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(54) Title: EPIDERMAL GROWTH FACTOR RECEPTOR MUTATIONS

(57) **Abrégé/Abstract:**

Mutations of the epidermal growth factor receptor (EGFr), of phosphatidylinositol 3'-kinase ("PI3K"), and of B-Raf are described. Methods of treating tumors containing mutated EGFr with human monoclonal antibodies against EGFr are described. Methods and kits for ascertaining the presence of one or more mutant EGFr, mutant PI3K, and/or mutant B-Raf in a sample and for treating disorders or conditions related to the presence of mutant EGFr, mutant PI3K, and/or mutant B-Raf are also described. Methods of treating tumors containing mutant EGFr, mutant PI3K, and/or mutant B-Raf are also described.



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WO 2006/091899 A3

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CECI EST LE TOME 1 DE 2
CONTENANT LES PAGES 1 À 94

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THIS SECTION OF THE APPLICATION/PATENT CONTAINS MORE THAN ONE VOLUME

THIS IS VOLUME 1 OF 2
CONTAINING PAGES 1 TO 94

NOTE: For additional volumes, please contact the Canadian Patent Office

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EPIDERMAL GROWTH FACTOR RECEPTOR MUTATIONS

FIELD

[002] The present application relates to epidermal growth factor receptor ("EGFr") mutations, to polynucleotides encoding mutant EGFr polypeptides, to vectors containing those polynucleotides, cells expressing those polynucleotides, and antibodies that bind to those polypeptides. The present application also relates to phosphatidylinositol 3'-kinase ("PI3K") mutations, to polynucleotides encoding mutant PI3K polypeptides, to vectors containing those polynucleotides, cells expressing those polynucleotides, and antibodies that bind to those polypeptides. The present application also relates to B-Raf mutations, to polynucleotides encoding mutant B-Raf polypeptides, to vectors containing those polynucleotides, cells expressing those polynucleotides, and antibodies that bind to those polypeptides. The present application also relates to methods of diagnosing cancer; methods of treating cancer using compounds reactive with mutant EGFr polypeptides, mutant PI3K polypeptides, or mutant B-Raf polypeptides; and methods and kits for predicting the usefulness of anti-EGFr specific binding agents, anti-PI3K specific binding agents, or anti-B-Raf specific binding agents in the treatment of tumors.

BACKGROUND

[003] Certain applications of monoclonal antibodies in cancer therapy rely on the ability of the antibody to specifically deliver to the cancerous tissues cytotoxic effector functions such as immune-enhancing isotypes, toxins or drugs. An alternative approach is to utilize monoclonal antibodies to directly affect the survival of tumor cells by depriving them of essential extracellular proliferation signals, such as those mediated by growth factors through their cell receptors. One of the attractive targets in this approach is the epidermal growth factor receptor (EGFr), which binds EGF and transforming growth factor α (TGF α) (see, e.g., Ullrich et al., Cell 61:203-212, 1990; Baselga et al., Pharmacol. Ther. 64: 127-154, 1994; Mendelsohn

et al., in *Biologic Therapy of Cancer* 607-623, Philadelphia: J.B. Lippincott Co., 1995; Fan et al., *Curr. Opin. Oncol.* 10: 67-73, 1998). Binding of EGF or TGF α to EGFr, a 170 kDa transmembrane cell surface glycoprotein, triggers a cascade of cellular biochemical events, including EGFr autophosphorylation and internalization, which culminates in cell proliferation (see, e.g., Ullrich et al., *Cell* 61:203-212, 1990).

[004] Several observations implicate EGFr in supporting development and progression of human solid tumors. EGF-r has been demonstrated to be overexpressed on many types of human solid tumors (see, e.g., Mendelsohn *Cancer Cells* 7:359 (1989), Mendelsohn *Cancer Biology* 1:339-344 (1990), Modjtahedi and Dean *Int'l J. Oncology* 4:277-296 (1994)). For example, EGF-r overexpression has been observed in certain lung, breast, colon, gastric, brain, bladder, head and neck, ovarian, and prostate carcinomas (see, e.g., Modjtahedi and Dean *Int'l J. Oncology* 4:277-296 (1994)). The increase in receptor levels has been reported to be associated with a poor clinical prognosis (see, e.g., Baselga et al. *Pharmacol. Ther.* 64: 127-154, 1994; Mendelsohn et al., *Biologic Therapy of Cancer* pp. 607-623, Philadelphia: J.B. Lippincott Co., 1995; Modjtahedi et al., *Intl. J. of Oncology* 4:277-296, 1994; Gullick, *Br. Medical Bulletin*, 47:87-98, 1991; Salomon et al., *Crit. Rev. Oncol. Hematol.* 19: 183-232, 1995). Both epidermal growth factor (EGF) and transforming growth factor-alpha (TGF- α) have been demonstrated to bind to EGF-r and to lead to cellular proliferation and tumor growth. In many cases, increased surface EGFr expression was accompanied by production of TGF α or EGF by tumor cells, suggesting the involvement of an autocrine growth control in the progression of those tumors (see, e.g., Baselga et al. *Pharmacol. Ther.* 64: 127-154, 1994; Mendelsohn et al., *Biologic Therapy of Cancer* pp. 607-623, Philadelphia: J.B. Lippincott Co., 1995; Modjtahedi et al., *Intl. J. of Oncology* 4:277-296, 1994; Salomon et al., *Crit. Rev. Oncol. Hematol.* 19: 183-232, 1995).

[005] Thus, certain groups have proposed that antibodies against EGF, TGF- α , and EGF-r may be useful in the therapy of tumors expressing or overexpressing EGF-r (see, e.g., Mendelsohn *Cancer Cells* 7:359 (1989),

Mendelsohn *Cancer Biology* 1:339-344 (1990), Modjtahedi and Dean *Int'l J. Oncology* 4:277-296 (1994), Tosi et al. *Int'l J. Cancer* 62:643-650 (1995)). Indeed, it has been demonstrated that anti-EGF-r antibodies blocking EGF and TGF- α binding to the receptor appear to inhibit tumor cell proliferation. At the same time, however, anti-EGF-r antibodies have not appeared to inhibit EGF and TGF- α independent cell growth (Modjtahedi and Dean *Int'l J. Oncology* 4:277-296 (1994)).

[006] Monoclonal antibodies specific to the human EGFr, capable of neutralizing EGF and TGF α binding to tumor cells and of inhibiting ligand-mediated cell proliferation in vitro, have been generated from mice and rats (see, e.g., Baselga et al., *Pharmacol. Ther.* 64: 127-154, 1994; Mendelsohn et al., in *Biologic Therapy of Cancer* 607-623, Philadelphia: J.B. Lippincott Co., 1995; Fan et al., *Curr. Opin. Oncol.* 10: 67-73, 1998; Modjtahedi et al., *Intl. J. Oncology* 4: 277-296, 1994). Some of those antibodies, such as the mouse 108, 225 (see, e.g., Aboud-Pirak et al., *J. Natl. Cancer Inst.* 80: 1605-1611, 1988) and 528 (see, e.g., Baselga et al., *Pharmacol. Ther.* 64: 127-154, 1994; Mendelsohn et al., in *Biologic Therapy of Cancer* 607-623, Philadelphia: J.B. Lippincott Co., 1995) or the rat ICR16, ICR62 and ICR64 (see, e.g., Modjtahedi et al., *Intl. J. Oncology* 4: 277-296, 1994; Modjtahedi et al., *Br. J. Cancer* 67:247-253, 1993; Modjtahedi et al., *Br. J. Cancer* 67: 254-261, 1993) monoclonal antibodies, were evaluated extensively for their ability to affect tumor growth in xenograft mouse models. Most of the anti-EGFr monoclonal antibodies were efficacious in preventing tumor formation in athymic mice when administered together with the human tumor cells (Baselga et al. *Pharmacol. Ther.* 64: 127-154, 1994; Modjtahedi et al., *Br. J. Cancer* 67: 254-261, 1993). When injected into mice bearing established human tumor xenografts, the mouse monoclonal antibodies 225 and 528 caused partial tumor regression and required the co-administration of chemotherapeutic agents, such as doxorubicin or cisplatin, for eradication of the tumors (Baselga et al. *Pharmacol. Ther.* 64: 127-154, 1994; Mendelsohn et al., in *Biologic Therapy of Cancer* 607-623, Philadelphia: J.B. Lippincott Co., 1995; Fan et al., *Cancer Res.* 53: 4637-4642, 1993; Baselga et al., *J. Natl. Cancer Inst.* 85: 1327-1333, 1993). A chimeric version of the 225

monoclonal antibody (C225), in which the mouse antibody variable regions are linked to human constant regions, exhibited an improved *in vivo* anti-tumor activity but only at high doses (see, e.g., Goldstein et al., Clinical Cancer Res. 1: 1311-1318, 1995; Prewett et al., J. Immunother. Emphasis Tumor Immunol. 19: 419-427, 1996). The rat ICR16, ICR62, and ICR64 antibodies caused regression of established tumors but not their complete eradication (Modjtahedi et al., Br. J. Cancer 67: 254-261, 1993). These results established EGFr as a promising target for antibody therapy against EGFr-expressing solid tumors and led to human clinical trials with the C225 monoclonal antibody in multiple human solid cancers (see, e.g., Baselga et al. Pharmacol. Ther. 64: 127-154, 1994; Mendelsohn et al., Biologic Therapy of Cancer pp. 607-623, Philadelphia: J.B. Lippincott Co., 1995; Modjtahedi et al., Intl. J. of Oncology 4:277-296, 1994).

[007] Certain advances in the biological arts made it possible to produce a fully human anti-EGFr antibody. Using mice transgenic for human immunoglobulin genes (Xenomouse™ technology, Abgenix, Inc.), human antibodies specific for human EGFr were developed (see, e.g., Mendez, Nature Genetics, 15: 146-156, 1997; Jakobovits, Advanced Drug Delivery Reviews, 31(1-2): 33-42, 1998; Jakobovits, Expert Opinion on Investigational Drugs, 7(4): 607-614, 1998; Yang et al., Crit. Rev. Oncol. Hematol. 38(1):17-23, 2001; WO98/24893; WO 98/50433). One such antibody, panitumumab, a human IgG2 monoclonal antibody with an affinity of 5×10^{-11} M for human EGFr, has been shown to block binding of EGF to the EGFr, to block receptor signaling, and to inhibit tumor cell activation and proliferation *in vitro* (see, e.g., WO98/50433; U.S. Patent No. 6,235,883). Studies in athymic mice have demonstrated that panitumumab also has *in vivo* activity, not only preventing the formation of human epidermoid carcinoma A431 xenografts in athymic mice, but also eradicating already-established large A431 tumor xenografts (see, e.g., Yang et al., Crit. Rev. Oncol. Hematol. 38(1):17-23, 2001; Yang et al., Cancer Res. 59(6):1236-43, 1999). Panitumumab has been considered for the treatment of renal carcinoma, colorectal adenocarcinoma, prostate cancer, and non small cell squamous lung

carcinoma, among other cancers (see, e.g., U.S. Patent Publication No. 2004/0033543), and clinical trials are underway with that antibody.

[008] In certain cell types, the binding of growth factors, such as EGFr, prevents apoptosis by stimulation of phosphatidylinositol 3-kinase ("PI3K") and B-Raf. PI3K activation triggers a molecular cascade leading to the downregulation of the central pathways controlling programmed cell death (Yao, R., Science 267:2003-2006, 1995). Members of the Raf family also have been identified as regulators of programmed cell death in mammals (Hunter, Cell 80:225-236, 1995). In Raf knockouts, mice lacking B-Raf showed disturbances in cell survival, while mice lacking Raf-1 or A-Raf did not show such disturbances (see, e.g., Pritchard, Curr. Biol. 6:614-617, 1996; Wojnowski, Nat. Genet. 16:293-297, 1997), indicating that B-Raf may possess specific functions in cell death regulation. Both PI3K and B-Raf are of interest in cell proliferation disorders, particularly cancer.

SUMMARY

[009] In certain embodiments, an isolated polypeptide comprising at least one amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 12, and SEQ ID NO: 13 is provided. In certain embodiments, an isolated polypeptide consisting of at least one amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 12, and SEQ ID NO: 13 is provided.

[010] In certain embodiments, an isolated polynucleotide encoding a polypeptide comprising at least one amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 12, and SEQ ID NO: 13 is provided. In certain embodiments, an isolated polynucleotide encoding a polypeptide consisting of at least one amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 12, and SEQ ID NO: 13 is provided.

[011] In certain embodiments, an isolated polypeptide comprising at least one amino acid sequence selected from SEQ ID NO: 15, SEQ ID NO: 16, and SEQ ID NO: 17 is provided. In certain embodiments, an isolated polypeptide consisting of at least one amino acid sequence selected from SEQ ID NO: 15, SEQ ID NO: 16, and SEQ ID NO: 17 is provided.

[012] In certain embodiments, an isolated polynucleotide encoding a polypeptide comprising at least one amino acid sequence selected from SEQ ID NO: 15, SEQ ID NO: 16, and SEQ ID NO: 17 is provided. In certain embodiments, an isolated polynucleotide encoding a polypeptide consisting of at least one amino acid sequence selected from SEQ ID NO: 15, SEQ ID NO: 16, and SEQ ID NO: 17 is provided.

[013] In certain embodiments, an isolated polypeptide comprising at least one amino acid sequence selected from SEQ ID NO: 19 and SEQ ID NO: 20 is provided. In certain embodiments, an isolated polypeptide consisting of at least one amino acid sequence selected from SEQ ID NO: 19 and SEQ ID NO: 20 is provided.

[014] In certain embodiments, an isolated polynucleotide encoding a polypeptide comprising at least one amino acid sequence selected from SEQ ID NO: 19 and SEQ ID NO: 20 is provided. In certain embodiments, an isolated polynucleotide encoding a polypeptide consisting of at least one amino acid sequence selected from SEQ ID NO: 19 and SEQ ID NO: 20 is provided.

[015] In certain embodiments, a vector comprising at least one isolated polynucleotide encoding a polypeptide comprising at least one amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 19, and SEQ ID NO: 20 is provided. In certain embodiments, a host cell comprising the vector is provided. In certain embodiments, a cell transformed with at least one isolated polynucleotide encoding a polypeptide comprising at least one amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO:

12, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 19, and SEQ ID NO: 20 is provided.

[016] In certain embodiments, a method of preparing a polypeptide is provided. In certain embodiments, the method comprises culturing a host cell comprising a vector that comprises at least one isolated polynucleotide encoding a polypeptide under conditions effective for polypeptide production. In certain embodiments, the method comprises culturing a cell comprising at least one isolated polynucleotide encoding a polypeptide under conditions effective for polypeptide production. In certain embodiments, the method further comprises isolating the polypeptide. In certain embodiments, a polypeptide prepared by the method is provided.

[017] In certain embodiments, a fusion protein comprising an isolated polypeptide comprising at least one amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 19, and SEQ ID NO: 20 fused to a heterologous polypeptide is provided.

[018] In certain embodiments, a specific binding agent which is capable of binding to an isolated polypeptide comprising at least one amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 19, and SEQ ID NO: 20 is provided. In certain embodiments, the specific binding agent is selected from at least one molecule selected from: an antibody, an antibody wherein the heavy chain and the light chain are connected by a linker, a single-Fv antibody, an immunologically functional immunoglobulin fragment, a Fab antibody, a Fab' antibody, a (Fab')₂ antibody, a monoclonal antibody, a polyclonal antibody, an anti-idiotypic antibody, a fully human antibody, a humanized antibody, a chimeric antibody, a CDR-grafted antibody, and an antibody that inhibits binding of EGF to an isolated polypeptide of comprising at least one amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10,

SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 20.

[019] In certain embodiments, a method of obtaining an antibody capable of binding at least one polypeptide comprising at least one amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, and SEQ ID NO: 13 is provided. In certain embodiments, the method comprises administering at least one polypeptide comprising at least one sequence selected from SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, and SEQ ID NO: 13 to an animal. In certain embodiments, the method further comprises obtaining an antibody capable of binding at least one polypeptide comprising at least one amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, and SEQ ID NO: 13 from the animal.

[020] In certain embodiments, a transgenic non-human animal comprising at least one polynucleotide encoding at least one amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 19, and SEQ ID NO: 20 is provided.

[021] In certain embodiments, a polynucleotide encoding at least one amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 19, and SEQ ID NO: 20 attached to a solid support is provided. In certain embodiments, a polypeptide comprising at least one amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID

NO: 16, SEQ ID NO: 17, SEQ ID NO: 19, and SEQ ID NO: 20 attached to a solid support is provided.

[022] In certain embodiments, an array of polynucleotides comprising at least one polynucleotide encoding at least one amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 19, and SEQ ID NO: 20 is provided. In certain embodiments, an array of polypeptides comprising at least one polypeptide comprising at least one amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 19, and SEQ ID NO: 20 is provided.

[023] In certain embodiments, a nucleic acid probe which hybridizes to a polynucleotide encoding a region of a mutant EGFr polypeptide is provided. In certain embodiments, the region comprises at least one EGFr mutation selected from L688P, Q701H, K745N, C781R, a histidine insertion between amino acids 771 and 772, T790M, L828stop, Q849R, F910L, and V948A. In certain embodiments, the nucleic acid probe hybridizes to a complement of the polynucleotide.

[024] In certain embodiments, a method of diagnosing a disease or condition which is related to one or more EGFr mutations in a subject is provided. In certain embodiments, a method of diagnosing a susceptibility to a disease or condition which is related to one or more EGFr mutations in a subject is provided. In certain embodiments, the method comprises determining the presence or amount of expression of a polypeptide comprising at least one amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 12, and SEQ ID NO: 13 in a sample from the subject. In certain embodiments, the method further comprises diagnosing a disease or condition which is related to one or more EGFr mutations based on the presence or amount of expression of the polypeptide. In certain embodiments, the method further comprises

diagnosing a susceptibility to a disease or condition which is related to one or more EGFr mutations based on the presence or amount of expression of the polypeptide.

[025] In certain embodiments, a method of determining the presence or absence of a polynucleotide encoding a mutant EGFr polypeptide in a sample is provided. In certain embodiments, a method of determining the presence or absence of a mutant EGFr polypeptide in a sample is provided. In certain embodiments, the method comprises exposing a sample to a probe which hybridizes to a polynucleotide encoding a region of a mutant EGFr polypeptide, wherein the region comprises at least one EGFr mutation selected from L688P, Q701H, K745N, C781R, a histidine insertion between amino acids 771 and 772, T790M, L828stop, Q849R, F910L, and V948A. In certain embodiments, the method further comprises determining the presence or absence of a polynucleotide encoding a mutant EGFr polypeptide in the sample. In certain embodiments, the method comprises determining the presence or absence of a mutant EGFr mutant EGFr polypeptide in the sample.

[026] In certain embodiments, a method of diagnosing an EGFr-related cancer in a subject is provided. In certain embodiments, the method comprises determining the presence or absence of at least one mutant EGFr polypeptide comprising at least one mutation selected from: L688P, Q701H, K745N, C781R, a histidine insertion between amino acids 771 and 772, T790M, L828stop, Q849R, F910L, and V948A in a sample from the subject. In certain embodiments, the method comprises determining the presence or absence of at least one mutant EGFr polynucleotide encoding a polypeptide comprising at least one mutation selected from: L688P, Q701H, K745N, C781R, a histidine insertion between amino acids 771 and 772, T790M, L828stop, Q849R, F910L, and V948A in a sample from the subject. In certain embodiments, the presence of the at least mutant EGFr polypeptide diagnoses an EGFr-related cancer in the subject. In certain embodiments, the presence of the at least one mutant EGFr polynucleotide diagnoses an EGFr-related cancer in the subject.

[027] In certain embodiments, a method of determining a likelihood of development of an EGFr-related cancer in a subject is provided. In certain embodiments, the method comprises determining the presence or absence of at least one mutant EGFr polypeptide comprising at least one mutation selected from: L688P, Q701H, K745N, C781R, a histidine insertion between amino acids 771 and 772, T790M, L828stop, Q849R, F910L, and V948A in a sample from the subject. In certain embodiments, the method comprises determining the presence or absence of at least one mutant EGFr polynucleotide encoding a polypeptide comprising at least one mutation selected from: L688P, Q701H, K745N, C781R, a histidine insertion between amino acids 771 and 772, T790M, L828stop, Q849R, F910L, and V948A in a sample from the subject. In certain embodiments, the presence of the at least mutant EGFr polypeptide is indicative of a likelihood of development of an EGFr-related cancer in the subject. In certain embodiments, the presence of the at least one mutant EGFr polynucleotide is indicative of a likelihood of development of an EGFr-related cancer in the subject.

[028] In certain embodiments, an EGFr-related cancer is non small cell lung carcinoma.

[029] In certain embodiments, a method of screening for a modulator of activity of at least one mutant EGFr polypeptide comprising at least one mutation selected from L688P, Q701H, K745N, C781R, a histidine insertion between amino acids 771 and 772, T790M, L828stop, Q849R, F910L, and V948A is provided. In certain embodiments, the method comprises contacting a cell with a test compound and detecting if the test compound modulates the activity of the mutant EGFr polypeptide. In certain embodiments, a compound identified by the method is provided. In certain embodiments, a method of treating a disease or condition which is related to at least one EGFr mutation selected from L688P, Q701H, K745N, C781R, a histidine insertion between amino acids 771 and 772, T790M, L828stop, Q849R, F910L, and V948A is provided. In certain embodiments, the method comprises administering the compound to a subject in need of treatment for the disease or condition which is related to at least one EGFr mutation.

[030] In certain embodiments, a method for treating a subject for a disease or condition which is related to at least one EGFr mutation is provided. In certain embodiments, the method comprises detecting at least one EGFr mutation in a polynucleotide from the subject, wherein detection of at least one EGFr mutation indicates that the patient has an increased susceptibility for developing a disease or condition which is related to at least one EGFr mutation. In certain embodiments, the method comprises detecting at least one EGFr mutation in a polynucleotide from the subject, wherein detection of at least one EGFr mutation indicates that the patient has a disease or condition which is related to at least one EGFr mutation. In certain embodiments, the method further comprises administering an antibody to the subject that specifically binds a mutant EGFr polypeptide. In certain embodiments, the antibody is a human antibody. In certain embodiments, the antibody is panitumumab or an antigen-binding region thereof.

[031] In certain embodiments, an EGFr mutation is selected from L688P, Q701H, K745N, C781R, a histidine insertion between amino acids 771 and 772, T790M, L828stop, Q849R, F910L, and V948A.

[032] In certain embodiments, a disease or condition which is related to at least one EGFr mutation is non small cell lung carcinoma.

[033] In certain embodiments, a method of treating a disease or condition which is related to at least one EGFr mutation is provided. In certain embodiments, the method comprises administering a polynucleotide antisense to the polynucleotide encoding at least one amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 19, and SEQ ID NO: 20 to a subject in need of such treatment.

[034] In certain embodiments, a method for establishing a mutant EGFr population profile in a specific population of individuals is provided. In certain embodiments, the method comprises determining the presence of at least one EGFr mutation in a genetic profile of the individuals in a population. In certain embodiments, the method further comprises establishing a relationship between mutant EGFr genetic profiles and specific characteristics

of the individuals. In certain embodiments, the specific characteristics of the individuals include a susceptibility to developing a disease or condition which is related to an EGFr mutation. In certain embodiments, the specific characteristics of the individuals include exhibiting a disease or condition which is related to an EGFr mutation.

[035] In certain embodiments, a method of predicting the efficacy of gefitinib treatment on a disease or condition in a subject is provided. In certain embodiments, the method comprises determining the presence or absence of EGFr mutation T790M in a mutant EGFr polypeptide of the subject. In certain embodiments, the presence of the EGFr mutation T790M in one or more mutant EGFr polypeptides indicates resistance to treatment with gefitinib.

[036] In certain embodiments, a method of determining responsiveness to treatment with an anti-EGFr antibody in a subject suffering from cancer is provided. In certain embodiments, the method comprises determining the presence or absence of EGFr mutation T790M in the subject. In certain embodiments, the antibody is panitumumab or cetuximab.

[037] In certain embodiments, a kit for detecting a polynucleotide encoding a mutant EGFr polypeptide in a subject is provided. In certain embodiments, the kit comprises a probe which hybridizes to a polynucleotide encoding a region of a mutant EGFr polypeptide, wherein the region comprises at least one EGFr mutation selected from L688P, Q701H, K745N, C781R, a histidine insertion between amino acids 771 and 772, T790M, L828stop, Q849R, F910L, and V948A. In certain embodiments, the kit further comprises two or more amplification primers. In certain embodiments, the kit further comprises a detection component. In certain embodiments, the kit further comprises a nucleic acid sampling component.

BRIEF DESCRIPTION OF THE FIGURES

[038] Figure 1 is a table showing a mutational analysis of non small cell lung carcinoma ("NSCLC") tumor samples from twenty patients, according to the work described in Example 1. EGFr exons 18, 19, 20, 21, and 23, PI3K exons 9 and 20, and B-Raf exon 15 from the genomic DNA of

each tumor were amplified, sequenced, and compared to wild-type EGFr, PI3K, or B-Raf sequence.

[039] Figure 2 is a table showing a mutational analysis of colorectal adenocarcinoma ("CRC") tumor samples from twenty patients, according to the work described in Example 1. EGFr exons 18, 19, 20, 21, and 23, PI3K exons 9 and 20, and B-Raf exon 15 from the genomic DNA of each tumor were amplified, sequenced, and compared to wild-type EGFr, PI3K, or B-Raf sequence.

[040] Figure 3 is a table showing a mutational analysis of NSCLC tumor samples from thirty-nine patients, according to the work described in Example 2. EGFr exons 18, 19, 20, 21, and 23 and B-Raf exons 11 and 15 from the genomic DNA of each tumor were amplified, sequenced, and compared to wild-type EGFr or B-Raf sequence.

[041] Figure 4 shows radioactive gel electrophoresis analyses of the inhibitory activity of gefitinib and panitumumab on wild-type and T790M EGFr autophosphorylation, according to the work described in Example 3.

[042] Figure 5 shows alignments of certain mutant EGFr polynucleotide and polypeptide sequences and certain PI3K polynucleotide and polypeptide sequences with the corresponding wild-type sequences.

[043] Figure 6 shows polynucleotide and polypeptide sequences for wild-type and mutant EGFr molecules.

[044] Figure 7 shows polynucleotide and polypeptide sequences for wild-type and mutant PI3K molecules.

[045] Figure 8 shows polynucleotide and polypeptide sequences for wild-type and mutant B-Raf molecules.

DETAILED DESCRIPTION OF CERTAIN PREFERRED EMBODIMENTS

[046] The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

Definitions

[047] Unless otherwise defined, scientific and technical terms used in connection with the present invention shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular.

[048] Generally, nomenclatures utilized in connection with, and techniques of, cell and tissue culture, molecular biology, and protein and oligo- or polynucleotide chemistry and hybridization described herein are those well known and commonly used in the art. Standard techniques are used for recombinant DNA, oligonucleotide synthesis, and tissue culture and transformation (e.g., electroporation, lipofection). Enzymatic reactions and purification techniques are performed according to the manufacturer's specifications or as commonly accomplished in the art or as described herein. The foregoing techniques and procedures are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification. See e.g., Sambrook et al. *Molecular Cloning: A Laboratory Manual* (2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989)). The nomenclatures utilized in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well known and commonly used in the art. Standard techniques are used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients.

[049] In this application, the use of "or" means "and/or" unless stated otherwise. Furthermore, the use of the term "including", as well as other forms, such as "includes" and "included", is not limiting. Also, terms such as "element" or "component" encompass both elements and components comprising one unit and elements and components that comprise more than one subunit unless specifically stated otherwise.

[050] As utilized in accordance with the present disclosure, the following terms, unless otherwise indicated, shall be understood to have the following meanings:

[051] The terms "isolated polynucleotide" and "isolated nucleic acid" are used interchangeably, and as used herein shall mean a polynucleotide of genomic, cDNA, or synthetic origin or some combination thereof, which by virtue of its origin (1) is not associated with all or a portion of a polynucleotide in which the "isolated polynucleotide" is found in nature, (2) is operably linked to a polynucleotide which it is not linked to in nature, or (3) does not occur in nature as part of a larger sequence.

[052] The terms "isolated protein" and "isolated polypeptide" are used interchangeably, and as referred to herein mean a protein of cDNA, recombinant RNA, or synthetic origin, or some combination thereof, which by virtue of its origin, or source of derivation, (1) is not associated with proteins found in nature, (2) is free of other proteins from the same source, e.g. free of murine proteins, (3) is expressed by a cell from a different species, or (4) does not occur in nature.

[053] The terms "polypeptide" and "protein" are used interchangeably and are used herein as a generic term to refer to native protein, fragments, peptides, or analogs of a polypeptide sequence. Hence, native protein, fragments, and analogs are species of the polypeptide genus.

[054] The terminology "X#Y" in the context of a mutation in a polypeptide sequence is art-recognized, where "#" indicates the location of the mutation in terms of the amino acid number of the polypeptide, "X" indicates the amino acid found at that position in the wild-type amino acid sequence, and "Y" indicates the mutant amino acid at that position. For example, the notation "L688P" with reference to the EGFr polypeptide indicates that there is a leucine at amino acid number 688 of the wild-type EGFr sequence, and that leucine is replaced with a proline in the mutant EGFr sequence.

[055] The terms "mutant EGFr polypeptide" and "mutant EGFr protein" are used interchangeably, and refer to an EGFr polypeptide comprising at least one EGFr mutation selected from L688P, Q701H, K745N,

C781R, a histidine insertion between amino acids 771 and 772, T790M, L828stop, Q849R, F910L, and V948A. Certain exemplary mutant EGFr polypeptides include, but are not limited to, allelic variants, splice variants, derivative variants, substitution variants, deletion variants, and/or insertion variants, fusion polypeptides, orthologs, and interspecies homologs. In certain embodiments, a mutant EGFr polypeptide includes additional residues at the C- or N-terminus, such as, but not limited to, leader sequence residues, targeting residues, amino terminal methionine residues, lysine residues, tag residues and/or fusion protein residues.

[056] The terms “mutant PI3K polypeptide” and “mutant PI3K protein” are used interchangeably, and refer to a PI3K polypeptide comprising at least one PI3K mutation selected from E542K, E545A, and H1047L. Certain exemplary mutant PI3K polypeptides include, but are not limited to, allelic variants, splice variants, derivative variants, substitution variants, deletion variants, and/or insertion variants, fusion polypeptides, orthologs, and interspecies homologs. In certain embodiments, a mutant PI3K polypeptide includes additional residues at the C- or N-terminus, such as, but not limited to, leader sequence residues, targeting residues, amino terminal methionine residues, lysine residues, tag residues and/or fusion protein residues.

[057] The terms “mutant B-Raf polypeptide” and “mutant B-Raf protein” are used interchangeably, and refer to a B-Raf polypeptide comprising at least one B-Raf mutation selected from V600E and K601E. Certain exemplary mutant B-Raf polypeptides include, but are not limited to, allelic variants, splice variants, derivative variants, substitution variants, deletion variants, and/or insertion variants, fusion polypeptides, orthologs, and interspecies homologs. In certain embodiments, a mutant B-Raf polypeptide includes additional residues at the C- or N-terminus, such as, but not limited to, leader sequence residues, targeting residues, amino terminal methionine residues, lysine residues, tag residues and/or fusion protein residues.

[058] The term "mutant EGFr fusion protein" refers to a fusion of one or more amino acids (such as a heterologous polypeptide) at the amino- or carboxyl-terminus of a mutant EGFr polypeptide.

[059] The term "mutant PI3K fusion protein" refers to a fusion of one or more amino acids (such as a heterologous polypeptide) at the amino- or carboxyl-terminus of a mutant PI3K polypeptide.

[060] The term "mutant B-Raf fusion protein" refers to a fusion of one or more amino acids (such as a heterologous polypeptide) at the amino- or carboxyl-terminus of a mutant B-Raf polypeptide.

[061] The term "naturally-occurring" as used herein as applied to an object refers to the fact that an object can be found in nature. For example, a polypeptide or polynucleotide sequence that is present in an organism (including viruses) that can be isolated from a source in nature and which has not been intentionally modified by man in the laboratory or otherwise is naturally-occurring.

[062] The term "operably linked" as used herein refers to the positioning of components such that they are in a relationship permitting them to function in their intended manner. A control sequence "operably linked" to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under conditions compatible with the control sequences.

[063] The term "control sequence" as used herein refers to polynucleotide sequences which are necessary to effect the expression and processing of coding sequences to which they are ligated. The nature of such control sequences differs depending upon the host organism; in prokaryotes, such control sequences generally include promoter, ribosomal binding site, and transcription termination sequences; in eukaryotes, generally, such control sequences include promoters and transcription termination sequences. The term "control sequences" is intended to include, at a minimum, all components whose presence is essential for expression and processing, and can also include additional components whose presence is advantageous, for example, leader sequences and fusion partner sequences.

[064] The term "polynucleotide" as referred to herein means a polymeric form of nucleotides of at least 10 bases in length, either ribonucleotides or deoxynucleotides or a modified form of either type of nucleotide. The term includes single and double stranded forms of DNA.

[065] The term "oligonucleotide" referred to herein includes naturally occurring and modified nucleotides linked together by naturally occurring, and non-naturally occurring oligonucleotide linkages.

Oligonucleotides are a polynucleotide subset generally comprising a length of 200 bases or fewer. Preferably oligonucleotides are 10 to 60 bases in length and most preferably 12, 13, 14, 15, 16, 17, 18, 19, or 20 to 40 bases in length. Oligonucleotides are usually single stranded, e.g. for probes, although oligonucleotides may be double stranded, e.g. for use in the construction of a gene mutant. Oligonucleotides of the invention can be either sense or antisense oligonucleotides.

[066] The terms "mutant EGFr polynucleotide", "mutant EGFr oligonucleotide," and "mutant EGFr nucleic acid" are used interchangeably, and refer to a polynucleotide encoding an EGFr polypeptide comprising at least one EGFr mutation selected from L688P, Q701H, K745N, C781R, a histidine insertion between amino acids 771 and 772, T790M, L828stop, Q849R, F910L, and V948A.

[067] The terms "mutant PI3K polynucleotide", "mutant PI3K oligonucleotide," and "mutant PI3K nucleic acid" are used interchangeably, and refer to a polynucleotide encoding a PI3K polypeptide comprising at least one PI3K mutation selected from E542K, E545A, and H1047L.

[068] The terms "mutant B-Raf polynucleotide", "mutant B-Raf oligonucleotide," and "mutant B-Raf nucleic acid" are used interchangeably, and refer to a polynucleotide encoding a B-Raf polypeptide comprising at least one B-Raf mutation selected from L688P, Q701H, K745N, C781R, a histidine insertion between amino acids 771 and 772, T790M, L828stop, Q849R, F910L, and V948A.

[069] The term "naturally occurring nucleotides" referred to herein includes deoxyribonucleotides and ribonucleotides. The term "modified nucleotides" referred to herein includes nucleotides with modified or

substituted sugar groups and the like. The term "oligonucleotide linkages" referred to herein includes oligonucleotide linkages such as phosphorothioate, phosphorodithioate, phosphoroselenoate, phosphorodiselenoate, phosphoroanilothioate, phosphoraniladate, phosphoroamidate, and the like. See e.g., LaPlanche et al. *Nucl. Acids Res.* 14:9081 (1986); Stec et al. *J. Am. Chem. Soc.* 106:6077 (1984); Stein et al. *Nucl. Acids Res.* 16:3209 (1988); Zon et al. *Anti-Cancer Drug Design* 6:539 (1991); Zon et al. *Oligonucleotides and Analogues: A Practical Approach*, pp. 87-108 (F. Eckstein, Ed., Oxford University Press, Oxford England (1991)); Stec et al. U.S. Patent No. 5,151,510; Uhlmann and Peyman *Chemical Reviews* 90:543 (1990).

An oligonucleotide can include a label for detection, if desired.

[070] The term "selectively hybridize" referred to herein means to detectably and specifically bind. Polynucleotides, oligonucleotides, and fragments thereof selectively hybridize to nucleic acid strands under hybridization and wash conditions that minimize appreciable amounts of detectable binding to nonspecific nucleic acids. High stringency conditions can be used to achieve selective hybridization conditions as known in the art and discussed herein. Generally, the nucleic acid sequence homology between polynucleotides, oligonucleotides, and fragments and a nucleic acid sequence of interest will be at least 80%, and more typically with preferably increasing homologies of at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, and 100%. Two amino acid sequences are homologous if there is a partial or complete identity between their sequences. For example, 85% homology means that 85% of the amino acids are identical when the two sequences are aligned for maximum matching. Gaps (in either of the two sequences being matched) are allowed in maximizing matching; gap lengths of 5 or less are preferred with 2 or less being more preferred. Alternatively and preferably, two protein sequences (or polypeptide sequences derived from them of at least 30 amino acids in length) are homologous, as this term is used herein, if they have an alignment score of more than 5 (in standard deviation units) using the program ALIGN with the mutation data matrix and a gap penalty of 6 or greater. See Dayhoff, M.O., in *Atlas of Protein Sequence and Structure*,

pp. 101-110 (Volume 5, National Biomedical Research Foundation (1972)) and Supplement 2 to that volume, pp. 1-10. The two sequences or parts thereof are more preferably homologous if their amino acids are greater than or equal to 50% identical when optimally aligned using the ALIGN program. The term "corresponds to" is used herein to mean that a polynucleotide sequence is homologous (i.e., is identical, not strictly evolutionarily related) to all or a portion of a reference polynucleotide sequence, or that a polypeptide sequence is identical to a reference polypeptide sequence. In contradistinction, the term "complementary to" is used herein to mean that the complementary sequence is homologous to all or a portion of a reference polynucleotide sequence. For illustration, the nucleotide sequence "TATAC" corresponds to a reference sequence "TATAC" and is complementary to a reference sequence "GTATA".

