The sequence of the mRNAs transcribed from the genes CTNNB1, and NRAS is described herein as SEQ ID NOs 4, and 5, respectively.

In another aspect, the invention relates to the use of a microarray and/or a kit as described above and herein for predicting the responsiveness of a human patient in need of anti-EGFR antibody treatment to an anti-EGFR antibody treatment.

In another aspect, the invention relates to the use of a composition for predicting responsiveness of a human patient in need of anti-EGFR antibody treatment to anti-EGFR antibody treatment. Such a composition comprises or consists of a first molecule from which the mutation status of PIK3CA can be deduced, a second molecule from which the mutation status of KRAS can be deduced, and a third molecule from which the mutation status of BRAF can be deduced.

The first molecule, the second molecule, and/or the third molecule are preferably genomic DNA, cDNA, mRNA, or cRNA or its fragments, such that using sequencing, real-time polymerase chain reaction, ligation chain reaction, Northern blotting, or microarray technology and the like, their nucleotide sequence can at least in part be determined. Based on the determined nucleotide sequence, the mutation status, a prediction can be made whether a subject from whom the composition is derived will be responsive to anti-EGFR antibody treatment.

In yet another aspect, the invention relates to the use of a stable cancer xenograft that was obtained by transplanting a fresh human tumor sample from a patient with colorectal cancer onto a mouse, in particular onto a nude mouse, and to propagate the tumor through several passages. Such engrafted mice can then be used in therapy experiments with, for instance, anti-EGFR antibodies. The tumor tissue from these mice can then be used for identifying genes that are suited for predicting the responsiveness of a human patient in need of anti-

EGFR antibody treatment. Other drugs can also be used, such as bevacizumab and/or oxaliplatin. Such mouse cancer xenografts are accepted models in the art of human cancer research.

In summary, a panel of 149 well-characterized patient-derived CRC xenografts was established. The xenograft models highly resemble the original primary tumor with respect to histology and genome-wide gene expression profiling.

The inventors have examined the mutation status in the three genes KRAS, BRAF and PIK3CA as a tool for the prediction of response/nonresponse towards monotherapy with an anti-EGFR antibody, e.g. cetuximab. The inventors' companion diagnostics (CDx) predicted response with sensitivity of 83 % and nonresponse with a specificity of 76 %. If exon 13 mutations are also linked with CE response, the CDx has a sensitivity of 94 % and a specificity of 67 %.

KRAS, BRAF, and PIK3CA mutation status combined has clinical utility higher than KRAS alone to individualize cetuximab treatment, also in the adjuvant setting, for patients with colorectal cancer of Dukes B and Dukes C.

Examples**MATERIALS AND METHODS****Experimental Aim**

Human tumor xenografts directly derived from patient cancer tissue are of vital importance for preclinical research as they realistically represent the heterogeneity and individuality of each malignoma with revealed similarity to the original patient carcinoma.

The inventors established 149 stably passageable colorectal cancer (CRC) xenograft models directly from fresh surgical colorectal tumor material. The xenografts provide a relevant model system that is close to the clinical situation and allow testing of antitumor agents in a fast and standardized manner. In 75 xenograft models, the inventors tested this approach and investigated the response/nonresponse towards targeted antibodies approved for the treatment of patients with advanced, metastatic CRC including cetuximab and bevacizumab as well as the standard chemotherapy agent oxaliplatin.

Bevacizumab (Avastin[®]) is a drug that blocks angiogenesis. It is used to treat various cancers, including colorectal cancer. Bevacizumab is a humanized monoclonal antibody that binds to vascular endothelial growth factor A (VEGF-A), which stimulates angiogenesis. Oxaliplatin (Eloxatin[®], Oxaliplatin Medac[®]), is [(1R,2R)-cyclohexane-1,2-diamine](ethanedioato-O,O')platinum(II) and is known in the art as a cancer chemotherapy drug.

Cetuximab (IMC-C225, Erbitux[®]) is a chimeric (mouse/human) monoclonal antibody, an epidermal growth factor receptor (EGFR) inhibitor.

Xenograft models provide sufficient tissue material for molecular studies of biomarkers that are predictive for response/nonresponse to therapy and can be used as companion diagnostics (CDx). In 67 xenograft models, the inventors analyzed mutation status of KRAS, BRAF and PIK3CA and investigated their relationships with various clinical and pathological characteristics as well as the response to therapies.

Patients

All fresh human tumor tissue samples originated from a multi-center prospective study "Molecular Signatures in Colorectal Cancer" (MSKK). 239 tumor tissue samples from

patients of all four Dukes stages (UICC I, II, III and IV) were collected during two years by a collaborating network of four clinics, using a standardized procedure. In parallel, comprehensive clinical data were collected for each patient and monitored. All included patients gave written informed consent prior to surgery. Clinical characteristics of 67 chemonaive patients with primary CRC are listed in table 2.

Establishment of xenografts

Shortly after surgery, original colorectal cancer tumor pieces were shipped in gentamicin containing RPMI-1640 medium to the mouse facility. After arrival at the mouse facilities they were transplanted onto immunodeficient mice and were further passaged until a stably grown tumor xenografts has developed. In this way a panel of 149 stably passagable colon cancer xenografts could be established as a permanent tumor models.

Surgical colorectal tumor samples were cut into pieces of 3 to 4 mm and transplanted within 30 min s.c. to 3 to 6 immunodeficient NOD/SCID mice (Taconic); the gender of the mice was chosen according to the donor patient. Additional tissue samples were immediately snap-frozen and stored at -80°C for genetic, genomic, and protein analyses. All animal experiments were done in accordance with the United Kingdom Co-ordinating Committee on Cancer Research regulations for the Welfare of Animals and of the German Animal Protection Law and approved by the local responsible authorities. Mice were observed daily for tumor growth. At a size of about 1 cm³, tumors were removed and passaged to naive NMRI: *nu/nu* mice (Charles River) for chemosensitivity testing. Tumors were passaged no more than 10 times. Numerous samples from early passages were stored in the tissue bank in liquid nitrogen and used for further experiments. Several rethawings led to successful engraftment in nude mice. All xenografts as well as the corresponding primary tumors were subjected to histological evaluation using snap-frozen, haematoxylin-eosin-stained tissue sections.

Testing of colorectal cancer drugs

75 xenograft models were used in therapy experiments testing responsiveness towards drugs approved in the treatment of patients with colorectal cancer including cetuximab as an anti-EGFR antibody, bevacizumab, and oxaliplatin. Each of the 75 tumors was transplanted onto 20 mice (5 controls and 5 for each drug). Models with treated-to-control ratios of relative

median tumor volumes of 20 % or lower were defined as responders. This resulted in response rates of 24 % for cetuximab, 4 % for bevacizumab and 7 % for oxaliplatin (Table 1). The chemotherapeutic response of the passagable tumors was determined in male NMRI: *nu/nu* mice. For that purpose, one tumor fragment each was transplanted s.c. to a number of mice. At palpable tumor size (50-100 mm³), 6 to 8 mice each were randomized to treatment and control groups and treatment was initiated. If not otherwise mentioned, the following drugs and treatment modalities were used: Bevacizumab (Avastin®; Genentech Inc., South San Francisco, CA, USA) 50 mg/kg/d, qd 7x2, I.p., Cetuximab (Erbix; Merck) 50 mg/kg/d, qd 7x2, i.p.; Oxaliplatin (Eloxatin, Sanofi-Aventis), 50 mg/kg/d, qd1-5, I.p. Doses and schedules were chosen according to previous experience in animal experiments and represent the maximum tolerated or efficient doses. The injection volume was 0.2 mL/20 g body weight.

Tumor size was measured in two dimensions twice weekly with a caliper-like instrument. Individual tumor volumes (*V*) were calculated by the formula: $V = (\text{length} + [\text{width}]^2) / 2$ and related to the values at the first day of treatment (relative tumor volume). Median treated to control (T/C) values of relative tumor volume were used for the evaluation of each treatment modality and categorized according to scores (– to ++++; see Table 3). The mean tumor doubling time of each xenograft model was calculated by comparing the size between 2- and 4-fold relative tumor volumes. Statistical analyses were done with the *U* test (Mann and Whitney) with $P < 0.05$. The body weight of mice was determined every 3 to 4 days and the change in body weight was taken as variable for tolerability.

Molecular characterization of Human Tumor Xenograft Samples

DNA and RNA extraction

Genomic DNA and total RNA were simultaneously extracted with AllPrep DNA/RNA Mini Kit (*automated protocol using the QIACube*) according to the manufacturer's instructions. DNA and RNA concentrations (ng/μl) were measured using UV spectrophotometer (Nanovue, GE Healthcare).

Mutation Analysis

Mutation analysis was conducted by allele-specific real-time PCR for KRAS, BRAF, PIK3CA, APC, CTNNβ1 and TP53 mutations with Custom TaqMan SNP Genotyping Assays

(Applied Biosystems). TaqManMGB assays were designed for: 8 substitutions in the KRAS gene (34G>A, 34G>T, 34G>C, 35G>A, 35G>T, 35G>C, 38 G>A and 436 G>A), the most frequent mutation in the BRAF gene (1799 T>A), 3 hotspots in the PIK3CA gene (1624G>A, 1633G>A, 3140A>G), most common substitution in the APC gene (4348 C->T), 2 hotspots in CTNN β 1 (121 A>G, 134 C>T) and 5 substitutions in TP53 (524 G>A, 742 C>T, 743 G>A, 818G>A, 844 C>T). Every well contained 20 ng of purified gDNA template. Samples were run in duplicates in the final volume of 10 μ l with TaqMan Universal PCR Master Mix (2x) No AmpErase UNG, 40 x working stock of SNP Genotyping Assay and blocking oligonucleotides (125 nmol/l). Competitive blocking oligonucleotides were added due to the presence of unspecific fluorescence signals originating from the adjacent mutations in codon 12 of the KRAS gene. Amplification was run in the ABI *Fast 7500* instrument. Standard TaqMan thermocycling conditions were used (10 min - 95°C, 40 cycles of. 15 s at 92 °C, 1 min at 60 °C) for KRAS mutation analysis, while for the BRAF anneal/extend step was prolonged to 90 s. A subset of critical samples was confirmed via dideoxy sequencing.

Primers, reporters and Blockers used in the real-time PCR are shown in table 5A and 5B.

Mutation status was assessed in tissue of 67 tumor xenografts, derived from chemonaive patients with primary tumors. The analysis was performed by allele-specific RT-PCR (Custom TaqMan SNP Genotyping Assays, Applied Biosystems) for the following hotspots:

KRAS: 34G>A, 34G>T, 34G>C, 35G>A, 35G>T, 35G>C, 38 G>A and 436 G>A;

BRAF: 1799 T>A, and

PIK3CA: 1624G>A, 1633G>A, 3140A>G.