[071] The following terms are used to describe the sequence relationships between two or more polynucleotide or amino acid sequences: "reference sequence", "comparison window", "sequence identity", "percentage of sequence identity", and "substantial identity". A "reference sequence" is a defined sequence used as a basis for a sequence comparison; a reference sequence may be a subset of a larger sequence, for example, as a segment of a full-length cDNA or gene sequence given in a sequence listing or may comprise a complete cDNA or gene sequence. Generally, a reference sequence is at least 18 nucleotides or 6 amino acids in length, frequently at least 24 nucleotides or 8 amino acids in length, and often at least 48 nucleotides or 16 amino acids in length. Since two polynucleotides or amino acid sequences may each (1) comprise a sequence (i.e., a portion of the complete polynucleotide or amino acid sequence) that is similar between the two molecules, and (2) may further comprise a sequence that is divergent between the two polynucleotides or amino acid sequences, sequence comparisons between two (or more) molecules are typically performed by comparing sequences of the two molecules over a "comparison window" to identify and compare local regions of sequence similarity. A "comparison window", as used herein, refers to a conceptual segment of at least 18 contiguous nucleotide positions or 6 amino acids wherein a

polynucleotide sequence or amino acid sequence may be compared to a reference sequence of at least 18 contiguous nucleotides or 6 amino acid sequences and wherein the portion of the polynucleotide sequence in the comparison window may comprise additions, deletions, substitutions, and the like (i.e., gaps) of 20 percent or less as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. Optimal alignment of sequences for aligning a comparison window may be conducted by the local homology algorithm of Smith and Waterman *Adv. Appl. Math.* 2:482 (1981), by the homology alignment algorithm of Needleman and Wunsch *J. Mol. Biol.* 48:443 (1970), by the search for similarity method of Pearson and Lipman *Proc. Natl. Acad. Sci. (U.S.A.)* 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package Release 7.0, (Genetics Computer Group, 575 Science Dr., Madison, Wis.), Geneworks, or MacVector software packages), or by inspection, and the best alignment (i.e., resulting in the highest percentage of homology over the comparison window) generated by the various methods is selected.

[072] The term "sequence identity" means that two polynucleotide or amino acid sequences are identical (i.e., on a nucleotide-by-nucleotide or residue-by-residue basis) over the comparison window. The term "percentage of sequence identity" is calculated by comparing two optimally aligned sequences over the window of comparison, determining the number of positions at which the identical nucleic acid base (e.g., A, T, C, G, U, or I) or residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the comparison window (i.e., the window size), and multiplying the result by 100 to yield the percentage of sequence identity. The terms "substantial identity" as used herein denotes a characteristic of a polynucleotide or amino acid sequence, wherein the polynucleotide or amino acid comprises a sequence that has at least 85 percent sequence identity, preferably at least 90 to 95 percent sequence identity, more usually at least 96, 97, 98, or 99 percent sequence identity as compared to a reference

sequence over a comparison window of at least 18 nucleotide (6 amino acid) positions, frequently over a window of at least 24-48 nucleotide (8-16 amino acid) positions, wherein the percentage of sequence identity is calculated by comparing the reference sequence to the sequence which may include deletions or additions which total 20 percent or less of the reference sequence over the comparison window. The reference sequence may be a subset of a larger sequence.

[073] As used herein, the twenty conventional amino acids and their abbreviations follow conventional usage. See *Immunology - A Synthesis* (2nd Edition, E.S. Golub and D.R. Gren, Eds., Sinauer Associates, Sunderland, Mass. (1991). The term "amino acid" or "amino acid residue," as used herein, refers to naturally occurring L amino acids or to D amino acids. The commonly used one- and three-letter abbreviations for amino acids are used herein (Bruce Alberts *et al.*, *Molecular Biology of the Cell*, Garland Publishing, Inc., New York (4th ed. 2002)). Stereoisomers (e.g., D-amino acids) of the twenty conventional amino acids, unnatural amino acids such as α -, α -disubstituted amino acids, N-alkyl amino acids, lactic acid, and other unconventional amino acids may also be suitable components for polypeptides of the present invention. Examples of unconventional amino acids include: 4-hydroxyproline, γ -carboxyglutamate, ϵ -N,N,N-trimethyllysine, ϵ -N-acetyllysine, O-phosphoserine, N-acetylserine, N-formylmethionine, 3-methylhistidine, 5-hydroxylysine, σ -N-methylarginine, and other similar amino acids and imino acids (e.g., 4-hydroxyproline). In the polypeptide notation used herein, the lefthand direction is the amino terminal direction and the righthand direction is the carboxy-terminal direction, in accordance with standard usage and convention.

[074] Similarly, unless specified otherwise, the lefthand end of single-stranded polynucleotide sequences is the 5' end; the lefthand direction of double-stranded polynucleotide sequences is referred to as the 5' direction. The direction of 5' to 3' addition of nascent RNA transcripts is referred to as the transcription direction. Sequence regions on the DNA strand having the same sequence as the RNA and which are 5' to the 5' end of the RNA

transcript are referred to as "upstream sequences". Sequence regions on the DNA strand having the same sequence as the RNA and which are 3' to the 3' end of the RNA transcript are referred to as "downstream sequences".

[075] As applied to polypeptides, the term "substantial identity" means that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 80 percent sequence identity, preferably at least 90 percent sequence identity, more preferably at least 95, 96, 97, or 98 percent sequence identity, and most preferably at least 99 percent sequence identity. Preferably, residue positions which are not identical differ by conservative amino acid substitutions. As discussed herein, minor variations in the amino acid sequences of antibodies or immunoglobulin molecules are contemplated as being encompassed by the present invention, providing that the variations in the amino acid sequence maintain at least 75%, more preferably at least 80%, 90%, 95%, and most preferably 99%. Conservative amino acid substitutions are those that take place within a family of amino acids that are related in their side chains. Genetically encoded amino acids are generally divided into families: (1) acidic=aspartate, glutamate; (2) basic=lysine, arginine, histidine; (3) non-polar=alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan; and (4) uncharged polar=glycine, asparagine, glutamine, cysteine, serine, threonine, tyrosine. More preferred families are: serine and threonine are aliphatic-hydroxy family; asparagine and glutamine are an amide-containing family; alanine, valine, leucine and isoleucine are an aliphatic family; phenylalanine, tryptophan, and tyrosine are an aromatic family, and cysteine and methionine as a sulfur-containing side chain family. For example, it is reasonable to expect that an isolated replacement of a leucine with an isoleucine or valine, an aspartate with a glutamate, a threonine with a serine, or a similar replacement of an amino acid with a structurally related amino acid will not have a major effect on the binding or properties of the resulting molecule, especially if the replacement does not involve an amino acid within a framework site. Preferred conservative amino acid substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine,

lysine-arginine, alanine-valine, glutamic acid-aspartic acid, cysteine-methionine, and asparagine-glutamine.

[076] Preferred amino acid substitutions are those which: (1) reduce susceptibility to proteolysis, (2) reduce susceptibility to oxidation, (3) alter binding affinity for forming protein complexes, (4) alter binding affinities, and (5) confer or modify other physicochemical or functional properties of such analogs. Analogs can include various muteins of a sequence other than the naturally-occurring peptide sequence. For example, single or multiple amino acid substitutions (preferably conservative amino acid substitutions) may be made in the naturally-occurring sequence (preferably in the portion of the polypeptide outside the domain(s) forming intermolecular contacts. A conservative amino acid substitution should not substantially change the structural characteristics of the parent sequence (e.g., a replacement amino acid should not tend to break a helix that occurs in the parent sequence, or disrupt other types of secondary structure that characterizes the parent sequence). Examples of art-recognized polypeptide secondary and tertiary structures are described in *Proteins, Structures and Molecular Principles* (Creighton, Ed., W. H. Freeman and Company, New York (1984)); *Introduction to Protein Structure* (C. Branden and J. Tooze, eds., Garland Publishing, New York, N.Y. (1991)); and Thornton et al. *Nature* 354:105 (1991).

[077] The term "analog" as used herein refers to polypeptides which are comprised of a segment of at least 25 amino acids that has substantial identity to a portion of an amino acid sequence of a naturally occurring polypeptide and which has at least one of the activities of the naturally occurring polypeptide. Typically, polypeptide analogs comprise a conservative amino acid substitution (or addition or deletion) with respect to the naturally-occurring sequence. Analogs typically are at least 20 amino acids long, preferably at least 50 amino acids long or longer, and can often be as long as a full-length naturally-occurring polypeptide.

[078] Peptide analogs are commonly used in the pharmaceutical industry as non-peptide drugs with properties analogous to those of the template peptide. Those types of non-peptide compound are termed "peptide

mimetics" or "peptidomimetics". Fauchere, *J. Adv. Drug Res.* 15:29 (1986); Veber and Freidinger *TINS* p.392 (1985); and Evans et al. *J. Med. Chem.* 30:1229 (1987). Such

compounds are often developed with the aid of computerized molecular modeling. Peptide mimetics that are structurally similar to therapeutically useful peptides may be used to produce an equivalent therapeutic or prophylactic effect. Generally, peptidomimetics are structurally similar to a paradigm polypeptide (i.e., a polypeptide that has a biochemical property or pharmacological activity), such as human antibody, but have one or more peptide linkages optionally replaced by a linkage selected from the group consisting of: --CH₂NH--, --CH₂S--, --CH₂-CH₂--, --CH=CH--(cis and trans), --COCH₂--, --CH(OH)CH₂--, and --CH₂SO--, by methods well known in the art. Systematic substitution of one or more amino acids of a consensus sequence with a D-amino acid of the same type (e.g., D-lysine in place of L-lysine) may be used to generate more stable peptides. In addition, constrained peptides comprising a consensus sequence or a substantially identical consensus sequence variation may be generated by methods known in the art (Rizo and Gierasch *Ann. Rev. Biochem.* 61:387 (1992);

for example, by adding internal cysteine residues capable of forming intramolecular disulfide bridges which cyclize the peptide.

[079] Preferred amino- and carboxy-termini of fragments or analogs occur near boundaries of functional domains. Structural and functional domains can be identified by comparison of the nucleotide and/or amino acid sequence data to public or proprietary sequence databases. Preferably, computerized comparison methods are used to identify sequence motifs or predicted protein conformation domains that occur in other proteins of known structure and/or function. Methods to identify protein sequences that fold into a known three-dimensional structure are known (see Bowie et al. *Science* 253:164 (1991)). Those of skill in the art can recognize sequence motifs and structural conformations that may be used to define structural and functional domains in accordance with the invention.

[080] The term "specific binding agent" refers to a natural or non-natural molecule that specifically binds to a target. Examples of specific

binding agents include, but are not limited to, proteins, peptides, nucleic acids, carbohydrates, lipids, and small molecule compounds. In certain embodiments, a specific binding agent is an antibody. In certain embodiments, a specific binding agent is an antigen binding region.

[081] The term “specific binding agent to a mutant EGFr polypeptide” refers to a specific binding agent that specifically binds any portion of a mutant EGFr polypeptide. In certain embodiments, a specific binding agent to a mutant EGFr polypeptide is an antibody to a mutant EGFr polypeptide. In certain embodiments, a specific binding agent to a mutant EGFr polypeptide is an antigen binding region.

[082] The term “specific binding agent to a mutant PI3K polypeptide” refers to a specific binding agent that specifically binds any portion of a mutant PI3K polypeptide. In certain embodiments, a specific binding agent to a mutant PI3K polypeptide is an antibody to a mutant PI3K polypeptide. In certain embodiments, a specific binding agent to a mutant PI3K polypeptide is an antigen binding region.

[083] The term “specific binding agent to a mutant B-Raf polypeptide” refers to a specific binding agent that specifically binds any portion of a mutant B-Raf polypeptide. In certain embodiments, a specific binding agent to a mutant B-Raf polypeptide is an antibody to a mutant B-Raf polypeptide. In certain embodiments, a specific binding agent to a mutant B-Raf polypeptide is an antigen binding region.

[084] The term “specifically binds” refers to the ability of a specific binding agent to bind to a target with greater affinity than it binds to a non-target. In certain embodiments, specific binding refers to binding for a target with an affinity that is at least 10, 50, 100, 250, 500, or 1000 times greater than the affinity for a non-target. In certain embodiments, affinity is determined by an affinity ELISA assay. In certain embodiments, affinity is determined by a BIAcore assay. In certain embodiments, affinity is determined by a kinetic method. In certain embodiments, affinity is determined by an equilibrium/solution method. In certain embodiments, an antibody is said to specifically bind an antigen when the dissociation constant

between the antibody and one or more of its recognized epitopes is $\leq 1 \mu\text{M}$, preferably $\leq 100 \text{ nM}$ and most preferably $\leq 10 \text{ nM}$.

[085] "Native antibodies and immunoglobulins" are usually heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light (L) chains and two identical heavy (H) chains. Each light chain is linked to a heavy chain by one covalent disulfide bond, while the number of disulfide linkages varies between the heavy chains of different immunoglobulin isotypes. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Each heavy chain has at one end a variable domain (VH) followed by a number of constant domains. Each light chain has a variable domain at one end (VL) and a constant domain at its other end; the constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light chain variable domain is aligned with the variable domain of the heavy chain. Particular amino acid residues are believed to form an interface between the light- and heavy-chain variable domains (Chothia *et al. J. Mol. Biol.* 186:651 (1985); Novotny and Haber, *Proc. Natl. Acad. Sci. U.S.A.* 82:4592 (1985); Chothia *et al., Nature* 342:877-883 (1989)).

[086] The term "antibody" refers to both an intact antibody and a antigen binding fragment thereof which competes with the intact antibody for specific binding. "Antigen binding fragment thereof" refers to a portion or fragment of an intact antibody molecule, wherein the fragment retains the antigen-binding function. Binding fragments are produced by recombinant DNA techniques, or by enzymatic or chemical cleavage of intact antibodies such as by cleavage with papain. Binding fragments include Fab, Fab', F(ab')₂, Fv, single-chain antibodies ("scFv"), Fd' and Fd fragments. Methods for producing the various fragments from monoclonal antibodies are well known to those skilled in the art (see, e.g., Pluckthun, 1992, *Immunol. Rev.* 130:151-188). An antibody other than a "bispecific" or "bifunctional" antibody is understood to have each of its binding sites be identical. An antibody substantially inhibits adhesion of a receptor to a counterreceptor when an excess of antibody reduces the quantity of receptor bound to counterreceptor by at least about 20%, 40%, 60%, or 80%, and more usually greater than

about 85%, 90%, 95%, 96%, 97%, 98%, or 99% (as measured in an in vitro competitive binding assay).

[087] An "isolated" antibody is one which has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials which would interfere with diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. In preferred embodiments, the antibody will be purified (1) to greater than 95% by weight of antibody as determined by the Lowry method, and terminal or internal amino acid sequencing by use of a spinning cup sequenator, or (2) to homogeneity by SDS-PAGE under reducing or nonreducing conditions using Coomassie blue or, preferably, silver stain. An isolated antibody includes the antibody *in situ* within recombinant cells since at least one component of the antibody's natural environment will not be present. Ordinarily, however, isolated antibody will be prepared by at least one purification step.

[088] The term "variable" refers to the fact that certain portions of the variable domains differ extensively in sequence among antibodies and are used in the binding and specificity of each particular antibody for its particular antigen. However, the variability is not evenly distributed throughout the variable domains of antibodies. It is concentrated in three segments called complementarity-determining regions (CDRs) or hypervariable regions both in the light-chain and heavy-chain variable domains. The more highly conserved portions of variable domains are called the framework (FR). The variable domains of native heavy and light chains each comprise four FR regions, largely adopting a β -sheet configuration, connected by three CDRs, which form loops connecting, and in some cases forming part of, the β -sheet structure. The CDRs in each chain are held together in close proximity by the FR regions and, with the CDRs from the other chain, contribute to the formation of the antigen-binding site of antibodies (see Kabat E.A., Wu, T.T., Perry, H., Gottesman, K. and Foeller, C. (1991) *Sequences of Proteins of Immunological Interest*, Fifth Edition. NIH Publication No. 91-3242; Kabat E.A. and Wu, T.T. (1991) *J. Immunol.*, 147, 1709-1719.)

The constant domains are not involved directly in binding an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody-dependent cellular toxicity.

[089] “Fv” is the minimum antibody fragment which contains a complete antigen-recognition and binding site. In a two-chain Fv species, this region consists of a dimer of one heavy- and one light-chain variable domain in tight, non-covalent association. In a single-chain Fv species, one heavy- and one light-chain variable domain can be covalently linked by a flexible peptide linker such that the light and heavy chains can associate in a “dimeric” structure analogous to that in a two-chain Fv species. It is in this configuration that the three CDRs of each variable domain interact to define an antigen-binding site on the surface of the VH-VL dimer. Collectively, the six CDRs confer antigen-binding specificity on the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

[090] The term “hypervariable region” when used herein refers to the amino acid residues of an antibody which are responsible for antigen-binding. The hypervariable region generally comprises amino acid residues from a “complementarity determining region” or “CDR” (e.g. residues 24-34 (L1), 50-62 (L2), and 89-97 (L3) in the light chain variable domain and 31-55 (H1), 50-65 (H2) and 95-102 (H3) in the heavy chain variable domain; Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)) and/or those residues from a “hypervariable loop” (e.g. residues 26-32 (L1), 50-52 (L2) and 91-96 (L3) in the light chain variable domain and 26-32 ((H1), 53-55 (H2) and 96-101 (H3) in the heavy chain variable domain; Chothia and Lesk *J. Mol. Biol* 196:901-917 (1987)). “Framework Region” or “FR” residues are those variable domain residues other than the hypervariable region residues as herein defined.

[091] The term “complementarity determining regions” or “CDRs,” when used herein, refers to parts of immunological receptors that make contact with a specific ligand and determine its specificity. The CDRs of immunological receptors are the most variable part of the receptor protein, giving receptors their diversity, and are carried on six loops at the distal end of

the receptor's variable domains, three loops coming from each of the two variable domains of the receptor.

[092] "Antibody-dependent cell-mediated cytotoxicity" and "ADCC" refer to a cell-mediated reaction in which non-specific cytotoxic cells that express Fc receptors (FcRs) (e.g. Natural Killer (NK) cells, neutrophils, and macrophages) recognize bound antibody on a target cell and subsequently cause lysis of the target cell. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII. Fc expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, *Annu. Rev. Immunol* 9:457-92 (1991). To assess ADCC activity of a molecule of interest, an *in vitro* ADCC assay, such as that described in US Patent No. 5,500,362, or 5,821,337 may be performed. Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed *in vivo*, e.g., in an animal model such as that disclosed in Clynes *et al. PNAS (USA)* 95:652-656 (1988).

[093] The term "epitope" includes any protein determinant capable of specific binding to an immunoglobulin and/or T-cell receptor. Epitopic determinants usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and usually have specific three dimensional structural characteristics, as well as specific charge characteristics.

[094] The term "agent" is used herein to denote a chemical compound, a mixture of chemical compounds, a biological macromolecule, or an extract made from biological materials.

[095] As used herein, the terms "label" or "labeled" refers to incorporation of a detectable marker, e.g., by incorporation of a radiolabeled amino acid or attachment to a polypeptide of biotinyl moieties that can be detected by marked avidin (e.g., streptavidin containing a fluorescent marker or enzymatic activity that can be detected by optical or colorimetric methods). In certain situations, the label or marker can also be therapeutic. Various methods of labeling polypeptides and glycoproteins are known in the art and

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

[096] The term "pharmaceutical agent or drug" as used herein refers to a chemical compound or composition capable of inducing a desired therapeutic effect when properly administered to a patient. Other chemistry terms herein are used according to conventional usage in the art, as exemplified by *The McGraw-Hill Dictionary of Chemical Terms* (Parker, S., Ed., McGraw-Hill, San Francisco (1985)).

[097] The term "antineoplastic agent" is used herein to refer to agents that have the functional property of inhibiting a development or progression of a neoplasm in a human, particularly a malignant (cancerous) lesion, such as a carcinoma, sarcoma, lymphoma, or leukemia. Inhibition of metastasis is frequently a property of antineoplastic agents.

[098] As used herein, "substantially pure" means an object species is the predominant species present (i.e., on a molar basis it is more abundant than any other individual species in the composition), and preferably a substantially purified fraction is a composition wherein the object species comprises at least about 50 percent (on a molar basis) of all macromolecular species present. Generally, a substantially pure composition will comprise more than about 80 percent of all macromolecular species present in the composition, more preferably more than about 85%, 90%, 95%, 96, 97, 98, or 99%. Most preferably, the object species is purified to essential homogeneity (contaminant species cannot be detected in the composition by conventional detection methods) wherein the composition consists essentially of a single macromolecular species.

[009] The term patient includes human and animal subjects.

[0100] The terms "mammal" and "animal" for purposes of treatment refers to any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, horses, cats, cows, etc. Preferably, the mammal is human.

[0101] The term "disease state" refers to a physiological state of a cell or of a whole mammal in which an interruption, cessation, or disorder of cellular or body functions, systems, or organs has occurred.

[0102] The terms "treat" or "treatment" refer to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) an undesired physiological change or disorder, such as the development or spread of cancer. For purposes of this invention, beneficial or desired clinical results include, but are not limited to, alleviation of symptoms, diminishment of extent of disease, stabilized (*i.e.*, not worsening) state of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. "Treatment" can also mean prolonging survival as compared to expected survival if not receiving treatment. Those in need of treatment include those already with the condition or disorder as well as those prone to have the condition or disorder or those in which the condition or disorder is to be prevented.

[0103] A "disorder" is any condition that would benefit from one or more treatments. This includes chronic and acute disorders or disease including those pathological conditions which predispose the mammal to the disorder in question. Non-limiting examples of disorders to be treated herein include benign and malignant tumors, leukemias, and lymphoid malignancies, in particular breast, rectal, ovarian, stomach, endometrial, salivary gland, kidney, colon, thyroid, pancreatic, prostate or bladder cancer. A preferred disorder to be treated in accordance with the present invention is a malignant tumor, such as cervical carcinomas and cervical intraepithelial squamous and glandular neoplasia, renal cell carcinoma (RCC), esophageal tumors, and carcinoma-derived cell lines.

[0104] A “disease or condition related to a mutant EGFr polypeptide” includes one or more of the following: a disease or condition caused by a mutant EGFr polypeptide; a disease or condition contributed to by a mutant EGFr polypeptide; a disease or condition that causes a mutant EGFr polypeptide; and a disease or condition that is associated with the presence of a mutant EGFr polypeptide. In certain embodiments, the disease or condition related to a mutant EGFr polypeptide may exist in the absence of the mutant EGFr polypeptide. In certain embodiments, the disease or condition related to a mutant EGFr polypeptide may be exacerbated by the presence of a mutant EGFr polypeptide. In certain embodiments, a disease or condition related to a mutant EGFr polypeptide is a cancer. Exemplary cancers include, but are not limited to, non small cell lung carcinoma, breast, colon, gastric, brain, bladder, head and neck, ovarian, and prostate carcinomas.

[0105] A “disease or condition related to a mutant PI3K polypeptide” includes one or more of the following: a disease or condition caused by a mutant PI3K polypeptide; a disease or condition contributed to by a mutant PI3K polypeptide; a disease or condition that causes a mutant PI3K polypeptide; and a disease or condition that is associated with the presence of a mutant PI3K polypeptide. In certain embodiments, the disease or condition related to a mutant PI3K polypeptide may exist in the absence of the mutation. In certain embodiments, the disease or condition related to a mutant PI3K polypeptide may be exacerbated by the presence of a mutant PI3K polypeptide. In certain embodiments, a disease or condition related to a mutant PI3K polypeptide is a cancer. Exemplary cancers include, but are not limited to, non small cell lung carcinoma, breast, colon, gastric, brain, bladder, head and neck, ovarian, and prostate carcinomas

[0106] A “disease or condition related to a mutant B-Raf polypeptide” includes one or more of the following: a disease or condition caused by a mutant B-Raf polypeptide; a disease or condition contributed to by a mutant B-Raf polypeptide; a disease or condition that causes a mutant B-Raf polypeptide; and a disease or condition that is associated with the presence of a mutant B-Raf polypeptide. In certain embodiments, the

disease or condition related to a mutant B-Raf polypeptide may exist in the absence of the mutation. In certain embodiments, the disease or condition related to a mutant B-Raf polypeptide may be exacerbated by the presence of a mutant B-Raf polypeptide. In certain embodiments, a disease or condition related to a mutant B-Raf polypeptide is a cancer. Exemplary cancers include, but are not limited to, non small cell lung carcinoma, breast, colon, gastric, brain, bladder, head and neck, ovarian, and prostate carcinomas.

[0107] In “combined therapy,” patients are treated with a specific binding agent for a target antigen in combination with a chemotherapeutic or antineoplastic agent and/or radiation therapy. The cancer is treated under protocol by the addition of a specific binding agent to a mutant EGFr polypeptide, a specific binding agent to a mutant PI3K polypeptide, and/or a specific binding agent to a mutant B-Raf polypeptide to standard first and second line therapy. Protocol designs will address effectiveness as assessed by reduction in tumor mass as well as the ability to reduce usual doses of standard chemotherapy. These dosage reductions will allow additional and/or prolonged therapy by reducing dose-related toxicity of the chemotherapeutic agent.

[0108] “Monotherapy” refers to the treatment of a disorder by administering immunotherapy to patients without an accompanying chemotherapeutic or antineoplastic agent.

Certain Embodiments

Polypeptides, Fragments, and Fusion Proteins

[0109] In certain embodiments, a deletion variant is a fragment of a full-length mutant EGFr polypeptide. In certain embodiments, such a fragment corresponds to an epitope of a mutant EGFr polypeptide. In certain embodiments, such a fragment is naturally-occurring (e.g., due to in vivo protease activity). In certain embodiments, such a fragment is chemically synthesized. In certain embodiments, such a fragment may be linked to a polypeptide to form a mutant EGFr fusion protein. In certain embodiments, such a fragment is at least 5, 6, 8 or 10 amino acids long. In certain embodiments, such a fragment is at least 14, at least 20, at least 50, or at least 70 amino acids long.

[0110] In certain embodiments, a deletion variant is a fragment of a full-length mutant PI3K polypeptide is provided. In certain embodiments, such a fragment corresponds to an epitope of a mutant PI3K polypeptide. In certain embodiments, such a fragment is naturally-occurring (e.g., due to in vivo protease activity). In certain embodiments, such a fragment is chemically synthesized. In certain embodiments, such a fragment may be linked to a polypeptide to form a mutant PI3K fusion protein. In certain embodiments, such a fragment is at least 5, 6, 8 or 10 amino acids long. In certain embodiments, such a fragment is at least 14, at least 20, at least 50, or at least 70 amino acids long.

[0111] In certain embodiments, a deletion variant is a fragment of a full-length mutant B-Raf polypeptide is provided. In certain embodiments, such a fragment corresponds to an epitope of a mutant B-Raf polypeptide. In certain embodiments, such a fragment is naturally-occurring (e.g., due to in vivo protease activity). In certain embodiments, such a fragment is chemically synthesized. In certain embodiments, such a fragment may be linked to a polypeptide to form a mutant B-Raf fusion protein. In certain embodiments, such a fragment is at least 5, 6, 8 or 10 amino acids long. In certain embodiments, such a fragment is at least 14, at least 20, at least 50, or at least 70 amino acids long.

[0112] In certain embodiments, a mutant polypeptide may be linked to at least one non-proteinaceous. Such groups include, but are not limited to, N-linked or O-linked carbohydrate chains, water-soluble polymers such as polyethylene glycol (PEG), and derivatives thereof (see for example U.S. Patent No. 4,179,337). Other chemical modifications within the meaning of this term include, but are not limited to, ethylene glycol/propylene glycol copolymers, carboxymethylcellulose, dextran, polyvinyl alcohol, and related molecules.

[0113] In certain embodiments, a mutant EGFr polypeptide may be modified at random positions within the molecule, or at predetermined positions within the molecule and may include one, two, three or more attached chemical moieties. In certain embodiments, a mutant EGFr polypeptide may also be modified at pre-determined positions in the

polypeptide, such as at the amino terminus, or at a selected lysine or arginine residue within the polypeptide. Other chemical modifications include, but are not limited to, a detectable label, such as an enzymatic, fluorescent, isotopic or affinity label to allow for detection and isolation of the mutant EGFr polypeptide.

[0114] In certain embodiments, a mutant PI3K polypeptide may be modified at random positions within the molecule, or at predetermined positions within the molecule and may include one, two, three or more attached chemical moieties. In certain embodiments, a mutant PI3K polypeptide may also be modified at pre-determined positions in the polypeptide, such as at the amino terminus, or at a selected lysine or arginine residue within the polypeptide. Other chemical modifications include, but are not limited to, a detectable label, such as an enzymatic, fluorescent, isotopic or affinity label to allow for detection and isolation of the mutant PI3K polypeptide.

[0115] In certain embodiments, a mutant B-Raf polypeptide may be modified at random positions within the molecule, or at predetermined positions within the molecule and may include one, two, three or more attached chemical moieties. In certain embodiments, a mutant B-Raf polypeptide may also be modified at pre-determined positions in the polypeptide, such as at the amino terminus, or at a selected lysine or arginine residue within the polypeptide. Other chemical modifications include, but are not limited to, a detectable label, such as an enzymatic, fluorescent, isotopic or affinity label to allow for detection and isolation of the mutant B-Raf polypeptide.

[0116] In certain embodiments, a mutant EGFr fusion protein is provided. In certain embodiments, a mutant EGFr polypeptide may be fused to a homologous polypeptide to form a homodimer or to a heterologous polypeptide to form a heterodimer. Exemplary heterologous polypeptides and peptides include, but are not limited to: an epitope to allow for the detection and/or isolation of the fusion protein; a transmembrane receptor protein or a portion thereof, such as an extracellular domain, a transmembrane domain, or an intracellular domain; a ligand or a portion thereof which binds to a

transmembrane receptor protein; an enzyme or portion thereof which is catalytically active; a polypeptide which promotes oligomerization, including, but not limited to a leucine zipper domain; a polypeptide which increases the stability of the fusion protein, including, but not limited to, an immunoglobulin constant region; and a polypeptide which has a therapeutic activity different from the mutant EGFr polypeptide. In certain embodiments, a mutant EGFr polypeptide or mutant EGFr fusion protein may be linked to an N-terminal methionine, which may be useful to allow expression in prokaryotic cells such as E. coli.

[0117] In certain embodiments, a mutant PI3K fusion protein is provided. In certain embodiments, a mutant PI3K polypeptide may be fused to a homologous polypeptide to form a homodimer or to a heterologous polypeptide to form a heterodimer. Exemplary heterologous polypeptides and peptides include, but are not limited to: an epitope to allow for the detection and/or isolation of the fusion protein; a transmembrane receptor protein or a portion thereof, such as an extracellular domain, a transmembrane domain, or an intracellular domain; a ligand or a portion thereof which binds to a transmembrane receptor protein; an enzyme or portion thereof which is catalytically active; a polypeptide which promotes oligomerization, including, but not limited to a leucine zipper domain; a polypeptide which increases the stability of the fusion protein, including, but not limited to, an immunoglobulin constant region; and a polypeptide which has a therapeutic activity different from the mutant PI3K polypeptide. In certain embodiments, a mutant P13K polypeptide or mutant P13K fusion protein may be linked to an N-terminal methionine, which may be useful to allow expression in prokaryotic cells such as E. coli.

[0118] In certain embodiments, a mutant B-Raf fusion protein is provided. In certain embodiments, a mutant B-Raf polypeptide may be fused to a homologous polypeptide to form a homodimer or to a heterologous polypeptide to form a heterodimer. Exemplary heterologous polypeptides and peptides include, but are not limited to: an epitope to allow for the detection and/or isolation of a mutant B-Raf fusion protein; a transmembrane receptor protein or a portion thereof, such as an extracellular domain, a

transmembrane domain, or an intracellular domain; a ligand or a portion thereof which binds to a transmembrane receptor protein; an enzyme or portion thereof which is catalytically active; a polypeptide which promotes oligomerization, including, but not limited to a leucine zipper domain; a polypeptide which increases the stability of the fusion protein, including, but not limited to, an immunoglobulin constant region; and a polypeptide which has a therapeutic activity different from the mutant B-Raf polypeptide. In certain embodiments, a mutant B-Raf polypeptide or mutant B-Raf fusion protein may be linked to an N-terminal methionine, which may be useful to allow expression in prokaryotic cells such as E. coli.

[0119] In certain embodiments, a heterologous or homologous polypeptide is fused to the amino-terminus of a mutant EGFr polypeptide. In certain embodiments, a heterologous or homologous polypeptide is fused to carboxy-terminus of a mutant EGFr polypeptide. In certain embodiments, one or more heterologous or homologous polypeptides or peptides is fused to both the amino- and the carboxy-termini of a mutant EGFr polypeptide. In certain embodiments, a polypeptide is fused directly to a mutant EGFr polypeptide. In certain embodiments, a polypeptide is fused to a mutant EGFr polypeptide via a linker or adapter molecule, as is known in the art. In certain such embodiments, the linker or adapter molecule is designed to contain a cleavage site for a protease to allow for the separation of the fused polypeptides.

[0120] In certain embodiments, a heterologous or homologous polypeptide is fused to the amino-terminus of a mutant PI3K polypeptide. In certain embodiments, a heterologous or homologous polypeptide is fused to carboxy-terminus of a mutant PI3K polypeptide. In certain embodiments, one or more heterologous or homologous polypeptides or peptides is fused to both the amino- and the carboxy-termini of a mutant PI3K polypeptide. In certain embodiments, a polypeptide is fused directly to a mutant PI3K polypeptide. In certain embodiments, a polypeptide is fused to a mutant PI3K polypeptide via a linker or adapter molecule, as is known in the art. In certain such embodiments, the linker or adapter molecule is designed to contain a

cleavage site for a protease to allow for the separation of the fused polypeptides.

[0121] In certain embodiments, a heterologous or homologous polypeptide is fused to the amino-terminus of a mutant B-Raf polypeptide. In certain embodiments, a heterologous or homologous polypeptide is fused to carboxy-terminus of a mutant B-Raf polypeptide. In certain embodiments, one or more heterologous or homologous polypeptides are fused to both the amino- and the carboxy-termini of a mutant B-Raf polypeptide. In certain embodiments, a polypeptide is fused directly to a mutant B-Raf polypeptide. In certain embodiments, a polypeptide is fused to a mutant B-Raf polypeptide via a linker or adapter molecule, as is known in the art. In certain such embodiments, the linker or adapter molecule is designed to contain a cleavage site for a protease to allow for the separation of the fused polypeptides.

Vectors, Host Cells, Transgenic Animals, and Protein Production and Purification

[0122] In certain embodiments, a vector comprising at least one polynucleotide encoding a mutant EGFr polypeptide is provided. In certain such embodiments, the mutant EGFr polypeptide comprises at least one amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, and SEQ ID NO: 13. In certain embodiments, mutant EGFr polypeptide comprises at least one EGFr mutation selected from: L688P, Q701H, K745N, C781R, a histidine insertion between amino acids 771 and 772, T790M, L828stop, Q849R, F910L, and V948A. In certain embodiments, the vector is an expression vector.

[0123] In certain embodiments, a vector comprising at least one polynucleotide encoding a mutant PI3K polypeptide is provided. In certain such embodiments, the mutant PI3K polypeptide comprises at least one amino acid sequence selected from SEQ ID NO: 15, SEQ ID NO: 16, and SEQ ID NO: 17. In certain embodiments, a mutant PI3K polypeptide comprises at least one PI3K mutation selected from: E542K, E545A, and H1047L. In certain embodiments, the vector is an expression vector.

[0124] In certain embodiments, a vector comprising at least one polynucleotide encoding a mutant B-Raf polypeptide is provided. In certain such embodiments, the mutant B-Raf polypeptide comprises an amino acid sequence selected from SEQ ID NO: 19 and SEQ ID NO: 20. In certain embodiments, a mutant B-Raf polypeptide comprises at least one B-Raf mutation selected from: V600E and K601E. In certain embodiments, the vector is an expression vector.

[0125] In certain embodiments, the expression vector may contain a promoter that is recognized by the host organism and operably linked to a nucleic acid molecule encoding a mutant EGFr. In certain embodiments, a native or heterologous promoter may be used depending on the host cell used for expression and the yield of protein desired.

[0126] Exemplary promoters for use with prokaryotic hosts include, but are not limited to, beta-lactamase and lactose promoter systems; alkaline phosphatase; a tryptophan (trp) promoter system; and hybrid promoters such as the tac promoter. In certain embodiments, other known bacterial promoters may be used. The sequences of known bacterial promoters have been published, thereby enabling one skilled in the art to ligate them to the desired nucleic acid sequence(s), using linkers or adapters as needed to supply any desired restriction sites.

[0127] Suitable promoters for use with yeast hosts are also well known in the art. In certain embodiments, yeast enhancers are advantageously used with yeast promoters. Suitable promoters for use with mammalian host cells are well known. Exemplary promoters for use with mammalian host cells include, but are not limited to, those obtained from the genomes of viruses such as polyoma virus, fowlpox virus, adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus and most preferably Simian Virus 40 (SV40). Exemplary mammalian promoters include, but are not limited to, heterologous mammalian promoters. Exemplary heterologous mammalian promoters include, but are not limited to, heat-shock promoters and the actin promoter.

[0128] Exemplary promoters which may be used for expressing mutant EGFr polynucleotides include, but are not limited to, the SV40 early promoter region (Benoist and Chambon (1981), *Nature*, 290:304-310); the CMV promoter; the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto et al. (1980), *Cell*, 22: 787-97); the herpes thymidine kinase promoter (Wagner et al. (1981), *Proc. Natl. Acad. Sci. U.S.A.*, 78: 1444-5); the regulatory sequences of the metallothionine gene (Brinster et al. (1982), *Nature*, 296: 39-42); prokaryotic expression vectors such as the beta-lactamase promoter (Villa-Kamaroff et al. (1978), *Proc. Natl. Acad. Sci. U.S.A.*, 75: 3727-31); and the tac promoter (DeBoer, et al. (1983), *Proc. Natl. Acad. Sci. U.S.A.*, 80: 21-25).

[0129] In certain embodiments, an enhancer sequence may be included in a vector to increase transcription in eukaryotic host cells. Exemplary enhancer sequences from mammalian genes include, but are not limited to, globin, elastase, albumin, alpha-feto-protein, and insulin. In certain embodiments, an enhancer from a virus is used. Exemplary enhancer sequences for the activation of eukaryotic promoters include, but are not limited to, the SV40 enhancer, the cytomegalovirus early promoter enhancer, the polyoma enhancer, and adenovirus enhancers. In certain embodiments, an enhancer may be spliced into the vector at a position 5' or 3' to the polypeptide coding region. In certain embodiments, the enhancer is located at a site 5' from the promoter. In certain embodiments, the enhancer is located at a site 3' from the end of the polypeptide coding region.

[0130] In certain embodiments, vectors are those which are compatible with at least one of bacterial, insect, and mammalian host cells. Exemplary vectors include, but are not limited to, pCRII, pCR3, and pcDNA3.1 (Invitrogen Company, San Diego, CA), pBSII (Stratagene Company, La Jolla, CA), pET15 (Novagen, Madison, WI), pGEX (Pharmacia Biotech, Piscataway, NJ), pEGFP-N2 (Clontech, Palo Alto, CA), pETL (BlueBacII; Invitrogen), pDSR-alpha (PCT Publication No. WO90/14363) and pFastBacDual (Gibco/BRL, Grand Island, NY).

[0131] Exemplary vectors include, but are not limited to, cosmids, plasmids and modified viruses compatible with the selected host cell. In

certain embodiments, the vectors may include plasmids including, but not limited to, Bluescript[®] plasmid derivatives (a high copy number ColE1-based phagemid, Stratagene Cloning Systems Inc., La Jolla CA), PCR cloning plasmids designed for cloning Taq-amplified PCR products (e.g., TOPO[™] TA Cloning[®] Kit, PCR2.1[®] plasmid derivatives, Invitrogen, Carlsbad, CA), and mammalian, yeast or virus vectors such as a baculovirus expression system (pBacPAK plasmid derivatives, Clontech, Palo Alto, CA). In certain embodiments, the recombinant molecules may be introduced into host cells via transformation, transfection, infection, electroporation, or other known techniques.

[0132] The term “transfection” refers to the taking up of an expression vector by a host cell whether or not any coding sequences are in fact expressed. Numerous methods of transfection are known to the ordinarily skilled artisan, including, but not limited to, CaPO₄ precipitation and electroporation. In certain embodiments, successful transfection is recognized when any indication of the operation of the transfected vector occurs within the host cell.

[0133] In certain embodiments, host cells may be prokaryotic host cells (such as *E. coli*) or eukaryotic host cells (such as a yeast cell, an insect cell, or a vertebrate cell). In certain embodiments, prokaryotic host cells such as *E. coli* produce unglycosylated protein; for example, unglycosylated shBCMA and unglycosylated shTACI, which may possess advantages over the glycosylated eukaryotic molecules. In certain embodiments, a host cell, when cultured under appropriate conditions, expresses a polypeptide of the invention which can subsequently be collected from the culture medium (if the host cell secretes it into the medium) or directly from the host cell producing it (if it is not secreted). In certain embodiments, selection of an appropriate host cell will take into account various factors, such as desired expression levels, polypeptide modifications that are desirable or necessary for activity, such as glycosylation or phosphorylation, and/or ease of folding into a biologically active molecule.

[0134] A number of suitable host cells are known in the art and many are available from the American Type Culture Collection (ATCC),

Manassas, VA. Exemplary host cells include, but are not limited to, mammalian cells, such as Chinese hamster ovary cells (CHO) (ATCC No. CCL61) CHO DHFR- cells (Urlaub et al. (1980), *Proc. Natl. Acad. Sci. USA* 97, 4216-20), human embryonic kidney (HEK) 293 or 293T cells (ATCC No. CRL1573), and 3T3 cells (ATCC No. CCL92). The selection of suitable mammalian host cells and methods for transformation, culture, amplification, screening and product production and purification are known in the art. Exemplary host cells include, but are not limited to, the monkey COS-1 (ATCC No. CRL1650) and COS-7 cell lines (ATCC No. CRL1651), and the CV-1 cell line (ATCC No. CCL70). Exemplary mammalian host cells include, but are not limited to, primate cell lines and rodent cell lines, including transformed cell lines. Exemplary host cells include, but are not limited to, normal diploid cells, cell strains derived from *in vitro* culture of primary tissue, stem cell lines, and primary explants. In certain embodiments, candidate cells may be genotypically deficient in the selection gene, or may contain a dominantly acting selection gene. Exemplary host cells include, but are not limited to, mouse neuroblastoma N2A cells, HeLa, mouse L-929 cells, 3T3 lines derived from Swiss, Balb-c or NIH mice, BHK or HaK hamster cell lines, which are available from the American Type Culture Collection, Manassas, VA). Each of these cell lines is known by and available to those skilled in the art of protein expression.

[0135] In certain embodiments, host cells may be bacterial cells. Exemplary bacterial host cells include, but are not limited to, various strains of *E. coli* (e.g., HB101, (ATCC No. 33694) DH5 α , DH10, and MC1061 (ATCC No. 53338)). Exemplary host cells also include, but are not limited to, various strains of *Pseudomonas* spp., *B. subtilis*, other *Bacillus* spp., and *Streptomyces* spp.

[0136] Many strains of yeast cells known to those skilled in the art are also available as host cells for expression of polypeptides. Certain such embodiments use commercially available expression systems, e.g., the Pichia Expression System (Invitrogen, San Diego, CA), following the manufacturer's instructions. In certain embodiments, such a system relies on the pre-pro-alpha sequence to direct secretion. In certain embodiments, transcription of

the insert is driven by the alcohol oxidase (AOX1) promoter upon induction by methanol. In certain embodiments, the host cell may be *Saccharomyces cerevisiae*.

[0137] In certain embodiments, plant cell systems may be used as host cells. In certain such embodiments, plant cell systems transfected with virus expression vectors (e.g., cauliflower mosaic virus, CaMV, or tobacco mosaic virus) are used.

[0138] In certain embodiments, a polynucleotide encoding a mutant EGFr polypeptide, a mutant PI3K polypeptide, and/or a mutant B-Raf polypeptide is cloned into a baculovirus expression vector, such as pVL1393 (PharMingen, San Diego, CA). In certain embodiments, such a vector can be used according to the manufacturer's directions (PharMingen) to infect *Spodoptera frugiperda* cells in sf9 protein-free media and to produce recombinant polypeptide. In certain embodiments, a mutant EGFr polypeptide, a mutant PI3K polypeptide, and/or a mutant B-Raf polypeptide is purified and concentrated from such media using a heparin-Sepharose^{*} column (Pharmacia).

[0139] In certain embodiments, insect cell systems may be used as host cells. Certain such systems are described, for example, in Kitts et al. (1993), *Biotechniques*, 14: 810-7, Lucklow (1993), *Curr. Opin. Biotechnol.*, 4: 564-72, and Lucklow et al. (1993), *J. Virol.*, 67: 4566-79. Exemplary insect cells include, but are not limited to, Sf-9 and Hi5 (Invitrogen, Carlsbad, CA).

[0140] In certain embodiments, transformation or transfection of a polynucleotide encoding a mutant EGFr polypeptide, a mutant PI3K polypeptide, and/or a mutant B-Raf polypeptide into a selected host cell may be accomplished by well known methods including methods such as calcium chloride, electroporation, microinjection, lipofection or the DEAE-dextran method. In certain embodiments, the method selected will in part be a function of the type of host cell to be used. These methods and other suitable methods are well known to the skilled artisan, and are set forth, for example, in Sambrook et al. *Molecular Cloning: A Laboratory Manual* (2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989)).

*Trademark

[0141] Host cells comprising (as by transformation or transfection) an expression vector encoding a mutant EGFr polypeptide, a mutant PI3K polypeptide, and/or a mutant B-Raf polypeptide may be cultured using standard media well known to the skilled artisan. In certain embodiments, the media may contain all nutrients necessary for the growth and survival of the cells. In certain embodiments, *E. coli* cells may be cultured in Luria Broth (LB) and/or Terrific Broth (TB). Exemplary media for culturing eukaryotic cells include, but are not limited to, RPMI 1640, MEM, DMEM, all of which may be supplemented with serum and/or growth factors according to the particular cell line being cultured. In certain embodiments, insect cells may be cultured in Grace's medium supplemented with yeastolate, lactalbumin hydrolysate, and/or fetal calf serum.

[0142] In certain embodiments, an antibiotic or other compound useful for selective growth of transfected or transformed cells is added as a supplement to the media. In certain embodiments, the compound to be used is chosen in view of the selectable marker element present on the plasmid with which the host cell was transformed. In certain embodiments, where the selectable marker element is kanamycin resistance, the compound added to the culture medium will be kanamycin. Exemplary compounds for selective growth include, but are not limited to, ampicillin, tetracycline and neomycin.

[0143] In certain embodiments, the amount of a mutant EGFr polypeptide, a mutant PI3K polypeptide, and/or a mutant B-Raf polypeptide produced by a host cell can be evaluated using standard methods known in the art. Exemplary methods include, but are not limited to, Western blot analysis, SDS-polyacrylamide gel electrophoresis, non-denaturing gel electrophoresis, HPLC separation, immunoprecipitation, and activity assays.

[0144] In certain embodiments, mutant EGFr polypeptides, mutant PI3K polypeptides, and/or mutant B-Raf polypeptides which are expressed in procaryotic host cells may be present in soluble form either in the periplasmic space or in the cytoplasm or in an insoluble form as part of intracellular inclusion bodies. In certain embodiments, mutant EGFr polypeptides, mutant PI3K polypeptides, and/or mutant B-Raf polypeptides can be extracted from the host cell using any standard technique known to the skilled artisan. In

certain embodiments, the host cells can be lysed to release the contents of the periplasm/cytoplasm by French press, homogenization, and/or sonication followed by centrifugation.

[0145] In certain embodiments, soluble forms of mutant EGFr polypeptides, mutant PI3K polypeptides, and/or mutant B-Raf polypeptides present either in the cytoplasm or released from the periplasmic space may be further purified using methods known in the art. In certain embodiments, mutant EGFr polypeptides, mutant PI3K polypeptides, and/or mutant B-Raf polypeptides are released from the bacterial periplasmic space by osmotic shock techniques.

[0146] If a mutant EGFr polypeptide, a mutant PI3K polypeptide, and/or a mutant B-Raf polypeptide has formed inclusion bodies, they may often bind to the inner and/or outer cellular membranes and thus will be found primarily in the pellet material after centrifugation. In certain embodiments, the pellet material may then be treated at pH extremes or with a chaotropic partner such as a detergent, guanidine, guanidine derivatives, urea, or urea derivatives in the presence of a reducing partner such as dithiothreitol at alkaline pH or tris carboxyethyl phosphine at acid pH to release, break apart, and solubilize the inclusion bodies. In certain embodiments, the mutant EGFr polypeptide, the mutant PI3K polypeptide, and/or the mutant B-Raf polypeptide may then be analyzed using gel electrophoresis, immunoprecipitation or the like. In certain embodiments, a mutant EGFr polypeptide, a mutant PI3K polypeptide, and/or a mutant B-Raf polypeptide may be isolated using standard methods such as those set forth below and in Marston et al. (1990), *Meth. Enz.*, 182: 264-75.

[0147] In certain embodiments, a mutant EGFr polypeptide, a mutant PI3K polypeptide, and/or a mutant B-Raf polypeptide may not be biologically active upon isolation. In certain embodiments, methods for "refolding" or converting the polypeptide to its tertiary structure and generating disulfide linkages, may be used to restore biological activity. In certain embodiments, the biological activity may be restored by exposing the solubilized polypeptide to a pH usually above 7 in the presence of a particular concentration of a chaotrope. The selection of chaotrope is very similar to the

choices used for inclusion body solubilization, but, in certain embodiments, the chaotrope is used at a lower concentration and is not necessarily the same as chaotropes used for the solubilization. In certain embodiments, the refolding/oxidation solution will also contain a reducing partner or the reducing partner plus its oxidized form in a specific ratio to generate a particular redox potential allowing for disulfide shuffling to occur in the formation of the protein's cysteine bridge(s). Exemplary redox couples include, but are not limited to, cysteine/cystamine, glutathione (GSH)/dithiobis GSH, cupric chloride, dithiothreitol(DTT)/dithiane DTT, and 2-mercaptoethanol(bME)/dithio-b(ME). In certain embodiments, a cosolvent may be used or may be needed to increase the efficiency of the refolding and exemplary repartners used for this purpose include, but are not limited to, glycerol, polyethylene glycol of various molecular weights, arginine, and related molecules.

[0148] In certain embodiments, mutant EGFr polypeptides, mutant PI3K polypeptides, and/or mutant B-Raf polypeptides may be prepared by chemical synthesis methods. In certain embodiments, the chemical synthesis method may incorporate solid phase peptide synthesis. In certain embodiments, the chemical synthesis methods may use techniques known in the art such as those set forth by Merrifield *et al.* (1963), *J. Am. Chem. Soc.*, 85: 2149; Houghten *et al.* (1985), *Proc Natl Acad. Sci. USA*, 82: 5132; and Stewart and Young (1984), *Solid Phase Peptide Synthesis*, Pierce Chemical Co., Rockford, IL. In certain embodiments, polypeptides may be synthesized with or without a methionine on the amino terminus. In certain embodiments, chemically synthesized mutant EGFr polypeptides, mutant PI3K polypeptides, and/or mutant B-Raf polypeptides may be oxidized using methods set forth in these references to form disulfide bridges. In certain embodiments, mutant EGFr polypeptides, mutant PI3K polypeptides, and/or mutant B-Raf polypeptides so prepared will retain at least one biological activity associated with a native or recombinantly produced mutant EGFr polypeptide, mutant PI3K polypeptide, and/or mutant B-Raf polypeptide .

[0149] In certain embodiments, transgenic animals may be used to express mutant EGFr polypeptides, mutant PI3K polypeptides, and/or mutant

B-Raf polypeptides. In certain embodiments, one may use a transgenic milk-producing animal (a cow or goat, for example) and obtain a glycosylated mutant EGFr polypeptide, a glycosylated mutant PI3K polypeptide, and/or a glycosylated mutant B-Raf polypeptide in the animal milk. In certain embodiments, plants are used to produce a glycosylated mutant EGFr polypeptide, a glycosylated mutant PI3K polypeptide, and/or a glycosylated mutant B-Raf polypeptide, as is known in the art.

[0150] In certain embodiments, one substantially purifies a mutant EGFr polypeptide. In certain embodiments, one substantially purifies a mutant PI3K polypeptide. In certain embodiments, one substantially purifies a mutant B-Raf polypeptide. Certain protein purification techniques are known to those of skill in the art. In certain embodiments, protein purification involves crude fractionation of polypeptide fractionations from non-polypeptide fractions. In certain embodiments, polypeptides are purified using chromatographic and/or electrophoretic techniques. Exemplary purification methods include, but are not limited to, precipitation with ammonium sulphate; precipitation with PEG; immunoprecipitation; heat denaturation followed by centrifugation; chromatography, including, but not limited to, affinity chromatography (e.g., Protein-A-Sepharose), ion exchange chromatography, exclusion chromatography, and reverse phase chromatography; gel filtration; hydroxylapatite chromatography; isoelectric focusing; polyacrylamide gel electrophoresis; and combinations of such and other techniques. In certain embodiments, a polypeptide is purified by fast protein liquid chromatography or by high pressure liquid chromatography (HPLC). In certain embodiments, purification steps may be changed or certain steps may be omitted, and still result in a suitable method for the preparation of a substantially purified polypeptide.

[0151] In certain embodiments, a mutant EGFr polypeptide, a mutant PI3K polypeptide, and/or a mutant B-Raf polypeptide may be prepared with one or more affinity tags, such as hexahistidine or other small peptide such as FLAG^{*} (Eastman Kodak Co., New Haven, CT) or myc (Invitrogen) at either the carboxyl or amino terminus and purified by a one-step affinity column. In certain embodiments, polyhistidine binds with great

*Trademark

affinity and specificity to nickel, thus an affinity column of nickel (such as the Qiagen® nickel columns) can be used for purification of polyhistidine-tagged specific binding partners. See for example, Ausubel et al., eds. (1993), *Current Protocols in Molecular Biology*, Section 10.11.8, John Wiley & Sons, New York. In certain embodiments, more than one purification step may be used.

[0152] In certain embodiments, one quantitates the degree of purification of a polypeptide preparation. Certain methods for quantifying the degree of purification are known to those of skill in the art. Certain exemplary methods include, but are not limited to, determining the specific binding activity of the preparation and assessing the amount of a polypeptide within a preparation by SDS/PAGE analysis. Certain exemplary methods for assessing the amount of purification of a polypeptide preparation comprise calculating the binding activity of a preparation and comparing it to the binding activity of an initial extract. In certain embodiments, the results of such a calculation are expressed as “fold purification.” The units used to represent the amount of binding activity depend upon the particular assay performed.

[0153] In certain embodiments, a polypeptide is partially purified. In certain embodiments, partial purification may be accomplished by using fewer purification steps or by utilizing different forms of the same general purification scheme. For example, in certain embodiments, a cation-exchange column chromatography performed utilizing an HPLC apparatus will generally result in a greater “fold purification” than the same technique utilizing a low-pressure chromatography system. In certain embodiments, methods resulting in a lower degree of purification may have advantages in total recovery of polypeptide, or in maintaining binding activity of a polypeptide.

[0154] In certain instances, the electrophoretic migration of a polypeptide can vary, sometimes significantly, with different conditions of SDS/PAGE. See, e.g., Capaldi et al., *Biochem Biophys Res Comm*, 76: 425 (1977). It will be appreciated that under different electrophoresis conditions, the apparent molecular weights of purified or partially purified polypeptide may be different.

Transgenic Animals

[0155] In certain embodiments, transgenic non-human animals comprising one or more polynucleotides encoding one or more mutant EGFr polypeptides, one or more mutant PI3K polypeptides, and/or one or more mutant B-Raf polypeptides are provided. In certain embodiments, non-human transgenic animals include, but are not limited to, rodents such as mice or rats, rabbits, goats, sheep, and other farm animals. Certain transgenic animals may be prepared using well known methods including, but not limited to, those described in U.S. Pat. No. 5,489,743 and in WO 94/28122.

[0156] In certain embodiments, animal transcriptional control regions which exhibit tissue specificity may be used to construct transgenic animals. Exemplary transcriptional control regions for use with tissue specific expression in transgenic animals include, but are not limited to, the elastase I gene control region which is active in pancreatic acinar cells (Swift et al. (1984), *Cell*, 38: 639-46; Ornitz et al. (1986), Cold Spring Harbor Symp. Quant. Biol. 50: 399-409; MacDonald (1987), *Hepatology*, 7: :425-515); the insulin gene control region which is active in pancreatic beta cells (Hanahan (1985), *Nature*, 315: 115-122); the immunoglobulin gene control region which is active in lymphoid cells (Grosschedl et al. (1984), *Cell*, 38: 647-58; Adames et al. (1985), *Nature*, 318: 533-8; Alexander et al. (1987), *Mol. Cell. Biol.*, 7: 1436-44); the mouse mammary tumor virus control region which is active in testicular, breast, lymphoid and mast cells (Leder et al. (1986), *Cell*, 45: 485-95); albumin gene control region which is active in liver (Pinkert et al. (1987), *Genes and Devel.*, 1: 268-76); the alphafetoprotein gene control region which is active in liver (Krumlauf et al. (1987), *Mol. Cell. Biol.*, 5: 1639-48; Hammer et al. (1987), *Science*, 235: 53-58); the alpha 1-antitrypsin gene control region which is active in the liver (Kelsey et al. (1987), *Genes and Devel.*, 1: 161-171); the beta-globin gene control region which is active in myeloid cells (Mogam et al. (1985), *Nature*, 315: 338-340; Kollias et al. (1986), *Cell*, 46: 89-94); the myelin basic protein gene control region which is active in oligodendrocyte cells in the brain (Readhead et al. (1987), *Cell*, 48: 703-712); the myosin light chain-2 gene control region which is active in skeletal muscle

(Sani (1985), *Nature*, 314: 283-286); and the gonadotropic releasing hormone gene control region which is active in the hypothalamus (Mason et al. (1986), *Science*, 234: 1372-8).

[0157] In certain embodiments, a non-human animal is provided in which a polynucleotide encoding a wild-type EGFr polypeptide has been disrupted (i.e., "knocked out") and replaced with one or more polynucleotides encoding a mutant EGFr polypeptide such that the level of expression of wild-type EGFr polypeptide is significantly decreased or completely abolished in the animal and the mutant EGFr polypeptide is expressed in the animal. In certain such embodiments, the animals may be prepared using techniques and methods such as those described in U.S. Pat. No. 5,557,032 or other techniques well known in the art. In certain embodiments, a non-human animal is provided in which the activity of the promoter for one or more mutant EGFr polypeptides is modulated (e.g., by using homologous recombination methods known in the art) to alter the level of expression of one or more mutant EGFr polypeptides. In certain such embodiments, the level of expression of a mutant EGFr polypeptide is increased. In certain such embodiments, the level of expression of the mutant EGFr polypeptide is decreased.

[0158] In certain embodiments, a non-human animal is provided in which a polynucleotide encoding a wild-type PI3K polypeptide has been disrupted (i.e., "knocked out") and replaced with one or more polynucleotides encoding a mutant PI3K polypeptide such that the level of expression of wild-type PI3K polypeptide is significantly decreased or completely abolished in the animal and the mutant PI3K polypeptide is expressed in the animal. In certain such embodiments, the animals may be prepared using techniques and methods such as those described in U.S. Pat. No. 5,557,032 or other techniques well known in the art. In certain embodiments, a non-human animal is provided in which the activity of the promoter for one or more mutant PI3K polypeptides is modulated (e.g., by using homologous recombination methods known in the art) to alter the level of expression of one or more mutant PI3K polypeptides. In certain such embodiments, the level of expression of the mutant PI3K polypeptide is increased. In certain such

embodiments, the level of expression of the mutant PI3K polypeptide is decreased.

[0159] In certain embodiments, a non-human animal is provided in which a polynucleotide encoding a wild-type B-Raf polypeptide have been disrupted (i.e., "knocked out") and replaced with one or more polynucleotides encoding a mutant B-Raf polypeptide such that the level of expression of wild-type B-Raf polypeptide is significantly decreased or completely abolished in the animal and the mutant B-Raf polypeptide is expressed in the animal. In certain such embodiments, the animals may be prepared using techniques and methods such as those described in U.S. Pat. No. 5,557,032 or other techniques well known in the art. In certain embodiments, a non-human animal is provided in which the activity of the promoter for one or more mutant B-Raf polypeptides is modulated (e.g., by using homologous recombination methods known in the art) to alter the level of expression of one or more mutant B-Raf polypeptides. In certain such embodiments, the level of expression of the mutant B-Raf polypeptide is increased. In certain such embodiments, the level of expression of the mutant B-Raf polypeptide is decreased.

[0160] In certain embodiments, a non-human transgenic animal can be used for drug candidate screening. In certain embodiments, the impact of a drug candidate on the non-human transgenic animal is measured. In certain embodiments, the ability of a drug candidate to increase the expression of a mutant EGFr polypeptide is measured. In certain embodiments, the ability of a drug candidate to decrease or prevent the expression of a mutant EGFr polypeptide is measured. In certain embodiments, the ability of a drug candidate to increase the activity of a mutant EGFr polypeptide is measured. In certain embodiments, the ability of a drug candidate to decrease or prevent the activity of a mutant EGFr polypeptide is measured. In certain embodiments, the ability of a drug candidate to decrease or prevent activation of a mutant EGFr polypeptide is measured. In certain embodiments, the ability of a drug candidate to increase activation of a mutant EGFr polypeptide is measured. In certain embodiments, the ability of a drug candidate to decrease or prevent

autophosphorylation of a mutant EGFr polypeptide is measured. In certain embodiments, the ability of a drug candidate to increase autophosphorylation of a mutant EGFr polypeptide is measured.

[0161] In certain embodiments, the ability of a drug candidate to decrease or prevent binding of one or more specific binding agents to a mutant EGFr polypeptide is measured. In certain embodiments, the ability of a drug candidate to increase binding of one or more specific binding agents to a mutant EGFr polypeptide is measured. In certain embodiments, the ability of a drug candidate to ameliorate a disease or condition related to mutant EGFr polypeptide is measured. In certain embodiments, the ability of a drug candidate to ameliorate a mutant EGFr polypeptide-related cancer is measured.

[0162] In certain embodiments, the ability of a drug candidate to increase the expression of a mutant PI3K polypeptide is measured. In certain embodiments, the ability of a drug candidate to decrease or prevent the expression of a mutant PI3K polypeptide is measured. In certain embodiments, the ability of a drug candidate to increase the activity of a mutant PI3K polypeptide is measured. In certain embodiments, the ability of a drug candidate to decrease or prevent the activity of a mutant PI3K polypeptide is measured. In certain embodiments, the ability of a drug candidate to decrease or prevent activation of a mutant PI3K polypeptide is measured. In certain embodiments, the ability of a drug candidate to increase activation of a mutant PI3K polypeptide is measured. In certain embodiments, the ability of a drug candidate to decrease or prevent autophosphorylation of a mutant PI3K polypeptide is measured. In certain embodiments, the ability of a drug candidate to increase autophosphorylation of a mutant PI3K polypeptide is measured.

[0163] In certain embodiments, the ability of a drug candidate to decrease or prevent binding of one or more specific binding agents to a mutant PI3K polypeptide is measured. In certain embodiments, the ability of a drug candidate to increase binding of one or more specific binding agents to a mutant PI3K polypeptide is measured. In certain embodiments, the ability of a drug candidate to ameliorate a disease or condition related to mutant

PI3K polypeptide is measured. In certain embodiments, the ability of a drug candidate to ameliorate a mutant PI3K polypeptide-related cancer is measured.

[0164] In certain embodiments, the ability of a drug candidate to increase the expression of a mutant B-Raf polypeptide is measured. In certain embodiments, the ability of a drug candidate to decrease or prevent the expression of a mutant B-Raf polypeptide is measured. In certain embodiments, the ability of a drug candidate to increase the activity of a mutant B-Raf polypeptide is measured. In certain embodiments, the ability of a drug candidate to decrease or prevent the activity of a mutant B-Raf polypeptide is measured. In certain embodiments, the ability of a drug candidate to decrease or prevent activation of a mutant B-Raf polypeptide is measured. In certain embodiments, the ability of a drug candidate to increase activation of a mutant B-Raf polypeptide is measured. In certain embodiments, the ability of a drug candidate to decrease or prevent autophosphorylation of a mutant B-Raf polypeptide is measured. In certain embodiments, the ability of a drug candidate to increase autophosphorylation of a mutant B-Raf polypeptide is measured.

[0165] In certain embodiments, the ability of a drug candidate to decrease or prevent binding of one or more specific binding agents to a mutant B-Raf polypeptide is measured. In certain embodiments, the ability of a drug candidate to increase binding of one or more specific binding agents to a mutant B-Raf polypeptide is measured. In certain such embodiments, the ability of a drug candidate to ameliorate a disease or condition related to mutant B-Raf polypeptide is measured. In certain embodiments, the ability of a drug candidate to ameliorate a mutant B-Raf polypeptide-related cancer is measured.

Specific Binding Agents and Antibodies

[0166] In certain embodiments, a specific binding agent to a mutant EGFr polypeptide is provided. In certain embodiments, a specific binding agent to a mutant PI3K polypeptide is provided. In certain embodiments, a specific binding agent to a mutant B-Raf polypeptide is provided. In certain

such embodiments, the specific binding agents are antibodies or antigen-binding fragments thereof.

[0167] In certain embodiments, monoclonal antibodies may be made using the hybridoma method first described by Kohler *et al.*, *Nature* 256: 495 (1975). In certain embodiments, monoclonal antibodies may be made by recombinant DNA methods (U.S. Patent No. 4,816,567).

[0168] In certain embodiments of the hybridoma method, a mouse or other appropriate host animal, including, but not limited to, a hamster or macaque monkey, is immunized to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the protein used for immunization. In certain embodiments, lymphocytes may be immunized *in vitro*. In certain embodiments, lymphocytes or lymphocytes enriched for B cells are fused with myeloma cells by an electrocell fusion process or by using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (Goding, *Monoclonal Antibodies: Principles and Practice*, pp.59-103, [Academic Press, 1996]).

[0169] In certain embodiments, hybridoma cells are seeded and grown in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, parental myeloma cells. In certain embodiments, if the parental myeloma cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine (HAT medium), which substances prevent the growth of HGPRT-deficient cells.

[0170] In certain embodiments, myeloma cells are selected that fuse efficiently, support stable high-level production of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. Exemplary myeloma cell lines include, but are not limited to, murine myeloma lines, such as those derived from MOP-21 and MC.-11 mouse tumors available from the Salk Institute Cell Distribution Center, San Diego, California USA, and SP-2 or X63-Ag8-653 cells available from the American Type Culture Collection, Rockville, Maryland USA. In certain embodiments, human myeloma and/or mouse-human heteromyeloma cell

lines are used for the production of human monoclonal antibodies (Kozbor, *J. Immunol.* 133: 3001 (1984); Brodeur *et al.*, *Monoclonal Antibody Production Techniques and Applications*, pp. 51-63, Marcel Dekker, Inc., New York, [1987]).

[0171] In certain embodiments, culture medium in which hybridoma cells are growing is assayed for production of monoclonal antibodies directed against the antigen. In certain embodiments, the binding specificity of monoclonal antibodies produced by hybridoma cells is determined by immunoprecipitation or by an *in vitro* binding assay. Exemplary binding assays include, but are not limited to, a radioimmunoassay (RIA), an enzyme-linked immunosorbent assay (ELISA), and the Scatchard analysis of Munson *et al.*, *Anal. Biochem.* 107: 220 (1980).

[0172] In certain embodiments, after hybridoma cells are identified that produce antibodies of the desired specificity, affinity, and/or activity, the cells may be subcloned by limiting dilution procedures and grown by standard methods (Goding, *Monoclonal Antibodies: Principles and Practice*, pp.59-103, Academic Press, 1996). Exemplary culture media for this purpose include, but are not limited to, DMEM and RPMI-1640 medium. In certain embodiments, hybridoma cells may be grown *in vivo* as ascites tumors in an animal.

[0173] In certain embodiments, monoclonal antibodies secreted by the subclones are suitably separated from the culture medium, ascites fluid, or serum by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

[0174] In certain embodiments, a polynucleotide encoding the monoclonal antibodies is readily isolated and sequenced using conventional procedures (*e.g.*, by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the monoclonal antibodies). In certain such embodiments, the hybridoma cells serve as a preferred source of such polynucleotide. In certain embodiments, isolated polynucleotide may be placed into expression vectors. In certain such embodiments, the expression vectors are transfected into host cells to obtain

the synthesis of monoclonal antibodies in the recombinant host cells. Exemplary host cells include, but are not limited to, *E. coli* cells, simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein. In certain embodiments, the polynucleotide may be modified, for example, by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide, creating a "chimeric" or "hybrid" antibody.

[0175] In certain embodiments, non-immunoglobulin polypeptides are substituted for the constant domains of an antibody. In certain embodiments, non-immunoglobulin polypeptides are substituted for the variable domains of one antigen-combining sites of an antibody to create a chimeric bivalent antibody comprising one antigen-combining site having specificity for a target antigen and another antigen-combining site having specificity for a different antigen.

[0176] In certain embodiments, chimeric or hybrid antibodies can be prepared *in vitro* using known methods in synthetic protein chemistry, including, but not limited to, those involving crosslinking agents. In certain such embodiments, immunotoxins can be constructed using a disulfide exchange reaction or by forming a thioether bond. Exemplary reagents for this purpose include, but are not limited to, iminothiolate and methyl-4-mercaptobutyrimidate.

[0177] In certain embodiments, human antibodies to a target antigen are provided. In certain embodiments, hybridoma technology is extended to create human antibodies using heteromyelomas (mouse x human hybrid myelomas) as fusion partners (see, e.g., Kozbor, *J. Immunol.* 133: 3001 (1984); Brodeur, *et al.*, *Monoclonal Antibody Production Techniques and Applications*, pp.51-63, Marcel Dekker, Inc., New York, 1987). In certain embodiments, human antibody-secreting cells can be immortalized by infection with the Epstein-Barr virus (EBV) (James and Bell, *J. Immunol. Methods* 100: 5-40 [1987]). In certain embodiments, the immortalization of human B cells can be achieved by introducing a defined combination of transforming genes (Hahn *et al.*, *Nature* 400: 464-468 [1999]).

[0178] In certain embodiments, transgenic animals (e.g. mice) that are capable, upon immunization, of producing a repertoire of human antibodies in the absence of endogenous immunoglobulin production are used to make human antibodies (see, e.g., Jakobovits *et al.*, *Nature* 362: 255-258 [1993]; Lonberg and Huszar, *Int. Rev. Immunol.* 13: 65-93 [1995]; Fishwild *et al.*, *Nat. Biotechnol.* 14: 845-851 [1996]; Mendez *et al.*, *Nat. Genet.* 15: 146-156 [1997]; Green, *J. Immunol. Methods* 231: 11-23 [1999]; Tomizuka *et al.*, *Proc. Natl. Acad. Sci. USA* 97: 722-727 [2000]; reviewed in Little *et al.*, *Immunol. Today* 21: 364-370 [2000]). It has been described that the homozygous deletion of the antibody heavy chain joining region (J_H) gene in chimeric and germ line mutant mice results in complete inhibition of endogenous antibody production (Jakobovits *et al.*, *Proc. Natl. Acad. Sci. USA* 90: 2551-2555 [1993]). Transfer of the human germ-line immunoglobulin gene array in such germ line mutant mice results in the production of human antibodies upon antigen challenge (Jakobovits *et al.*, *Nature* 362: 255-258 [1993]).

[0179] Mendez *et al.* (*Nature Genetics* 15: 146-156 [1997]) have generated a line of transgenic mice designated as "Xenomouse® II" that, when challenged with an antigen, generates high affinity fully human antibodies. This was achieved by germ-line integration of megabase human heavy chain and light chain loci into mice with deletion into endogenous J_H segment. The XenoMouse® II harbors 1,020 kb of human heavy chain locus containing approximately 66 V_H genes, complete D_H and J_H regions and three different constant regions (μ , δ and γ), and also harbors 800 kb of human κ locus containing 32 V _{κ} genes, J _{κ} segments and C _{κ} genes. In certain embodiments, the antibodies produced in those mice closely resemble those seen in humans in all respects, including gene rearrangement, assembly, and repertoire. In certain embodiments, the human antibodies are preferentially expressed over endogenous antibodies due to a deletion in the endogenous J_H segment that prevents gene rearrangement in the murine locus.