Altogether, the inventors observed at least one mutation in 40 of the 67 (60 %) xenograft models. There were 32 single mutations: 24 in KRAS, 6 in BRAF and 2 in PIK3CA. In 8 of the 67 (12 %) CRC models, the inventors observed mutations in two genes, 7 in KRAS and PIK3CA and 1 in BRAF and PIK3CA. KRAS and BRAF mutations were mutually exclusive.

Mutations in codon 12 of KRAS were most frequent and observed in 21 (31 %) of the models. Of these the 35 G>T mutation occurred in 11 models. There were 6 mutations in codon 13 (9 %) and 2 mutations in codon 146 (3 %) of KRAS. No difference was observed in the frequency of PIK3CA mutations between exons 9 (7 %) and 20 (7 %).

The corresponding primary patient tumors showed the same mutation profile.

Controls

Every 96-well plate was composed of 40 patients samples in duplicates, 4 negative controls (WT), 8 positive controls (titrations), 4 non – template controls (NTC, DNA substituted with DNase-free water).

As negative control (WT) served genomic DNA isolated from lymphocytes of peripheral blood system of a healthy donor that underwent colonoscopy. As positive control we used serial dilutions of DNA isolated from the cell lines harboring mutations in the investigated hotspots (A549 – KRAS 34 G>A; NCI-H385 - KRAS 34 G>T; HuP-T3 - KRAS 34 G>C; A427 – KRAS 35 G>A, CTNN β 1 121 A>G; SW620 - KRAS 35 G>T, TP53 818 G>A; RPMI-8226 - KRAS 35 G>C; HCT-116 - KRAS 38 G>A; ML-2 - KRAS 436 G>A; HT-29 – BRAF 1799 T>A; T84 – PIK3CA 1624 G>A; HCT-15 - PIK3CA 1633 G>A; SKOV3 – PIK3CA 3140 A>G; KLE – TP53 524 G >A; OVCAR3 – TP53 743 G>A, HuP-T3 – TP53 844 C>T) in wild-type genomic DNA (see above) that mimics different tumor cell content in the sample (100 %; 50 %; 20 %; 10 %; 5 %; 2 %; 1 %; 0.5 %)

Gene expression analysis - identification of the source tumor of a xenograft based on RNA expression

As part of the validation of the xenograft model, we examined whether the original tumor corresponding to the xenograft was identifiable based on RNA expression patterns. It is our hypothesis that even if this identification is complicated by marked differences between native and xenograft samples, the subset of RNA markers that causes these differences can be identified, such that after exclusion of this subset from the comparison, the identification of the source becomes possible.

As a proxy for the source tumor we use a snap frozen sample removed at the same time as the engrafted material. Identification of the source can then be based on similarities between the xenograft and this original tumor sample. To compare a xenograft to its source, we place each RNA microarray measuring more than 54.000 transcripts in a high dimensional Euclidian space to establish a concept of distance. We consider the source of a xenograft identified,

when the xenograft's RNA measurement is closer in distance to the measurement of the native sample from the same source than to all other samples in a sufficiently large comparison set.

The inventors used Affymetrix U133 Plus 2.0 GeneChip measurements from 254 samples, consisting of 127 pairs, each composed of a native sample of a colorectal tumor and one of its xenografts. After condensation of the complete set with FARMS/INI, 18018 informative probesets were identified. To identify probesets that are specific to the difference of xenograft vs. native, we calculated the Wilcoxon rank sum statistic between the group of xenografts and original samples for each probeset.

After removing 20 % of RNA probesets that strongly distinguished the xenograft from the primary tumor, 67 % and more of the tumors were properly identified as pairs that were closer to each other than to any other sample among the 254 (=127*2).

Response to Cetuximab as an anti-EGFR-antibody

The majority of the cetuximab responders are wild type in KRAS, BRAF and PIK3CA. This corresponds to a sensitivity S+ of 83% (15/18). Three of the cetuximab responder (17 %) harbor mutations in the KRAS gene. Of those one mutation was found in codon 12 and two in codon 13. The 38G>A mutation in codon 13 was recently linked with improved response to CE (De Roock et al. JAMA 2010, 304:1812). If the exon 13 mutations are linked with CE response in the inventors' dataset an improved S+ of 94 % will result.

Nonresponse to Cetuximab as an anti-EGFR-antibody

28 of the 49 cetuximab non-responders exhibited KRAS mutations. If only KRAS mutations are considered 57 % of the non-responders can be identified. The specificity (S-) can be significantly improved to 76 % (37/49) if BRAF and PIK3CA mutations are determined in addition to KRAS mutations (see Tab. 4). If exon 13 mutations are linked with response to cetuximab, S- will decrease to 67 %.

Prospective Prediction of Response to Cetuximab as an anti-EGFR-antibody

The inventors also investigated how well response can be predicted from the mutation data. A machine learning approach was used to estimate unbiased prospective prediction rates for response to cetuximab based on the mutation profile. In each of a large number of bootstrap loops, a new predictor was trained on a new randomly selected subset of the tumors and applied to the remaining tumors. KRAS, BRAF and PIK3CA mutations achieve a S+ of 84 % (95 % CI: 59 % and 96 %) and a S- of 68 % (95 % CI: 54 % and 80 %).

Tables

Table 1: Treatment scheme and response rates of the 75 pharmacologically characterized xenograft models. Treatment groups consist of 5 animals each. T/C values represent the treated-to-control ratios of relative median tumor volumes.

Drug	Treatment scheme	Responder T/C < 20% (> 80% tumor growth inhibition) Number (%)	
Oxaliplatin	qd1-5 50 mg/kg/d	5/75	7
Cetuximab	qd 7x2 50 mg/kg/d	18/75	24
Bevacizumab	qd 7x2 50 mg/kg/d	3/75	4

Table 2: Mutation distribution in a group of 67 xenograft models in respect to the patient characteristics

Characteristic	Total	BRAF		KRAS		PIK3CA		Double	
		Mut	%	Mut	%	Mut	%	Mut	%
Total n pts	67	7	10%	31	46%	10	15%	8	12%
Gender									
Male	31	2	6%	12	39%	4	13%	3	10%
Female	36	5	14%	19	53%	6	17%	5	14%
Age									
<60 yrs old	15	0	-	7	47%	2	13%	2	13%
>60 yrs old	52	7	13%	24	46%	8	15%	6	12%
Location									
Colon	46	7	15%	18	39%	9	20%	7	15%
Rectum	21	0	-	13	62%	1	5%	1	5%
UICC Stage									
I	8	1	13%	5	63%	2	25%	2	25%
II	22	2	9%	5	23%	1	5%	0	0%
III	28	3	11%	18	64%	6	21%	5	18%
IV	9	1	11%	3	33%	1	11%	1	11%

Table 3 A: Mutation status of responders to cetuximab as anti-EGFR antibody: T/C values represent the treated-to-control ratios of relative median tumor volumes; *Rating: If T/C: > 50 %: “-”, 36-50 %: “+”, 21-35 %: “++”, 6-20 %: “+++”, < 5 %: “++++”

Model- No.	Passage	Response to						Mutation Status		
		Oxaliplatin		Cetuximab		Bevacizumab		KRAS	BRAF	PIK3CA
		T/C	Rating	T/C	Rating	T/C	Rating			
8169	P2	19,7	+++	7,6	+++	39,4	+	WT	WT	WT
8233	P3	68,4	-	8,9	+++	31,1	++	WT	WT	WT
8439	P2	84,3	-	19,6	+++	20,6	+++	38 G>A	WT	WT
8583	P3	65,5	-	17,2	+++	43,1	+	WT	WT	WT
8525	P3	40	+	12	+++	38,7	+	WT	WT	WT
8660	P3	19,4	+++	7	+++	19,4	+++	WT	WT	WT
8676	P3	86,4	-	17,3	+++	34,9	++	38 G>A	WT	WT
8445	P3	35,5	++	5,3	+++	47,4	+	WT	WT	WT
8704	P3	37,7	+	8,6	+++	20,3	++	WT	WT	WT
8754	P3	116,7	-	5,3	+++	63,2	-	WT	WT	WT
8710	P4	37,7	+	13,1	+++	41,4	+	WT	WT	WT
8818	P4	61,0	-	17,5	+++	43,9	+	WT	WT	WT
8060	P3	40,9	+	4,5	++++	46,7	+	WT	WT	WT
8623	P2	69,3	-	2,7	++++	102,7	-	WT	WT	WT
8521	P3	68,3	-	1,7	++++	41,0	+	WT	WT	WT
8555	P3	61,8	-	3,6	++++	80	-	WT	WT	WT
8858	P4	15,9	+++	1,2	++++	20,3	++	34 G>A	WT	WT
8815	P4	66,3	-	3,2	++++	21,0	++	WT	WT	WT

Table 3 B. Mutation status of non-responders to cetuximab as anti-EGFR antibody: T/C values represent the treated-to-control ratios of relative median tumor volumes; *Rating: If T/C: > 50 %: “-”, 36-50 %: “+”, 21-35 %: “++”, 6-20 %: “+++”, < 5 %: “++++”

Model- No.	Passage	Response to						Mutation Status		
		Oxaliplatin		Cetuximab		Bevacizumab		KRAS	BRAF	PIK3CA
		T/C	Rating	T/C	Rating	T/C	Rating			
8260	P2	61,5	-	72,7	-	66,6	-	WT	1799 T>A	WT
8213	P3	54,7	-	94,7	-	63,8	-	WT	1799 T>A	WT
8183	P3	72,9	-	67,8	-	103,4	-	35 G>A	WT	WT
8433	P2	34,7	++	79,2	-	23,6	++	38 G>A	WT	WT
8434	P2	93	-	61	-	88,2	-	35 G>T	WT	3140 A>G
8435	P2	85,9	-	81,4	-	37,2	+	436 G>A	WT	1633 G>A
8424	P2	58,3	-	58,3	-	33,3	++	WT	WT	WT
8469	P2	68,4	-	55,2	-	57,9	-	35 G>T	WT	WT
8610	P2	53,6	-	62,5	-	50	+	35 G>A	WT	WT
8649	P2	71	-	79,3	-	82,8	-	35 G>T	WT	1633 G>A
8683	P2	90,3	-	120	-	70,3	-	35 G>T	WT	1633 G>A
8611	P2	71,4	-	74,7	-	88,7	-	35 G>T	WT	WT
8522	P3	37	+	64,4	-	38	+	WT	WT	WT
8632	P3	30,2	++	62,5	-	87,5	-	WT	WT	WT

8734	P3	83,3	-	79,2	-	67,8	-	WT	WT	1624 G>A
8722	P3	90	-	73,1	-	56	-	WT	WT	WT
8421	P3	95,3	-	81	-	54,2	-	WT	1799 T>A	WT
8537	P3	37	+	60,9	-	57,2	-	WT	1799 T>A	WT
8764	P3	77,3	-	82,1	-	54,6	-	38 G>A	WT	WT
8729	P3	31,5	++	53,2	-	64,3	-	35 G>T	WT	WT
8428	P3	75	-	88	-	66,7	-	35 G>T	WT	WT
8690	P3	52,2	-	89,7	-	53,6	-	WT	WT	WT
8732	P3	67,7	-	51,6	-	47,4	+	38 G>A	WT	WT
8823	P2	43,2	+	69,3	-	31,4	++	WT	1799 T>A	3140 A>G
8723	P3	60,9	-	65,7	-	50	+	34 G>A	WT	3140 A>G
8833	P3	48,5	+	69,7	-	54,6	-	WT	1799 T>A	WT
8795	P3	61,5	-	51,2	-	58,2	-	35 G>T	WT	3140 A>G
8814	P3	38,8	+	63,4	-	38,4	+	WT	1799 T>A	WT
8847	P3	88,3	-	93	-	34,5	++	35 G>T	WT	WT
8207	P2	56,7	-	43,7	+	24,5	++	WT	WT	WT
8209	P3	42,2	+	36,9	+	16,5	+++	35 G>A	WT	WT

8440	P2	45,7	+	48,6	+	41,2	+	WT	WT	WT
8397	P2	25,4	++	40,8	+	36,6	+	35 G>A	WT	WT
8541	P2	76,6	-	42,4	+	90	-	WT	WT	WT
8613	P2	52,2	-	45,6	+	108,7	-	WT	WT	WT
8509	P3	56,6	-	39,6	+	50,9	-	35 G>C	WT	WT
8497	P3	43,9	+	38,1	+	40,4	+	WT	WT	WT
8652	P3	45,2	+	46,7	+	31	++	WT	WT	WT
8862	P3	22,3	++	49,2	+	21,4	++	436 G>A	WT	3140 A>G
8873	P4	28,6	++	47,7	+	52,4	-	35 G>T	WT	WT
8724	P4	57,1	-	42,7	+	22,2	++	38 G>A	WT	WT
8389	P3	51,5	+	24,8	++	64,6	-	35 G>C	WT	WT
8311	P2	22,2	++	22	++	26	++	35 G>C	WT	WT
8578	P2	58,1	-	32	++	26,7	++	35 G>C	WT	WT
8591	P2	186,2	-	35,3	++	53	-	WT	WT	WT
8590	P3	62,2	-	27	++	54,5	-	35 G>A	WT	WT
8733	P3	4,7	++++	28	++	24,32	++	35 G>T	WT	WT
8784	P3	22,5	++	27,1	++	35,42	++	35 G>C	WT	WT
8760	P3	72,2	-	33,8	++	48,65	+	WT	WT	1633 G>A

Table 4: Cross tabulation table. It shows the relation between mutation status in 3 analyzed genes (KRAS, BRAF and PIK3CA) and response towards cetuximab as an anti-EGFR antibody. It allows calculating the prediction of CE response with a sensitivity (S+) of 83 % and a specificity (S-) of 76 % (S+: the percentage of responders who are wild type; S-: the percentage of mutated nonresponders). PPV (positive predictive value) = 55 %, NPV (negative predictive value) = 92,5 %

Mutation status	Cetuximab response observed		Total
	Responders	Nonresponders	
Mutated	3	37	40
Wildtype	15	12	27
Total	18	49	67

Assay Name	Target Mutation	Forward Primer Seq.	Reverse Primer Seq.	Reporter 1 Sequence	Reporter 2 Sequence	Blocking Oligonucleotide sequence
KRAS_ex2-121a	34 G>A	AGGCCTGCTGAAAAATGACTG AATAT	GCTGTATCGTCAAAGGCACTCT T	TTGGAGCTGGTGGC GTA	TAGTTGGAGCTAG TGGCGTA	TAGTTGGAGCTAGT GGCCTAG
KRAS_ex2-121t	34 G>T	AGGCCTGCTGAAAAATGACTG AATAT	GCTGTATCGTCAAAGGCACTCT T	TTGGAGCTGGTGGC GTA	TTGGAGCTTGTGG CGTA	TAGTTGGAGCTTGT GGCCTAG
KRAS_ex2-121c	34 G>C	AGGCCTGCTGAAAAATGACTG AATAT	GCTGTATCGTCAAAGGCACTCT T	TTGGAGCTGGTGGC GTA	TTGGAGCTCGTGG CGTA	-
KRAS_ex2-122a	35 G>A	AGGCCTGCTGAAAAATGACTG AATAT	GCTGTATCGTCAAAGGCACTCT T	TTGGAGCTGGTGGC GTA	TTGGAGCTGATGG CGTA	TAGTTGGAGCTGAT GGCCTAG
KRAS_ex2-122t	35 G>T	AGGCCTGCTGAAAAATGACTG AATAT	GCTGTATCGTCAAAGGCACTCT T	TTGGAGCTGGTGGC GTA	TTGGAGCTGTTGG CGTA	TAGTTGGAGCTGTT GGCCTAG
KRAS_ex2-122c	35 G>C	AGGCCTGCTGAAAAATGACTG AATAT	GCTGTATCGTCAAAGGCACTCT T	TTGGAGCTGGTGGC GTA	TTGGAGCTGCTGG CGTA	TAGTTGGAGCTGCT GGCCTAG
KRAS_ex2-131t	37 G>T	AGGCCTGCTGAAAAATGACTG AATAT	GCTGTATCGTCAAAGGCACTCT T	TTGGAGCTGGTGGC GTA	TTGGAGCTGGTTG CGTA	-
KRAS_132a_teipar	38 G>A	AGGCCTGCTGAAAAATGACTG AATAT	GCTGTATCGTCAAAGGCACTCT T	TGGTGGCGTAGGC A	CTGGTGACGTAGG CA	-
BRAF	1799 T>A	CTACTGTTTTCCTTTACTTACT ACACCTCAGA	ATCCAGACAACCTGTTCAAAC TGATG	CTAGCTACAGTGAA ATC	TAGCTACAGAGAA ATC	-
KRAS_146a	436 G>A	GGCTCAGGACTTAGCAAGAA GTTAT	GCAGAAAACAGATCTGTATT TATTTACAGTGT	TCTTGTCTTTTGCTG ATGTT	TCTTGTCTTTTGTTG ATGTT	-
PIKex9-1624	1624 G>A	AGCTCAAAGCAAATTTCTACAC GAGAT	GCACCTACCTGTGACTCCATA GAAA	CCTCTCTCTGAAAT CA	CCTCTCTCTAAAA TCA	-
PIKex9-1633	1633 G>A	TCAAAGCAAATTTCTACACGA GATCCT	GCACCTACCTGTGACTCCATA GAAA	CTCTCTGAAAATCAC TGAGCAG	CTCTGAAAATCACT AAGCAG	-
PIKex20-3140	3140 A>G	GCAAGAGGCTTTGGAGTATTT CATG	GCTGTTTAATTGTGTGGAAG ATCCAA	CCACCATGATGTGC ATC	CACCATGACGTGC ATC	-
CTNNex3-134	134C >T	GGAATCCATTCTGGTGCCACT AC	CCTCTTCTCCTCAGGATTGCCCT T	CCACTCAGAGAAG GAG	CACTCAGAAAAGG AG	-
CTNNex3-121	121A >G	CTGGCAGCAACAGTCTTACCT	GGATTGCCCTTTACCACCTCAGA GAAAG	CTGGTGCCACTACC ACA	TGGTGCCACTGCCC ACA	-

Table 5A

Target Mutation nt	Blocking Oligonucleotide sequence
34 G>A	TAGTTGGAGCTAGTGGCGTAG
34 G>T	TAGTTGGAGCTTGTGGCGTAG
34 G>C	-
35 G>A	TAGTTGGAGCTGATGGCGTAG
35 G>T	TAGTTGGAGCTGTTGGCGTAG
35 G>C	TAGTTGGAGCTGCTGGCGTAG
37 G>T	-
38 G>A	-
1799 T>A	-
436 G>A	-
134C>T	-
121A>G	-

Table 5B

Figures

Figure 1: Transplantation of tumor pieces onto highly immunodeficient NOD/SCID mice immediately upon arrival. Passages performed until development of stable growing human xenograft.

Figure 2: Results of the transplantation of 239 clinical samples. Relatively high engraftment rate of 62 % was obtained. 10 % of samples were refused as a result of unsterility, which is a natural problem of colon tissue.

Figure 3: Recognition of primary tumor / xenograft pairs originating from the same patient.

The inventors ordered mRNAs by increasing p-value in a Wilcoxon rank sum test for difference between xenografts and primary tumors along the x axis. The falling (lighter) curve shows the p-value (right scale). The right vertical bar marks the p value of 5 %, the left vertical bar marks the Bonferroni adjusted p value for multiple testing. The black curve (scale on the left) shows the fraction of same-source primary tumor/xenograft pairs that were identified after leaving out probesets with p-values below the threshold on the x-axis.

Specifically, the x-axis lists all 18018 probesets by increasing p-value in the Wilcox test: it starts on the left with p-values smaller than $1e-10$, for the probesets most different between xenografts and native samples. The falling (lighter) curve with its negative-logarithmic scale on the right y-axis shows only the higher p-values as they increase from $1e-10$ to 1 – p-values below $1e-10$ are omitted. The right vertical bar marks a threshold of 5 % without adjusting for multiple testing of 18018 probesets. A Bonferroni-adjusted threshold is shown as a left vertical bar. The black curve with its scale on the left y-axis, shows the fraction of same-source xenograft-native pairs that were identified after leaving out the number of probesets named on the x-axis. In other word, the x-axis shows the number of probesets that are removed from consideration before source identification is attempted, their removal is due to high native vs. xenograft difference, independent of their value for source characterization.

Figure 4: Mutations distribution in 67 xenograft models

Starting with the top right pie piece, going clockwise:

24/67 KRAS, 36 %; 6/67 BRAF, 9 %; 2/67 PIK3CA, 3 %; 8/67 PIK3CA + KRAS, 10 %; 1/67 PIK3CA + BRAF, 2 %; 27/67 WT, 40 %.

Sequences

In the following sequences, nucleotides whose mutation status can be determined according to the invention are written in bold; nucleotides, whose mutation status is preferably being determined according to the invention are written in bold and are also underlined.