[0180] In certain embodiments, a transgenic animal comprising human immunoglobulin genes (e.g., the Xenomouse® II (Abgenix, Inc.)) may be immunized with an antigen of particular interest, such as a mutant EGFr

polypeptide, a mutant PI3K polypeptide, and/or a mutant B-Raf polypeptide. In certain embodiments, sera from those immunized animals is screened for antibody reactivity against the initial antigen. In certain embodiments, lymphocytes are isolated from lymph nodes or spleen cells and may further be enriched for B cells by selecting for CD138-negative and CD19+ cells. In certain embodiments, those B cell cultures (BCCs) are fused to myeloma cells to generate hybridomas as detailed above. In certain embodiments, those B cell cultures are screened further for reactivity against the initial antigen. Such screening includes, but is not limited to, ELISA, a competition assay with known antibodies that bind the antigen of interest, and in vitro binding to transiently transfected CHO cells expressing the antigen. In certain embodiments, single B cells secreting antibodies of interest are identified by a specific hemolytic plaque assay. In certain such embodiments, cells targeted for lysis are sheep red blood cells (SRBCs) coated with the antigen. In certain such embodiments, the formation of a plaque indicates specific antigen-mediated lysis of the target cells, and thus the presence of a B cell culture secreting the immunoglobulin of interest and complement. In certain such embodiments, the single antigen-specific plasma cell in the center of the plaque can be isolated and used for isolation of mRNA.

[0181] In certain embodiments, the polynucleotide encoding the variable region of the antibody secreted can be cloned using reverse-transcriptase PCR. In certain embodiments, the cloned polynucleotide is further inserted into a suitable expression vector, such as a vector cassette such as a pcDNA, or a pcDNA vector containing the constant domains of immunoglobulin heavy and light chain. In certain embodiments, the generated vector is transfected into host cells, (i.e., CHO cells), and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences.

[0182] In certain embodiments, phage display technology is used to produce human antibodies and antibody fragments *in vitro*, from immunoglobulin variable (V) domain gene repertoires from unimmunized donors (see, e.g., McCafferty *et al.*, *Nature* 348: 552-553 [1990]; reviewed in

Kipriyanov and Little, *Mol. Biotechnol.* 12: 173-201 [1999]; Hoogenboom and Chames, *Immunol. Today* 21: 371-378 [2000]). In certain such embodiments, antibody V domain genes are cloned in-frame into either a major or minor coat protein gene of a filamentous bacteriophage, such as M13 or fd, and displayed as functional antibody fragments on the surface of the phage particle. In certain embodiments, the filamentous particle contains a single-stranded DNA copy of the phage genome, and selections based on the functional properties of the antibody also result in selection of the gene encoding the antibody exhibiting those properties. Phage display can be performed in a variety of formats, including, but not limited to, those identified in the following documents: Johnson and Chiswell, *Current Opinion in Structural Biology* 3: 564-571 [1993]; Winter *et al.*, *Annu. Rev. Immunol.* 12: 433-455 [1994]; Dall'Acqua and Carter, *Curr. Opin. Struct. Biol.* 8: 443-450 [1998]; and Hoogenboom and Chames, *Immunol. Today* 21: 371-378 [2000]. Sources of V-gene segments for phage display include, but are not limited to, a small random combinatorial library of V genes derived from the spleens of immunized mice (Clackson *et al.*, (*Nature* 352: 624-628 [1991]) and a repertoire of V genes from unimmunized human donors (Marks *et al.*, *J. Mol. Biol.* 222: 581-597 (1991), or Griffiths *et al.*, *EMBO J.* 12: 725-734 (1993)).

[0183] In certain embodiments, in a natural immune response, antibody genes accumulate mutations at a high rate (somatic hypermutation). In certain embodiments, some of the changes introduced confer higher affinity. In certain embodiments, B cells displaying high-affinity surface immunoglobulin are preferentially replicated and differentiated during subsequent antigen challenge. In certain embodiments, that natural process can be mimicked by employing the technique known as "chain shuffling" (Marks *et al.*, *Bio/Technol.* 10: 779-783 [1992]). In certain such embodiments, the affinity of "primary" human antibodies obtained by phage display can be improved by sequentially replacing the heavy and light chain V region genes with repertoires of naturally occurring variants (repertoires) of V domain genes obtained from unimmunized donors, allowing the production of antibodies and antibody fragments with affinities in the nM range. In certain embodiments, a very large phage antibody repertoire is constructed (also

known as "the mother-of-all libraries"), as described by Waterhouse *et al.*, *Nucl. Acids Res.* 21: 2265-2266 (1993). In certain such embodiments, a high affinity human antibody is directly isolated from a large phage library (see, e.g., Griffiths *et al.*, *EMBO J.* 13: 3245-3260 (1994)). In certain embodiments, gene shuffling can be used to derive human antibodies from rodent antibodies, where the human antibody has similar affinities and specificities to the starting rodent antibody. In certain such embodiments, the heavy or light chain V domain gene of rodent antibodies obtained by phage display technique is replaced with a repertoire of human V domain genes, creating rodent-human chimeras (also referred to as "epitope imprinting"). In certain embodiments, selection of variable regions by the antigen results in isolation of human variable regions capable of restoring a functional antigen-binding site, i.e. the epitope governs (imprints) the choice of partner. In certain embodiments, when the process is repeated in order to replace the remaining rodent V domain, a human antibody is obtained which has no framework or CDR residues of rodent origin (see PCT patent application WO 93/06213, published 1 April 1993).

Arrays

[0184] In certain embodiments, microarrays comprising one or more polynucleotides encoding one or more mutant EGFr polypeptides are provided. In certain embodiments, microarrays comprising one or more polynucleotides complementary to one or more polynucleotides encoding one or more mutant EGFr polypeptides are provided. In certain embodiments, microarrays comprising one or more polynucleotides encoding one or more mutant PI3K polypeptides are provided. In certain embodiments, microarrays comprising one or more polynucleotides complementary to one or more polynucleotides encoding one or more mutant PI3K polypeptides are provided. In certain embodiments, microarrays comprising one or more polynucleotides encoding one or more mutant B-Raf polypeptides are provided. In certain embodiments, microarrays comprising one or more polynucleotides complementary to one or more polynucleotides encoding one or more mutant B-Raf polypeptides are provided.

[0185] In certain embodiments, the presence or absence of one or more mutant EGFr polynucleotides in two or more cell or tissue samples is assessed using microarray technology. In certain embodiments, the quantity of one or more mutant EGFr polynucleotides in two or more cell or tissue samples is assessed using microarray technology. In certain such embodiments, the cell or tissue is treated prior to the assessment, and the ability of the treatment to affect the quantity of the one or more mutant EGFr polynucleotides is also assessed.

[0186] In certain embodiments, the presence or absence of one or more mutant PI3K polynucleotides in two or more cell or tissue samples is assessed using microarray technology. In certain embodiments, the quantity of one or more mutant PI3K polynucleotides in two or more cell or tissue samples is assessed using microarray technology. In certain such embodiments, the cell or tissue is treated prior to the assessment, and the ability of the treatment to affect the quantity of the one or more mutant PI3K polynucleotides is also assessed.

[0187] In certain embodiments, the presence or absence of one or more mutant B-Raf polynucleotides in two or more cell or tissue samples is assessed using microarray technology. In certain embodiments, the quantity of one or more mutant B-Raf polynucleotides in two or more cell or tissue samples is assessed using microarray technology. In certain such embodiments, the cell or tissue is treated prior to the assessment, and the ability of the treatment to affect the quantity of the one or more mutant B-Raf polynucleotides is also assessed.

[0188] In certain embodiments, the presence or absence of one or more mutant EGFr polypeptides in two or more cell or tissue samples is assessed using microarray technology. In certain such embodiments, mRNA is first extracted from a cell or tissue sample and is subsequently converted to cDNA, which is hybridized to the microarray. In certain such embodiments, the presence or absence of cDNA that is specifically bound to the microarray is indicative of the presence or absence of the mutant EGFr polypeptide. In certain such embodiments, the expression level of the one or more mutant EGFr polypeptides is assessed by quantitating the amount of cDNA that is

specifically bound to the microarray. In certain such embodiments, the cell or tissue is treated prior to the assessment, and the ability of the treatment to affect expression of the one or more mutant EGFr polypeptides is also assessed.

[0189] In certain embodiments, the presence or absence of one or more mutant PI3K polypeptides in two or more cell or tissue samples is assessed using microarray technology. In certain such embodiments, mRNA is first extracted from a cell or tissue sample and is subsequently converted to cDNA, which is hybridized to the microarray. In certain such embodiments, the presence or absence of cDNA that is specifically bound to the microarray is indicative of the presence or absence of the mutant PI3K polypeptide. In certain such embodiments, the expression level of the one or more mutant PI3K polypeptides is assessed by quantitating the amount of cDNA that is specifically bound to the microarray. In certain such embodiments, the cell or tissue is treated prior to the assessment, and the ability of the treatment to affect expression of the one or more mutant PI3K polypeptides is also assessed.

[0190] In certain embodiments, the presence or absence of one or more mutant B-Raf polypeptides in two or more cell or tissue samples is assessed using microarray technology. In certain such embodiments, mRNA is first extracted from a cell or tissue sample and is subsequently converted to cDNA, which is hybridized to the microarray. In certain such embodiments, the presence or absence of cDNA that is specifically bound to the microarray is indicative of the presence or absence of the mutant B-Raf polypeptide. In certain such embodiments, the expression level of the one or more mutant B-Raf polypeptides is assessed by quantitating the amount of cDNA that is specifically bound to the microarray. In certain such embodiments, the cell or tissue is treated prior to the assessment, and the ability of the treatment to affect expression of the one or more mutant B-Raf polypeptides is also assessed.

[0191] In certain embodiments, microarrays comprising one or more mutant EGFr polypeptides are provided (see, e.g., McBeath et al., Science, 289:1760-1763, 2000). In certain embodiments, candidate specific binding

agents to one or more mutant EGFr polypeptides are screened using a mutant EGFr polypeptide microarray. In certain embodiments, candidate compounds for modulating the activity of a mutant EGFr polypeptide are screened using a mutant EGFr polypeptide microarray. In certain such embodiments, the ability of candidate compounds to decrease or prevent autophosphorylation of mutant EGFr polypeptides is assessed. In certain such embodiments, the ability of candidate compounds to increase autophosphorylation of mutant EGFr polypeptides is assessed.

[0192] In certain embodiments, microarrays comprising one or more mutant PI3K polypeptides are provided (see, e.g., McBeath et al., Science, 289:1760-1763, 2000). In certain embodiments, candidate specific binding agents to one or more mutant PI3K polypeptides are screened using a mutant PI3K polypeptide microarray. In certain embodiments, candidate compounds for modulating the activity of a mutant PI3K polypeptide are screened using a mutant PI3K polypeptide microarray. In certain such embodiments, the ability of candidate compounds to decrease or prevent autophosphorylation of mutant PI3K polypeptides is assessed. In certain such embodiments, the ability of candidate compounds to increase autophosphorylation of mutant PI3K polypeptides is assessed.

[0193] In certain embodiments, microarrays comprising one or more mutant B-Raf polypeptides are provided (see, e.g., McBeath et al., Science, 289:1760-1763, 2000). In certain embodiments, candidate specific binding agents to one or more mutant B-Raf polypeptides are screened using a mutant B-Raf polypeptide microarray. In certain embodiments, candidate compounds for modulating the activity of a mutant B-Raf polypeptide are screened using a mutant B-Raf polypeptide microarray. In certain such embodiments, the ability of candidate compounds to decrease or prevent autophosphorylation of mutant B-Raf polypeptides is assessed. In certain such embodiments, the ability of candidate compounds to increase autophosphorylation of mutant B-Raf polypeptides is assessed.

[0194] In certain embodiments, microarrays comprising one or more specific binding agents to one or more mutant EGFr polypeptides are provided. In certain such embodiments, the presence or absence of one or

more mutant EGFr polypeptides in a cell or tissue is assessed. In certain such embodiments, the quantity of one or more mutant EGFr polypeptides in a cell or tissue is assessed.

[0195] In certain embodiments, microarrays comprising one or more specific binding agents to one or more mutant PI3K polypeptides are provided. In certain such embodiments, the presence or absence of one or more mutant PI3K polypeptides in a cell or tissue is assessed. In certain such embodiments, the quantity of one or more mutant PI3K polypeptides in a cell or tissue is assessed.

[0196] In certain embodiments, microarrays comprising one or more specific binding agents to one or more mutant B-Raf polypeptides are provided. In certain such embodiments, the presence or absence of one or more mutant B-Raf polypeptides in a cell or tissue is assessed. In certain such embodiments, the quantity of one or more mutant B-Raf polypeptides in a cell or tissue is assessed.

Certain Methods

[0197] In certain embodiments, a method of obtaining an antibody capable of binding at least one mutant EGFr polypeptide is provided. In certain embodiments, a method of obtaining an antibody capable of binding at least one mutant PI3K polypeptide is provided. In certain embodiments, a method of obtaining an antibody capable of binding at least one mutant B-Raf polypeptide is provided. In certain embodiments, a method of obtaining an antibody capable of binding at least one mutant EGFr polypeptide is provided, comprising administering at least one mutant EGFr polypeptide to an animal, and obtaining an antibody capable of binding at least one mutant EGFr polypeptide from the animal. In certain embodiments, a method of obtaining an antibody capable of binding at least one mutant PI3K polypeptide is provided, comprising administering at least one mutant PI3K polypeptide to an animal, and obtaining an antibody capable of binding at least one mutant PI3K polypeptide from the animal. In certain embodiments, a method of obtaining an antibody capable of binding at least one mutant B-Raf polypeptide is provided, comprising administering at least one mutant B-Raf polypeptide to an animal, and obtaining an antibody capable of binding at

least one mutant B-Raf polypeptide from the animal. In certain such embodiments, the antibody is a human antibody.

[0198] In certain embodiments, a method of obtaining an antibody capable of binding at least one polypeptide comprising at least one amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO:3, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 19, and SEQ ID NO: 20 is provided, comprising administering at least one polypeptide comprising at least one sequence selected from SEQ ID NO: 2, SEQ ID NO:3, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 19, and SEQ ID NO: 20 to an animal; and obtaining an antibody capable of binding at least one polypeptide comprising at least one amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO:3, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 19, and SEQ ID NO: 20 from the animal is provided. In certain such embodiments, the antibody is a human antibody.

[0199] In certain embodiments, a method of obtaining an antibody capable of binding at least one polypeptide comprising at least one amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO:3, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 19, and SEQ ID NO: 20 is provided, comprising administering at least one fragment of at least one polypeptide comprising at least one sequence selected from SEQ ID NO: 2, SEQ ID NO:3, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 19, and SEQ ID NO: 20 to an animal, wherein the at least one fragment comprises at least one mutation; and obtaining an antibody capable of binding at least one

polypeptide comprising at least one amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO:3, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 19, and SEQ ID NO: 20 from the animal is provided. In certain such embodiments, the antibody is a human antibody.

[0200] In certain embodiments, a method of diagnosing a disease or condition which is related to one or more EGFr mutations in a subject is provided. In certain embodiments, a method of diagnosing a disease or condition which is related to one or more PI3K mutations in a subject is provided. In certain embodiments, a method of diagnosing a disease or condition which is related to one or more B-Raf mutations in a subject is provided. In certain embodiments, a method of diagnosing a disease or condition which is related to one or more EGFr mutations in a subject comprises: (a) determining the presence or amount of expression of a mutant EGFr polypeptide in a sample from the subject; and (b) diagnosing a disease or condition which is related to one or more EGFr mutations based on the presence or amount of expression of the polypeptide. In certain embodiments, a method of diagnosing a disease or condition which is related to one or more EGFr mutations in a subject comprises: (a) determining the presence or amount of transcription or translation of a mutant EGFr polynucleotide in a sample from the subject; and (b) diagnosing a disease or condition which is related to one or more EGFr mutations based on the presence or amount of transcription or translation of the polynucleotide. In certain embodiments, the disease or condition is cancer.

[0201] In certain embodiments, a method of diagnosing a disease or condition which is related to one or more EGFr mutations in a subject comprises: (a) determining the presence or amount of expression of a polypeptide comprising at least one amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 12, and SEQ ID NO: 13 in a sample from the subject; and (b) diagnosing a disease or condition which is related to one or more EGFr mutations based on the presence or

amount of expression of the polypeptide. In certain embodiments, a method of diagnosing a disease or condition which is related to one or more EGFr mutations in a subject comprises: (a) determining the presence or amount of transcription or translation of a polynucleotide encoding at least one amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 12, and SEQ ID NO: 13 in a sample from the subject; and (b) diagnosing a disease or condition which is related to one or more EGFr mutations based on the presence or amount of transcription or translation of the polynucleotide. In certain embodiments, the disease or condition is cancer.

[0202] In certain embodiments, a method of diagnosing a disease or condition which is related to one or more PI3K mutations in a subject is provided. In certain embodiments, a method of diagnosing a disease or condition which is related to one or more PI3K mutations in a subject comprises: (a) determining the presence or amount of expression of a mutant PI3K polypeptide in a sample from the subject; and (b) diagnosing a disease or condition which is related to one or more PI3K mutations based on the presence or amount of expression of the polypeptide. In certain embodiments, a method of diagnosing a disease or condition which is related to one or more PI3K mutations in a subject comprises: (a) determining the presence or amount of transcription or translation of a mutant PI3K polynucleotide in a sample from the subject; and (b) diagnosing a disease or condition which is related to one or more PI3K mutations based on the presence or amount of transcription or translation of the polynucleotide. In certain embodiments, the disease or condition is cancer.

[0203] In certain embodiments, a method of diagnosing a disease or condition which is related to one or more PI3K mutations in a subject comprises: (a) determining the presence or amount of expression of a polypeptide comprising at least one amino acid sequence selected from SEQ ID NO: 15, SEQ ID NO: 16, and SEQ ID NO: 17 in a sample from the subject; and (b) diagnosing a disease or condition which is related to one or more PI3K mutations based on the presence or amount of expression of the

polypeptide. In certain embodiments, a method of diagnosing a disease or condition which is related to one or more PI3K mutations in a subject comprises: (a) determining the presence or amount of transcription or translation of a polynucleotide encoding at least one amino acid sequence selected from SEQ ID NO: 15, SEQ ID NO: 16, and SEQ ID NO: 17 in a sample from the subject; and (b) diagnosing a disease or condition which is related to one or more PI3K mutations based on the presence or amount of transcription or translation of the polynucleotide. In certain embodiments, the disease or condition is cancer.

[0204] In certain embodiments, a method of diagnosing a disease or condition which is related to one or more B-Raf mutations in a subject is provided. In certain embodiments, a method of diagnosing a disease or condition which is related to one or more B-Raf mutations in a subject comprises: (a) determining the presence or amount of expression of a mutant B-Raf polypeptide in a sample from the subject; and (b) diagnosing a disease or condition which is related to one or more B-Raf mutations based on the presence or amount of expression of the polypeptide. In certain embodiments, a method of diagnosing a disease or condition which is related to one or more B-Raf mutations in a subject comprises: (a) determining the presence or amount of transcription or translation of a mutant B-Raf polynucleotide in a sample from the subject; and (b) diagnosing a disease or condition which is related to one or more B-Raf mutations based on the presence or amount of transcription or translation of the polynucleotide. In certain embodiments, the disease or condition is cancer.

[0205] In certain embodiments, a method of diagnosing a disease or condition which is related to one or more B-Raf mutations in a subject comprises: (a) determining the presence or amount of expression of a polypeptide comprising at least one amino acid sequence selected from SEQ ID NO: 19, and SEQ ID NO: 20 in a sample from the subject; and (b) diagnosing a disease or condition which is related to one or more B-Raf mutations based on the presence or amount of expression of the polypeptide. In certain embodiments, a method of diagnosing a disease or condition which is related to one or more B-Raf mutations in a subject comprises: (a)

determining the presence or amount of transcription or translation of a polynucleotide encoding at least one amino acid sequence selected from SEQ ID NO: 19 and SEQ ID NO: 20 in a sample from the subject; and (b) diagnosing a disease or condition which is related to one or more B-Raf mutations based on the presence or amount of transcription or translation of the polynucleotide. In certain embodiments, the disease or condition is cancer.

[0206] In certain embodiments, a method of diagnosing a susceptibility to a disease or condition which is related to one or more EGFr mutations in a subject is provided. In certain embodiments, a method of diagnosing a susceptibility to a disease or condition which is related to one or more EGFr mutations in a subject comprises: (a) determining the presence or amount of expression of a mutant EGFr polypeptide in a sample from the subject; and (b) diagnosing a susceptibility to a disease or condition which is related to one or more EGFr mutations based on the presence or amount of expression of the polypeptide. In certain embodiments, a method of diagnosing a susceptibility to a disease or condition which is related to one or more EGFr mutations in a subject comprises: (a) determining the presence or amount of transcription or translation of a mutant EGFr polynucleotide in a sample from the subject; and (b) diagnosing a susceptibility to a disease or condition which is related to one or more EGFr mutations based on the presence or amount of transcription or translation of the polynucleotide. In certain embodiments, the disease or condition is cancer.

[0207] In certain embodiments, a method of diagnosing a susceptibility to a disease or condition which is related to one or more EGFr mutations in a subject comprises: (a) determining the presence or amount of expression of a polypeptide comprising at least one amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 12, and SEQ ID NO: 13 in a sample from the subject; and (b) diagnosing a susceptibility to a disease or condition which is related to one or more EGFr mutations based on the presence or amount of expression of the polypeptide. In certain embodiments, a method of diagnosing a susceptibility to a disease

or condition which is related to one or more EGFr mutations in a subject comprises: (a) determining the presence or amount of transcription or translation of a polynucleotide encoding at least one amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 12, and SEQ ID NO: 13 in a sample from the subject; and (b) diagnosing a susceptibility to a disease or condition which is related to one or more EGFr mutations based on the presence or amount of transcription or translation of the polypeptide. In certain embodiments, the disease or condition is cancer.

[0208] In certain embodiments, a method of diagnosing a susceptibility to a disease or condition which is related to one or more PI3K mutations in a subject is provided. In certain embodiments, a method of diagnosing a susceptibility to a disease or condition which is related to one or more PI3K mutations in a subject comprises: (a) determining the presence or amount of expression of a mutant PI3K polypeptide in a sample from the subject; and (b) diagnosing a susceptibility to a disease or condition which is related to one or more PI3K mutations based on the presence or amount of expression of the polypeptide. In certain embodiments, a method of diagnosing a susceptibility to a disease or condition which is related to one or more PI3K mutations in a subject comprises: (a) determining the presence or amount of transcription or translation of a mutant PI3K polynucleotide in a sample from the subject; and (b) diagnosing a susceptibility to a disease or condition which is related to one or more PI3K mutations based on the presence or amount of transcription or translation of the polynucleotide. In certain embodiments, the disease or condition is cancer.

[0209] In certain embodiments, a method of diagnosing a susceptibility to a disease or condition which is related to one or more PI3K mutations in a subject comprises: (a) determining the presence or amount of expression of a polypeptide comprising at least one amino acid sequence selected from SEQ ID NO: 15, SEQ ID NO: 16, and SEQ ID NO: 17 in a sample from the subject; and (b) diagnosing a susceptibility to a disease or condition which is related to one or more PI3K mutations based on the presence or amount of expression of the polypeptide. In certain

embodiments, a method of diagnosing a susceptibility to a disease or condition which is related to one or more PI3K mutations in a subject comprises: (a) determining the presence or amount of transcription or translation of a polynucleotide encoding at least one amino acid sequence selected from SEQ ID NO: 15, SEQ ID NO: 16, and SEQ ID NO: 17 in a sample from the subject; and (b) diagnosing a susceptibility to a disease or condition which is related to one or more PI3K mutations based on the presence or amount of transcription or translation of the polypeptide. In certain embodiments, the disease or condition is cancer.

[0210] In certain embodiments, a method of diagnosing a susceptibility to a disease or condition which is related to one or more B-Raf mutations in a subject is provided. In certain embodiments, a method of diagnosing a susceptibility to a disease or condition which is related to one or more B-Raf mutations in a subject comprises: (a) determining the presence or amount of expression of a mutant B-Raf polypeptide in a sample from the subject; and (b) diagnosing a susceptibility to a disease or condition which is related to one or more B-Raf mutations based on the presence or amount of expression of the polypeptide. In certain embodiments, a method of diagnosing a susceptibility to a disease or condition which is related to one or more B-Raf mutations in a subject comprises: (a) determining the presence or amount of transcription or translation of a mutant B-Raf polynucleotide in a sample from the subject; and (b) diagnosing a susceptibility to a disease or condition which is related to one or more B-Raf mutations based on the presence or amount of transcription or translation of the polynucleotide. In certain embodiments, the disease or condition is cancer.

[0211] In certain embodiments, a method of diagnosing a susceptibility to a disease or condition which is related to one or more B-Raf mutations in a subject comprises: (a) determining the presence or amount of expression of a polypeptide comprising at least one amino acid sequence selected from SEQ ID NO: 19 and SEQ ID NO: 20 in a sample from the subject; and (b) diagnosing a susceptibility to a disease or condition which is related to one or more B-Raf mutations based on the presence or amount of expression of the polypeptide. In certain embodiments, a method of

diagnosing a susceptibility to a disease or condition which is related to one or more B-Raf mutations in a subject comprises: (a) determining the presence or amount of transcription or translation of a polynucleotide encoding at least one amino acid sequence selected from SEQ ID NO: 19 and SEQ ID NO: 20 in a sample from the subject; and (b) diagnosing a susceptibility to a disease or condition which is related to one or more B-Raf mutations based on the presence or amount of transcription or translation of the polypeptide. In certain embodiments, the disease or condition is cancer.

[0212] In certain embodiments, a method of determining the presence or absence of a polynucleotide encoding a mutant EGFr polypeptide is provided. In certain embodiments, a method of determining the presence or absence of a polynucleotide encoding a mutant EGFr polypeptide in a sample comprises (a) exposing a sample to a probe which hybridizes to a polynucleotide encoding a region of a mutant EGFr polypeptide, wherein the region comprises at least one EGFr mutation selected from L688P, Q701H, K745N, C781R, a histidine insertion between amino acids 771 and 772, T790M, L828stop, Q849R, F910L, and V948A, and (b) determining the presence or absence of a polynucleotide encoding a mutant EGFr polypeptide in the sample. In certain embodiments, a method of determining the presence or absence of a mutant EGFr polypeptide in a sample comprises (a) exposing a sample to a probe which hybridizes to a polynucleotide encoding a region of a mutant EGFr polypeptide, wherein the region comprises at least one EGFr mutation selected from L688P, Q701H, K745N, C781R, a histidine insertion between amino acids 771 and 772, T790M, L828stop, Q849R, F910L, and V948A, and (b) determining the presence or absence of a mutant EGFr polypeptide in the sample.

[0213] In certain embodiments, a method of determining the presence or absence of a polynucleotide encoding a mutant PI3K polypeptide is provided. In certain embodiments, a method of determining the presence or absence of a polynucleotide encoding a mutant PI3K polypeptide in a sample comprises (a) exposing a sample to a probe which hybridizes to a polynucleotide encoding a region of a mutant PI3K polypeptide, wherein the region comprises at least one PI3K mutation selected from E542K, E545A,

and H1047L, and (b) determining the presence or absence of a polynucleotide encoding a mutant PI3K polypeptide in the sample. In certain embodiments, a method of determining the presence or absence of a mutant PI3K polypeptide in a sample comprises (a) exposing a sample to a probe which hybridizes to a polynucleotide encoding a region of a mutant PI3K polypeptide, wherein the region comprises at least one PI3K mutation selected from E542K, E545A, and H1047L, and (b) determining the presence or absence of a polynucleotide encoding a mutant PI3K polypeptide in the sample.

[0214] In certain embodiments, a method of determining the presence or absence of a polynucleotide encoding a mutant B-Raf polypeptide is provided. In certain embodiments, a method of determining the presence or absence of a polynucleotide encoding a mutant B-Raf polypeptide in a sample comprises (a) exposing a sample to a probe which hybridizes to a polynucleotide encoding a region of a mutant B-Raf polypeptide, wherein the region comprises at least one B-Raf mutation selected from V600E and K601E, and (b) determining the presence or absence of a polynucleotide encoding a mutant B-Raf polypeptide in the sample. In certain embodiments, a method of determining the presence or absence of a mutant B-Raf polypeptide in a sample comprises (a) exposing a sample to a probe which hybridizes to a polynucleotide encoding a region of a mutant B-Raf polypeptide, wherein the region comprises at least one B-Raf mutation selected from V600E and K601E, and (b) determining the presence or absence of a mutant B-Raf polypeptide in the sample.

[0215] In certain embodiments, a method of screening for a modulator of activity of at least one mutant EGFr polypeptide is provided. In certain embodiments, a method of screening for a modulator of activity of at least one mutant EGFr polypeptide comprises contacting a cell expressing at least one polynucleotide encoding a mutant EGFr polypeptide with a test compound; and detecting if the test compound modulates the activity of the mutant EGFr polypeptide. In certain such embodiments, the test compound increases the activity of the EGFr polypeptide. In certain such embodiments, the test compound decreases the activity of the EGFr polypeptide. In certain

such embodiments, a test compound identified to decrease the activity of the EGFr polypeptide can be used to treat a disease or condition which is related to at least one mutant EGFr polypeptide. In certain such embodiments, a test compound identified to increase the activity of the EGFr polypeptide can be used to treat a disease or condition which is related to at least one mutant EGFr polypeptide.

[0216] In certain embodiments, a method of screening for a modulator of activity of at least one mutant PI3K polypeptide is provided. In certain embodiments, a method of screening for a modulator of activity of at least one mutant PI3K polypeptide comprises contacting a cell expressing at least one polynucleotide encoding a mutant PI3K polypeptide with a test compound; and detecting if the test compound modulates the activity of the mutant PI3K polypeptide. In certain such embodiments, the test compound increases the activity of the PI3K polypeptide. In certain such embodiments, the test compound decreases the activity of the PI3K polypeptide. In certain such embodiments, a test compound identified to decrease the activity of the PI3K polypeptide can be used to treat a disease or condition which is related to at least one mutant PI3K polypeptide. In certain such embodiments, a test compound identified to increase the activity of the PI3K polypeptide can be used to treat a disease or condition which is related to at least one mutant PI3K polypeptide.

[0217] In certain embodiments, a method of screening for a modulator of activity of at least one mutant B-Raf polypeptide is provided. In certain embodiments, a method of screening for a modulator of activity of at least one mutant B-Raf polypeptide comprises contacting a cell expressing at least one polynucleotide encoding a mutant B-Raf polypeptide with a test compound; and detecting if the test compound modulates the activity of the mutant B-Raf polypeptide. In certain such embodiments, the test compound increases the activity of the B-Raf polypeptide. In certain such embodiments, the test compound decreases the activity of the B-Raf polypeptide. In certain such embodiments, a test compound identified to decrease the activity of the B-Raf polypeptide can be used to treat a disease or condition which is related to at least one mutant B-Raf polypeptide. In certain such embodiments, a

test compound identified to increase the activity of the B-Raf polypeptide can be used to treat a disease or condition which is related to at least one mutant B-Raf polypeptide.

[0218] In certain embodiments, a method for treating a subject for a disease or condition which is related to at least one EGFr mutation is provided. In certain embodiments, a method for treating a subject for a disease or condition which is related to at least one EGFr mutation is provided and the method comprises:

- (a) detecting at least one EGFr mutation in a polynucleotide from the subject, wherein detection of at least one EGFr mutation indicates that the patient has an increased susceptibility for developing a disease or condition which is related to at least one EGFr mutation; and
- (b) administering an antibody to the subject that specifically binds a mutant EGFr polypeptide.

[0219] In certain such embodiments, the antibody is a human antibody. In certain such embodiments, the antibody is panitumumab or an antigen binding region thereof.

[0220] In certain embodiments, a method for treating a subject for a disease or condition which is related to at least one EGFr mutation is provided and the method comprises:

- (a) detecting at least one EGFr mutation in a polynucleotide from the subject, wherein detection of at least one EGFr mutation indicates that the patient has a disease or condition which is related to at least one EGFr mutation; and
- (b) administering an antibody to the subject that specifically binds a mutant EGFr polypeptide.

[0221] In certain such embodiments, the antibody is a human antibody. In certain such embodiments, the antibody is panitumumab or an antigen binding region thereof.

[0222] In certain embodiments, a method for treating a subject for a disease or condition which is related to at least one EGFr mutation is provided, wherein at least one of the EGFr mutations is selected from L688P,

Q701H, K745N, C781R, a histidine insertion between amino acids 771 and 772, T790M, L828stop, Q849R, F910L, and V948A.

[0223] In certain embodiments, a method for treating a subject for a disease or condition which is related to at least one EGFr mutation is provided, wherein the disease or condition which is related to at least one EGFr mutation is non small cell lung carcinoma.

[0224] In certain embodiments, a method for treating a subject for a disease or condition which is related to at least one EGFr mutation is provided, comprising administering a polynucleotide antisense to a mutant EGFr polynucleotide to a subject in need of such treatment.

[0225] In certain embodiments, a method for establishing a mutant EGFr population profile in a specific population of individuals is provided comprising: (a) determining the presence of at least one EGFr mutation in a genetic profile of the individuals in a population; and (b) establishing a relationship between mutant EGFr genetic profiles and the individuals. In certain such embodiments, the specific characteristics of the individuals include a susceptibility to developing a disease or condition which is related to an EGFr mutation. In certain such embodiments, the specific characteristics of the individuals include exhibiting a disease or condition which is related to an EGFr mutation.

[0226] In certain embodiments, a method of predicting the efficacy of gefitinib treatment on a disease or condition in a subject is provided, comprising determining the presence or absence of EGFr mutation T790M in a mutant EGFr polypeptide of the subject, wherein the presence of the EGFr mutation T790M in one or more mutant EGFr polypeptides indicates resistance to treatment with gefitinib.

[0227] In certain embodiments, a method of determining responsiveness to treatment with an anti-EGFr antibody in a subject suffering from cancer is provided, comprising determining the presence or absence of EGFr mutation T790M in the subject. In certain such embodiments, the antibody is panitumumab or cetuximab.

In certain embodiments, a kit for detecting a polynucleotide encoding a mutant EGFr polypeptide in a subject is provided. In certain such

embodiments, the kit comprises a probe which hybridizes to a polynucleotide encoding a region of a mutant EGFr polypeptide, wherein the region comprises at least one EGFr mutation selected from L688P, Q701H, K745N, C781R, a histidine insertion between amino acids 771 and 772, T790M, L828stop, Q849R, F910L, and V948A. In certain embodiments, the kit further comprises two or more amplification primers. In certain embodiments, the kit further comprises a detection component. In certain embodiments, the kit further comprises a nucleic acid sampling component.

[0228] The following examples, including the experiments conducted and results achieved are provided for illustrative purpose only and are not to be construed as limiting upon the present invention.

EXAMPLES

EXAMPLE 1

IDENTIFICATION OF EGFR, PI3K, AND B-RAF MUTATIONS IN NON SMALL CELL LUNG CARCINOMA AND COLORECTAL ADENOCARCINOMA TUMOR SAMPLES

[0229] To identify mutations in EGFr, phosphatidylinositol 3'-kinase ("PI3K") and B-Raf associated with non small cell lung carcinoma ("NSCLC"), specific exons of EGFr, PI3K, and B-Raf were isolated and amplified from NSCLC tumor samples. Double-blinded tumor samples from twenty patients enrolled in a first line NSCLC trial comparing chemotherapeutic treatment alone (carboplatin/paclitaxel) versus chemotherapeutic treatment combined with panitumumab, a human anti-EGFr antibody (Amgen), were obtained prior to patient treatment with chemotherapy and/or panitumumab. To identify mutations in EGFr and PI3K associated with colorectal adenocarcinoma ("CRC"), specific exons of EGFr and PI3K were isolated and amplified from twenty CRC patient tumors. Double-blinded tumor samples from twenty patients enrolled in a first line CRC trial comparing chemotherapeutic treatment alone (carboplatin/paclitaxel) versus chemotherapeutic treatment combined with panitumumab, a human anti-EGFr antibody (Amgen), were obtained prior to patient treatment with chemotherapy and/or panitumumab. Each isolated exon was sequenced to identify any alterations from the wild-type sequences for those exons.

[0230] NSCLC tumor samples from twenty patients (Table 1) and CRC tumor samples from twenty patients (Table 2) were collected. A portion of each tumor sample was stained to identify the amount of EGFr expression of the tumor and rated for staining on a three-point scale (where 3 is the greatest degree of staining). At least 10% of each tumor sample demonstrated a staining level of three or greater. Tumor tissue was separated from adjacent normal tissue, necrotic debris, and stroma by macro dissection of formalin-fixed, paraffin-embedded tissue sections. Trimmed samples were fixed on microscope slides and stored at room temperature.