Homo sapiens phosphoinositide-3-kinase, catalytic, alpha polypeptide (PIK3CA), mRNA (SEQ ID NO. 1)

ATGCCTCCACGACCATCATCAGGTGAACGTGTTGGGGCATCCACTTGATGCCCCAAGAATCCTAGTAGAATGTTTA
CTACCAAATGGAATGATAGTGACTTTAGAATGCCTCCGTGAGGCTACATTAATAACCATAAAGCATGAACATTTT
AAAGAAGCAAGAAAATACCCCTCCATCAACTTCTTCAAGATGAATCTTCTTACATTTTCGTAAGTGTACTCAA
GAAGCAGAAAGGGAAGAATTTTTTGGATGAAACAAGACGACTTTGTGACCTTCGGCTTTTCAACCCTTTTTAAAA
GTAATTGAACCAGTAGGCAACCGTGAAGAAAAGATCCTCAATCGAGAAAATTGGTTTTGCTATCGGCATGCCAGTG
TGTGAATTTGATATGGTTAAAGATCCAGAAGTACAGGACTTCCGAAGAAAATATTCTGAACGTTTGTAAAGAAGCT
GTGGATCTTAGGGACCTCAATTCACCTCATAGTAGAGCAATGTATGTCTATCCTCCAAATGTAGAATCTTCACCA
GAATTGCCAAAGCACATATATAATAAATTAGATAAAGGGCAAATAATAGTGGTGATCTGGGTAATAGTTTTCTCCA
AATAATGACAAGCAGAAGTATACTCTGAAAATCAACCATGACTGTGTACCAGAACAAGTAATTGCTGAAGCAATC
AGGAAAAAACTCGAAGTATGTTGCTATCCTCTGAACAACTAAAACCTCTGTGTTTTAGAAATATCAGGGCAAGTAT
ATTTTAAAGTGTTGGATGTGATGAATACTTCTAGAAAAATATCCTCTGAGTCAGTATAAGTATATAAGAAGC
TGTATAATGCTTGGGAGGATGCCCAATTTGATGTTGATGGCTAAAGAAAGCCTTTATCTCAACTGCCAATGGAC
TGTTTTACAATGCCATCTTATTCCAGACGCATTTCCACAGCTACACCATATATGAATGGAGAAAACATCTACAAAA
TCCCTTTGGGTTATAAATAGTGCACTCAGAATAAAAAATTCTTTGTGCAACCTACGTGAATGTAAATATTCGAGAC
ATTGATAAGATCTATGTTTGAACAGGTATCTACCATGGAGGAGAACCCTTATGTGACAATGTGAACACTCAAAGA
GTACCTTGTTCCAATCCCAGGTGGAATGAATGGCTGAATTATGATATATACATTCCTGATCTTCCTCGTGCTGCT
CGACTTTGCCTTTCCATTTGCTCTGTTAAAGGCCGAAAGGGTGCTAAAGAGGAACACTGTCCATTGGCATGGGGA
AATATAAAGCTTGTGTTGATTACACAGACACTCTAGTATCTGGAAAAATGGCTTTGAATCTTTGGCCAGTACCTCAT
GGATTAGAAGATTTGCTGAACCCCTATTGGTGTTACTGGATCAAATCCAAATAAAGAAAACCTCCATGCTTAGAGTTG
GAGTTTGACTGGTTCAGCAGTGTGGTAAAGTTCCAGATATGTCAGTGATTGAAGAGCATGCCAATTGGTCTGTA
TCCCAGAAAGCAGGATTTAGCTATTCCCACGCAGGACTGAGTAACAGACTAGCTAGAGACAATGAATTAAGGGAA
AATGACAAAGAACAGCTCAAAGCAATTTCTACACGAGATCCTCTCTCTGAAATCACTGAGCAGGAGAAAGATTTT
CTATGGAGTCACAGACACTATTGTGTAAGTATCCCCGAAATTTCTACCCAAATTTGCTTCTGTCTGTTAAATGGAAT
TCTAGAGATGAAGTAGCCAGATGTATTGCTTGGTAAAAGATTGGCCTCCAATCAAACCTGAACAGGCTATGGAA
CTTCTGGACTGTAATTACCCAGATCCTATGGTTCGAGGTTTTGCTGTTTCGGTGCTTGGAAAAATATTTAACAGAT
GACAACTTTCTCAGTATTTAATTCAGCTAGTACAGGTCTTAAATATGAACAATATTTGGATAACTTGCTTGTG
AGATTTTACTGAAGAAAGCATTGACTAATCAAAGGATTGGGCACCTTTTCTTTGGCATTATAAACTGAGATG
CACATAAAACAGTTAGCCAGAGGTTTGGCTGCTTTTGGAGTCTTATTGTCGTGCATGTGGGATGATTTTGAAG
CACCTGAATAGGCAAGTCAGGCAATGGAAAAGCTTAATACTAAGTACATTCTCAAACAGGAGAGAAGGAT
GAAACACAAAAGGTACAGATGAAGTTTTTAGTTGAGCAAAATGAGGCGACCAGATTTTCATGGATGCTCTACAGGGC
TTTCTGTCTCCTCTAAACCTGCTCATCAACTAGGAAACCTCAGGCTTGAAGAGTGTCGAATTATGTCCTCTGCA
AAAAGGCCACTGTGGTTGAATTGGGAGAACCCAGACATCATGTGAGAGTTACTGTTTCAGAACAAATGAGATCATC
TTTAAAAATGGGGATGATTTACGGCAAGATATGCTAACACTTCAAATATTCGTATTATGGAAAAATATCTGGCAA
AATCAAGGTCTTGATCTTCGAATGTTACCTTATGGTTGTCTGTCAATCGGTGACTGTGTGGGACTTATTGAGGTG
GTGCGAAATTCTCACACTATTATGCAATTCAGTGCAAAGGCGGCTTGAAGGTGCACTGCAGTTCAACAGCCAC
ACACTACATCAGTGGCTCAAAGACAAGAACAAGGAGAAATATATGATGCAGCCATTGACCTGTTTACACGTTCA
TGTGCTGGATACTGTGTAGCTACCTTCATTTTGGGAATTGGAGATCGTCACAATAGTAACATCATGGTGAAAGAC
GATGGACAACCTGTTTCATATAGATTTTGGACACTTTTTGGATCACAAGAAGAAAAAATTTGGTTATAAACGAGAA
CGTGTGCCATTTGTTTTGACACAGGATTTCTTAATAGTGATTAGTAAAGGAGCCCAAGAAATGCACAAAGACAAGA
GAATTTGAGAGGTTTTCAGGAGATGTGTTACAAGGCTTATCTAGCTATTTCGACAGCATGCCAATCTCTTCATAAAT
CTTTTCTCAATGATGCTTGGCTCTGGAATGCCAGAATACAATCTTTTGATGACATTGCATACATTGAAAGACC
CTAGCCTTAGATAAACTGAGCAAGAGGCTTTGGAGTATTTTCATGAAACAAATGAATGATGCACATCATGGTGGC
TGGACAACAAAAATGGATTGGATCTTCCACACAATTAACAGCATGCATTGAACTGAAAAGATAACTGAGAAAAT
GAAAGCTCACTCTGGATTCCACACTGCACTGTTAATAACTCTCAGCAGGCAAAGACCGATTGCATAGGAATTGCA
CAATCCATGAACAGCATTAGAATTTACAGCAAGAACAGAAATAAAATACTATATAATTTAAATAATGTAAACGCA
AACAGGGTTTGATAGCACTTAACTAGTTTATTTCAAATAAAGCTTTAGAATAATGCGCAATTTTCATGTTATGC
CTTAAGTCCAAAAAGGTAAACTTTGAAGATTGTTTGATCTTTTTTTAAAAAACAAAACAAAAATCCCCA
AAATATATAGAAATGATGGAGAAGGAAAAAAAAAAAAAAAAAAAA

Homo sapiens v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRAS), transcript variant b, mRNA (SEQ ID NO. 2)

ATGACTGAATATAAACTTGTGGTAGTTGGAGCTGGTGGCGTAGGCAAGAGTGCCTTGACGATACAGCTAATTCAG
AATCATTTTTGTGGACGAATATGATCCAACAATAGAGGATTCTACAGGAAGCAAGTAGTAATTGATGGAGAAACC
TGTCTCTTGGATATTCTCGACACAGCAGGTCAAGAGGAGTACAGTGCAATGAGGGACCAGTACATGAGGACTGGG
GAGGGCTTTCTTTGTGTATTTGCCATAAATAATACTAAATCATTTGAAGATATTCACCATTATAGAGAACAAATT
AAAAGAGTTAAGGACTCTGAAGATGTACCTATGGTCCTAGTAGGAAATAAATGTGATTTGCCCTCTAGAACAGTA
GACACAAAACAGGCTCAGGACTTAGCAAGAAGTTATGGAATTCCTTTTTATTGAAACATCAGCAAAGACAAGACAG
GGTGTGATGATGCCTTCTATACATTAGTTCGAGAAATTGCAAAAACATAAAAGAAAAGATGAGCAAAGATGGTAAA
AAGAAGAAAAAGAAGTCAAAGACAAAGTGTGTAATTATGTAAATACAATTTGTAATTTTTTCTTAAGGCATACTA
GTACAAGTGGTAATTTTTGTACATTACACTAAATTATTAGCATTTGTTTTAGCATTACCTAATTTTTTCTCTGCT
CCATGCAGACTGTTAGCTTTTACCTTAAATGCTTATTTTAAAATGACAGTGGAGTTTTTTTTTCTCTAAGTGC
CAGTATTTCCAGAGTTTTGGTTTTTGAAGTAGCAATGCCTGTGAAAAAGAACTGAATACCTAAGATTTCTGTCT
TGGGGTTTTTGGTGCATGCAGTTGATTACTTCTTATTTTTCTTACCAATTGTGAATGTTGGTGTGAAACAAATTA
ATGAAGCTTTTGAATCATCCCTATTCTGTGTTTTATCTAGTCACATAAATGGATTAATTACTAATTTTCAGTTGAG
ACCTTCTAATTGGTTTTTACTGAAACATTGAGGGAACACAAATTTATGGGCTTCTCTGATGATGATTCTTCTTAGGC
ATCATGTCCTATAGTTTGTTCATCCCTGATGAATGTAAAGTTACACTGTTTCACAAAGGTTTTGTCTCCTTTCCACT
GCTATTAGTCATGGTCACTCTCCCCAAAATATTATATTTTTTCTATAAAAAAGAAAAAATGGAaaaaaattacaa
GGCAATGGAACTATTATAAGGCCATTTCTTTTTCACATTAGATAAATTAATAAAAGACTCCTAATAGCTTTTCT
CTGTTAAGGCAGACCCAGTATGAAATGGGGATTATTATAGCAACCATTTTGGGGCTATATTTACATGCTACTAAA
TTTTTATAATAATTGAAAAGATTTTAAACAAGTATAAAAAATTCTCATAGGAATTAATGTAGTCTCCCTGTGTCA
GACTGCTCTTTCATAGTATAACTTTAAATCTTTTCTCAACTGAGTCTTTGAAGATAGTTTTAATTCTGCTTGT
GACATTAAGAGATTATTTGGGCCAGTTATAGCTTATTAGGTGTTGAAGAGACCAAGGTTGCAAGGCCAGGCCCTG
TGTGAACCTTTGAGCTTTTCATAGAGAGTTTTACAGCATGGACTGTGTCCACGGTCATCCAGTGTGTGCATGCA
TTGGTTAGTCAAAATGGGGAGGGACTAGGGCAGTTTGGATAGCTCAACAAGATACAATCTCACTCTGTGGTGGTC
CTGCTGACAAATCAAGAGCATTGCTTTTTGTTTTCTTAAGAAAACAAACTCTTTTTTAAAAATTACTTTTAAATATT
AACTCAAAAGTTGAGATTTTGGGGTGGTGGTGTGCCAAGACATTAATTTTTTTTTTAAACAATGAAGTGAAGAAAG
TTTTACAATCTCTAGGTTTGGCTAGTTCTCTTAACACTGGTTAAATTAACATTGCATAAACACTTTTCAAGTCTG
ATCCATATTTAATAATGCTTTAAATAAAAAATAAAAAACAATCCTTTTGATAAATTTAAATGTTACTTATTTTAA
AATAAATGAAGTGAGATGGCATGGTGAGGTGAAAGTATCACTGGACTAGGAAGAAGGTGACTTAGGTTCTAGATA
GGTGTCTTTTAGGACTCTGATTTTGGAGACATCACTTACTATCCATTTCTTCATGTTAAAAAGAGTCATCTCAAA
CTCTTAGTTTTTTTTTTTACAACTATGTAATTTATATCCATTTACATAAGGATACACTTATTTGTCAAGCTCA
GCACATCTGTAAATTTTTTAACCTATGTTACCACTCTTCAGTGCCAGTCTTGGGCAAAATGTGTCAAGAGGTGA
AGTTTATATTTGAATATCCATTCTCGTTTTAGGACTCTTCTTCCATATTAGTGTCTGCTTGCCTCCCTACCTTCC
ACATGCCCCATGACTTGATGCAGTTTTTAATACTTGTAAATCCCCTAACCATAAGATTTACTGCTGCTGTGGATAT
CTCCATGAAGTTTTCCCACTGAGTCACATCAGAAATGCCCTACATCTTATTTCTCAGGGCTCAAGAGAATCTGA
CAGATACCATAAAGGGATTTGACCTAATCACTAATTTTCAGGTGGTGGCTGATGCTTTGAACATCTCTTTGCTGC
CCAATCCATTAGCGACAGTAGGATTTTCAAACCTGGTATGAATAGACAGAACCCTATCCAGTGGAAGGAGAATT
TAATAAAGATAGTGCTGAAAGAATTCCTTAGGTAATCTATAACTAGGACTACTCCTGGTAACAGTAATACATTCC
ATTGTTTTAGTAACCAGAAATCTTCATGCAATGAAAAATACTTTAATTCATGAAGCTTACTTTTTTTTTTTGGTG
TCAGAGTCTCGCTCTTGTCAACCCAGGCTGGAATGCAGTGGCGCCATCTCAGCTCACTGCAACCTCCATCTCCCAG
GTTCAAGCGATTCTCGTGCTCGGCCCTCTGAGTAGCTGGGATTACAGGCGTGTGCCACTACACTCAACTAATTT
TTGTATTTTTTAGGAGAGACGGGGTTTTCACCTGTTGGCCAGGCTGGTCTCGAACTCCTGACCTCAAGTGATTAC
CCACCTTGGCCTCATAAACCTGTTTTGCAGAACTCATTTATTTCAGCAAAATATTTATTGAGTGCTACCAGATGCC
AGTCACCGCACAAAGGCACTGGGTATATGGTATCCCCAAACAAGAGACATAATCCCGGTCTTAGGTAGTGCTAGT
GTGGTCTGTAATATCTTACTAAGGCCTTTGGTATACGACCCAGAGATAACACGATGCGTATTTTAGTTTTGCAAA
GAAGGGGTTTTGGTCTCTGTGCCAGCTCTATAATTGTTTTGCTACGATTCCTGAACTCTTCGATCAAGCTACT
TTATGTAAATCACTTCATTGTTTTAAAGGAATAAACTTGATTATATTGTTTTTTATTTGGCATAACTGTGATTC
TTTTAGGACAATTACTGTACACATTAAGGTGATGTGATATTCATATTGACCCAAATGTGTAATATTCAGTT
TTCTCTGCATAAGTAATTAATAATACTTAAAAATTAATAGTTTTATCTGGGTACAAATAAACAGGTGCCTGAAC
TAGTTCACAGACAAGGAACTTCTATGTAAAAATCACTATGATTTCTGAATTGCTATGTGAACTACAGATCTTT
GGAACACTGTTTAGGTAGGGTGTAAAGACTTACACAGTACCTCGTTTTCTACACAGAGAAAAGAAATGGCCATACTT
CAGGAAGTGCAGTGCTTATGAGGGGATATTTAGGCCTCTTGAATTTTTGATGTAGATGGGCATTTTTTTTAAAGGTA
GTGGTTAATTACCTTTATGTGAACCTTTGAATGGTTTAAACAAAAGATTTGTTTTTGTAGAGATTTTAAAGGGGGAG
AATTCATAGAAATAAATGTTACCTAATTATTACAGCCTTAAAGACAAAAATCCTTGTTGAAGTTTTTTTTAAAAAA
GCTAAATTACATAGACTTAGGCATTAACATGTTTGTGGAAGAATATAGCAGACGTATATTGTATCATTTGAGTGA
ATGTTCCCAAGTAGGCATTCTAGGCTCTATTTAACTGAGTCACACTGCATAGGAATTTAGAACCTAATTTTTATA
GGTTATCAAACTGTTGTCAACATTGCACAATTTTGTCTAATATATACATAGAACTTTGTGGGGCATGTTAAG
TTACAGTTTGCACAAGTTCATCTCATTTGTATTCCATTGATTTTTTTTTTCTTCTAAACATTTTTTCTTCAAACA
GTATATAACTTTTTTTTAGGGGATTTTTTTTTTAGACAGCAAAAACTATCTGAAGATTTCCATTTGTCAAAAAGTAA

TGATTTCTTGATAATTGTGTAGTAATGTTTTTTAGAACCCAGCAGTTACCTTAAAGCTGAATTTATATTTAGTAA
CTTCTGTGTTAATACTGGATAGCATGAATTCTGCATTGAGAACTGAATAGCTGTCATAAAATGAAACTTTCTTT
CTAAAGAAAGATACTCACATGAGTTCTTGAAGAATAGTCATAACTAGATTAAGATCTGTGTTTTAGTTTAATAGT
TTGAAGTGCCTGTTTGGGATAATGATAGGTAATTTAGATGAATTTAGGGGAAAAAAAAGTTATCTGCAGATATGT
TGAGGGCCCATCTCTCCCCCACACCCCCACAGAGCTAACTGGGTACAGTGTTTTATCCGAAAGTTTCCAATTC
CACTGTCTTGTGTTTTTCATGTTGAAAATACTTTTTGCATTTTTTCTTTGAGTGCCAAATTTCTTACTAGTACTATTT
CTTAATGTAACATGTTTACCTGGAATGTATTTTAACTATTTTTGTATAGTGTAACCTGAAACATGCACATTTTTGT
ACATTGTGCTTTCTTTTGTGGGACATATGCAGTGTGATCCAGTTGTTTTCCATCATTGTTGCGCTGACCTAGG
AATGTTGGTCATATCAAACATTAAAAATGACCACTCTTTTAATTGAAATTAACCTTTTAAATGTTTATAGGAGTAT
GTGCTGTGAAGTGATCTAAAATTTGTAATATTTTTGTGCATGAACTGTACTACTCCTAATTATTGTAATGTAATAA
AAATAGTTACAGTGACAAAAA