Table 1: NSCLC Patient Samples

Histology Number	Patient Number	Clinical Trial Patient Number
04H-361 JH-2	16914	4146
04H-362 JLM-2	16917	4178
04H-366 JKH-1	16928	4103
04H-368 DC-2	16935	4133
04H-370 WRW-2	16941	4140
04H-423 GHB S-1	17093	4113
04H-453 DSP S-1	17183	4130
04H-487 MMH S-1	17255	4118
04H-488 NSP S-1	17258	4121
04H-489 JDE S-1	17261	4135
04H-496 BAH S-1	17282	4161
04H-499 JMW S-1	17291	4143
04H-511 LRR S-1	17327	4182
04H-512 GLP S-1	17330	4183
04H-523 RLL S-1	17363	4116
04H-524 FPJ S-1	17366	4120
04H-525 DJK S-1	17369	4122
04H-526 JMS S-1	17372	4129
04H-593 KMW-1	17891	4101
04H-595 REG-1	17897	4123

Table 2: CRC Patient Samples

Histology Number	Patient Number	Clinical Trial Patient Number
04H-537 MLB S-1	17380	9006
04H-538 TAO S-1	17383	9021
04H-540 RRK S-1	17389	9001
04H-541 HJB S-1	17392	9002
04H-542 PJW-1	17395	9003
04H-543 JWJ S-1	17398	9004
04H-546 RFH S-1	17407	9011
04H-547 WCD S-1	17410	9014
04H-548 LKW S-1	17413	9024
04H-550 DGA S-1	17419	9038
04H-551 TLR S-1	17422	9020
04H-552 KS S-1	17425	9037
04H-556 MJJ S-1	17437	9015
04H-557 MLR S-1	17440	9034
04H-559 PH S-1	17446	9040
04H-563 AMF S-1	17458	9033
04H-565 RCR S-1	17464	9029
04H-566 GC S-1	17467	9039
04H-567 GWB S-1	17470	9013
04H-568 HLB S-1	17473	9019

[0231] Genomic DNA was prepared from the sample slides using the Pinpoint Slide DNA Isolation System (Zymo Research, Orange, CA) according to the manufacturer's protocol. The final isolated genomic DNA product was dissolved in 500 μ L water. The sequences corresponding to exons 18, 19, 20, 21, and 23 of human EGFr, exons 9 and 20 of human PI3K, and exon 15 of human B-Raf were amplified by PCR using primers specific for each exon. Primer sequences for each exon were designed using the

intron sequences 5' and 3' to each exon in the wild-type EGFr cDNA sequence (Genbank Accession No. AC006977; SEQ ID NO: 55). The genomic wild-type EGFr nucleotide sequence is found at Genbank Accession No. AC073324. The wild-type EGFr polypeptide sequence is found at Genbank Accession No. AAS83109 (SEQ ID NO: 1). The forward primer for EGFr exon 18 was 5'-GGG CCA TGT CTG GCA CTG CTT TCC- 3' (SEQ ID NO: 22), and the reverse primer for EGFr exon 18 was 5'-GAA ATA TAC AGC TTG CAA GGA CTC -3' (SEQ ID NO: 23). The forward primer for EGFr exon 19 was 5'-AAT ATC AGC CTT AGG TGC GGC TCC -3' (SEQ ID NO: 24), and the reverse primer for EGFr exon 19 was 5'-GAG AAA AGG TGG GCC TGA GGT TC-3' (SEQ ID NO: 25). The forward primer for EGFr exon 20 was 5'-CTG CGT AAA CGT CCC TGT GCT AGG TC-3' (SEQ ID NO: 26) and the reverse primer for EGFr exon 20 was 5'-GCA CGC ACA CAC ATA TCC CCA TGG C-3' (SEQ ID NO: 27). The forward primer for EGFr exon 21 was 5'-GCA TGA ACA TGA CCC TGA ATT CGG-3' (SEQ ID NO: 28) and the reverse primer for EGFr exon 21 was 5'-CCT GCA TGT GTT AAA CAA TAC AGC-3' (SEQ ID NO: 29). The forward primer for EGFr exon 23 was 5'-TCA TTC ATG ATC CCA CTG CCT TC-3' (SEQ ID NO: 30), and the reverse primer for EGFr exon 23 was 5'-CAG CTG TTT GGC TAA GAG CAG CC-3' (SEQ ID NO: 31).

[0232] The wild-type PI3K polypeptide sequence is found at Genbank Accession No. U79143 (SEQ ID NO: 14). The wild-type PI3K cDNA sequence is shown in Figure 7 (SEQ ID NO: 58). The forward primer for PI3K exon 9 was 5'-CTG TAA ATC ATC TGT GAA TCC AGA GGG G-3' (SEQ ID NO: 32), and the reverse primer for PI3K exon 9 was 5'-GTA AAT TCT GCT TTA TTT ATT CCA ATA GGT ATG G-3' (SEQ ID NO: 33). The forward primer for PI3K exon 20 was 5'-CTA CGA AAG CCT CTC TAA TTT TGT GAC ATT TGA GC-3' (SEQ ID NO: 34), and the reverse primer for PI3K exon 20 was 5'-CTT GCT GTA AAT TCT AAT GCT GTT CAT GGA TTG TGC-3' (SEQ ID NO: 35). The wild-type B-Raf polypeptide sequence is found at Genbank Accession No. NM004333 (SEQ ID NO: 18). The wild-type B-Raf cDNA sequence is shown in Figure 8 (SEQ ID NO: 60). The forward primer for B-Raf exon 11 was 5'-GGG GAT CTC TTC CTG TAT CCC TCT CAG GC-

3' (SEQ ID NO: 36), and the reverse primer for B-Raf exon 11 was 5'-GTT TAT TGA TGC GAA CAG TGA ATA TTT CC-3' (SEQ ID NO: 37). The forward primer for B-Raf exon 15 was 5'-CAT AAT GCT TGC TCT GAT AGG-3' (SEQ ID NO: 38), and the reverse primer for B-Raf exon 15 was 5'-GTA ACT CAG CAG CAT CTC AG-3' (SEQ ID NO: 39).

[0233] PCR was performed using Taq DNA polymerase (Roche Diagnostics Corp) and the following conditions: 5 µL of 10x Taq buffer, 0.5 µL of 24 mM MgCl₂, 1 µL genomic DNA (approximately 0.5 ng), 7 µL of 2.5 mM dNTPs, 1 µL Taq polymerase (5U) and 29.5 µL ddH₂O were combined and mixed. Six µL of combined primer stock (10 µM of each each) was added to each tube. The cycle protocol was 1 cycle of 4 minutes at 93 °C, 10 seconds at 93 °C, 30 seconds at 62 °C, 30 seconds at 72 °C for 35 cycles, and 1 cycle of 4 min at 72 °C. At the end of the reaction the temperature was held at 4 °C.

[0234] The PCR products for each individual exon were pooled and gel-purified. The purified amplified exon sequences were subcloned into a pCR2.1 vector using a TOPO^{*}-TA Cloning Kit (Invitrogen Corp) according to the manufacturer's instructions. *E. coli* colonies containing the vector and insert exon of interest were picked by a Genetix Colony Picker. Those colonies were grown overnight in liquid medium. Plasmid DNA from each overnight bacterial culture was isolated using a QIAGEN 9600, 3000, or 8000 Bio-robot (Qiagen) according to the manufacturer's instructions.

[0235] Isolated plasmid DNA containing each exon was sequenced using a BigDye 3.1 Terminator Kit (Applied Biosystems, Inc.) according to the manufacturer's instructions. Sequencing data was collected using a 3700, 3100, or 3730 Genetic Analyzer (Applied Biosystems, Inc.), and analyzed using the SeQuencher program (GeneCodes Corp.). The exon sequences from the patient samples were compared to the wild-type exon sequences. The results are shown schematically in Figures 1 and 2.

[0236] The mutational analysis of the NSCLC patient tumor samples (Figure 1) identified several mutations in EGFr: two different mutations in exon 18 of EGFr in two different patients (Q701H (SEQ ID NO: 40, which encodes the polypeptide of SEQ ID NO: 3) and L688P (SEQ ID

* Trademark

NO: 41, which encodes the polypeptide of SEQ ID NO: 2)); a 15 base pair deletion (SEQ ID NO: 42, which encodes the polypeptide of SEQ ID NO: 4) and a mutation (K745N (SEQ ID NO: 43, which encodes the polypeptide of SEQ ID NO: 5)) in exon 19 of EGFr in two different patients; three different mutations in exon 20 of EGFr in three different patients (C781R (SEQ ID NO: 44, which encodes the polypeptide of SEQ ID NO: 6), T790M (SEQ ID NO: 45, which encodes the polypeptide of SEQ ID NO: 8), and a histidine insertion between amino acids 771 and 772 (SEQ ID NO: 46, which encodes the polypeptide of SEQ ID NO: 7)); one mutation (Q849R (SEQ ID NO: 47, which encodes the polypeptide of SEQ ID NO: 10)) in exon 21 of EGFr in a single patient; and two different mutations in exon 23 of EGFr in two different patients (V948A (SEQ ID NO: 48, which encodes the polypeptide of SEQ ID NO: 13) and F910L (SEQ ID NO: 49, which encodes the polypeptide of SEQ ID NO: 12)). Analysis of the PI3K exons in the NSCLC patient samples identified a single mutation (E545A (SEQ ID NO: 50, which encodes the polypeptide of SEQ ID NO: 16)) in exon 9 of PI3K that was observed in seven different patients and no mutations in exon 20 of PI3K. Analysis of B-Raf exon 15 also identified a single mutation (V600E (SEQ ID NO: 51, which encodes the polypeptide of SEQ ID NO: 19)) in two different patients.

[0237] The mutational analysis of the CRC patient tumor samples, in contrast, did not identify any mutations of EGFr in the twenty CRC patients (Figure 2). Thirteen of the twenty patients had the same E545A mutation (SEQ ID NO: 50, which encodes the polypeptide of SEQ ID NO: 16) in exon 9 of PI3K that had been previously identified in the NSCLC patient samples. In addition, the mutation E542K (SEQ ID NO: 53, which encodes the polypeptide of SEQ ID NO: 15) was identified in three other patients in that exon. One mutation (H1047L (SEQ ID NO: 54, which encodes the polypeptide of SEQ ID NO: 17)) was identified in exon 20 of PI3K, in a single patient.

[0238] Thus twelve different EGFr mutations, one PI3K mutation, and one B-Raf mutation were identified in the NSCLC patient tumor samples, while three PI3K mutations and no EGFr mutations were identified in the CRC patient tumor samples.

EXAMPLE 2

EXPANDED NON SMALL CELL LUNG CARCINOMA MUTATIONAL ANALYSIS

[0239] An expanded mutational study of thirty-nine additional NSCLC patient tumor samples was performed. Double-blinded tumor samples from thirty-nine patients enrolled in a first line NSCLC trial comparing chemotherapeutic treatment alone (carboplatin/paclitaxel) versus chemotherapeutic treatment combined with panitumumab, a human anti-EGFr antibody (Amgen), were obtained prior to patient treatment with chemotherapy and/or panitumumab. Using the identical DNA isolation, amplification, sub-cloning, and analysis procedures as set forth in Example 1, EGFr exons 18, 19, 20, 21, and 23, and B-Raf exons 11 and 15 were analyzed for the presence of mutations. The thirty-nine samples are detailed in Table 3, and the results of the analyses of those samples appear in Figure 3.

Table 3: NSCLC Patient Samples for Expanded Study

Histology Number	Patient Number	Clinical Trial Patient Number
04H-424 JAQ S-2	17096	4119
04H-425 JZ-2	17099	4228
04H-426 PAP-2	17102	4233
04H-427 SFD-2	17105	4239
04H-428 AMB S-2	17108	4167
04H-429 ELH S-2	17111	4273
04H-430 HDD S-2	17114	4144
04H-431 CMW S-2	17117	4213
04H-432 JL S-2	17120	4165
04H-433 RC S-2	17123	4170
04H-434 RZ S-2	17126	4219
04H-435 GK S-2	17129	4265
04H-436 RT S-2	17132	4248
04H-437 MMF S-2	17135	4240

04H-438 JDR S-2	17138	4179
04H-439 LC S-2	17141	4256
04H-440 GLP S-2	17144	4275
04H-441 MHR S-2	17147	4206
04H-442 JEF S-2	17150	4222
04H-443 HBA S-2	17153	4223
04H-444 DT S-2	17156	4231
04H-447 CD S-2	17165	4207
04H-449 DWB S-2	17171	4164
04H-450 DLR S-2	17174	4211
04H-454 NPJ S-2	17186	4136
04H-456 NEN S-2	17192	4151
04H-461 LWF S-2	17207	4218
04H-479 MAT S-2	17231	4229
04H-482 GPH S-2	17240	4221
04H-484 JP S-2	17246	4156
04H-493 JS S-2	17273	4208
04H-497 JMP S-2	17285	4189
04H-503 SAS S-2	17303	4254
04H-504 JDD S-2	17306	4152
04H-507 RWR S-2	17315	4157
04H-510 CSL S-2	17324	4180
04H-513 ALF S-2	17333	4205
04H-515 VIT S-2	17339	4149
04H-522 VAB S-2	17360	4257

[0240] The results of the analysis identified no mutations in EGFr exons 20, or 23. A single mutation was identified in EGFr exon 18 (L688P (SEQ ID NO: 41, which encodes the polypeptide of SEQ ID NO: 2)) in four different patient samples. A single 15 base pair deletion (SEQ ID NO: 42, which encodes the polypeptide of SEQ ID NO: 4) in EGFr exon 19 was identified in a single patient sample. Two mutations were identified in EGFr

exon 21 (L858R (SEQ ID NO: 61, which encodes the polypeptide of SEQ ID NO: 11) and L828stop (SEQ ID NO: 56, which encodes the polypeptide of SEQ ID NO: 9)), each in two different patients. No mutations were identified in B-Raf exon 11. One mutation, K601E (SEQ ID NO: 57, which encodes the polypeptide of SEQ ID NO: 20), was identified in B-Raf exon 15 in a single patient sample. Of the observed mutations, two had been previously identified in Example 1 (L688P in EGFr exon 18 and the 15 base pair deletion in EGFr exon 19), and three were newly identified (L858R and L828stop in EGFr exon 21, and K601E in B-Raf exon 15). In all, nine confirmed mutations in the EGFr gene were identified in eight NSCLC patient samples, and one confirmed mutation in the B-Raf gene was identified in one NSCLC patient.

EXAMPLE 3

ANALYSIS OF AUTOPHOSPHORYLATION CAPABILITY OF MUTANT EGFR POLYPEPTIDE

[0241] Typically, EGFr undergoes an autophosphorylation event as a precursor to internalization upon binding to a ligand such as EGF or TGF- α . Accordingly, certain EGFr mutant polypeptides identified in Example 2 were studied to determine inhibition of EGF-induced EGFr phosphorylation in vitro.

[0242] Chinese hamster ovary cell lines overexpressing wild-type (SEQ ID NO: 1) or mutated EGFr polypeptide were constructed. Cells from each line were plated and treated with 0-2 μ M of either panitumumab or gefitinib (IressaTM, 4-quinazolinamine, N-(3-chloro-4-fluorophenyl)-7-methoxy-6-[3-4-morpholin) propoxy], a small molecule kinase inhibitor) prior to stimulation with EGF. The IC₅₀ for EGF-induced autophosphorylation was calculated for the gefitinib and panitumumab-treated samples (Table 4). The raw electrophoresis data for wild-type EGFr and the T790M mutant EGFr polypeptides are shown in Figure 4.

Table 4: IC50 for EGFr Autophosphorylation after Treatment with Gefitinib or Panitumumab

EGFr Mutation	gefitinib pretreatment IC50 (nM)	panitumumab pretreatment IC50 (nM)
none (wild-type)	14.6	0.23
15 base pair deletion in exon 19	1.4	0.17
L858R in exon 21	3.2	0.18
T790M in exon 20	> 2000	0.23

[0243] As shown in Table 4, both gefitinib and panatumumab were effective in preventing EGFr autophosphorylation at low concentration for the wild-type EGFr and the 15 base pair deletion and L858R EGFr mutants. Autophosphorylation of the T790M mutant EGFr polypeptide, however, was not inhibited by gefitinib (IC50 > 2000 nM), yet was effectively inhibited by panitumumab (IC50 of 0.23 nM). Thus, panitumumab may be a more efficacious treatment than gefitinib for NSCLC patients having a T790M mutation in EGFr exon 20 than gefitinib.

EXAMPLE 4

CORRELATION OF MUTATIONAL ANALYSIS WITH PANITUMUMAB EFFICACY

[0244] After the mutational analysis of Example 2, the results of the study were unblinded for the patients in which mutations were observed (Table 5). Clinical data was assessed by an investigator every six weeks using the Response Evaluation Criteria In Solid Tumors (RECIST), which provides guidelines for identifying improvement, stable disease, or progressive disease based on tumor size (see Therasse et al., February 2000, "New Guidelines to Evaluate the Response to Treatment in Solid Tumors," J. Natl. Cancer Inst. 92(3): 205-216).

Table 5: NSCLC Patient Samples

Identified Mutation	Gender	Smoking History	Treatment	% EGFr with staining level of 3 or greater)	Response
15 base pair deletion exon 19	male	never	chemo	60	stable disease
L688P Exon 18	female	former	chemo	50	stable disease
L688P Exon 18	male	former	chemo	80	partial response
L688P Exon 18	male	former	chemo	10	stable disease
T790M Exon 20	male	former	chemo + panitumumab	10	stable disease
L858R Exon 21	male	former	chemo + panitumumab	90	stable disease
Q701H Exon 18	female	never	chemo + panitumumab	20	progressive disease
15 base pair deletion exon 19	female	never	chemo + panitumumab	40	partial response

The results demonstrate that panitumumab in combination with chemotherapy yielded stable disease for at least 12 weeks for those patients with a T790M mutation in EGFr Exon 20 and a L858R mutation in EGFr Exon 21. Using the chemotherapy/panitumumab combination therapy, a partial response was observed in a patient with a 15 base pair deletion in EGFr exon 19. In contrast, a patient with the same 15 base pair deletion in EGFr exon 19 achieved only stable disease with chemotherapeutic treatment alone.

[0245] Recent studies have identified several EGFr mutations in tumors from NSCLC that display sensitivity to the EGFr tyrosine kinase inhibitors gefitinib (Iressa™ (AstraZeneca) and erlotinib (Tarceva™ (Genentech), N-(3-ethynylphenyl)-6,7-bis(2-methoxyethoxy)-4-quinazolinamine). Lynch et al. (2004, "Activating Mutations in the Epidermal Growth Factor Receptor Underlying Responsiveness of Non-Small-Cell Lung Cancer to Gefitinib," New England J. Med. 350(21): 2129-39) found that the following EGFr mutations were associated with the susceptibility of NSCLC

patient tumors to treatment with gefitinib: deletions in the amino acid 746-753 region, L858R, L861Q, and G719C. Paez et al. (2004, "EGFR Mutations in Lung Cancer: Correlation with Clinical Response to Gefitinib Therapy," *Science* 304: 1497-1500) had similar findings to Lynch et al., Identifying tumors with EGFr mutations L858R, G719S, and various deletion mutations between amino acids 746 and 759 as being susceptible to treatment with gefitinib. Pao et al., 2004 ("EGF receptor gene mutations are common in lung cancers from "never smokers" and are associated with sensitivity of tumors to gefitinib and erlotinib," *Proc. Natl. Acad. Sci. USA* 101(36): 13306-13311), found that similar EGFr mutations (E746-A750 deletion, L747-S752 deletion, L858R, and R776C/L858R) were associated with susceptibility of NSCLC tumors to treatment with gefitinib or erlotinib.

[0246] Like those studies, the studies discussed in Examples 1 and 2 also identified the 15 base pair deletion mutant in exon 19, and L858R in exon 21 as EGFr mutations associated with NSCLC tumors. Of the data for which unblinded patient outcomes were available, the tumors containing either of those two mutations or T790M were inhibited by panitumumab in combination with chemotherapy. The T790M mutation, however, was not previously identified in the gefitinib/erlotinib experiments. In vitro studies demonstrate that while autophosphorylation of T790M EGFr mutants is effectively inhibited at very low concentrations of panitumumab, gefitinib is not an effective inhibitor of autophosphorylation of that mutant EGFr. Thus, panitumumab combination therapy and not gefitinib may be an effective treatment for T790M EGFr mutants.

APPENDIX A**Page 1 of 2****Identification and Preclinical Characterization of EGFr Somatic Gene Mutations from a Panitumumab Phase 2 NSCLC Clinical Trial: Discovery of a Novel Mutation with Panitumumab Sensitivity and Gefitinib Resistance**

Freeman D,^{1*} Juan T,^{1*} Sarosi I,¹ Crawford J,² Sandler A,³ Schiller J,⁴ Prager D,⁵ Johnson D,³ Jerian S,¹ Radinsky R¹ (*These authors contributed equally)

¹Amgen Inc., Thousand Oaks, CA; ²Duke University Medical Center, Durham, NC; ³Vanderbilt-Ingram Cancer Center, Nashville, TN; ⁴University of Wisconsin, Madison, WI; ⁵UCLA Medical Center, Los Angeles, CA

Background: Understanding the different mechanisms between EGFr tyrosine kinase inhibitors and monoclonal antibodies may lead to insight into the EGFr pathway and the treatment modality likely to result in clinical benefit. Panitumumab, a fully human monoclonal antibody, directed against the EGFr extracellular domain is currently being studied in a 1st line NSCLC trial comparing chemotherapy (carboplatin/paclitaxel) vs chemotherapy plus panitumumab (n=175; primary endpoint = time to progression). 60 pts were assessed for EGFr gene mutations. The objective of this study was to determine if panitumumab has differential activity vs. gefitinib against novel EGFr mutations.

Methods: Genomic DNA was isolated from dissected FFPE tumor sections (pretreatment), PCR performed on EGFr gene exons 18, 19, 20, 21, and 23, PCR products pooled, subcloned, and >30 colonies per exon were sequenced and resolved on a Genetic Analyzer. Subsequent PCR and genomic DNA sequencing confirmed the existence of mutations. These data were linked to clinical outcome data (investigator assessed per RECIST every 6 weeks). To determine inhibition of EGF-induced EGFr autophosphorylation in vitro, WT and mutant EGFr overexpressing CHO cells were treated with 0-2 uM of either panitumumab or gefitinib prior to EGF stimulation.

Results: Sixty NSCLC pts revealed 5 different somatic EGFr gene mutations in 8 pts.

APPENDIX A

Page 2

Patient	Gender	Smoking History	Treatment	% EGFr (3+)	Mutation	Response*
1	Male	Never	Chemo	60	15 base pair Deletion Exon 19	SD
2	Female	Former	Chemo	50	Point Mutation Exon 18	SD
3	Male	Former	Chemo	80	Point Mutation Exon 18	PR
4	Male	Former	Chemo	10	Point Mutation Exon 18	SD
5	Male	Former	Chemo + panitumumab	10	Point Mutation Exon 20	SD
6	Male	Former	Chemo + panitumumab	90	Point Mutation Exon 21	SD
7	Female	Never	Chemo + panitumumab	20	Point Mutation Exon 18	PD
8	Female	Never	Chemo + panitumumab	40	15 base pair Deletion Exon 19	PR

* PR=partial response, SD= stable disease, PD= progressive disease

The IC₅₀ for EGF-induced autophosphorylation of EGFr wildtype, exon 19 deletion, exon 21 point mutation and novel exon 20 mutation was 14.6, 1.4, 3.2 and >2000nM for cells pretreated with gefitinib and 0.23, 0.17, 0.18 and 0.23nM for cells pretreated with panitumumab.

Conclusion: Eight pts harbored EGFr somatic gene mutations. In EGFr overexpressing CHO cells, panitumumab inhibited EGF-induced EGFR autophosphorylation regardless of mutational status. A novel EGFr mutation in exon 20 is associated with in-vitro sensitivity to panitumumab, resistance to gefitinib and clinical benefit in a patient who experienced stable disease for 2 cycles (~12 weeks) in response to treatment with panitumumab + chemotherapy.

APPENDIX B

Page 1 of 2

Analysis of EGFr Gene Mutations in NSCLC Patients Treated with Panitumumab Plus Paclitaxel and Carboplatin or Chemotherapy Alone

Freeman D,^{1*} Juan T,^{1*} Sarosi I,¹ Crawford J,² Sandler A,³ Schiller J,⁴ Prager D,⁵ Johnson D,³ Moss S,¹ Radinsky R¹ (*These authors contributed equally)

¹ Amgen Inc., Thousand Oaks, CA; ² Duke University Medical Center, Durham, NC; ³ Vanderbilt-Ingram Cancer Center, Nashville, TN; ⁴ University of Wisconsin, Madison, WI; ⁵ UCLA Medical Center, Los Angeles, CA

Background: Panitumumab is a fully human monoclonal antibody directed against the epidermal growth factor receptor (EGFr). Recent data suggest that somatic gene mutations in the EGFr kinase domain are associated with sensitivity to small molecule tyrosine kinase inhibitors (TKIs) in a subset of non-small cell lung carcinoma (NSCLC) patients (pts). To determine the association, if any, of EGFr gene mutations with pt outcome to panitumumab treatment, the EGFr gene was sequenced in the tumors of 60 pts with NSCLC enrolled in a randomized phase 2 study comparing the efficacy and safety of paclitaxel and carboplatin (chemotherapy) versus panitumumab plus chemotherapy. Eligible pts had stage IIIb/IV disease and tumors with EGFr expression of 1+, 2+ or 3+ in $\geq 10\%$ of tumor cells as shown by immunohistochemistry. Tumor response was assessed by investigators every 6 weeks using RECIST criteria. This study has completed enrollment (n = 175), and treatment is ongoing.

Methods: Tumor tissue (pretreatment) was separated from adjacent normal tissue, necrotic debris and stroma by dissection of formalin-fixed, paraffin-embedded tissue sections under a light microscope. Genomic DNA was isolated and PCR was performed on exons 18, 19, 20, 21, and 23 of the EGFr gene. PCR products were pooled and subcloned, and a minimum of 30 colonies per exon per pt was analyzed. Subsequent PCR and sequencing of the purified genomic DNA was used confirm the existence of a mutation. Mutational analysis of the EGFr was performed using fluorescent dye-terminator chemistry and resolved on a Genetic Analyzer.

Results: DNA sequencing of the 60 NSCLC pts revealed somatic EGFr gene mutations in 8 pts total, 4 from the chemotherapy arm and 4 pts from the chemotherapy plus panitumumab arm.

Patient	Treatment	% EGFr (3+)	Mutation	Response*
1	Chemo	60	15 base pair Deletion Exon 19	SD

APPENDIX B

Page 2

2	Chemo	50	Point Mutation Exon 18	SD
3	Chemo	80	Point Mutation Exon 18	PR
4	Chemo	10	Point Mutation Exon 18	SD
5	Chemo + panitumumab	10	Point Mutation Exon 20	SD
6	Chemo + panitumumab	90	Point Mutation Exon 21	SD
7	Chemo + panitumumab	20	Point Mutation Exon 18	PD
8	Chemo + panitumumab	40	15 base pair Deletion Exon 19	PR

*PR=partial response, SD= stable disease, PD= progressive disease

Conclusions: Sixty NSCLC pts were evaluated for EGFr somatic gene mutations. Eight patients were found to harbor EGFr gene mutations, 3 pts were observed to have partial response or stable disease with panitumumab plus chemotherapy, 1 pt did not respond to panitumumab plus chemotherapy. Four pts were observed to have partial response or stable disease with chemotherapy alone. The outcome of the 60 pts will be presented.

DEMANDE OU BREVET VOLUMINEUX

LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVET COMPREND PLUS D'UN TOME.

CECI EST LE TOME 1 DE 2
CONTENANT LES PAGES 1 À 94

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THIS IS VOLUME 1 OF 2
CONTAINING PAGES 1 TO 94

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NOM DU FICHER / FILE NAME :

NOTE POUR LE TOME / VOLUME NOTE:

CLAIMS

1. A fusion protein comprising an isolated polypeptide comprising the amino acid sequence of SEQ ID NO: 8 fused to a heterologous polypeptide.
2. The fusion protein of claim 1, wherein the isolated polypeptide consists of the amino acid sequence of SEQ ID NO: 8.
3. An isolated polynucleotide encoding the fusion protein of claim 1 or 2.
4. A vector comprising the isolated polynucleotide of claim 3.
5. A host cell comprising the vector of claim 4.
6. A cell transformed with the isolated polynucleotide of claim 3.
7. A method of preparing the fusion protein defined in claim 1 or 2, comprising culturing the host cell of claim 5 under conditions effective for polypeptide production.
8. The method of claim 7, further comprising isolating the fusion protein.
9. A method of preparing the fusion protein defined in claim 1 or 2, comprising culturing the cell of claim 6 under conditions effective for polypeptide production.
10. The method of claim 9, further comprising isolating the fusion protein.
11. A method of obtaining an antibody capable of binding a fusion protein as defined in claim 1 or 2, comprising:
administering a fusion protein as defined in claim 1 or 2 to an animal;
and
obtaining an antibody capable of binding the fusion protein from the animal.
12. The polynucleotide of claim 3 attached to a solid support.
13. The fusion protein of claim 1 or 2 attached to a solid support.

14. An array of polynucleotides comprising an isolated polynucleotide encoding a polypeptide comprising the amino acid sequence of SEQ ID NO: 8, or encoding a polypeptide consisting of the amino acid sequence of SEQ ID NO: 8.

15. An array of polypeptides comprising an isolated polypeptide comprising the amino acid sequence of SEQ ID NO: 8, or consisting of the amino acid sequence of SEQ ID NO: 8.

16. A method of screening for a modulator of activity of a mutant EGFr polypeptide, T790M comprising:

(a) contacting a cell transformed with an isolated polynucleotide encoding a polypeptide comprising the amino acid sequence of SEQ ID NO: 8, or encoding a polypeptide consisting of the amino acid sequence of SEQ ID NO: 8, or a cell comprising a vector which comprises said isolated polynucleotide, with a test compound; and

(b) detecting if the test compound modulates the activity of the mutant EGFr polypeptide.

17. A method of determining responsiveness to treatment with panitumumab or cetuximab in a subject suffering from cancer comprising determining the presence or absence of EGFr mutation T790M of SEQ ID NO: 8 in the subject, wherein the presence of EGFr mutation T790M indicates that the cancer will be responsive to panitumumab or cetuximab treatment, and wherein the cancer is non small cell lung cancer (NSCLC).

18. A method of determining resistance to gefitinib treatment but not panitumumab treatment in a subject suffering from non small cell lung cancer comprising determining the presence or absence of EGFr mutation T790M of SEQ ID NO: 8 in the cancer, wherein the presence of EGFr mutation T790M indicates that the cancer will be resistant to gefitinib treatment but not panitumumab treatment.

FIGURE 1 . Result of mutational analysis of NSCLC patient tumor samples.

ATB #	EGFR E18	EGFR E19	EGFR E20	EGFR E21	EGFR E23	PIK3 E09	PIK3 E20	b-raf E15
16914	WT (9)	WT (21)	N.D.	WT (8)	WT (6)	TBD	TBD	TBD
16917	N.D.	WT (12)	N.D.	WT (17)	WT (6)	TBD	TBD	TBD
16928	WT (8)	WT (38)	WT (14)	WT (46)	WT (17)	TBD	TBD	TBD
16935	WT (3)	WT (23)	C781R (9/35)	WT (36)	V948A (12/30)	TBD	TBD	TBD
16941	WT (15)	WT (16)	N.D.	N.D.	N.D.	TBD	TBD	TBD
17093	WT (62)	WT (9)	HIS insert (2/28)	WT (2)	N.D.	TBD	TBD	TBD
17183	WT (69)	WT (45)	WT (7)	Q849R (3 of 38)	WT (36)	E545A (4 of 41)	N.D.	WT (11)
17255	WT (80)	WT (36)	WT (66)	WT (12)	WT (21)	WT (8)	WT (2)	WT (9)
17258	WT (89)	WT (18)	WT (11)	WT (5)	WT (3)	WT (14)	N.D.	WT (82)
17261	WT (197)	WT (22)	N.D.	WT (6)	WT (12)	WT (13)	WT (2)	WT (16)
17282	WT (69)	WT (44)	WT (56)	WT (75)	WT (63)	E545A (4 of 42)	WT (5)	WT (49)
17291	WT (96)	WT (23)	WT (62)	WT (25)	WT (23)	E545A (16 of 146)	WT (3)	WT (19)
17327	Q701H (11/195)	WT (2)	WT (107)	WT (23)	WT (15)	WT (3)	N.D.	WT (6)
17330	WT (89)	WT (31)	WT (39)	WT (114)	WT (27)	E545A (7 of 30)	WT (7)	WT (39)
17363	L688P (19/69)	15-bp del (4/26)	WT (40)	WT (24)	WT (21)	WT (29)	WT (33)	WT (5)
17366	WT (39)	WT (26)	T790M (27/140)	WT (5)	WT (3)	WT (26)	N.D.	N.D.
17369	WT (128)	WT (22)	WT (34)	WT (29)	WT (49)	N.D.	WT (4)	TBD
17372	WT (30)	WT (109)	WT (17)	WT (36)	F910L (7 of 84)	E545A (5 of 43)	WT (1)	WT (122)
17891	WT (34)	K745N (5 of 44)	WT (6)	WT (43)	WT (128)	E545A (38 of 160)	N.D.	V600E (7/44)
17897	WT (142)	WT (48)	WT (29)	WT (64)	WT (49)	E545A (2 of 31)	N.D.	V600E (7/71)

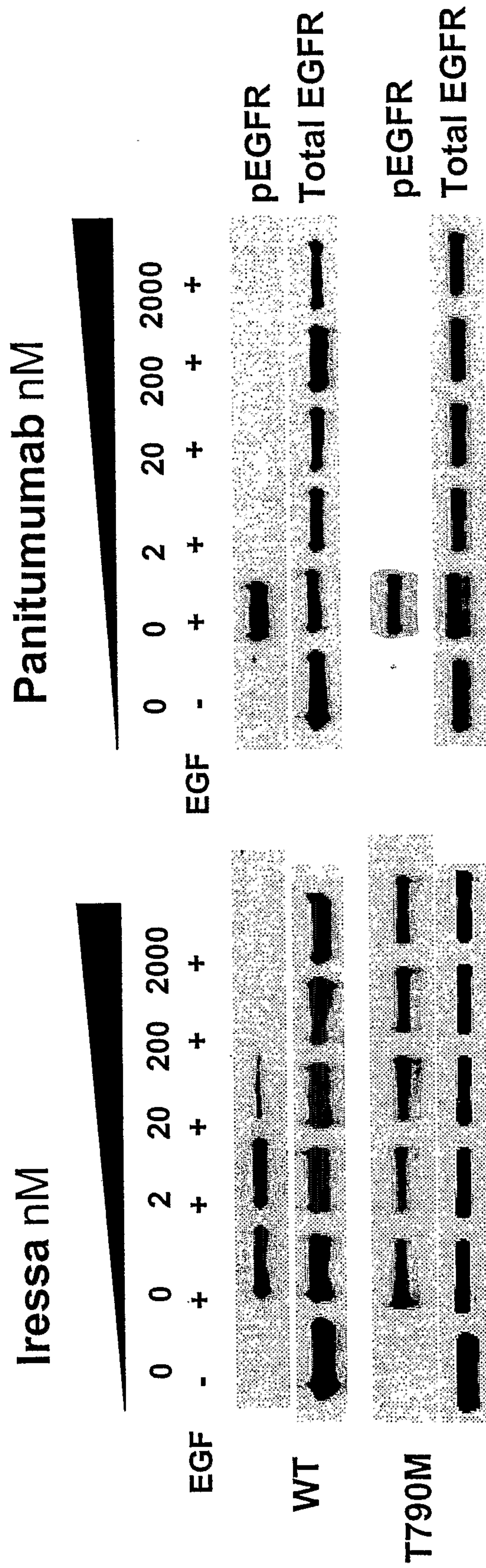
FIGURE 2 . Result of mutational analysis of CRC patient tumor samples.