**Homo sapiens v-raf murine sarcoma viral oncogene homolog B1 (BRAF), mRNA
(SEQ ID NO. 3)**

ATGGCGGGCGCTGAGCGGTGGCGGTGGTGGCGGGCGGGAGCCGGGCCAGGCTCTGTTCAACGGGGACATGGAGCCC
GAGGCCGGCGCCGGCGCCGGCGCCGGCGCTCTTCGGCTGCGGACCCTGCCATTCCGGAGGAGGTGTGGAATATC
AAACAAATGATTAAGTTGACACAGGAACATATAGAGGCCCTATTGGACAAATTTGGTGGGGAGCATAATCCACCA
TCAATATATCTGGAGGCCTATGAAGAATACACCAGCAAGCTAGATGCACTCCAACAAAAGAGAACAACAGTTATTG
GAATCTCTGGGGAACGGAAGTATTTTTCTGTTTCTAGCTCTGCATCAATGGATACCGTTACATCTTCTCTCTCT
TCTAGCCTTTTCAGTGCTACCTTCATCTCTTTTCAGTTTTTCAAATCCCACAGATGTGGCACGGAGCAACCCCAAG
TCACCACAAAACCTATCGTTAGAGTCTTCTGCCCAACAAACAGAGGACAGTGGTACCTGCAAGGTGTGGAGTT
ACAGTCCGAGACAGTCTAAAGAAAGCACTGATGATGAGAGGTCTAATCCCAGAGTGCTGTGCTGTTTACAGAATT
CAGGATGGAGAGAAGAAACCAATTGGTTGGGACACTGATATTTCTGGCTTACTGGAGAAGAATTGCATGTGGAA
GTGTTGGAGAATGTTCCACTTACAACACACAACCTTTGTACGAAAAACGTTTTTCCACTTAGCATTTTGTGACTTT
TGTCGAAAGCTGCTTTTCCAGGGTTTCCGCTGTCAAACATGTGGTTATAAATTTACCAGCGTTGTAGTACAGAA
GTTCCACTGATGTGTGTTAATTATGACCAACTTGATTTGCTGTTTGTCTCCAAGTTCTTTGAACACCACCCAATA
CCACAGGAAGAGGCGTCTTAGCAGAGACTGCCCTAACATCTGGATCATCCCCTCCGCACCCGCCTCGGACTCT
ATTGGGCCCCAAATTCTCACCAGTCCGTCTCCTTCAAATCCATTCCAATTCCACAGCCCTTCCGACCAGCAGAT
GAAGATCATCGAAATCAATTTGGGCAACGAGACCGATCCTCATCAGCTCCAATGTGCATATAAACACAATAGAA
CCTGTCAATATTGATGACTTGATTAGAGACCAAGGATTTCTGTTGATGGAGGATCAACCACAGGTTTGTCTGCT
ACCCCCCTGCCTCATTACCTGGCTCACTAACTAACGTGAAAGCCTTACAGAAATCTCCAGGACCTCAGCGAGAA
AGGAAGTCATCTTCATCCTCAGAAGACAGGAATCGAATGAAAACACTTGGTAGACGGGACTCGAGTGATGATTGG
GAGATTCTGATGGGCAGATTACAGTGGGACAAAGAATTGGATCTGGATCATTTGGAACAGTCTACAAGGGAAAG
TGGCATGGTGATGTGGCAGTGAAAATGTTGAATGTGACAGCACCTACACCTCAGCAGTTACAAGCCTTCAAAAAT
GAAGTAGGAGTACTCAGGAAAACACGACATGTGAATATCCTACTCTTCATGGGCTATTCCACAAAGCCACAACCTG
GCTATTGTTACCCAGTGGTGTGAGGGCTCCAGCTTGATACCATCTCCATATCATTGAGACCAAATTTGAGATG
ATCAAACCTATAGATATTGCACGACAGACTGCACAGGGCATGGATTACTTACACGCCAAGTCAATCATCCACAGA
GACCTCAAGAGTAATAATATATTTCTTCATGAAGACCTCAGATAAAAAATAGGTGATTTTGGTCTAGCTACAGTG
AAATCTCGATGGAGTGGGTCCCATCAGTTTGAACAGTTGTCTGGATCCATTTTGTGGATGGCACCAGAAGTCATC
AGAATGCAAGATAAAAAATCCATACAGCTTTTCAGTCAGATGTATATGCATTTGGAATTGTTCTGTATGAATTGATG
ACTGGACAGTTACCTTATTCAAACATCAACAACAGGGACCAGATAATTTTTATGGTGGGACGAGGATACCTGTCT
CCAGATCTCAGTAAGGTACGGAGTAACTGTCCAAAAGCCATGAAGAGATTAATGGCAGAGTGCCCTCAAAAAGAAA
AGAGATGAGAGACCACTCTTTCCCCAAATTTCTCGCTCTATTGAGCTGCTGGCCCGCTCATTGCCAAAATTCAC
CGCAGTGCATCAGAACCCTCCTTGAATCGGGCTGGTTTCCAAACAGAGGATTTTAGTCTATATGCTTGTGCTTCT
CCAAAACACCCATCCAGGCAGGGGATATGGTGCCTTCTGTCCACTGAAACAAATGAGTGAGAGAGTTTCAGG
AGAGTAGCAACAAAAGGAAAATAAATGAACATATGTTTGCTTATATGTTAAATGAAATAAAATACTCTCTTTTTT
TTTAAGGTGAACCAAGAACACTTGTGTGGTTAAAGACTAGATATAATTTTTTCCCCAACTAAAATTTATACTTA
ACATTGGATTTTTTAACATCCAAGGGTTAAAATACATAGACATTGCTAAAAATTTGGCAGAGCCTCTCTAGAGGCT
TTACTTTCTGTTCCGGGTTTGTATCATTCACTTGGTTATTTTTAAGTAGTAACTTCAGTTTCTCATGCACTTTT
GTTGCCAGCTATCACATGTCCACTAGGGACTCCAGAAGAAGACCCTACCTATGCCTGTGTTTGCAGGTGAGAAGT
TGGCAGTCGGTTAGCCTGGGTAGATAAGGCAAACCTGAACAGATCTAATTTAGGAAGTCAGTAGAATTTAATAAT
TCTATTATTATTCTTAATAATTTTTCTATAACTATTTCTTTTATAACAATTTGGAAAATGTGGATGTCTTTTAT
TTCCTTGAAGCAATAAACTAAGTTTCTTTTTATAAAAA

Homo sapiens catenin (cadherin-associated protein), beta 1, 88kDa (CTNNB1), transcript variant 2, mRNA (SEQ ID NO. 4)

ATGGCTACTCAAGCTGATTTGATGGAGTTGGACATGGCCATGGAACCAGACAGAAAAGCGGCTGTTAGTCACTGG
CAGCAACAGTCTTACCTGGACTCTGGAATCCATTCTGGTGCCACTACCACAGCTCCTTCTCTGAGTGGTAAAGGC
AATCCTGAGGAAGAGGATGTGGATACCTCCCAAGTCTGTATGAGTGGGAACAGGGATTTTCTCAGTCCTTCACT
CAAGAACAAGTAGCTGATATTGATGGACAGTATGCAATGACTCGAGCTCAGAGGGTACGAGCTGCTATGTTCCCT
GAGACATTAGATGAGGGCATGCAGATCCCATCTACACAGTTTGATGCTGCTCATCCCACTAATGTCCAGCGTTTG
GCTGAACCATCACAGATGCTGAAACATGCAGTTGTAAACTTGATTAACATCAAGATGATGCAGAACTTGCCACA
CGTGCAATCCCTGAACTGACAAAACCTGCTAAATGACGAGGACCAGGTGGTGGTTAATAAGGCTGCAGTTATGGTC
CATCAGCTTTCTAAAAAGGAAGCTTCCAGACACGCTATCATGCGTTCTCCTCAGATGGTGTCTGCTATTGTACGT
ACCATGCAGAATACAAATGATGTAGAAACAGCTCGTTGTACCGCTGGGACCTTGCATAACCTTTCCCATCATCGT
GAGGGCTTACTGGCCATCTTTAAGTCTGGAGGCATTCTGCCCCTGGTGAAAATGCTTGGTTTACCAGTGGATTCT
GTGTTGTTTTATGCCATTACAACTCTCCACAACCTTTTATTACATCAAGAAGGAGCTAAAATGGCAGTGCGTTTA
GCTGGTGGGCTGCAGAAAATGGTTGCCTTGCTCAACAAAACAAATGTTAAATTCTTGGCTATTACGACAGACTGC
CTTCAAATTTTAGCTTATGGCAACCAAGAAAGCAAGCTCATCATACTGGCTAGTGGTGGACCCCAAGCTTTAGTA
AATATAATGAGGACCTATACTTACGAAAAAATACTGTGGACCACAAGCAGAGTGCTGAAGGTGCTATCTGTCTGC
TCTAGTAATAAGCCGGCTATTGTAGAAGCTGGTGGAAATGCAAGCTTTAGGACTTCACCTGACAGATCCAAGTCAA
CGTCTTGTTTCAGAACTGTCTTTGGACTCTCAGGAATCTTTCAGATGCTGCAACTAAACAGGAAGGGATGGAAGGT
CTCCTTGGGACTCTTGTTTCAGCTTCTGGGTTTCAGATGATATAAATGTGGTCACCTGTGCAGCTGGAATTCCTTCT
AACCTCACTTGCAATAATTATAAGAACAAGATGATGGTCTGCCAAGTGGGTGGTATAGAGGCTCTTGTGCGTACT
GTCCTTCGGGCTGGTGACAGGGAAGACATCACTGAGCCTGCCATCTGTGCTCTTCGTCATCTGACCAGCCGACAC
CAAGAAGCAGAGATGGCCAGAATGCAGTTCGCCTTCACTATGGACTACCAGTTGTGGTTAAGCTCTTACACCCA
CCATCCCCTGGCCTCTGATAAAGGCTACTGTTGGATTGATTCGAAATCTTGCCCTTTGTCCCACAAATCATGCA
CCTTTGCGTGAGCAGGGTGCCATTCCACGACTAGTTGAGTTGCTTGTTCGTGCACATCAGGATACCCAGCGCCGT
ACGTCCATGGGTGGGACACAGCAGCAATTTGTGGAGGGGGTCCGCATGGAAGAAATAGTTGAAGGTTGTACCGGA
GCCCTTCACATCCTAGCTCGGGATGTTTACAACCGAATTGTTATCAGAGGACTAAATACCATTCCATTGTTTGTG
CAGCTGCTTTATTCTCCCATTTGAAAACATCCAAAGAGTAGCTGCAGGGGTCTCTGTGAACTTGCTCAGGACAAG
GAAGCTGCAGAAGCTATTGAAGCTGAGGGAGCCACAGCTCCTCTGACAGAGTTACTTCACTCTAGGAATGAAGGT
GTGGCGACATATGCAGCTGCTGTTTTGTTCCGAATGTCTGAGGACAAGCCACAAGATTACAAGAAACGGCTTTCA
GTTGAGCTGACCAGCTCTCTCTTCAGAACAGAGCCAATGGCTTGAATGAGACTGCTGATCTTGGACTTGATATT
GGTGCCCGAGGAGAACCCCTTGATATCGCCAGGATGATCCTAGCTATCGTTCTTTTCACTCTGGTGGATATGGC
CAGGATGCCTTGGGTATGGACCCCATGATGGAACATGAGATGGGTGGCCACCACCCTGGTGTCTGACTATCCAGTT
GATGGGCTGCCAGATCTGGGGCATGCCAGGACCTCATGGATGGGCTGCCAGGTGACAGCAATCAGCTGGCC
TGGTTTGATACTGACCTGTAAATCATCCTTTAGCTGTATTGTCTGAACTTGCATTGTGATTTGGCCTGTAGAGTTG
CTGAGAGGGCTCGAGGGGTGGGCTGGTATCTCAGAAAAGTGCTGACACACTAACCAAGCTGAGTTTCTTATGGGA
ACAATTGAAGTAACTTTTTGTTCTGGTCTTTTTGGTTCGAGGAGTAACAATACAAATGGATTTTGGGAGTGACT
CAAGAAGTGAAGAATGCACAAGAATGGATCACAAGATGGAATTTATCAAACCTAGCCTTGCTTGTAAATTTTT
TTTTTTTTTTTTTTAAGAATATCTGTAATGGTACTGACTTTGCTTGTCTTGAAGTAGCTCTTTTTTTTTTTTTTT
TTTTTTTTTTTGCAGTAACTGTTTTTTAAGTCTCTCGTAGTGTTAAGTTATAGTGAATACTGCTACAGCAATTTCT
AATTTTTAAGAATTGAGTAATGGTGTAGAACACTAATTCATAATCACTCTAATTAATTGTAATCTGAATAAAGTG
TAACAATTGTGTAGCCTTTTTGTATAAAATAGACAAATAGAAAATGGTCCAATTAGTTTCTTTTTTAATATGCTT
AAAATAAGCAGGTGGATCTATTTTCATGTTTTTGATCAAAAACATTTGGGATATGTATGGGTAGGGTAAATCAGT
AAGAGGTGTTATTTGGAACCTTGTTTTGGACAGTTTACCAGTTGCCTTTTATCCCAAAGTTGTTGTAACCTGCTG
TGATACGATGCTTCAAGAGAAAATGCGGTTATAAAAAATGGTTTCAGAAATAAACTTTTAATTTCATTGATTC