ATB #	EGFR E18	EGFR E19	EGFR E20	EGFR E21	EGFR E23	PIK3 E9	PIK3 E20	b-raf E15
17380	WT (67)	WT (113)	WT (169)	NO PCR PRODUCT	WT (34)	E545A (9/145)	N.D.	TBD
17383	WT (47)	WT (56)	WT (82)	WT (34)	WT (101)	E545A (3 of 106)	WT (49)	TBD
17389	WT (78)	WT (41)	WT (208)	WT (17)	WT (39)	E545A (3 of 11)	WT (7)	TBD
17392	WT (199)	WT (90)	WT (38)	WT (43)	WT (55)	E545A (1 of 4)	WT (13)	TBD
17395	WT (225)	N.D.	WT (191)	WT (19)	WT (5)	WT (8)	WT (3)	TBD
17398	WT (76)	WT (70)	WT (138)	WT (26)	WT (22)	E545A (1 of 30)	WT (13)	TBD
17407	WT (94)	WT (8)	WT (57)	WT (45)	WT (6)	E545A (8 of 151)	WT (4)	TBD
17410	WT (141)	WT (68)	WT (37)	WT (41)	WT (51)	E545A (1 of 15)	WT (142)	TBD
17413	WT (143)	WT (29)	WT (43)	WT (33)	WT (17)	WT (17)	WT (16)	TBD
17419	WT (99)	WT (48)	WT (58)	WT (30)	WT (2)	E545A (1 of 17)	WT (21)	TBD
17422								
17425	WT (77)	WT (77)	WT (56)	WT (34)	WT (23)	E545A (1 of 38)	H1047 (42 of 110)	TBD
17437	WT (93)	WT (20)	WT (46)	WT (38)	WT (54)	NO PCR PRODUCT	WT (8)	TBD
17440	WT (132)	WT (103)	WT (20)	WT (45)	WT (37)	E545A (2 of 37)	N.D.	TBD
17446	WT (31)	WT (50)	WT (40)	WT (29)	WT (8)	E542K (1 of 25)	WT (23)	TBD
17458	WT (192)	WT (32)	WT (40)	WT (41)	WT (32)	E545A (1 of 19)	WT (3)	TBD
17464	WT (221)	NO PCR PRODUCT	N.D.	WT (33)	WT (5)	E542K (4 of 46)	WT (76)	TBD
17467	WT (115)	WT (34)	WT (61)	WT (39)	WT (10)	E545A (3 of 48)	N.D.	TBD
17470	WT (79)	WT (54)	WT (134)	WT (20)	WT (107)	E545A (4 of 31)	NO PCR PRODUCT	TBD
17473	WT (66)	WT (112)	WT (16)	WT (13)	WT (31)	E542K (1 of 32)	WT (5)	TBD

FIGURE 3: Results of Expanded Mutational Analysis of NSCLC Patient Tumor Samples

ATB Number	EGFR Exon18	EGFR Exon19	EGFR Exon20	EGFR Exon21	EGFR Exon23	b-raf Exon11	b-raf Exon15
17096	WT (36)	WT (75)	WT (41)	WT (43)	WT (49)	WT (54)	WT (84)
17099	WT (52)	WT (107)	WT (39)	L858R (13 of 81)	WT (59)	WT (43)	WT (49)
17102	WT (73)	15bp deletion (43 of 63)	WT (85)	WT (26)	WT (17)	WT (21)	WT (77)
17105	WT (50)	WT (22)	WT (89)	WT (18)	WT (37)	WT (46)	WT (10)
17108	WT (94)	WT (9)	WT (60)	WT (42)	WT (43)	WT (30)	WT (116)
17111	WT (30)	WT (51)	WT (7)	WT (20)	WT (54)	WT (49)	WT (138)
17114	WT (35)	WT (90)	WT (51)	WT (36)	WT (27)	WT (66)	WT (26)
17117	WT (20)	WT (2)	WT (24)	WT (47)	WT (34)	WT (79)	WT (53)
17120	WT (90)	WT (130)	WT (13)	No product	WT (56)	WT (47)	WT (44)
17123	WT (87)	WT (33)	No product	No product	WT (21)	No product	WT (32)
17126	WT (29)	WT (31)	WT (69)	WT (28)	WT (25)	WT (57)	WT (30)
17129	L688P (15 of 145)	WT (40)	WT (9 of 95)	WT (64)	WT (25)	WT (38)	WT (55)
17132	WT (24)	WT (23)	WT (91)	WT (12)	WT (74)	WT (47)	WT (62)
17135	WT (87)	No product	No product	WT (4)	WT (90)	WT (9)	WT (58)
17138	WT (23)	No product	WT (33)	WT (35)	WT (24)	WT (7)	WT (46)
17141	L688P (11 of 148)	No product	WT (17)	WT (18)	WT (26)	WT (20)	WT (116)
17144	WT (93)	WT (22)	WT (63)	WT (49)	WT (6)	No product	WT (16)
17147	WT (19)	WT (6)	WT (29)	WT (49)	WT (31)	WT (65)	WT (163)
17150	WT (29)	N.D.	N.D.	WT (22)	WT (9)	WT (74)	WT (102)
17153	WT (21)	WT (81)	WT (90)	WT (25)	WT (52)	WT (23)	WT (10)
17156	WT (92)	No product	No product	No product	WT (3)	No product	WT (8)
17165	WT (89)	N.D.	WT (100)	WT (69)	WT (30)	WT (14)	WT (7)
17171	WT (24)	WT (8)	WT (40)	WT (36)	N.D.	WT (71)	WT (89)
17174	L688P (12 of 68)	No product	WT (5)	N.D.	WT (21)	WT (92)	WT (12)
17186	WT (67)	WT (20)	No product	WT (13)	WT (10)	WT (54)	WT (66)
17192	WT (19)	N.D.	WT (90)	WT (5)	WT (3)	WT (116)	WT (5)
17207	WT (13)	WT (20)	WT (33)	WT (30)	WT (19)	WT (56)	WT (84)
17231	WT (92)	No product	WT (131)	No product	WT (20)	WT (8)	No product
17240	WT (2)	WT (6)	WT (94)	WT (30)	WT (6)	WT (85)	K601E (19 of 184)
17246	WT (34)	WT (9)	WT (40)	WT (38)	WT (3)	WT (54)	WT (70)
17273	WT (8)	WT (32)	WT (44)	WT (19)	WT (39)	WT (47)	WT (44)
17285	L688P (4 of 103)	WT (27)	WT (25)	L828 Stop (8 of 185)	WT (20)	WT (28)	WT (28)
17303	WT (16)	No product	WT (26)	WT (28)	WT (17)	WT (75)	WT (73)
17306	WT (88)	WT (13)	WT (37)	WT (102)	WT (10)	WT (23)	No product
17315	WT (29)	No product	WT (92)	WT (95)	WT (28)	WT (31)	WT (24)
17324	WT (91)	N.D.	WT (25)	L858R (35 of 238)	WT (2)	WT (71)	N.D.
17333	No product	N.D.	No product	WT (82)	WT (17)	WT (84)	WT (4)
17339	WT (33)	WT (18)	No product	L828 Stop (6 of 132)	N.D.	WT (93)	WT (71)
17360	WT (62)	WT (2)	No product	WT (53)	WT (89)	WT (15)	WT (2)

Clinically Observed EGFr Mutation- Differential Sensitivity to Iressa and Panitumumab (in vitro)



EGFr gene mutation (T790M) found in a NSCLC patient that responded to Paclitaxel / Carboplatin + panitumumab combination therapy

FIGURE 4

FIGURE 5A

Exon20.txt x His

```
1 GAAGCCTACGTGATGGCCAGCGTGGACA...ACCCCCACGTGTGCCGCCT 47
  |||||||||||||||||||||||||||||||||||||||||||||||||||
1 GAAGCCTACGTGATGGCCAGCGTGGACAACCACCCCCACGTGTGCCGCCT 50

48 GCTGGGCATCTGCCTCACCTCCACCGTGCAACTCATCACGCAGCTCATGC 97
  |||||||||||||||||||||||||||||||||||||||||||||||||||
51 GCTGGGCATCTGCCTCACCTCCACCGTGCAACTCATCACGCAGCTCATGC 100

98 CCTTCGGCTGCCTCCTGGACTATGTCCGGGAACACAAAGACAATATTGGC 147
  |||||||||||||||||||||||||||||||||||||||||||||||||||
101 CCTTCGGCTGCCTCCTGGACTATGTCCGGGAACACAAAGACAATATTGGC 150

148 TCCCAGTACCTGCTCAACTGGTGTGTGCAGATCGCAAAG 186 SEQ ID NO: 52
  |||||||||||||||||||||||||||||||||||||||||||||||||||
151 TCCCAGTACCTGCTCAACTGGTGTGTGCAGATCGCAAAG 189 SEQ ID NO: 59
```

Exon20.pep x His.pep

```
1 EAYVMASVDN.PHVCRLLGICLTSTVQLITQLMPFGCLLDYVREHKDNIG 49
  ||||||||||| |||||||||||||||||||||||||||||||||||||||
1 EAYVMASVDNHPHVCRLLGICLTSTVQLITQLMPFGCLLDYVREHKDNIG 50

50 SQYLLNWCVQIAK 62 SEQ ID NO: 62
  |||||||||||
51 SQYLLNWCVQIAK 63 SEQ ID NO: 21
```

FIGURE 5B

Exon20.txt x T790M.txt

```
      .      .      .      .
1 GAAGCCTACGTGATGGCCAGCGTGGACAACCCCCACGTGTGCCGCCTGCT 50
  |||
1 GAAGCCTACGTGATGGCCAGCGTGGACAACCCCCACGTGTGCCGCCTGCT 50

      .      .      .      .
51 GGGCATCTGCCTCACCTCCACCGTGCAACTCATCACGCAGCTCATGCCCT 100
  |||
51 GGGCATCTGCCTCACCTCCACCGTGCAACTCATCATGCAGCTCATGCCCT 100

      .      .      .      .
101 TCGGCTGCCTCCTGGACTATGTCCGGGAACACAAAGACAATATTGGCTCC 150
  |||
101 TCGGCTGCCTCCTGGACTATGTCCGGGAACACAAAGACAATATTGGCTCC 150

      .      .      .
151 CAGTACCTGCTCAACTGGTGTGTGCAGATCGCAAAG 186 SEQ ID NO: 63
  |||
151 CAGTACCTGCTCAACTGGTGTGTGCAGATCGCAAAG 186 SEQ ID NO: 64
```

Exon20.pep x T790M.pep

```
      .      .      .      .
1 EAYVMASVDNPHVCRLLGICLTSTVQLITQLMPFGCLLDYVREHKDNIGS 50
  |||
1 EAYVMASVDNPHVCRLLGICLTSTVQLIMQLMPFGCLLDYVREHKDNIGS 50

      .
51 QYLLNWCVQIAK 62 SEQ ID NO: 65
  |||
51 QYLLNWCVQIAK 62 SEQ ID NO: 66
```

FIGURE 5C

Exon18.txt x Q701H.txt

```
1 CTTGTGGAGCCTCTTACACCCAGTGGAGAAGCTCCCAACCAAGCTCTCTT 50
  |||||
1 CTTGTGGAGCCTCTTACACCCAGTGGAGAAGCTCCCAACCACGCTCTCTT 50

51 GAGGATCTTGAAGGAAACTGAATTCAAAAAGATCAAAGTGCTGGGCTCCG 100
  |||||
51 GAGGATCTTGAAGGAAACTGAATTCAAAAAGATCAAAGTGCTGGGCTCCG 100

101 GTGCGTTCGGCACGGTGTATAAG 123 SEQ ID NO: 67
  |||||
101 GTGCGTTCGGCACGGTGTATAAG 123 SEQ ID NO: 68
```

Exon18.pep x Q701H.pep

```
1 LVEPLTPSGEAPNQALLRILKETEFKKIKVLGSGAFGTVYK 41 SEQ ID NO: 69
  |||||
1 LVEPLTPSGEAPNHALLRILKETEFKKIKVLGSGAFGTVYK 41 SEQ ID NO: 70
```

FIGURE 5D

Exon18.txt x L688P.txt

```
      .      .      .      .
1 CTTGTGGAGCCTCTTACACCCAGTGGAGAAGCTCCCAACCAAGCTCTCTT 50
  | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1 CCTGTGGAGCCTCTTACACCCAGTGGAGAAGCTCCCAACCAAGCTCTCTT 50

      .      .      .      .
51 GAGGATCTTGAAGGAACTGAATTCAAAAAGATCAAAGTGCTGGGCTCCG 100
  | | | | | | | | | | | | | | | | | | | | | | | | | | | |
51 GAGGATCTTGAAGGAACTGAATTCAAAAAGATCAAAGTGCTGGGCTCCG 100

      .      .
101 GTGCGTTCGGCACGGTGTATAAG 123 SEQ ID NO: 71
    | | | | | | | | | | | | | | | |
101 GTGCGTTCGGCACGGTGTATAAG 123 SEQ ID NO: 72
```

Exon18.pep x L688P.pep

```
      .      .      .      .
1 LVEPLTPSGEAPNQALLRILKETEFKKIKVLGSGAFGTVYK 41 SEQ ID NO: 73
  | | | | | | | | | | | | | | | | | | | | | | | | | | | |
1 PVEPLTPSGEAPNQALLRILKETEFKKIKVLGSGAFGTVYK 41 SEQ ID NO: 74
```

FIGURE 5E

PI3K-Exon9.txt x E545A.txt

```
      .           .           .           .
1  GTAACAGACTAGCTAGAGACAATGAATTAAGGGAAAATGACAAAGAACAG 50
   |||
1  GTAACAGACTAGCTAGAGACAATGAATTAAGGGAAAATGACAAAGAACAG 50

      .           .           .           .
51 CTCAAAGCAATTTCTACACGAGATCCTCTCTCTGAAATCACTGAGCAGGA 100
   |||
51 CTCAAAGCAATTTCTACACGAGATCCTCTCTCTGAAATCACTGCGCAGGA 100

      .           .
101 GAAAGATTTTCTATGGAGTCACAG.... 124 SEQ ID NO: 75
   |||
101 GAAAGATTTTCTATGGAC.CACAGGTAA 127 SEQ ID NO: 76
```

PI3K-Exon9.pep x E545A.pep

```
      .           .           .           .
1  NRLARDNELRENDKEQLKAISTRDPLSEITEQEKDFLWSH. 40 SEQ ID NO: 77
   |||
1  NRLARDNELRENDKEQLKAISTRDPLSEITAQEKDFLWTTG 41 SEQ ID NO: 78
```

FIGURE 5F

PI3K-Exon20.txt x H1047L.txt

```
1 GTTTCAGGAGATGTGTTACAAGGCTTATCTAGCTATTCGACAGCATGCCA 50
  |||
1 GTTTCAGGAGATGTGTTACAAGGCTTATCTAGCTATTCGACAGCATGCCA 50

51 ATCTCTTCATAAATCTTTTCTCAATGATGCTTGGCTCTGGAATGCCAGAA 100
  |||
51 ATCTCTTCATAAATCTTTTCTCAATGATGCTTGGCTCTGGAATGCCAGAA 100

101 CTACAATCTTTTGATGACATTGCATACATTCGAAAGACCCTAGCCTTAGA 150
  |||
101 CTACAATCTTTTGATGACATTGCATACATTCGAAAGACCCTAGCCTTAGA 150

151 TAAAACTGAGCAAGAGGCTTTGGAGTATTTTCATGAAACAAATGAATGATG 200
  |||
151 TAAAACTGAGCAAGAGGCTTTGGAGTATTTTCATGAAACAAATGAATGATG 200

201 CACATCATGGTGGCTGGACAACAAAATGGATTGGATCTTCCACACAATT 250
  |||
201 CACTTCATGGTGGCTGGACAACAAAATGGATTGGATCTTCCACACAATT 250

251 AAACAGCATGCATTGAACTGA 271 SEQ ID NO: 79
  |||
251 AAACAGCATGCATTGAACTGA 271 SEQ ID NO: 80
```

PI3K-Exon20.pep x H1047L.pep

```
1 FQEMCYKAYLAIRQHANLFINLFSMMLGSGMPELQSFDDIAYIRKTLALD 50
  |||
1 FQEMCYKAYLAIRQHANLFINLFSMMLGSGMPELQSFDDIAYIRKTLALD 50

51 KTEQEALEYFMKQMNDAAHGGWTTKMDWIFHTIKQHALN* 90 SEQ ID NO: 81
  |||
51 KTEQEALEYFMKQMNDALHGGWTTKMDWIFHTIKQHALN* 90 SEQ ID NO: 82
```

FIGURE 6A

(SEQ ID NO: 55) Wild-type EGFR cDNA

ATGCGACCTCCGGGACGGCCGGGCGAGCGCTCCTGGCGCTGCTGGCGCTCTGCCGGCGAGTCGGGCTCTGGAGGAAAGAAAGTTTGCCCAAGGCA 100
 CGAGTAACAAGCTCAGCGAGTTGGGCACTTTTGAAGATCATTTTTCAGCCTCCAGAGGATGTTCAATAACTGTGAGTGGTCTTGGGAATTTGGAAAT 200
 TACCTATGTGCAGAGGAATTAATGATCTTTTCTTTAAAGACCATCCAGGAGTGGCTGGTTATGTCTCATTTGCCCTCAACACACAGTGGAGCGAATTCCT 300
 TTGGAAACACTGCAGATCATCAGAGGAATAATGTACTACGAAATTCCTATGCTTACGAGCTTATCTAATATGATGCAAAATAAACCAGGACTGAAGG 400
 AGCTGCCCATGAGAAATTTACAGGAATCCTTGCAATGGCGCGTTCAGCAACACCTGGCAGCTGCCAAAAGTGTGATCCAAAGTGGAGCTGTGG 500
 AGTCAGCAGTGACTTTCTCAGCAACATGTCTGATGGACTTCCAGAAACACCTGGGCGCTGCCGAGTGTCCGGCGCTGCCAGTCCCAAGTCCCAAGTGTGCCACA 600
 GGTGCAGGAGAGGAACTGCCAGAAACTGACCAAAATCATCTGTGCCAGAGTGTCCGGCGCTGCCGAGTCCCAAGTCCCAAGTGTGCCACA 700
 ACCAGTGTGTGAGGCTGCACAGGCCCCCGGAGAGGAGTGTCCCTGGTCTGCCGCAAAATTCGAGACGAAGCCACGTGCAAGTCCCAAGTGTGCCACA 800
 CATGCTCTACAAACCCACACGTACAGATGGATGTGAACCCGAGGGCAATAACAGCTTTTGGTGCACCTGGTGAAGAGTGTCCCGTAAATATATGTG 900
 GTGACAGATCACGGCTCGTGTCCGAGCCTGTGGGCGGACAGCTATGAGATGGAGGAAGACGGCGTCCGAAAGTGAAGTGTCCCGTAAATATATGTG 1000
 GCAAGTGTGTACGGAATAGGTAATTTGGTGAATTTAAGACTCACCTCTCATAAATGCTACGAATATTAACACCTTCAAAACCTGCACCTCCATCAGTGG 1100
 CGATCTCCACATCTCTGCCGTGGCATTTAGGGTGACTCTTTCACACATACCTCTCCCTGTGATCCACAGGAACCTGGATATTTGAAACCTGTAAAGGAA 1200
 ATCACAGGGTTTGTGCTGATTCAGGCTTGGCTGAAACAGGACGGACCTCCATGCTTTGAGAACTTAGAAATCATACGGCGCAGGACCAAGCAACATG 1300
 GTCAGTTTCTCTTGCAGTCTCAGCCTGAACATACCTTGGGATACGCTCCCTCAAGGAGATAAGTGTGAGATGTGATAATTTTCAGGAACAA 1400
 AAATTTGTGCTATGCAATAACATAAACTGAAATAAACTGTTTGGACCTCCGGTCAGAAACCAAAATTAAGCAACAGAGGTGAACACAGCTGCAAG 1500
 GCCACAGGCCAGTCTGCCATGCTTGTGCTCCCGGAGGGCTGTCTGGGCGCCGAGGCCAGGGACTGCGTCTCTTGGCGGAATGTACGCCGAGGCAAGG 1600
 AATGCGTGGACAAGTGCAGCTTCTGAGAGGTGAGCCAAAGGAGTTTGTGGAGAACTCTGAGTGCATACAGTGCACCCAGAGTGCCTGCTCAGGCCAT 1700
 GAAACATACCTGCACAGGACGGGACCAAGACAACTGTATCCAGTGTGCCCACTACATTTGACGGCCCCCACTGCGTCAAGACCTGCCCGGAGGAGTCA 1800
 GGAGAAACAACACCTGCTGTGGAAGTACGACACCGCGCCATGTGTGCCACCTGTCACCTCAAACTGCGATGCACTAGGATGCACTGGGCCAGGCTCTT 1900
 AAGGCTGTCCAAACGAATGGGCTAAGATCCCGTCCATCGCCACTGGGCGCTCTCTTGTCTGTGTGGTGGCCCTTGGGATCGGCCCTCTT 2000
 CATGCGAAGGCCCAATCGTTCGGAAAGCGCACGCTGCGGAGGCTGTGAGGAGGAGCTTGTGGAGCTCTTACACCCAGTGGAGAGCTGCCCAAC 2100
 CAAGCTCTCTTGAAGGATCTTGAAGAACTGAATTCAAAGATCAAGTCTGGGCTCCGGTGCCTTGGCACGGTGTATAGGACTCTGGATCCCA 2200
 AAGGTGAGAAAGTTAAATTCCTCGTCTGCTATCAAGGAATTAAGAGAAAGCAACATCTCCGAAAGCCAAAGAAATCTCGATGAAGCTACCTGATGGC 2300
 CAGCGTGGACAAACCCACGTGTGCCCTGTGCGCATCTGCCCTCACCTCCAGTCACTCATCAGCAGCTCATGCTTCCGCTGCTCCCTGGAC 2400
 TATGTCCGGGAACACAAAGACAATATTGGCTCCAGTACCTGCTCAACTGGTGTGTCAGATCGCAAAAGGCGATGAACCTTGGAGGACCGTCCGCTGG 2500
 TGCACCGGACCTGGCAGCCAGGAACGTACTGGTGAAACACCGCAGCATGTCAAGATCACAGATTTTGGGCTGGCCAAAATGCTGGTGGTGGGAAGAA 2600
 AGAATACCATGCAGAGGCAAGTGCCCTATCAAGTGGATGGCATTTGGAATCAATTTTACACAGAAATCTATACCCACAGAGTGTCTGGAGCTAC 2700
 GGGTGACCCGTTTGGGAGTTGATGACCTTTGGATCCAAAGCATATGACGGAAATCCCTGCCAGCGAGATCTCTCCATCTCTGGAGAAAGGAAACGCTCC 2800
 CTCAGCCACCCATATGTACCATCGATGTCTACATGATCATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGAT 2900
 ATTCTCCAAATGGCCCGAGACCCCGAGCTACCTTGTCTATTCAGGGGATGAAAGAAATGCAATTTGCCAAAGTCTACAGACTCCAACCTTCTACCGTGCC 3000
 CTGATGGATGAAGAGACATGGACGACGTGGTGGATGCCAGAGTACCTCATCCACAGAGGCTTCTTCAGCAGCCCTCCACGTCACGGACTCCCC 3100
 TCCTGAGCTCTCTGAGTGCACACAGCAACAATTCACCCGTGGCTTGCATTTGATAGAAATGGGCTGCAAGCTGTCCCATCAAGGAAGACAGCTTCTTGCA 3200
 GCGATACAGCTCAGACCCACAGGCGCTTGTGAGTGGACAGCATAGACGACACCTTCTCTCCAGTGCCTGAATACATAAACCAAGTCCGTTCCCAAAGG 3300
 CCCGCTGGCTCTGTGCAGAAATCTCTGTCTATCACAAATCAGCCTCTGAACCCCGCAGCAGAGACCCACACTACAGGACCCCAAGGACCTGCAGTGG 3400
 GCAACCCCGAGTATCTCAACATGTCTCAGCCACTGTGTCAACAGCAATTCGACAGCCCTGCCCCACTGGGCCCCAGAAAGGACGACCAAAATAGCCT 3500
 GGACAAACCTGACTACAGCAGGACTCTTTTCCCAAGGAAGCCAAATGGCATCTTTAAGGGCTCCACAGCTGAAATAATGCAGAAATACCTAAGGGTC 3600
 GCGCCACAAAGCAGTGAATTTATTTGGAGCATGA 3633

FIGURE 6B

(SEQ ID NO: 1) Wild-type EGFR amino acid

MRPSGTAGAA	LLALLAALCP	ASRALEKKV	CQGTSNKLTQ	LGTFEDHFLS	50
LQRMFNCEV	VLGNLEITYV	QRYVDLSFLK	TIQEVAGYVL	IALNTVERIP	100
LENLQIIRGN	MYEENSAYALA	VLSDYDANKT	GLKELPMRNL	QEILHGAVRF	150
SNNPALCNVE	SIQWRDIVSS	DFLSNMSMDF	QNHLSGCQKC	DPSCPNGSCW	200
GAGEENCQKL	TKIICAQQCS	GRCRGKSPSD	CCHNQCAAGC	TGPRESDECLV	250
CRKFRDEATC	KDTCPPMLLY	NPTYQMDVN	PEGKYSFGAT	CVKKCPRNVV	300
VTDHGSCVRA	CGADSYEMEE	DGVRKCKKCE	GPCRKVCNGI	GIGEFKDSLS	350
INATNIKHFK	NCTSIISGDLH	ILPVAFRGDS	FTHTPPLDPQ	ELDLIKTVKE	400
ITGFLLIQAW	PENRTDLHAF	ENLEIIRGRT	KOHGQFSLAV	VSLNITSLGL	450
RSLKEISDGD	VIIISGNKNLC	YANTINWKKL	FGTSGQTKI	ISNRGENSCK	500
ATGQVCHALC	SPEGCWGPEP	RDCVSCRNV	RGRECVDKCK	LLEGEPRFV	550
ENSECIQCHP	ECLPQAMNIT	CTGRGPDNCI	QCAHYIDGPH	CVKTCPAGVM	600
GENNTLVWKY	ADAGHVCHLC	HPNCTYGCTG	PGLGCGPTNG	PKIPSIATGM	650
VGALLLLLVV	ALGIGLFMRR	RHIVRKRTL	RLQERELVE	PLTPSGEAPN	700
QALLRILKET	EFKKIKVLGS	GAFGTVYKGL	WIPEGEKVKI	PVAIKELREA	750
TSPKANKEIL	DEAYVMASVD	NPHVCRLLGI	CLTSTVQLIT	QIMPFGCLLD	800
YVREHKDNIG	SQYLLNWCVQ	IAKGMNYLE	RRLVHRDLAA	RNVLVKTPQH	850
VKITDFGLAK	LLGAEKEYH	AEGKVPIKW	MALESILHRI	YTHQSDVWSY	900
GVTVWELMTF	GSKPYDGIPA	SEISSILEKG	ERLPQPPICT	IDVYMIMVKC	950
WMIDADSRPK	FRELIIIEFSK	MARDPQRYLV	IQGDERMHL	SPTDSNFYRA	1000
LMDEEDMDV	VDADYLLIPQ	QGFSSPSTS	RTPLLSLSA	TSNNSTVACI	1050
DRNGLQSCPI	KEDSFLQYS	SDPTGALTED	SIDDTFLPVP	EYINQSVPKR	1100
PAGSVQNPVY	HNQPLNPAPS	RDPHYQDPHS	TAVGNPEYLN	TVQPTCVNST	1150
FDSPAHAQK	GSHQISLDNP	DYQQDFFPKE	AKPNGIFKGS	TAENAEYLRV	1200
APQSSEFIGA					1210

FIGURE 6C

(SEQ ID NO: 41) EGFR (L688P) cDNA

ATGCGACCCCTCCGGGACGGCCGGGCGCAGCGCTCCTGGCGCTGCGCTCTGCCCGGCGAGTCGGGCTCTGGAGGAAAAAGATTGCGCAAGGCA 100
 CGAGTAACAAGCTCAGCAGTTGGGCACTTTTGAAGATCATTTTCTCAGCCTCCAGAGGATGTTCAATAACTGTGAGGTGCTCTTGGAAATTTGGAAT 200
 TACCTATGTGCAGAGGAATTATGATCTTTCTTTAAAGACCATCCAGAGGTGGTGGTTATGTCTCTCATTTGCCCTCAACACACAGTGGAGCGGAATTCCT 300
 TTGGAAAAACCTGCAGATCATCAGAGGAAATATGTACTACGAAATTCCTATGCTTAGCAGTCTTATCTAATGATGCAAAATAAAACCGGACTGAAGG 400
 AGCTGCCCATGAGAAATTTACAGGAAATCCTGCAATGGCGCCGTGCGGTTTCAGCAACCAACCCCTGCCCTGTCAACGTGGAGAGCATCCAGTGGCGGGACAT 500
 AGTCAGCAGTGACTTTCTCAGCAACATGTCGATGGACTTCCAGAACCACTGGGCACTGCCAAAGTGTGATCCAAGCTGTCCCAATGGGAGCTGCTGG 600
 GGTGCAGGAGAGGAACTGCCAGAAACTGACCAAAATCATCTGTGCCAGCATGTCTCCGGCGCTGCCGTGGCAAGTCCCAAGTGTGCAAGTGTGCTGCCACA 700
 ACCAGTGTGCTGCAGGCTGCACAGGCCCGCGGAGAGCGACTGCCCTGGTCTGCCGCAATTCGAGACGAAAGCCACGTGCAAGGACACCTTGCCCCCACT 800
 CATGCTCTACAACCCACACGTACCAAGATGGATGTGAACCCGAGGGCAATACAGCTTTGGTGCCACCTGCGTGAAGAAAGTGTCCCGTAATATATGTG 900
 GTGACAGATCACGGCTCGTGCCTCCGAGCCTGTGGGCGCGACAGCTATGAGATGGAGAACGCGCTCCGCAAGTGTAAAGAAAGTGCAGAGGCGCTTGCC 1000
 GCAAGTGTGTAAACGGAATAGGTATTTGGTGAATTTAAAGACTCAGTCTCCATAAATGCTACGAATATTAAACACTTCAAAAACGTGCACCTCCATCAGTGG 1100
 CGATCTCCACATCTTCCCGGTGGCATTTAGGGGTGACTCTTTCACACATACCTCCCTCTGTGATCCACAGGAACCTGGATAATTCTGAAAACCGTAAAGGAA 1200
 ATCACAGGGTTTCTGCTGATTCAGGCTTGGCCTGAAACACAGGACGACCTCCATGCTTGTGAGAACCTAGAAAATCATACGCGGAGGACCAAGCAACATG 1300
 GTCAGTTTCTCTTGTGAGTCTGAGCTGAGCTCCCTCAAGGAGATAAGTGTGAGATGTGATAAATTTTCAGGAAACAA 1400
 AAATTTGTGCTATGCAAAATACAAATAAACTGGAAACCTCCGGTCAGAAAAACCAAAATTAAGCAACAGAGGTGAAAAACAGCTGCAAG 1500
 GCCACAGGCCAGGTCTGCCATGCCCTTGTGCTCCCGAGGGCTGCTGGGGCCCGAGGCCAGGGACTGCGTCTCTTGGCCGGAATGTCAGCCGAGGCAAGG 1600
 AATGCGTGGACAAAGTGCAGCTTCTGGAGGGTGAAGCAAGGGAGTTTGTGGAGAACTCTGAGTGCATACAGTGCCACCCAGAGTGCTTCCCTCAGGCCAT 1700
 GAACATCACCTGCACAGGACGGGACCAAGACAACTGTATCCAGTGTGCCACTACATTTGACGGCCCCCACTGCGTCAAGACCTGCCCCGAGGAGTCAATG 1800
 GGAGAAACAAACCTGTGCTGGAAGTACGCAGACGCCGGCCATGTGTGCCACTCCAAACTGCACTACGGATGCACCTGGGCAAGTGGCTCTTT 1900
 AAGGCTGTCCAAACGAATGGCCCTAAGATCCCGTCCATCGCCACTGGGATGGTGGGGCCCTCCTCTTTGCTGTGTGTGGCTTGGGATCGGCCCTCTT 2000
 CATGCGAAGCGCCACATCGTTTCGGAAGCGCACGCTGCGGAGGCTGCTGAGAGAGGAGCCTGTGGAGCCTTTACACCCAGTGGAGAGAGTCCCAAC 2100
 CAAGCTCTCTTGAGGATCTTGAAGGAAACTGAATTCAAAAAGATCAAGTGTGGCTCCGGTTCGGCACGGTGTATAAGGACTCTGGATCCAG 2200
 AAGTGTAGAAAGTTAAATTCCTCCGTCCCTATCAAGGAATTAAGAGAACCAACATCTCCGAAAGCCAAAGAAATCTCTGATGAAGCCTACGTGATGGC 2300
 CAGCGTGGACAAACCCACAGTGTGCCCTGTGGGCTATCTGCCCTCACCTCCAGTCACTCATCAGCAGCTCATGCCCTTCGGCTGCCCTCCCTGGAC 2400
 TATGTCCGGGAACACAAAGACAAATATTGGCTCCAGTACCTGCTCACTGGTGTGCAGATCGCAAGGGCATGAACCTACTTGGAGGACCGTCCCTGG 2500
 TGCACCGCGACCTGGCAGCCAGGAACGTACTGGTGAACACACCGCAGCATGTCAAGATCACAGATTTTGGGCTGGCCAAACTGTGCTGGTGGGAGAGAA 2600
 AGAATACCATGCAGAGGAGCAAAAGTGCCTATCAAGTGGATGGCATTTTACACAGAAATCTATACCCACAGAGTGAATGTCTGGAGCTAC 2700
 GGGGTGACCCGTTTGGGAGTTGATGACCTTTGGATCCAAAGCCATATGACGGAATCCCTGCCAGCGAGATCTCTCCATCTGGAGAAAAGGAAACGCCCTCC 2800
 CTCAGCCACCCATATGTACCATCGATGTCTACATGATCATGGTCAAGTGTGATGATAGACGAGATAGTCCCAAGTTCCTGAGTTCAGTTCATCGA 2900
 ATTCTCCAAAATGGCCCGAGACCCCGAGCTACCTTGTCAATTCAGGGGATGAAAGAAATGCAATTTGCCAAGTCTTACAGACTCCAATTTCTACCGTGCC 3000
 CTGATGGATGAAGAACATGGACGACGCTGTGGATGCCGACGATACCTCATCCACAGCGGCTTCTTACAGAGCCCTCCACGTCAAGGACTCCCC 3100
 TCCCTGAGCTCTGTGAGTGCAACCAAGCAAAATTCACCGTGGCTTGATAGAAATGGGCTGCAAAAGCTGTCCCATCAAGGAAGACAGCTTCTTGCA 3200
 GCGATACAGCTCAGACCCCAAGCGGCCCTTGACTGAGGACAGCATAGACGACACCTTCTCCAGTGCCTGAATACATAAACCAAGTCCGTTCCTCCAAAAGG 3300
 CCGCTGGCTCTGTGCAGAACTCTGTCTATACATCAGCTCTGAACCCCGCGCCAGCAGAGACCCACATACAGGACCCCAAGCACTGCAGTGG 3400
 GCAACCCCGAGTATCTCAACACTGTCCAGCCCACTGTGTCAACAGCACATTCGACAGCCCTGCCCACTGGGCCCCAGAAAGCAGCCCAATTAGCCT 3500
 GGACAACCCCTGACTACCAAGCAGGACTTCTTTTCCCAAGGAAAGCCAAATGGCATCTTTAAGGGCTCCACAGCTGAAAAATGCAGAAATACCTAAGGTC 3600
 GCGCCACAAAGCAGTGAAATTTATTGGAGCATGA 3633

FIGURE 6D

(SEQ ID NO: 2) EGFR(L688P) amino acid

MRPSGTAGAA	LLALLAALCP	ASRALEKKV	CQGTSNKLTQ	LGTFEDHFLS	50
LQRMFNACEV	VLGNLEITYV	QRNYDLSFLK	TIOEVAGYVL	IALNTVERIP	100
LENLQIIRGN	MYYENSYALA	VLSDNYDANKT	GLKELPMRNL	QEILHGAVRF	150
SNNPALCNVE	SIQWRDIVSS	DFLSNMSMDF	QNHIGSCQKC	DPSCPNGSCW	200
GAGEENCQKL	TKIICAQQCS	GRCRGKSPSD	CCHNQCAAGC	TGPRESDCIV	250
CRKFRDEATC	KDTCPPMLY	NPTTYQMDVN	PEGKYSFGAT	CVKKCPRNVV	300
VTDHGSCVRA	CGADSYEMEE	DGVRKCKKCE	GPCRKVCNGI	GIGEFKDSLS	350
INATNIKHFK	NCTSIISDLH	ILPVAFRGDS	FTHTPPLDPQ	ELDILKTVKE	400
ITGFLLIQAW	PENRTDLHAF	ENLEIIRGRT	KQHGQFSLAV	VSLNITSIGL	450
RSLKEISDGD	VIIISGNKNLC	YANTINWKKL	FGTSGQTKI	ISNRGENSCK	500
ATGQVCHALC	SPEGCWGPEP	RDCVSCRNVS	RGRECVDKCK	LLEGEPRFV	550
ENSECICQHP	ECLPQAMNIT	CTGRGPDNCI	QCAHYIDGPH	CVKTCPAGVM	600
GENNTLVWKY	ADAGHVCHLC	HPNCTYGCTG	PGLEGCPTNG	PKIPSIATGM	650
VGALLLLLVV	ALGIGLFMRR	RHIVRKRTL	RLQEREPVE	PLTPSGEAPN	700
QALLRILKET	EKKIKVLGS	GAFGTVYKGL	WIPEGEVKI	PVAIKELREA	750
TSPKANKEIL	DEAYVMASVD	NPHVCRLLGI	CLTSTVQLIT	QLMPFGCLLD	800
YVREHKDNIG	SQYLLNWCVQ	IAGGMNYLED	RRLVHRDLAA	RNVLVKTPQH	850
VKITDFGLAK	LLGAEEKEYH	AEGGKVPKIK	MALESILHRI	YTHQSDVWSY	900
GVTVWELMTF	GSKPYDGIPA	SEISSILEKG	ERLPQPPICT	IDVYIMIMVKC	950
WMIDADSRPK	FRELIIIEFSK	MARDPQRYLV	IQDERMHLP	SPTDSNFYRA	1000
LMDEEDMDDV	VDADERYLIPQ	QGFSSPSTS	RTPLLSSLSA	TSNNSTVACI	1050
DRNGLQSCPI	KEDSFLQYS	SDPTGALTED	SIDDTFLPVP	EYINQSVPKR	1100
PAGSVQNPVY	HNQPLNPAPS	RDPHYQDPHS	TAVGNPEYLN	TVQPTCVNST	1150
FDSPAHAQK	GSHQISLDNP	DYQQDFFPKE	AKPNGIFKGS	TAENAEYLRV	1200
APQSSEFIGA					1210

FIGURE 6E

(SEQ ID NO: 40) EGFR (Q701H) cDNA

ATGCGACCTCCGGGACGGCCGGGGCAGCGCTCCTGGCGCTGCTGGCTGCGCTCTGCCCGGCGAGTCGGGCTCTGGAGGAAAAGAAAGTTTGCCCAAGGCA 100
 CGAGTAACAAGCTCAGCGAGTTGGGCACATTTTGAAGATCATTTTCTCAGCTCCAGAGGATGTTCAATAACTGTGAGGTGGTCTCTTGGAAATTTGGAAAT 200
 TACCTATGTGCAGAGGAATTATGATCTTTTCCCTTAAAGACCATCCAGGAGTGGTGTATGTCTCATTTGCCCTCAACACACAGTGGAGCGAATTCCT 300
 TTGGAAAACCTGCAGATCATCAGAGGAAATATGTACTACGAAAATTCCTATAGCTTTATAGCAGTCTTAATACTATGATGCAAAATAAAACCGGACTGAAGG 400
 AGCTGCCCATGAGAAAATTTACAGGAAATCCTGCGATGGCGCCGTGCGGTTACGCAACACCTGCCCTGTGCAACGTGGAGAGCATCCAGTGGCGGACAT 500
 AGTCAGCAGTGAATTTCTCAGCAACATGTGCGATGGACTTCCAGAACCATCCAGGAGTGTCCGCGCTGCAAGCTGTGATCCAAAGTGTCCCAATGGGAGCTGCTGG 600
 GGTGAGGAGAGGAACCTGCCAGAACTGACCAAAATCATCTGTGCCAGCATGTCTCCGGCGCTGCCGAGTCCCAAGTGTGATCCCAAGTGTCCCAAGTGTGCTG 700
 ACCAGTGTGCTGAGGCTGCACAGGCCCCCGGAGAGCGACTGCTGTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT 800
 CATGCTCTACAAACCCACACCTACAGATGATGTGAACCCCGAGGCAATACAGCTTTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT 900
 GTGACAGATCACGGCTCGTGGCTCCGAGCTGTGAGCTGTGAGCTGTGAGCTGTGAGCTGTGAGCTGTGAGCTGTGAGCTGTGAGCTGTGAGCTGTGAGCTGTG 1000
 GCAAGTGTGTAACGGAATAGGTAATTTAGGGTGACTCCTTACACATACTCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT 1100
 CGATCTCCACATCTCTGCTGATTTAGGCTTGGCTGAAACACAGGACGGACCTCCATGCTTTCAGAACTAGAAATCATACGCGGAGGACCAAGCAACATG 1200
 ATCACAGGGTTTTTGTGCTGATTTAGGCTTGGCTGAAACACAGGACGGACCTCCATGCTTTCAGAACTAGAAATCATACGCGGAGGACCAAGCAACATG 1300
 GTGAGTTTCTCTTGCAGTCTGCTGAGCTGAAACATAAATAAATGGAATAAATGTTTGGGACCTCCGCTCAGAAAACCAAAATTAAGCAACAGAGGTGAAACAGCTGCAAG 1400
 AAATTTGTGCTATGCAAAATACATAAATAAATGGAATAAATGTTTGGGACCTCCGCTCAGAAAACCAAAATTAAGCAACAGAGGTGAAACAGCTGCAAG 1500
 GCCACAGGCCAGGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT 1600
 AATGCGTGGACAAAGTGCAAGCTTCTGGAGGCTGAGCCAGACAACTGTATCCAGTGTGCCACCTGTGCCATCCAAACTGACCTGAGTGTGAGTGTGAGTGTG 1700
 GAACATCACCTGCACAGGACGGGACCAAGACAACTGTATCCAGTGTGCCACCTGTGCCATCCAAACTGACCTGAGTGTGAGTGTGAGTGTGAGTGTGAGTGTG 1800
 GGAGAAACACACCTGTGCTGGAAGTACGACACCGCGGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT 1900
 AAGGCTGTCCAAACGAAATGGGCTTAAGATCCCGTCCATCGCCACTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT 2000
 CATGCGAAGCGCCACATCGTTGGAAGCGCACGCTGCGGAGGCTGTGCGAGAGGAGCTTGTGGAGCTCTTACACCCAGTGTGCTGCTGCTGCTGCTGCTGCTGCT 2100
 CACGCTCTCTTGAGGATCTTGAAGGAACTGAATTCAAAAGATCAAGTGTGGCTCCGGTGTGGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT 2200
 AAGTGAGAAAGTTAAATTTCCCGTCCGCTATCAAGGAATTAAGAGAGCAACATCTCCGAAAGCCAAACAAGAAATCTCGATGAAGCCCTACGTGATGGC 2300
 CAGCGTGGACAAACCCCAAGTGTGCGGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT 2400
 TATGTCCGGGAACACAAAGACAAATATTTGGCTCCAGTACCTGCTCAACTGGTGTGTCAGATCGCAAAAGGGCATGAACCTACTTGGAGGACCGTCCGCTTGG 2500
 TGCACCGGACCTGGCAGCCAGGAACGTAATGTTGAAGGAACTGAATTCAAAAGATCAAGTGTGGCTCCGGTGTGGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT 2600
 AGAATACCATGCAGAGGAGGCAAGTGCCTATCAAGTGGATGGCATTTGGAATCAATTTTACACAGAAATCTATACCCAGAGTGTGATGTCTGGAGCTAC 2700
 GGGTGACCGTTTGGAGTTGATGACCTTTGGATCCAAAGCCATATGACGGAATCCCTGCCAGCGAGATCTCTCCATCCTGGAGAAAGGAGAACGCCCTCC 2800
 CTCAGCCACCCATATGTACCATCGATGTCTACATGATCAATGGTCAAGTGTGATGATAGACGAGATAGTCCCAAGTTCCTGAGTGTGATCATCCGA 2900
 ATTCTCCAAAATGGCCCGAGACCCCAAGCTTGTCTATTCAGGGGATGAAAGAAATGCAATTTGCCAAAGTCTACAGACTCCAACTTCTACCGTGCC 3000
 CTGATGGATGAAGAGACATGGACGACGTGGTGGATGCCGACGAGTACCTCATCCACAGAGGCTTCTTCAGCAGCCCTCCACGTCCAGGACTCCCC 3100
 TCCGTGAGCTCTGTGAGTGAACCCAGCAACAATTCACCGTGGCTTGCATTTGATAGAAAATGGGCTGCAAGCTGTCCATCAAGGAAAGACAGCTTCTTGCA 3200
 GCGATACAGCTCAGACCCACAGGCGCTTGTGAGGACAGCATAGACGACACCTTCTCCAGTGCCTGAATACATAAACCAGTCCGTCCCAAAAGG 3300
 CCCGTGGCTCTGTGCAGAAATCCTGTCTATCACAAATCAGCCCTGTGAACCCCGCCAGCAGAGACCCACACTACCAGGACCCCCACAGCAGCTGCAGTGG 3400
 GCAACCCCGAGTATCTCAACACTGTCTCAGCCACCTGTGTCAACAGCATTCGACAGCCCTGCGCCACAGAAAAGGACGACCAAAATAGCCT 3500
 GGACAAACCTGACTACAGCAGGACTTCTTTCCCAAGGAAGCCAAATGGCATCTTTAAGGGCTCCACAGCTGAAAATGCAGAAATACCTAAGGGTC 3600
 GCGCCACAAAGCAGTGAAATTTATTGGAGCATGA 3633

FIGURE 6F

(SEQ ID NO: 3)	EGFR(Q701H)	amino acid	
MRPSGTAGAA	LLALLAALCP	ASRALEEKKV	CQGTSNKLTQ
LQRMFNANCEV	VLGNLEITYV	QRNYDLSFLK	TIQEVAGYVL
LENLQIIRGN	MYYENSYALA	VLSNYDANKT	GLKELPMRNL
SNNPALCNVE	SIQWRDIVSS	DFLSNMSMDF	QNHLSGCQKC
GAGEENCQKL	TKIICAQOCS	GRCRGKSPSD	CCHNQCAAGC
CRKFRDEATC	KDTCPPPLMLY	NPTTYQMDVN	PEGKYSFGAT
VTDHGSCVRA	CGADSYEMEE	DGVRKCKKCE	GPCRKVCNGI
INATNIKHFK	NCTSIISGDLH	ILPVAFRGDS	FTHTPPPLDPQ
ITGFLLIQAW	PENRTDLHAF	ENLEIIRGRT	KQHGQFSLAV
RSLSKEISDGD	VIIISGNKNLC	YANTINWKKL	FGTSGQKTKI
ATGQVCHALC	SPEGCWGPEP	RDCVSCRNVS	RGRECVDKCK
ENSECIIQCHP	ECLPQAMNIT	CTGRGPDNCI	QCAHYIDGPH
GENNTLVWKY	ADAGHVCHLC	HPNCTYGCTG	PGLEGCPNG
VGALLLLLVV	ALGIGLFMR	RHIVRKRTL	RLQERELVE
HALLRIILKET	EFKKIKVLGS	GAFGTVYKGL	WIPEGEKVKI
TSPKANKEIL	DEAYVMASVD	NPHVCRLLGI	CLTSTVQLIT
YVREHKDNIG	SQYLLNWCVQ	IAKGMNYLED	RRLVHRDLAA
VKITDFGLAK	LLGAEKEYH	AEGKVPIKW	MALESILHRI
GVTVWELMTF	GSKPYDGIPA	SEISSILEKG	ERLPQPPICT
WMIDADSRPK	FRELIIIEFSK	MARDPQRYLV	IQDERMHLP
LMDEEDMDV	VDADLEYLIPQ	QGFFSSPSTS	RTPLLSSLSA
DRNGLQSCPI	KEDSEFLQYS	SDPTGALTED	SIDDTFLPVP
PAGSVQNPVY	HNQPLNPAPS	RDPHYQDPHS	TAVGNPEYLN
FDSPAHAQK	GSHQISLDNP	DYQQDFFPKE	AKPNGIFKGS
APQSSEFFIGA			

FIGURE 6G

(SEQ ID NO: 42) EGFR 15-bp deletion cDNA

ATGCGACCTCCGGGACGGCCGGGCGCTCTGGCGCTGCTGGCGAGTCGGGCTCTGGAGGAAAGAAAGTTTGCCAAAGCA 100
 CGAGTAACAAGCTCAGCGAGTTGGGCACTTTTGAAGATCATTTTCTCAGCCTCCAGAGGATGTTCAATAACTGTGAGGTGCTCTTGGGAATTTGGAAT 200
 TACCTATGTGCAGAGGAATTATGATCTTTCTCTTAAAGACCATCCAGAGGTGGTGGTTATGTCTCTCATTTGCCCTCAACACACAGTGGAGCGAATTCCT 300
 TTGGAAAACCTGCAGATCATCAGAGGAATATGTACTACGAAATTCCTATGCCCTTAGCAGTCTTATCTAATGATGCAATAATAAACCGGACTGAAGG 400
 AGCTGCCCATGAGAAATTTACAGGAAATCCTGCATGGCGCGTTCAGCAACCAACCTGCGGAGCTGCCAAAAGTGTGATCCAAAGTGTCCCAATGGGAGCTGCTGG 500
 AGTCAGCAGTGACTTTCTCAGCAACATGTCTGATGGACTTCCAGAACTTCAGACTGTCTCCGGGCGTCCGCTGGCAAGTCCCAAGTGTCCCAATGGGAGCTGCTGG 600
 GGTGAGGAGAGGAACTGCCAGAAAACCTGACCAAAATCATCTGTGCCAGAGTCTCCGGGCGTCCGCTGGCAAGTCCCAAGTGTCCCAATGGGAGCTGCTGG 700
 ACCAGTGTGTCAGGCTGCACAGGCCCCCGGAGAGCGACTGCCCTGGTCTGCCGCAAAATTCGAGAGCAAGCCACGTGCAAGGACACCTGCCCCCACT 800
 CATGCTCTACAAACCCACACGTACCATGATGGATGTGAACCCCGAGGGCAATACAGCTTTGGTGCCACCTGCTGAAAGTGTCCCGTAAATATATGTG 900
 GTGACAGATCAGGGTCTGTGCTCCGAGCTTGTGGGCGGACAGCTATGAGATGAGGAAGACGGCGTCCGCAAGTGTAAAGTGTGCAAGGGCCCTTGCC 1000
 GCAAGTGTGAACGGAAATAGGTATTTGGTGAAATTTAAAGACTCACTCTCCATAAATGCTACGAATATTAACACTTCAAAAACCTGCAACCTCCATCAGTGG 1100
 CGATCTCCACATCCTGCCGTGGCATTTAGGGGTGACTCTTCACACATCTCTGATCCACAGGAACCTGGATAATTTCTGAAAACCTGCAACCTCCATCAGTGG 1200
 ATCACAGGGTTTGTGCTGATTCAGGCTTGGCTGAAACAGGACGGACTCTCATGCTTTTGAGAACCTAGAAAATCATACGGCGGAGGACCAAGCAACATG 1300
 GTGAGTTTCTCTTGCTGCTGCTGAGCTGAGCTGAAACATATCCTTGGGATTAACGCTCCCTCAAGGAGATAAGTGTGAGATGTGATAATTTTCAGGAAACAA 1400
 AAATTTGTGCTATGCAATAACAATAAATGGAATAAATGTTTGGGACCTCCGGTCAGAAAACCAAAATATATAAGCAACAGAGGTGAAAACAGCTGCAAG 1500
 GCCACAGGCCAGGTCTGCCATGCCCTTGTGCTCCCGAGGGCTGTGCGGCGCCGAGGACTGCGTCTCTTGGCGGAATGTGAGGTGAAAACAGCTGCAAG 1600
 AATGCGTGGACAAAGTCAAGCTTCTGGAGGTGAGCCAAAGGAGTTTGTGAGAACTCTGAGTGCAATACAGTCCACCCAGAGTGCCTGCTCAGGCCAT 1700
 GAACATCACTGCACAGGACGGGACCAAGCAACTGTATCCAGTGTGCCACTACATATGACGGGCGCCCACTGCTCAAGACCTGCCCGCAGGAGTCAATG 1800
 GGAGAAAACAACCTGGTCTGGAAAGTACGACGCGGCCATGTGTGCCACTGTGCCATCCAAACTGCACCTACGGATGCACCTGGGCGCAGGTCTTG 1900
 AAGGCTGTCCAAACGAATGGGCTAAGATCCCGTCCATCGGCACTGGGAGGCTTGTGGAGCCCTCTTACACCCAGTGGAGAGCTCCCAAC 2000
 CATGCGAAGCGCCACATCGTTGGAAGCGCACGCTGCGGAGGCTGTGCAAGGAGGAGCTTGTGGAGCCCTCTTACACCCAGTGGAGAGCTCCCAAC 2100
 CAAGCTCTCTTGAGGATCTTGAAAGGAACCTGAATTCAAAAGATCAAAAGTGTGGGCTCCGGTCCGTCGCTTGGCAAGGACTCTGGATCCCAG 2200
 AAGGTGAGAAAAGTTAAATTCCTGCTGCTATCAAAACATCTCCGAAAGCCAAACAGGAATCCTCGATGAAGCTACGTGATGGCCAGCTGGACAACCC 2300
 CCACGTGTGCCCTGCTGGGCACTGCTCCCTCACTCCACCGTGAACCTCATACGCACTCATGCGCTTCGGCTGCTCGATGAAGCTACGTGATGGCCAGCTGG 2400
 AAAGACAATATTGGCTCCAGTACCTGCTCAACTGTGTGCAAGGCAATGAACCTACTTGGAGGACCGTCTGCTGGTGGTGGTGGTGGTGGTGGTGGTGG 2500
 CAGCCAGGAAAGTACTGTGTAAGAACACCGCAGCATGTCAAGATCAAGATTTTGGGCTGGCCAAACTGTGCTGGTGGGAGAGAAAGAAATACCATGCAGA 2600
 AGGAGGCAAAAGTGCCTATCAAGTGGATGGCATTTGGAATCAATTTTACACAGAAATCTATACCCACAGAGTGTGCTGGGAGTACGGGTGACCCGTTTGG 2700
 GAGTTGATGACCTTTGGATCCAAAGCCATATGACGGAAATCCCTGCCAGCGAGATCTCTCCATCTGAGAAAGGAGAACGCCCTCCCTCAGCCACCATAT 2800
 GTACCATCGATGTACATGATCATGGTCAAGTGTGATGATAGACGCAATAGTCCCAAGTTCCTGAGTTGATCATCGAATTTCTCAAAATGGC 2900
 CCGAGACCCCGAGCTACCTTGTCTATTACGGGGATGAAAAGATGCAATTTGCCAAGTCTACAGACTCCAATTTCTACCGTCCCTGATGGATGAAGAA 3000
 GACATGGACGACGTGGTGGATGCCGACGAGTACCTCATCCACAGCAGGGCTTTTTCAGCAGCCCTCCACGTCAAGGACTCCCTCCCTGAGCTCTCTGA 3100
 GTGCAACAGCAACAAATCCACCGTGGCTTGCAATTTGATAGAAATGGGCTGCAAAAGCTGTCCCATCAAGGAAGACAGCTTCTTGCAGCGATACAGCTCAGA 3200
 CCCCACAGGCGCTTGAATGAGGACAGCATAGACGACACTTCTCCCTCCAGTCCGTTCCAAAAGGCCCGCTGGCTGGCTCTGTG 3300
 CAGAACTCTGTCTATCAATCAATCAGCTCTGAAACCCCGGCGCCAGCAGAGACCCACATACAGGACCCACAGCACTGCAAGTGGGCAACCCCGAGTATC 3400
 TCAACACTGTCCAGCCCTGTGTCAACAGCACATTCGACAGCCCTGCCACTGGGCGCAGAAAGGAGCCCAAAATTAGCTGGACAACCCCTGACTA 3500
 CCAGCAGGACTTCTTTCCCAAGGAAGCCAAATGGCATCTTTAAGGGCTCCACAGCTGAAAATGCAGAAATACCTAAGGTGCGGCCCAACAAGCAGT 3600
 GAATTTATTGGAGCATGA 3618

FIGURE 6H

(SEQ ID NO: 4) EGFR 15bp deletion amino acid

MRPSGTAGAA	LLALLAALCP	ASRALEEKV	CQGTSNKLTQ	LGTFEDHFLS	50
LQRMFNCEV	VLGNLEITYV	QRNYDLSFLK	TIQEVAGYVL	IALNTVERIP	100
LENLQIIRGN	MYYENSYALA	VLSNYDANKT	GLKELPMRNL	QEILHGAVRF	150
SNNPALCNVE	SIQWRDIVSS	DFLSNMSMDF	QNHLGSCQKC	DPSCPNGSCW	200
GAGEENCQKL	TKIICAQQCS	GRCRGKSPSD	CCHNQCAAGC	TGPRESDCLV	250
CRKFRDEATC	KDTCPPPLMLY	NPTTYQMDVN	PEGKYSFGAT	CVKKCPRNYV	300
VTDHGSCVRA	CGADSYEMEE	DGVRKCKKCE	GPCRKVCNGI	GIGEFKDSLS	350
INATNIKHFK	NCTSIISGDLH	ILPVAFRGDS	FTHTPPLDPQ	ELDILKTVKE	400
ITGFLLIQAW	PENRTDLHAF	ENLEIIRGRT	KQHGQFSLAV	VSLNITSLSGL	450
RSLSKEISDGD	VIIISGNKNLC	YANTINWKKL	FGTSGQTKI	ISNRGENSCK	500
ATGQVCHALC	SPEGCWGPEP	RDCVSCRNV	RGRECVDKCK	LLEGEPRFV	550
ENSECIIQCHP	ECLPQAMNIT	CTGRGPDNCI	QCAHYIDGPH	CVKTCPAGVM	600
GENNTLVWKY	ADAGHVCHLC	HPNCTYGCTG	PGLEGCPYNG	PKIPSIATGM	650
VGALLLLLVV	ALGIGLFMR	RHIVRKRTL	RLQERELVE	PLTPSGEAPN	700
QALLRILKET	EFKKIKVLGS	GAFGTVYKGL	WIPEGEKVKI	PVAIKTSPKA	750
NKEILDEAYV	MASVDNPHVC	RLIGICLTST	VQLITQLMPF	GCLLDYVREH	800
KDNIGSQVLL	NWCVQIAKGM	NYLEDRLVH	RDLAARNVLV	KTPQHVKITD	850
FGLAKLLGAE	EKEYHAEQK	VPIKWMALES	ILHRIYTHQS	DVWSYGVTVW	900
ELMTFGSKPY	DGIPASEISS	ILEKGERLPQ	PPICTIDVYM	IMVKCWMIDA	950
DSRPKFRELI	IEFSKMARDP	QRYLVIQDE	RMHLPSPTDS	NFYRALMDEE	1000
DMDDVVDAD	YLIPOQGFSS	SPSTSRTPLL	SSLSATSNNS	TVACIDRNL	1050
QSCPIKEDSF	LQRYSSDPTG	ALTEDSIDDT	FLPVPEYINQ	SVPKRPAGSV	1100
QNPVYHNQPL	NPAPSRDPHY	QDPHSTAVGN	PEYLNVTQPT	CVNSTFDSPA	1150
HWAQKGSQHI	SLDNPDYQQD	FFPKEAKPNG	IFKGSTAENA	EYLRVAPQSS	1200
EFIGA					1205

(SEQ ID NO:	: 43)	EGFR (K745N)	cDNA

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FIGURE 6J

(SEQ ID NO: 5)	EGFR(K745N)	amino acid	
MRPSGTAGAA	LLALLAALCP	ASRALEEKV	CQGTSNKLTQ
LQRMFNCEV	VLGNLEITYV	QRNYDLSFLK	TIQEVAGYVL
LENLQIIRGN	MYYENSYALA	VLSNYDANKT	GLKELPMRNL
SNNPALCNVE	SIQWRDIVSS	DFLSNMSMDF	QNHLSGCQKC
GAGEENCQKL	TKIICAQQCS	GRCRGKSPSD	CCHNQCAAGC
CRKFRDEATC	KDTCPPPLMLY	NPTYQMDVN	PEGKYSFGAT
VTDHGSCVRA	CGADSYEMEE	DGVRKCKKCE	GPCRKVCNGI
INATNIKHFK	NCTISISGDLH	ILPVAFRGDS	FTHTPPLDPQ
ITGFLLIQAW	PENRTDLHAF	ENLEIIRGRT	KQHGQFSLAV
RSLKEISDGD	VIIISGNKNC	YANTINWKKL	FGTSGQTKI
ATGQVCHALC	SPEGCWGPEP	RDCVSCRNV	RGRECVDKCK
ENSECICQHP	ECLPQAMNIT	CTGRGPDNCI	QCAHYIDGPH
GENNTLVWKY	ADAGHVCHLC	HPNCTYGCTG	PGLEGCPNG
VGALLLLLVV	ALGIGLFMRR	RHIVRKRTL	RLQERELVE
QALLRILKET	EFKKIKVLGS	GAFGTVYKGL	WIPEGEKVKI
TSPKANKEIL	DEAYVMASVD	NPHVCRLLGI	CLTSTVOLIT
YVREHKDNIG	SQYLLNWCVQ	IAKGMNYLED	RRLVHRDLAA
VKITDFGLAK	LLGAEEKEYH	AEGKVPIKW	MALESILHRI
GVTVWELMTF	GSKPYDGIPA	SEISSILEKG	ERLPQPPICT
WMIDADSRPK	FRELIIIEFSK	MARDPQRYLV	IQGDERMHLP
LMDEEDMDDV	VDADLEYLIPQ	QGFFSSPSTS	RTPLLSSL
DRNGLQSCPI	KEDSFLQRY	SDPTGALTED	SIDDTFLPVP
PAGSVQNPVY	HNQPLNPAPS	RDPHYQDPHS	TAVGNPEYLN
FDSPAHWAQK	GSHQISLDNP	DYQQDFFPKE	AKPNGIFKGS
APQSSEFIGA			

50 100 150 200 250 300 350 400 450 500 550 600 650 700 750 800 850 900 950 1000 1050 1100 1150 1200 1210

FIGURE 6K

(SEQ ID NO: 44) EGFR (C781R) cDNA

ATGCGACCTCCGGGACGGCCGGGCGCGCTCTGGCGCTCGCTGCGGCGAGTCGGCTCTGGAGGAAAGAAAGTTTGCCAAAGGCA 100
 CGAGTAACAAGCTCAGCGAGTTGGGCACTTTTGAAGATCAATTTCTCAGCCTCCAGAGGATGTTCAATAACTGTGAGGTGTCCTTGGGAATTTGGAAT 200
 TACCTATGTGCAGAGGAATTATGATCTTTCTCTTAAAGACCATCCAGAGGTGGTGGTTATGTCTCTCAATTGCTTCAACACACAGTGGAGCGAATTCCT 300
 TTGGAAAACCTGCAGATCATCAGAGGAATAATGTACTACGAAATATCCCTTAGCAGTCTTATCTAATATGATGCAATAATAAACCCGGACTGAAG 400
 AGCTGCCATGAGAAATTTACAGGAAATCTTGCAATGGCGCGTGGTTTCAGCAACCAACCTGTGCAACGTGGAGAGCATCCAGTGGCGGACAT 500
 AGTCAGCAGTGACTTTCTCAGCAACATGTCGATGGACTTCCAGAACCACTGGGCGAGTCCCAAAAGTGTGATCCAAAGCTGTCCCAATGGGAGCTGCTGG 600
 GGTGCAGGAGAGGAACCTGCCAGAAACTGACCAAAATCATCTGTGCCAGAGTCTCCGGCGCTGCTCCCAAGTCCCAAGTGTGCAAGTGTGCAAGTGTG 700
 ACCAGTGTGCTGAGGCTGCACAGGCCCGCGGAGAGCGACTGCCCTGGTCTGCCGCAAAATTCGAGAGCAAGCACTGCAAGTGTGCAAGTGTGCAAGTGTG 800
 CATGCTCTACAAACCCACACGTACAGATGGATGGATGTAACCCGAGGCAATATACAGCTTTGGTGCCACCTGCTGTAAGAGTGTGCAAGTGTGCAAGTGTG 900
 GTGACAGATCACGGCTCGTGCTCCGAGCTTGGTGAATTTAAGACTCACTCTCCATAAATGCTACGAATATTAACACTTCAAAAACCTGCAAGTGTGCAAGTGTG 1000
 GCAAGTGTGTAACGGAATAGGTAATTTAGGGTGACTCTCTTACACATACTCTCTCTGATCCAGGAACTGGATAATTTGGAATAATTTGGAATAATTTG 1100
 CGATCTCCACATCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT 1200
 ATCAGAGGTTTCTGCTGATTCAGGCTTGGCTGAAACACAGGACCTCATGCTCTTGGAGAACCTAGAAATCATACGCGCAGGACCAAGCAACATG 1300
 GTCAGTTTCTCTTGCAGTCTGTCAGCTTGAACATACATCTTGGGATACGCTCCCTCAAGGAGATAAGTGTGAGATGTGATATTTTCAAGAAACAA 1400
 AAATTTGTGCTATGCAAAATACAAATAAATGGAATAAATGTTGGGACCTCCGGTCAGAAACCAAAATTTATAAGCAACAGAGGTGAAACACAGCTGCAAG 1500
 GCCACAGGCCAGGCTCTGCCATGCTTGTGCTCCCGGAGGCTGCTGGGCGCCGAGGCCAGGACTGCGTCTTTGCCGGAATGTGAGCCGAGGCGAGG 1600
 AATGCGTGGACAAGTCAAGCTTCTGGAGGCTGAGCCAAAGGAGTTTGTGGAGAACTCTGAGTGCATACAGTGCACCCAGAGTGCCTGCTCAGGCCAT 1700
 GAACATCACTGCACAGGACGGGACACAGACAACTGTATCCAGTGTGCCACTACATTTGACGGCCCACTGCTGCAAGTGTGCAAGTGTGCAAGTGTGCAAG 1800
 GGAGAAACAAACCTGGTCTGGAAAGTACGACAGCCGCGCATGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT 1900
 AAGGCTGTCCAACGAATGGGCTAAGATCCCGTCCATCGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT 2000
 CATGCGAAGGCGCCACATCGTTTCGGAAGCGCACGCTGCGGAGGCTGCTGCAAGGAGGAGCTTGTGGAGCCCTTTACACCCAGTGGAGAACTCCCAAC 2100
 CAAGCTCTTTGAGGATCTTGAAGGAACTGAATTCAGAAAGATCAAAAGATCAAAAGTCTGGGCTCCGGTCCGTAAGCAAGGAAATCTCTGATGAAAG 2200
 AAGGTGAGAAAGTTAAATTTCCCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT 2300
 CAGCGTGGACAAACCCACGCTGTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT 2400
 TATGTCCGGGAACACAAAGACAAATATTTGGCTCCAGTACCTGCTCAACTGTGTGCTCAAGTGTGCTCAAGTGTGCTCAAGTGTGCTCAAGTGTGCTCAAG 2500
 TGCAACCGGACCTGGCAGCCAGGAACGTACTGTGTAAGTGGATGGCATTTGAATCAATTTTACAGAAATCTATACCCAGAGTGTGATGTCTGGAGCTAC 2600
 AGAATACCATGCAAGAGGCAAAAGTGCCTATCAAGTGGATGGCATTTGAATCAATTTTACAGAAATCTATACCCAGAGTGTGATGTCTGGAGCTAC 2700
 GGGGTGACCGTTTGGAGTTGATGACCTTTGGATCCAAAGCCATATGACGGAATCCCTGCCAGCGAGATCTCCCTCCATCTCTGGAGAAAGGAGAACGCTCC 2800
 CTCAGCCACCCATATGTACCATCGATGTCTACATGATCATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGATGAT 2900
 ATTCTCCAAAATGGCCCGAGACCCCGAGCTACCTTGTCAATTCAGGGGATGAAAGATGCAATTTGCCAAGTCTACAGACTCCAACTTCTACCGTGCC 3000
 CTGATGGATGAAGAGACATGGACGACGCTGGTGGATGCCGACGAGTACCTCATCCACAGCAGGGCTTCTTACAGCAGCCCTCCACGTACGGACTCCCC 3100
 TCCCTGAGCTCTCTGAGTGCACACAGCAACAAATTCACCCGCTTGCATTTGATAGAAATGGGCTGCAGAGTGTCTCCCATCAAGGAAGACAGCTTCTTGCA 3200
 GCGATACAGCTCAGACCCCAAGCGGCTTGAATGAGCAGCATAGACGACACTTCTCCAGTGCCTGAATACATAAACAGTCCGTTCCCAAAAGG 3300
 CCCGCTGGCTCTGTGCAGAAATCTGTCTATACAAATCAGCTCTGAACCCCGCGCCAGCAGAGACCCACACTACAGGACCCCAAGAGTGTGAGTGG 3400
 GCAACCCCGAGTATCTCAACACTGTTCAGCCCACTGTGTCAACAGCATAATCGACAGCCCTGCGCCCAAGAAAGGAGCAGCCCAATTAGCCT 3500
 GGACAACCTGACTACAGCAGGACTTCTTTCCCAAGGAAGCCAAATGTCATCTTTAAGGGCTCCACAGCTGAAATATGCAGAAATACCTAAGGCTC 3600
 GCGCCACAAAGCAGTGAATTTATTTGGAGCATGA 3633

FIGURE 6L

(SEQ ID NO: 6)	EGFR(C781R)	amino acid	
MRPSGTAGAA	LLALLAALCP	ASRALEEKKV	CQGTSNKLTQ
LQRMFNCEV	VLGNLEITYV	QRNYDLSFLK	TIQEVAGYVL
LENLQIIRGN	MYYENSYALA	VL SNYDANKT	GLKELPMRNL
SNNPALCNVE	SIQWRDIVSS	DFLSNMSMDF	QNHGSCQKC
GAGEENCQKL	TKIICAQQCS	GRCRGKSPSD	CCHNQCAAGC
CRKFRDEATC	KDTCPPMLLY	NPTTYQMDVN	PEGKYSFGAT
VTDHGSCVRA	CGADSYEMEE	DGVRKCKKCE	GPCRKVCNGI
INATNIKHFK	NCTSIGDLH	ILPVAFRGDS	FTHTPPLDPO
ITGFLLIQAW	PENRTDLHAF	ENLEIIRGRT	KQHGQFSLAV
RSLEISDGD	VIIISGNKNC	YANTINWKKL	FGTSGQKTKI
ATGQVCHALC	SPEGCWGPEP	RDCVSCRNV	RGRECVDKCK
ENSECIQCHP	ECLPQAMNIT	CTGRGPDNCI	QCAHYIDGPH
GENNTLVWKY	ADAGHVCHLC	HPNCTYGCTG	PGLEGCTNG
VGALLLLLVV	ALGIGLFMRR	RHIVRKRTL	RLLOERELVE
QALLRLILKET	EFKKIKVLGS	GAFGTVYKGL	WIPEGEKVKI
TSPKANKEIL	DEAYVMASVD	NPHVCRLLGI	RLTSTVQLIT
YVREHKDNIG	SQYLLNWCVQ	IAKGMNILED	RRLVHRDLAA
VKITDFGLAK	LLGAEEKEYH	AEGKVPIKW	MALESILHRI
GVTVWELMTF	GSKPYDGIPA	SEISSILEKG	ERLPQPPICT
WMIDADSRPK	FRELIIIEFSK	MARDPQRYLV	IQGDERMHLP
LMDEEDMDDV	VDADYLLIPQ	QGFFSSPSTS	RTPLLSLSA
DRNGLQSCPI	KEDSFLQYS	SDPTGALTED	SIDDTFLPVP
PAGSVQNPVY	HNQPLNPAPS	RDPHYQDPHS	TAVGNPEYLN
FDSPAHAQAQ	GSHQISLDNP	DYQQDFFPKE	AKPNGIFKGS
APQSSEFIGA			

(SEQ ID NO: 46) EGFR HIS insertion after amino acid position 771 cDNA

23/50

FIGURE 6N

(SEQ ID NO: 7) EGFR HIS insertion after amino acid position 771 amino acid

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MRPSGTAGAA  L L A L L A A L C P  A S R A L E E K K V  C Q G T S N K L T Q  L G T F E D H F L S  50
L Q R M F N N C E V  V L G N L E I T Y V  Q R N Y D L S F L K  T I Q E V A G Y V L  I A L N T V E R I P  100
L E N L Q I I R G N  M Y Y E N S Y A L A  V L S N Y D A N K T  G L K E L P M R N L  Q E I L H G A V R F  150
S N N P A L C N V E  S I Q W R D I V S S  D F L S N M S M D F  Q N H L G S C Q K C  D P S C P N G S C W  200
G A G E E N C Q K L  T K I I C A Q Q C S  G R C R G K S P S D  C C H N Q C A A G C  T G P R E S D C L V  250
C R K F R D E A T C  K D T C P P L M L Y  N P T Y Q M D V N  P E G K Y S F G A T  C V K K C P R N Y V  300
V T D H G S C V R A  C G A D S Y E M E E  D G V R K C K K C E  G P C R K V C N G I  G I G E F K D S L S  350
I N A T N I K H F K  N C T S I S G D L H  I L P V A F R G D S  F T H T P P L D P Q  E L D I L K T V K E  400
I T G F L L I Q A W  P E N R T D L H A F  E N L E I I R G R T  K Q H G Q F S L A V  V S L N I T S L G L  450
R S L K E I S D G D  V I I S G N K N L C  Y A N T I N W K K L  F G T S G Q K T K I  I S N R G E N S C K  500
A T G Q V C H A L C  S P E G C W G P E P  R D C V S C R N V S  R G R E C V D K C K  L L E G E P R E F V  550
E N S E C I Q C H P  E C L P Q A M N I T  C T G R G P D N C I  Q C A H Y I D G P H  C V K T C P A G V M  600
G E N N T L V W K Y  A D A G H V C H L C  H P N C T Y G C T G  P G L E G C P T N G  P K I P S I A T G M  650
V G A L L L L L V V  A L G I G L F M R R  R H I V R K R T L R  R L L Q E R E L V E  P L T P S G E A P N  700
Q A L L R I L K E T  E F K K I K V L G S  G A F G T V Y K G L  W I P E G E K V K I  P V A I K E L R E A  750
T S P K A N K E I L  D E A Y V M A S V D  N H P H V C R L L G  I C L T S T V Q L I  T Q L M P F G C L L  800
D Y V R E H K D N I  G S Q Y L L N W C V  Q I A K G M N Y L E  D R R L V H R D L A  A R N V L V K T P Q  850
H V K I T D F G L A  K L L G A E E K E Y  H A E G G K V P I K  W M A L E S I L H R  I Y T H Q S D V W S  900
Y G V T V W E L M T  F G S K P Y D G I P  A S E I S S I L E K  G E R L P Q P P I C  T I D V Y M I M V K  950
C W M I D A D S R P  K F R E L I I E F S  K M A R D P Q R Y L  V I Q G D E R M H L  P S P T D S N F Y R  1000
A L M D E E D M D D  V V D A D E Y L I P  Q Q G F F S S P S T  S R T P L L S S L S  A T S N N S T V A C  1050
I D R N G L Q S C P  I K E D S F L Q R Y  S S D P T G A L T E  D S I D D T F L P V  P E Y I N Q S V P K  1100
R P A G S V Q N P V  Y H N Q P L N P A P  S R D P H Y Q D P H  S T A V G N P E Y L  N T V Q P T C V N S  1150
T F D S P A H W A Q  K G S H Q I S L D N  P D Y Q Q D F F P K  E A K P N G I F K G  S T A E N A E Y L R  1200
V A P Q S S E F I G  A
1211