Homo sapiens neuroblastoma RAS viral (v-ras) oncogene homolog (NRAS), mRNA (SEQ ID NO. 5)

ATGACTGAGTACAACTGGTGGTGGTTGGAGCAGGTGGTGGTGGGAAAAGCGCACTGACAATCCAGCTAATCCAG
AACCCTTTGTAGATGAATATGATCCCACCATAGAGGATTCTTACAGAAAACAAGTGGTTATAGATGGTGAAACC
TGTTTGTGGACATACTGGATACAGCTGGACAAGAAGAGTACAGTGCCATGAGAGACCAATACATGAGGACAGGC
GAAGGCTTCTCTGTGTATTTGCCATCAATAATAGCAAGTCATTTGCGGATATTAACCTCTACAGGGAGCAGATT
AAGCGAGTAAAAGACTCGGATGATGTACCTATGGTGCTAGTGGGAAACAAGTGTGATTTGCCAACAAAGGACAGTT
GATACAAAACAAGCCCACGAACCTGGCCAAGAGTTACGGGATTCCATTATTGAAACCTCAGCCAAGACCAGACAG
GGTGTGGAAGATGCTTTTTACACACTGGTAAGAGAAATACGCCAGTACCGAATGAAAAAATCAACAGCAGTGAT
GATGGGACTCAGGGTTGTATGGGATGGCATGTGTGGTGATGTAACAAGATACTTTTAAAGTTTTGTGAGAAAAG
AGCCACTTTCAAGCTGCAGTGCACACCTGGTCTGCTGCTTCCCTGGAGGAGAAGTATTCCTGTTGCTGTCTTCAGT
CTCACAGAGAAGCTCCTGCTACTTCCCCAGCTCTCAGTAGTTTAGTACAATAATCTCTATTTGAGAAGTTCTCAG
AATAACTACCTCCTCACTTGGCTGTCTGACCAGAGAATGCACCTCTTGTTACTCCCTGTTATTTTTCTGCCCTGG
GTTCTTCCACAGCACAAACACACCTCTGCCACCCAGGTTTTTCATCTGAAAAGCAGTTTCATGTCTGAAACAGAG
AACCACACCGCAAACGTGAAATTCTATTGAAAACAGTGTCTTGAGCTCTAAAGTAGCAACTGCTGGTGATTTTTT

TTTTCTTTTTACTGTTGAACTTAGAACTATGCTAATTTTTGGAGAAATGTCATAAATTACTGTTTTGCCAAGAAT
ATAGTTATTATTGCTGTTTGGTTTGTATAATGTTATCGGCTCTATTCTCTAAACTGGCATCTGCTCTAGATTC
ATAAATACAAAAATGAATACTGAATTTTGAGTCTATCCTAGTCTTCACAACCTTGACGTAATTAATCCAACCTT
CACAGTGAAGTGCCTTTTTCTAGAAAGTGGTTTGTAGACTTCCTTTATAATATTTTCACTGGAATAGATGTCTCAA
AAATCCTTATGCATGAAATGAATGTCTGAGATACGTCTGTGACTTATCTACCATTTGAAGGAAAGCTATATCTATT
TGAGAGCAGATGCCATTTTGTACATGTATGAAATTGGTTTTCCAGAGGCCTGTTTTGGGGCTTTCCCAGGAGAAA
GATGAAACTGAAAGCACATGAATAATTTCACTTAATAATTTTTACCTAATCTCCACTTTTTTCATAGGTTACTAC
CTATACAATGTATGTAATTTGTTTCCCCTAGCTTACTGATAAACCTAATATTTCAATGAACTTCCATTTGTATTCA
AATTTGTGTCATACCAGAAAGCTCTACATTTGCAGATGTTCAAAATATTGTAAAACTTTGGTGCATTGTTATTTAA
TAGCTGTGATCAGTGATTTTCAAACCTCAAATATAGTATATTAACAAATTACATTTTCACTGTATATCATGGTAT
CTTAATGATGTATATAATTGCCTTCAATCCCCTTCTCACCCACCTCTACAGCTTCCCCACAGCAATAGGGGC
TTGATTATTTTCACTTGAGTAAAGCATGGTGCTAATGGACCAGGGTCACAGTTTCAAACTTGAACAATCCAGTTA
GCATCACAGAGAAAGAAATTTCTTCTGCATTTGCTCATTGCACCAGTAACCTCCAGCTAGTAATTTTGTAGGTAGC
TGCAGTTAGCCCTGCAAGGAAAGAAGAGGTCAGTTAGCACAAACCTTTACCATGACTGGAAAACTCAGTATCAC
GTATTTAAACATTTTTTTTTCTTTTGTAGCCATGTAGAACTCTAAATTAAGCCAATATTTCTCATTTGAGAAATGAGG
ATGTCTCAGCTGAGAAACGTTTTAAATTTCTTTTATTCTAATGTTCTTTGAAGGGTTTAAAAACAAGATGTTGAT
AAATCTAAGCTGATGAGTTTGTCTAAAAACAGGAAGTTGAAATTGTTGAGACAGGAATGGAAAAATATAATTAATTG
ATACCTATGAGGATTTGGAGGCTTGGCATTTTAATTTGCAGATAATACCCTGGTAATTTCTCATGAAAAATAGACT
TGGATAACTTTTTGATAAAAGACTAATTCAAAATGGCCACTTTGTTCTGTCTTTAATATCTAAATACCTTACTGA
GGTCTCTCATCTTCTATATTATGAATTTTCATTTATTAAGCAAATGTCATATTACCTTGAAATTCAGAAGAGAAG
AAACATATACTGTGTCCAGAGTATAATGAACCTGCAGAGTTGTGCTTCTTACTGCTAATTTCTGGGAGCTTTTACA
GTACTGTCTCATCTTTGTAAATGGAAATTTCTGCTTTTCTGTTTCTGCTCCTTCTGGAGCAGTGCTACTCTGTAATT
TTCCTGAGGCTTATCACCTCAGTCATTTCTTTTTTAAATGTCTGTGACTGGCAGTGATTCTTTTTCTTAAAAATC
TATTAAATTTGATGTCAAATTAGGGAGAAAGATAGTTACTCATCTTGGGCTCTTGTGCCAATAGCCCTTGTATGT
ATGTACTTAGAGTTTTTCCAAGTATGTTCTAAGCACAGAAAGTTTCTAAATGGGGCCAAAATTCAGACTTGAGTATG
TTCTTTGAATACCTTAAGAAGTTACAATTAGCCGGGCATGGTGGCCCGTGCTGTAGTCCCAGCTACTTGAGAGG
CTGAGGCAGGAGAATCACTTCAACCCAGGAGGTGGAGGTTACAGTGAGCAGAGATCGTGCCACTGCACTCCAGCC
TGGGTGACAAGAGAGACTTGTCTCAAAAAAAAAGTTACACCTAGGTGTGAATTTTGGCACAAAGGAGTGACAAA
CTTATAGTTAAAAGCTGAATAACTTCAGTGTGGTATAAAACGTGGTTTTTAGGCTATGTTTGTGATTGCTGAAAA
GAATTTCTAGTTTACCTCAAAATCCTTCTCTTTCCCCAAATTAAGTGCTGGCCAGCTGTCATAAATTACATATTC
CTTTTGGTTTTTTTTTAAAGGTTACATGTTCAAGAGTGAAAATAAGATGTTCTGTCTGAAGGCTACCATGCCGGATC
TGTAATGAACCTGTTAAATGCTGTATTTGCTCCAACGGCTTACTATAGAATGTTACTTAATACAATATCATACT
TATTACAATTTTTTACTATAGGAGTGTAATAGGTAATAATTAATCTCTATTTTAGTGGGCCCATGTTTAGTCTTTCA
CCATCCTTTAACTGCTGTGAATTTTTTTGTCTGACTTGAAGCAAGGATAGAGAAACACTTTAGAGATATGTG
GGGTTTTTTTTTACCATTCCAGAGCTTGTGAGCATAATCATATTTGCTTTATATTTATAGTCATGAACTCCTAAGTT
GGCAGCTACAACCAAGAACCAAAAAATGGTGCGTTCTGCTTCTTGTAATTCATCTCTGCTAATAAATTATAAGAA
GCAAGGAAATTAGGGAAATATTTTATTTGGATGGTTTCTATAAACAAGGGACTATAATTTCTTGACATTATTT
TTCATCTTTGCTGTTTCTTTGAGCAGTCTAATGTGCCACACAATTATCTAAGGTATTTGTTTTCTATAAGAATTG
TTTTAAAGTATTCTTGTTACCAGAGTAGTTGTATTATATTTCAAACGTAAGATGATTTTTTAAAGCCTGAGTA
CTGACCTAAGATGGAATTGTATGAACTCTGCTCTGGAGGGAGGGAGGATGTCCGTGGAAGTTGTAAGACTTTTTA
TTTTTTTTGTGCCATCAAATATAGGTAAAAATAATTGTGCAATTCTGCTGTTTAAACAGGAACCTATTGGCCTCCTT
GGCCCTAAATGGAAGGGCCGATATTTTAAAGTTGATTATTTTATTGTAAATTAATCCAACCTAGTTCTTTTTAATT
TGGTTGAATGTTTTTTCTTGTTAAATGATGTTTAAAAAATAAAAACTGGAAGTTCTTGGCTTAGTCATAATTTCTT
ATATTCA

Claims

1. A method for predicting the responsiveness of a human patient in need of anti-EGFR antibody treatment to such treatment based on a tumor sample, comprising:
 - Determining the mutation status of PIK3CA, KRAS, and BRAF in the tumor sample, and
 - Concluding, based on the mutation status of PIK3CA, KRAS, and BRAF, whether the patient is responsive to anti-EGFR antibody treatment.
2. The method of claim 1, wherein determining the mutation status of PIK3CA comprises determining whether at least one mutations is present that is chosen from the group consisting of: 1624G>A, 1633G>A, and 3140A>G.
3. The method of claim 1 or 2, wherein determining the mutation status of KRAS comprises determining whether at least one mutations is present that is chosen from the group consisting of: 34G>A, 34G>T, 34G>C, 35G>A, 35G>T, 35G>C, 38 G>A and 436 G>A.
4. The method of claims 1 to 3, wherein determining the mutation status of BRAF comprises determining whether a T>A mutations is present at position 1799 of the BRAF gene.
5. The method of claims 1 to 4, wherein a wild type mutation status of PIK3CA, KRAS, and BRAF allows concluding that the patient is responding to anti-EGFR antibody treatment.
6. The method of claims 1 to 5, wherein a wild type mutation status of PIK3CA, KRAS, BRAF and KRAS 35G>A mutation allow concluding that the patient is responding to anti-EGFR antibody treatment.
7. The method of claims 1 to 5, wherein at least one mutation in one of the genes PIK3CA, KRAS and BRAF allow concluding that the patient is not responding to anti-EGFR antibody treatment.
8. The method of claims 1 to 7, wherein the anti-EGFR antibody is cetuximab, panitumumab or IMC-11F8.

9. The method of claim 1 to 8, wherein the tumor sample is from a colorectal cancer of any of Dukes stage A to D.

10. The method of claims 1 to 9, wherein determining the mutation status is performed using genomic DNA, cDNA, mRNA, or cRNA or its fragments.

11. The method of claims 1 to 10, wherein determining the mutation status is performed using sequencing, real-time polymerase chain reaction, ligation chain reaction, Northern blotting, High Resolution Melt (HRM) analysis, Scorpions PCR, COLD-PCR, Allele Specific PCR, Single-Stranded Conformation Polymorphism Analysis, Denaturing High Performance Liquid Chromatography or microarray technology.

12. Use of a microarray for predicting the responsiveness of a human patient in need of anti-EGFR antibody treatment to such treatment, wherein the microarray comprises:

- probes for determining the mutation status of PIK3CA, KRAS, and BRAF in a biological sample.

13. Use of a kit for predicting the responsiveness of a human patient in need of anti-EGFR antibody treatment to such treatment, wherein the kit comprises:

- means for determining the mutation status of PIK3CA, KRAS, and BRAF in a biological sample.

14. Use claim 13, wherein the means for determining the mutation status of PIK3CA, KRAS, and BRAF is chosen from the group consisting of primers, probes, and blocker oligonucleotides, in particular for use in real-time polymerase chain reaction, sequencing, ligation chain reaction, or microarray technology.

15. Use of a composition for predicting responsiveness of a human patient in need of anti-EGFR antibody treatment to such treatment, wherein the composition comprises

- a first molecule from which the mutation status of PIK3CA can be deduced,
- a second molecule from which the mutation status of KRAS can be deduced, and
- a third molecule from which the mutation status of BRAF can be deduced.

16. Use of claim 15, wherein the first molecule, the second molecule, and/or the third molecule are chosen from the group consisting of genomic DNA, cDNA, mRNA, or cRNA or its fragments.

17. Use of a cancer xenograft transplanted onto a mouse for finding genes that can be used for predicting responsiveness of a human patient in need of anti-EGFR antibody treatment to such treatment, in particular wherein the genes are PIK3CA, KRAS, and/or BRAF.

Figure 1

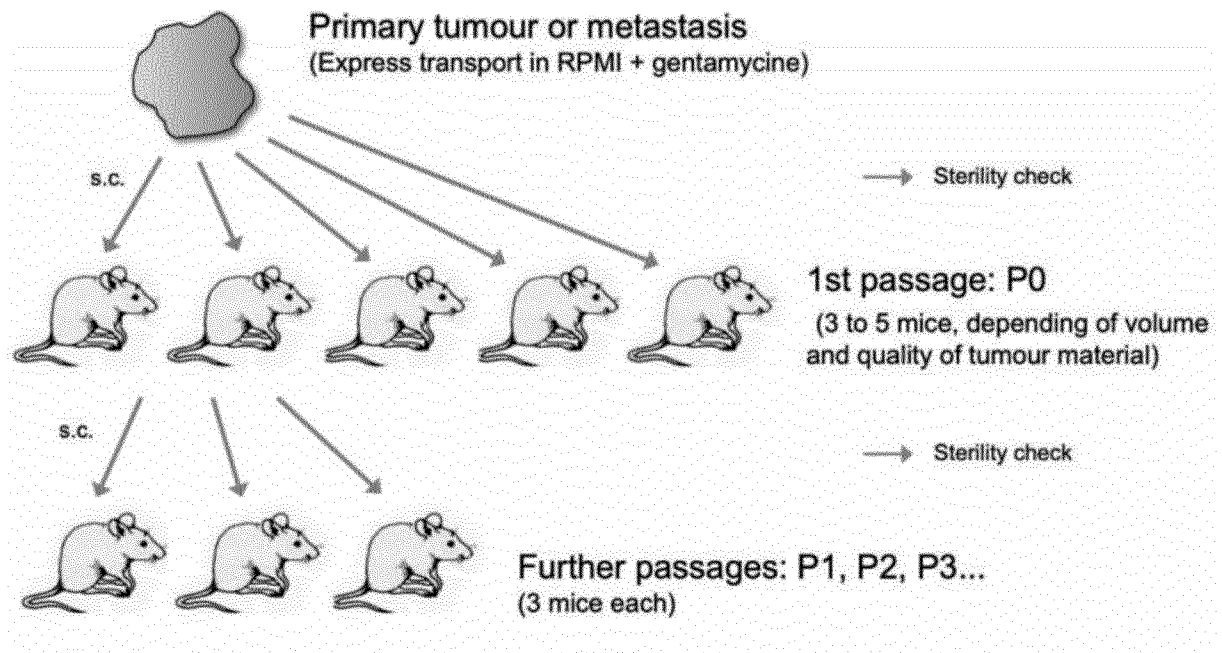


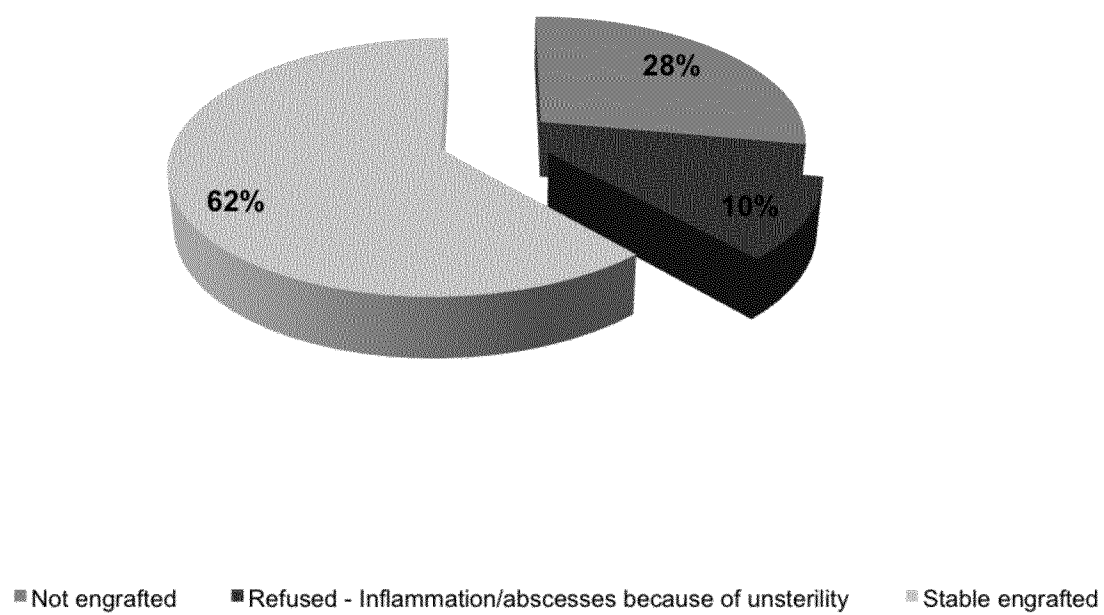
Figure 2

Figure 3

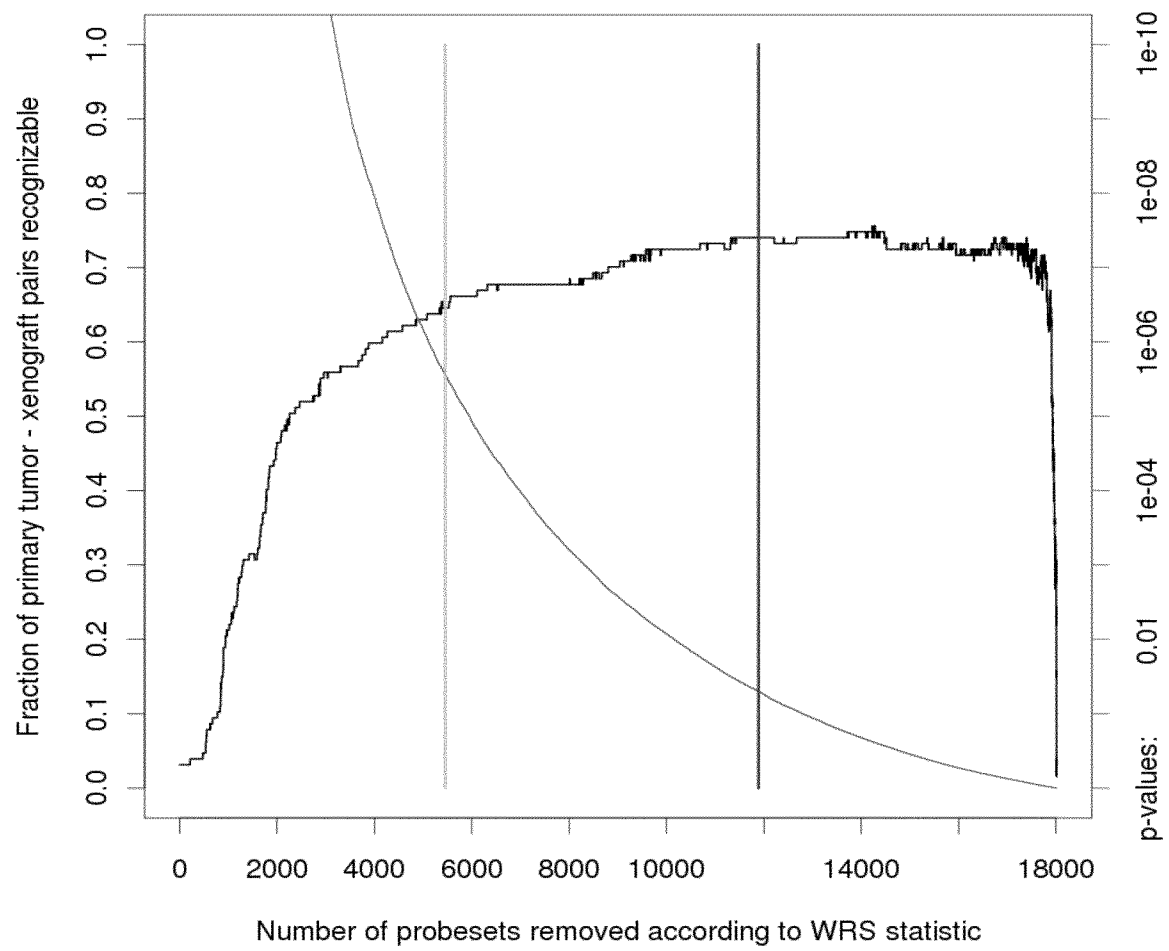


Figure 4