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(SEQ ID NO: 45)	EGFR (T790M)	cDNA
1	ATG	ATG
2	CTG	CTG
3	TGG	TGG
4	CTG	CTG
5	CTG	CTG
6	CTG	CTG
7	CTG	CTG
8	CTG	CTG
9	CTG	CTG
10	CTG	CTG
11	CTG	CTG
12	CTG	CTG
13	CTG	CTG
14	CTG	CTG
15	CTG	CTG
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120	CTG	CTG
121	CTG	CTG
122	CTG	CTG
123	CTG	CTG
124	CTG	CTG
125	CTG	CTG
126	CTG	CTG
127	CTG	CTG
128	CTG	CTG
129	CTG	CTG
130	CTG	

25/50

FIGURE 6P

(SEQ ID NO: 8) EGFR(T790M) amino acid

MRPSGTAGAA	LLALLAALCP	ASRALEEKKV	CQGTSNKLTQ	LGTFEDHFLS	50
LQRMFNCEV	VLGNLEITYV	QRNYDLSFLK	TIQEVAGYVL	IALNTVERIP	100
LENLQIIRGN	MYYENSYALA	VLSDYDANKT	GLKELPMRNL	QEILHGAVRF	150
SNNPALCNVE	SIQWRDIVSS	DFLSNMSMDF	QNHLSGCQKC	DPSCPNGSCW	200
GAGEENCQKL	TKIICAQQCS	GRCRGKSPSD	CCHNQCAAGC	TGPRESDCLV	250
CRKFRDEATC	KDTCPPPLMLY	NPTYQMDVN	PEGKYSFGAT	CVKKCPRNVV	300
VTDHGSCVRA	CGADSYEMEE	DGVRKCKKCE	GPCRKVCNGI	GIGEFKDSLS	350
INATNIKHFK	NCTSIISGDLH	ILPVAFRGDS	FTHTPPLDPQ	ELDILKTVKE	400
ITGFLLLIQAW	PENRTDLHAF	ENLEIIRGRT	KOHGQFSLAV	VSLNITSGL	450
RSIKEISDGD	VIIISGNKNC	YANTINWKKL	FGTSGQKTKI	ISNRGENSCK	500
ATGQVCHALC	SPEGCWGPEP	RDCVSCRNV	RGRECVDKCK	LLEGEPREFV	550
ENSECICQCHP	ECLPQAMNIT	CTGRGPDNCI	QCAHYIDGPH	CVKTCPAGVM	600
GENNTLVWKY	ADAGHVCHLC	HPNCTYGCTG	PGLGCGPTNG	PKIPSIATGM	650
VGALLLLLVV	ALGIGLFMRR	RHIVRKRTL	RLQERELVE	PLTPSGEAPN	700
QALLRILKET	EFKKIKVLGS	GAFGTVYKGL	WIPEGEKVKI	PVAIKELREA	750
TSPKANKKEIL	DEAYVMASVD	NPHVCRLLGI	CLTSTVQLIM	QLMPFGCLLD	800
YVREHKDNIG	SOYLLNWCVQ	IAKGMNYLED	RRLVHRDLAA	RNVLVKTPQH	850
VKITDFGLAK	LLGAEKEYH	AEGKVPIKW	MALESILHRI	YTHQSDVWSY	900
GVTVWELMTF	GSKPYDGIPA	SEISSILEKG	ERLPQPPICT	IDVYMIMVKC	950
WMIDADSRPK	FRELIIEFSK	MARDPQRYLV	IQDERMHLP	SPTDSNFYRA	1000
LMDEEDMDDV	VDADYLLIPQ	QGFSSPSTS	RTPLLSLSA	TSNNSTVACI	1050
DRNGLQSCPI	KEDSFLQRY	SDPTGALTED	SIDDTFLPVP	EYINQSVPKR	1100
PAGSVQNPVY	HNQPLNPAPS	RDPHYQDPHS	TAVGNPEYLN	TVQPTCVNST	1150
FDSPAHAQK	GSHQISLDNP	DYQQDFFPKE	AKPNGIFKGS	TAENAEYLRV	1200
APQSSEFIGA					1210

(SEQ ID NO: 56) EGFR L828stop cDNA

27/50

FIGURE 6R

(SEQ ID NO: 9) EGFR L828stop amino acid

MRPSGTAGAALLALLAALCPASRALEEKKKVCQGTSNKLTQLGTFEDHFLS
 LQRMFNCEVVLGNLEITYVQRNYDLSEFLKTIQEVAGYVLIALNTVERIP
 LENLQIIRGNMYEENSALAVLSNYDANKTGLKELPMRNLQEIILHGAVRF
 SNNPALCNVESIQWRDIVSSDFLSNMSMDFQNHLSGCQKCDPSCPNGSCW
 GAGEENCQKLTKIICAQQC SGRCRGKSPSDCCCHNQCAAGCTGPRESDCLV
 CRKFRDEATCKDTCPPMLYNPTTYQMDVNPBGKYSFGATCVKKCPRNYV
 VTDHGSCVRACGADSYEMEEDGVRKCKCEGPCRKVCNCGIGIGEEFKDSLS
 INATNIKHFKNCTISISGDLHILPVAFRGDSFTHTPPLDPQELDILKTVKE
 ITGFLLIQAWPENRTDLHAFENLEIIRGRTKQHGFSLAVVSLNITSLGL
 RSLKEISDGDVIIISGNKNLCYANTINWKKLFGTSGQTKIISNRGENSCK
 ATQVCHALCSPEGCWGPEPRDCVSCRNVSRGRECVDKCKLLEGEPRFV
 ENSECIQCHPECLPQAMNITCTGRGPDNCIQCAHYIDGPHCVKTCPAGVM
 GENNTLVWKYADAGHVCHLCHPNCTYGTGPGLEGCPNTPGPKIPSIATGM
 VGALLLLLVVALGIGLFMRRRHIVRKRTRLRRLQERELVEPLTPSGEAPN
 QALLRILKETEFKKIKVLGSGAFGTVYKGLWIPEGEKVKIPVAIKELREA
 TSPKANKEILLDEAYVMAVSDNPHVCRLLGICLTSTVQLITQLMPFGCLLD
 YVREHKDNIGSQYLLNWCVCQIAKGMNY

(SEQ ID NO: 47)	EGFR (Q849R)	CDNA
1	ATG	ATG
2	CTG	CTG
3	CTG	CTG
4	CTG	CTG
5	CTG	CTG
6	CTG	CTG
7	CTG	CTG
8	CTG	CTG
9	CTG	CTG
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130	CTG	

29/50

FIGURE 6T

(SEQ ID NO: 10) EGFR(Q849R) amino acid

MRPSGTAGAAALLAALCPASRALEEKKVCQGTSNKLTQLGTFEDHFLS
LQRMFNNCVVLGNLEITYVQRYDLSFLKTIQEVAGYVLLIALNTVERIP
LENLQIIIRGNMYENSYALAVLSNYDANKTGLKELPMRNLQEILHGAVRF
SNNPALCNVESIQWRDIVSSDFLSNMMDFFQNHLSGCKQKCDPSCPNGSCW
GAGEENCQKLTKIIICAQQCSGRCRGKSPSDCCHNQCAAGCTGTPRESDCLV
CRKFRDEATCKDTCPPPLMLYNPTTYQMDVNPEGKYSFGATCVKKCPRNYV
VTDHGSCVRACGADSYEMEEEDGVRKCKCEGPCRKVCNGIGIGEFKDSLS
INATNIKHFKNCTSI SGDLHILPVAFRGDSFTHTPPLDPQELDILKTVKE
ITGFLLIQAWPENRTDLHAFENLEIIIRGTRKQHGQFSLAVVSLNITSGL
RSLKEISDGDVIIISGNKNLCYANTINWKKLFGTSGQTKIIISNRGENSCK
ATGQVCHALCSPEGCGPEPRDCVSCRNVSRGRECVCKLLEGEPRFV
ENSECIIQCHPECLPQAMNITCTGRGPDNCIQCAHYIDGPHCVKTCPAGVM
GENNTLVWKYADAGHVCHLCHPNCTYGTGPGLEGCPTNGPKIPIATGM
VGALLLLLVVALGIGLFMRRRHIVRKRTRLRLLQERELVEPLTPSGEAPN
QALLRIILKETEFKKIKVLGSGAFGTVYKGLWIPEGEKVKIPVAIKELREA
TSPKANKEILDEAYVMASVDNPHVCRLLGICLTSTVQLITQLMPFGCLLD
YVREHKDNIQSQYLLNWCVQIAKGMNYLEDRLVHRDLAARNVLVKTPRH
VKITDFGLAKLLGAEKEYHAEGGKVPKWMALLESILHRIYTHQSDVWSY
GVTWVELMTFGSKPYDGI PASEISSILEKGERLPQPPICTIDVYIMVKC
WMIDADSRPKFRELIIEFSKMARDPQRYLVIQDERMHLPSPDTSNFYRA
LMDEEDMDDVDADEYLIIPQQGFFSSPSTSRTPLLSSLSATSNNSTVACI
DRNGLQSCPIKEDSFLQRYSSDPTGALTEDSIDDTFLPVPEYINQSVPKR
PAGSVQNPVYHNQPLNPAPSRDPHYQDPHSTAVGNPEYLNVTVOPTCVNST
FDSPAHWAAQKGSHQISLDNPDYQQDFFPKEAKPNGIFKGSTAENAEYLRV
APQSSEFIGA

(SEQ ID NO: 61)	EGFR (L858R)	cDNA
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2	CTG	CTG
3	CTG	CTG
4	CTG	CTG
5	CTG	CTG
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124	CTG	CTG
125	CTG	CTG
126	CTG	CTG
127	CTG	CTG
128	CTG	CTG
129	CTG	CTG
130	CTG	

31/50

FIGURE 6V

(SEQ ID NO: 11) EGFR(L858R) amino acid

MRPSGTAGAAALLAALCPASRALEEKKVCQGTSNKLTQLGTFEDHFLS
 LQRMENNCEVVLGNLEITYVQRYNDLSFLKTIQEVAGYVLLIALNTVERIP
 LENLQIIRGNMYENSYALAVLSNYDANKTGLKELPMRNLQELHGAVERF
 SNNPALCNVESIQWRDIVSSDFLSNMSMDFQNHLSGCQKCDPSCPNGSCW
 GAGEENCQKLTKIICAQQCSCRCRGKSPSDCCHNQCAAGCTGPRESDCLV
 CRKFRDEATCKDTCPPMLYNPTTYQMDVNPGEKYSFGATCVKKCPRNYV
 VTDHGSCVRACGADSYEMEEDGVRKCKCEGPCRKVCNGIGIGIEFKDSLS
 INATNIKHFNCTSSISGDLHILPVAFRGDSFTHTPPLDPQELDILKTVE
 ITGFLLIQAWPENRTDLHAFENLEIIRGRTKQHGFSLAVSLNITSLGL
 RSLKEISDGDVIIISGNKNLCYANTINWKKLFGTSGQTKIIISNRGENSCK
 ATGQVCHALCSPEGWCWPEPRDCVSCRNVSRGRECVDKCKLLEGEPRFV
 ENSECIQCHPECLPQAMNITCTGRGPDNCIQCAHYIDGPHCVKTCPAGVM
 GENNTLVWKYADAGHVCHLCHPNCTYGTGPGLEGCPNGPKIPSIATGM
 VGALLLLLVVALGIGLFMRRRHIVRKRTRLRRLQERELVEPLTPSGEAPN
 QALLRILKETEFKKIKVLGSGAFGTVYKGLWIPEGEKVKIPVAIKELREA
 TSPKANKEILDEAYVMAVDNPHVCRLLGICLTSTVQLITQLMPFGCLLD
 YVREHKDNIGSQYLLNWCVQIAKGMNYLEDRRLVHRDLAARNVLVKTPQH
 VKITDFGRAKLLGAEKEYHAEGKVPIKWMALLESILHRIYTHQSDVWSY
 GVTVWELMTFGSKPYDGI PASEISSILEKGERLPQPPICTIDVYMIMVKC
 WMIDADSRPKFRELIIEF SKMARDPQRYLVIQDERMHLPSPDTSNIFYRA
 LMDEEDMDVDVDADEYLIPQQGFFSSPSTSRTPLLSSLATSNNSTVACI
 DRNGLQSCPIKEDSFLQRYSSDPTGALTEDSIDDTFLPVPEYINQSVPKR
 PAGSVQNPVYHNQPLNPAPSRDPHYQDPHSTAVGNPEYLNVTQPTCVNST
 FDSFAHWAQKGSHQISLDNPDYQQDFFPKEAKPNGIFKFGSTAENAEYLRV
 APQSSEFFIGA

(SEQ ID NO: 49)	EGFR (F910L)	cDNA
1	ATG	ATG
2	CTG	CTG
3	CTG	CTG
4	CTG	CTG
5	CTG	CTG
6	CTG	CTG
7	CTG	CTG
8	CTG	CTG
9	CTG	CTG
10	CTG	CTG
11	CTG	CTG
12	CTG	CTG
13	CTG	CTG
14	CTG	CTG
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16	CTG	CTG
17	CTG	CTG
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121	CTG	CTG
122	CTG	CTG
123	CTG	CTG
124	CTG	CTG
125	CTG	CTG
126	CTG	CTG
127	CTG	CTG
128	CTG	CTG
129	CTG	CTG
130	CTG	

33/50

FIGURE 6X

(SEQ ID NO: 12) EGFR(F910L) amino acid

MRPSGTAGAAALLAALCPASRALEEKKVCQGTSNKLTQLGTFEDHFLS
LQRMFNNECVLGNLEITYVQORNYDLSFLKTIQEVAGYVLIALNTVERIP
LENLQIIRGNMYENSYALAVLSNYDANKTGLKELPMRNLQEILHGAVRF
SNNPALCNVESIQWRDIVSSDFLSNMSMDFQNHLSGCQKCDPSCPNGSCW
GAGEENCQKLTKEIICAAQCSGRCRGKSPSDCCHNQCAAGCTGPRESDCLV
CRKFRDEATCKDTCPPMLYNPTTYQMDVNPEGKYSFGATCVKCKCPRNYV
VTDHGSCVRACGADSYEMEEDGVRKCKCEGPCRKVCNGIGIGEEFKDSLS
INATNIKHFKNCTSI SGDLHILPVAFRGDSFTHTPPPLDPQELDILKTVKE
ITGFLLIQAWPENRTDLHAFENLEIIRGRTKQHGQFSLAVVSLNITSGL
RSLKEISDGDVLI SGKNLCYANTINWKKLFGTSGQTKIISNRGENSCK
ATGQVCHALCSPEGCWGPEPRDCVSCRNVSRGRECVDKCKLLEGEPREFV
ENSECIQCHPECLPQAMNITCTGRGPDNCIOCAHYIDGPHCVKTCPAGVM
GENNTLVWKYADAGHVCHLCHPNCTYGTGPGLEGCPTNGPKIPSIATGM
VGALLLLLVVALGIGLFMRRRHIVRKRTRLRLLQERELVEPLTPSGEAPN
QALLRILKETEFKKIKVLGSGAFGTVYKGLWIPEGEKVKIPVAIKELREA
TSPKANKEILDEAYVMA SVDNPHVCRLLGICLTSTVQLITQLMPFGCLLD
YVREHKDNIGSQYLLNWCVQIAKGMNYLEDRRLLVHRDLAARNVLVKTPQH
VKITDFGLAKLLGAEKEYHAEGGKVP IKWMALESILHRIYTHQSDVWSY
GVTVWELMTLGSKPYDGI PASEISSILEKGERLPQPPICTIDVYMIMVKC
WMIDADSRPKFFRELII EFSKMARDPQRYLVIQDERMHLPSPTDSNFYRA
LMDEEDMDDVVDAD EYLIPQQGFSSPSTSRTPLLSSLSATSNSTVACI
DRNGLQSCPIKEDSFLQRYSSDPTGALTEDSIDDTFLPVPEYINQSVPKR
PAGSVQNPVYHNQPLNPAPSRDPHYQDPHSTAVGNPEYLNVTQPTCVNST
FDSPAHWAAQKGSHQISLDNPDYQQDFFPKKAKPNGIFKGSTAENAEYLRV
APQSSEFIGA

FIGURE 6Y

(SEQ ID NO: 48) EGFR (V948A) cDNA

ATGCGACCCCTCCGGGACGGCCGGGGCAGCGCTCCTGGCGCTGCTGGCTCTGCCCCGGCGAGTCGGGCTCTGGAGGAAAAGAAAGTTTGTGCCAAGGCA
 CGAGTAAACAAGCTCACGCAAGTTGGGCACCTTTTGAAGATCATTTTCTCAGCCTCCAGAGGATGTTCAATACTGTAGGTGGTCTCTTGGAAATTTGGAAAT
 TACCTATGTGCAGAGGAATTATGATCTTTTCCCTTCTTAAAGACCATCCAGAGGTGGCTGTTATGTCTTATGCTTATCTAACTATGATGCAAAATAAACCAGGACTGAAGG
 TTGGAAAACCTGCAGATCATCAGAGGAAATATGTACTACGAAAATTCCTATGCTTACAGCTTATCTAACTATGATGCAAAATAAACCAGGACTGAAGG
 AGCTGCCCCATGAGAAAATTTACAGGAAATCCTGTCATGGCGCCGTGCGTTTCAGCAACAACCTGCCCCGTGCAACCTGTGGAGAGCATCCAGTGGCGGACAT
 AGTCAGCAGTGACTTTTCTCAGCAACATGTGCGATGGACTTCCAGAACCACTGGGCGAGTGCCTCCGGCGCTGCAAGTGTGATCCAAAGTGTCCCAATGGGAGCTGCTGG
 GGTGCAGGAGAGGAAACTGCCAGAAACTGACCAAAATCATCTGTGCCAGCAGTGTCTCCGGCGCTGCAAGTGTGATCCCAAGTGTCCCAATGGGAGCTGCTGG
 ACCAGTGTGTCAGGCTGCACAGGCCCCCGGAGAGCGACTGCCGTGTCCTCCGCAATTCAGAGACGAAGCCACGTGCAAGGACACCTGCCCCCCTCAT
 CATGCTCTACAAACCCACCGTACCAGATGGATGTGAACCCCGAGGGCAATACAGCTTTTGGTGGCCACCTGCGTGAAGAAGTGTCCCGTAATTATGTG
 GTGACAGATCACGGCTCTGTGCTCCGAGCTCGAGCTATGAGATGGAGGAAGACGGCTCCGCAAGTGTAAAGAGTGTGCAAGAGTGTGCGAAGGGCTTGGC
 GCAAAGTGTGTAACGGAATAGGTAATTGGTGAATTTAAAGACTCACCTCCATAAATGCTACGAATATTAACACTTCAAAAACCTGCACCTCCATCAGTGG
 CGATCTCCACATCTCGCGGTGGCATTTAGGGGTGACTCCTTCACACATACTCCTCTCGGATCCACAGGAACCTGGATAATTTCTGAAAACCTGTAAAGGAA
 ATCACAGGGTTTGTGCTGATTCAGGCTTGGCCTGAAAACAGGACGGACCTCCATGCCCTTTGAGAACCTAGAAAATCATACGCGGAGGACCAAGCAACATG
 GTCAGTTTCTCTTGTGAGTCTGTCAGCTGAGCTGAACATATCTTTGGGATTAACGCTCCCTCAAGGAGATAAGTGTGAGATGTGATAATTTTCAGGAAACAA
 AAATTTGTGCTATGCAAAATACAAATAAATACTGGAAATACTGTTTGGGACCTCCGGTCAGAAAACCAAAATATATAAGCAACAGAGGTGAAAACAGCTGCAAG
 GCCACAGGCCAGGCTCTGCCATGCTTGTGCTCCCGAGGGCTGCTGGGGCCCGGAGCCAGGGACTGCGTCTCTTTGCCGGAATGTCAAGCCAGGAGGAGG
 AATGCGTGGACAAGTGAAGCTTCTGAGGGGTGAGCCAAAGGAGTTTGTGGAGAACTCTGAGTGCATACAGTGCACCCAGAGTCCCTGCTCAGGCCAT
 GAACATCACCTGCACAGGACGGGACAGACAACTGTATCCAGTGTGCCACTACATTGACGGCCCCACCTGCGTCAAGACCTGCCCCGAGGAGTGCATG
 GGAGAAAACAACCTGGTCTGGAAAGTACGCAGACGCCGGCCATGTGTGCCACTCAAACCTGACCTACGGATGCACCTGGGCCAGTGGCCAGGTCTTTG
 AAGGCTGTCCAAACGAATGGGCCTAAGATCCCGTCCATCGCCACTGGGATGGTGGGCCCTCCTCTTGTGCTGCTGGTGGGCCCTGGGATCGGCCCTCTT
 CATGCCAAAGGCCACATCGTTCCGAAAGCCACGCTGCGGAGGCTGCTGCAGGAGAGGAGCTTGTGGAGCTTGTGGGACCTCCGTCAGAAAGCAAGCTCCCCAAC
 CAAGCTCTCTTGAGGATCTTTGAAAGGAACTGAATTCAAAAGATCAAAAGTGTGGCTCCGGTCCGTTCCGACGCTCGGTGTAAGGGAATCTTGATCCAG
 AAGGTGAGAAAAGTTAAAATTTCCCTCGCTATCAAGGAATTAAGAGAAAGCAACATCTCCGAAAGCCAAAGAAATCTCTGATGAAGGACTCTGGATCCAG
 CAGCGTGGACAAACCCACGCTGTGCCCTGCTGGGCACTGCGCTCACCTCCAGCTCATCACGAGCTCATGCCCCATCGGAGATCCCTGGAGAAAGGAAACGCCTCC
 TATGTCCGGGAACAAAGACAAATATTGGCTCCAGTACCTGCTCAACTGGTGTGTCAGATCGCAAGGCAATGAACCTACTTTGGAGGACCCGTGCTGGTGG
 TGCACCGGACCTGGCAGCCAGGAACGTACTGGTGAATAACACCGCAGCATGTCAAGATCACAGATTTTGGGCTGGCCAAAACCTGCTGGGTGCGGAAAGAGAA
 AGAATACCATGCAGAGGAGGCAAGTGCCTATCAAGTGGATGGCAATTGGAATCAATTTTACACAGATCTATACCCACAGAGTGTGTGGAGCTAC
 GGGTGACCCGTTTGGAGTTGATGACCTTTGGATCCAGCCATATGACGGAATCCCTGCCAGCGAGATCTCTCATCTGGAGAAAGGAAACGCCTCC
 CTCAGCCACCCATATGTACCATCGATGTCTACATGATCATGGCCAAAGTGTGATGATAGACGCGAGATAGTCGCCCAAAGTTCCGTGAGTTGATCATCGA
 ATTCTCCAAAATGGCCCGAGACCCCGAGCTACCTTGTCTATTGAGGGGATGAAAGATGCTATTGCCAAGTCTTACAGACTCCAACTTCTACCGTGGC
 CTGATGGATGAAGAAGACATGGACGACGTGGTGGATGCCGACGAGTACCTCATCCACAGCGGGCTTCTTCAGCAGCCCCCTCCACGTCAAGGACTCCCC
 TCCTGAGCTCTCTGAGTGAACCCAGCAACAATTTCCACCGTGGCTTGCATTGATAGAAAATGGGCTGCAAGCTGTCCCATCAAGGAAGACAGCTTCTTTGCA
 GCATACAGCTCAGACCCACAGCGCCCTTGACTGAGGACAGCATAGACGACACTTCTCCAGTGCCTGAATACATAAACCAAGTCCGTTCCTCCAAAGG
 CCGCTGGCTCTGTGAGAAATCCTGTCTATCACAAATCAGCCTCTGAACCCCGCGCCAGCAGAGACCCACACTACAGGACCCCCACAGCACTGCAGTGG
 GCAACCCCGAGTATCTCAACACTGTCCAGCCACTGTGTCAACAGCACATTCGACAGCCCTGCCACTGGGCCAGAAAAGGACGCCAACCAATTAGCCT
 GGACAACCCCTGACTACAGCAGGACTTCTTTCCCAAGGAAGCCAAATGGCATCTTTTAAGGGCTCCACAGCTGAAAATGCAAGATACTTAAGGGTC
 GCGCCACAAAGCAGTGAATTTATTGGAGCATGA

FIGURE 6Z

(SEQ ID NO: 13) EGFR (V948A) amino acid

MRPSGTAGAAALLAALCPASRALEKKKVCQGTSNKLLTQLGTFEDHFLS
LQRMFNCEVVLGNLEITYVQRYNVDLSFLKTIQEVAGYVLIALNTVERIP
LENLQIIRGNMYEYNSYALAVLSNYDANKTGLKELPMRNLQEIILHGAVRF
SNNPALCNVESIQWRDIVSSDFLSNMMDFFQNLGSCQKCDPSCPNGSCW
GAGEENCQKLTKIICAQQC SGRCRGKSPSDCCHNQCAAGCTGPRESDCLV
CRKFRDEATCKDTCPPMLYNPTTYQMDVNPGEKYSEFGATCVKKCPRNYV
VTDHGSCVRACGADSYEMEEDGVRKCKCEGPCRVKVCNNGIGIGEEFKDSLS
INATNIKHFKNCTSI SGDLHILPVAFRGDSFTHTPPLDPQELDILKTVE
ITGFLLIQAWPENRTDLHAFENLEIIRGR TKHQGFSLAVVSLNITSLGL
RSLKEISDGDVII SGKNLCYANTINWKKLFGTSGQTKIIISNRGENSCK
ATGQVCHALCSPEGCWGPEPRDCVSCRNVSRGECVDKCKLLEGEPRFEV
ENSECIQCHPECLPQAMNITCTGRGPDNCIQCAHYIDGPHCVKTCPAGVM
GENNTLVWKYADAGHVCHLCHPNCTYGTGPGLEGCTNGPKIPSIATGM
VGALLLLLVVALGIGLFMRRRHIVRKRTRLRRLLOERELVEPLTPSGEAPN
QALLRILKETEFKKIKVLGSGAFGTVYKGLWIPEGEKVKIPVAIKELREA
TSPKANKEILDEAYVMAVDNPHVCRLLGICLTSTVQLITQLMPFGCLLD
YVREHKDNIGSQYLLNWCVOIAKGMNyleDRRLVHRDLAARNVLVKTPOH
VKITDFGLAKLLGAEKEYHAEGGKVP IKWMALESILHRIYTHQSDVWSY
GVTVWELMTFGSKPYDGI PASEISSILEKGERLPQPPICTIDVYMIMAKC
WMIDADSRPKFRELIIEFSKMARDPQRYLVIQDERMHLPSPDTSNFYRA
LMDEEDMDDVDADAYLI PQQGFSSPSTSRTPLLSSL SATSNNSTVACI
DRNGLQSCPIKEDSFLQRYSSDPTGALTEDSIDDTFLPVPEYINQSVPKR
PAGSVQNPVYHNQPLNPAPSRDPHYQDPHSTAVGNPEYLN TVQPTCVNST
FDSPA HWAQKGSHQISLDNPDYQQDFFPKAKPNGIFKGSTAENAEYLRV
APQSSEFIGA

FIGURE 7A

(SEQ ID NO: 58) Wild-type PI3K cDNA

ATGCCCTCCAAGACCATCATCAGGTGAACTGTGGGGCATCCACTTGTATGCCCCCAAGAAATCCCTAGTAGAATGTTTACTACCAAAATGGAATGATAGTGACTTT
 TAGAATGCCCTCCGTGAGGCTACATTAATAAACAAGCAATGAACATAAAGCAAGCAAGAAATACCCCTCCATCAACTTCTTCAAGATGAATCTTC
 TTACATTTTTCGTAAGTGTACCCCAAGAGCAGAAAGGAAGAAATTTTGTGATGAACAAGACGACTTTGTGACCTTTCGGCTTTTTCACCCCTTTTFAAAA
 GTAATTGAACCAAGTAGGCAACCGTGAAAGAAAGATCTCAATCGAGAAATTTGGTTTGTCTATCGGCATGCCAGTGTGAATTTGATATGGTTAAAGATC
 CAGAAGTACAGGACTTCCGAAAGAAATATTCTGAACGTTTGTAAAGAAAGCTGTGGATCTTAGGGACCTCAATTCACCTCATAGTAGAGCAATGTATGTCTTA
 TCCTCCAAATGTAGAATCTTCAACCAGAAATTGCCAAAATCAACCATGACTGTGTACCAAGCAAAAGTAATTGCTGAAGCAATCAGGAAAAAACCTCGAAGTATGTTGC
 AATAATGACAAGCAGAAAGTATACCTGTGTTTGTGTTTGTAGAAATATCAGGGCAAGTATATTTTAAAGTGTGTGGATGTGAATACCTTCTAGAAAATAATCTCT
 TATCCTCTGAAACAACATAAATCTCTGTGTTTGTGTTTGTAGAAATATCAGGGCAAGTATATTTTAAAGTGTGTGGATGTGAATACCTTCTAGAAAATAATCTCT
 GAGTCAGTATAAGTATAAAGAAAGCTGTATAATGCTTTGGGAGGATGCCCAATTTGATGTTGATGGCTAAAGAAAGCCCTCTATTTCTCAACTGCCAATGGAC
 TGTTTTACAAATGCCATCTTATTTCCAGACGCATTTCCACAGCTACCATATATGAATGGAGAAACATCTACAAAATCCCTTTTGGGTTATATAAATAGTGCAC
 TCAGAAATAAATAATCTTTTGTGCAACCTACGTGAATGTAAATATTTCAGACATTTGATAAGATCTATGTTCCGAACAGGTATCTACCATGGAGGAAACCCCTT
 ATGTGACAAATGTGAACACTCAAGAGTACCTTTGTTCCAAATCCAGGTGGAATGAATGGCTGAATTAATGATATATACATTTCTGATCTTCTGCTGCTGCT
 CGACTTTGCTTTCATTTGCTCTGTTTAAAGGCCGAAAGGTGCTFAAGAGGAAACACTGTCCCATTTGGCATGGGAAATATAAACTTGTGTTGATTTACACAG
 ACACCTAGTATCTGGAAATAATGGCTTTGAAATCTTTTGGCCAGTACCTCATGGACTAGAAAGATTTGCTGAACCTTATTTGGTGTACTGGATCAAAATCCAAA
 TAAAGAAACTCCATGCTTAGAGTTGGAGTTTGACTGGTTTCAGCAGTGTGGTAAAGTTCCCAAGATATGTCAAGTGAATGAAGAGCATGCCAATTTGGTCTGTA
 TCCCGAGAGCAGGATTTAGCTATTCCACGCGAGGACTGAGTAACAGACTAGCTAGAGACATGAATTAAGGGAATAATGACAAAGAACAGCTCAAGCAA
 TTCTACACGAGATCCCTCTCTCTGAAATCTCACTGAGCAGGAGAAAGATTTTCTATGGATCAAGACACTATTTGTGTAACTATCCCCGAAATTTCTACCCAA
 ATTGCTTCTGTCTGTAAATGGAAATTTCTAGAGATGAAGTAGCCAGATGTATTGCTTGGTAAAGATTTGCCCTCCAAATCAAACTGAACAGGCTATGGAA
 CTCTCGGACTGTAAATTACCCAGATCTTATGGTTTCGAGGTTTGTGCTGTTGGAATACTTTGCTTGTGAGATTTTACTGAAGAAAGCATTTGACTAATCAAGGATTTGGCCTTTT
 AGCTAGTACAGGCTCTAAATAATGAACAAATATTGGATAACTTTGCTTGTGAGATTTTGGCTTGTGGCTGCTTATTTGGAGTCTTATTTGGGATGTATTTGAAG
 CTTTTGGCATTTAAATCTGAGATGCACAAATAAACAAGTTAGCCAGAGGTTTGGCTGCTTAACTTAACTGACATTTCTAAACAGGAGAAAGGATGAAACACAAAAGGTACAGTGAAGT
 CACCTGAATAGGCAAGTCGAGGCAATGGAAAGCTCATTAACCTGACATTTCTAAACAGGAGAAAGGATGAAACACAAAAGGTACAGTGAAGT
 TTTTAGTTGAGCAAAATGAGGCGACAGATTTTCATGGATGCTTACAGGGCTTTCTGTCTCTTAAACCTGCTCATCACTAGCAACTAGGAAACCTCAGGCTTGA
 AGAGTGTGCAATTATGTCTCTGCAAAAAGGCCACTGTGGTTGAAATTTGGGAGAAACCCAGACATCATGTCAAGTTACTGTTTTCAGAACAAATGAGATCATC
 TTTAAATAATGGGGATGATTTACGGCAAGATATGCTAACACTTCAAAATATTCGTATTATGGAAATAATCTGGCAAAATCAAGTCTTTGATCTTCGAATGT
 TACCTTATGGTTGTCTCAATTCGGTGACTGTGTGGACTTATTGAGGTGGTGGAAATTTCTACACATATATGCAAAATTCAGTGAAGTGAAGGCGGCTTGAA
 AGGTGCACATGCAGTTCAACAGCCACACATACATCAGTGGCTCAAGACAAAGAAATATATATGATGCAGCCATTTGACCTGTTTTACACAGTTCA
 TGTGCTGGATACCTGTGTAGCTACCTTCAATTTTGGGAATTTGGAGATCGTCACAAATAGTAACATCATGTTGAAAGACGATTTCTTAATAGTATAGTAAAGG
 TTGGACACTTTTTTGGATCACAAAGAAAGAAATAATTTGGTTATAAACGAGAACGTGTGCCATTTGTTTTCAGACAGGATTTCTTAATAGTATAGTAAAGG
 AGCCCAAGAAATGCACAAAGACAAAGAAATTTGAGAGGTTTTCAGGAGATGTGTACAAAGGCTTATCTAGCTATTCGACAGCATGCCAATCTCTTCAATAAT
 CTTTCTCAATGATGCTTGGCTCTGGAAATGCCAGAACTACAATCTTTTGTGATGACATTTGAAAGACCTTAGCCTTAGATATAAATGAGCAAG
 AGGCTTTTGGAGTATTTTCATGAACAAATGAATGATGCACATCATGGTGGCTGGACAAACAAATGGATTTGGATCTTCCACACAAATTAACACAGCATGCATTT
 GAACTGA

FIGURE 7B

(SEQ ID NO: 14) Wild-type PI3K amino acid

MPPRSSGELWGIHLMPPRIIVECLLPNGMIVTLECLREATLITIKHSELF
KEARKYPLHQLQDESSYIFVSVTQEAEREFEFFDETRRLCDLRLFQPFLLK
VIEPVGNREEKIILNREIGFAIGMPVCEFFDMVKDPEVQDFRRNIIINVCKEA
VDLRDLNSPHSRAMYVYPPNVESSEPELPKHIYNKLDKGQIIIVVIWIVSP
NNDKQKYTLKINHDCVPEQVIAEAIKKKTRSMLLSSSEQLKLCVLEYQGY
ILKVCGCDEYFLEKYPLSQYKYIRSCIMLGRMPNMLMAKESLYSQLPMD
CFTMPSYSRRISTATPYMNGETSTKSLWVINSALRIKILCATYVNVNIRD
IDKIYVRTGIYHGGEPLCDNVNTQRPVPCSNPRWNEWLNVDIYIPDLPRAA
RLCLSIKSVKGRKGAKKEHCPLAWGNINLFDYTDTLVSGKMALNLWVPVPH
GLEDLLNPIGVTGSNPNETPCLELEFDWFSSVVKFPDMSVIEEHANWSV
SREAGFSYSHAGLSNRLARDNELRENDKEQLKAISTRDPLSEITEQEKDF
LWSHRHYCVTIPEILPKLLLSVKWNSRDEVAQMYCLVKDWPPIKPEQAME
LLDCNYPDPMPVRGFAVRCLLEKYLTDKLSQYLIQLVQVLKYEQYLDNLLV
RFLKKKALTNRIGHFFFWHLKSEMHNTVTSQRFGLLLESYCRACGMYLK
HLNRQVEAMEKLIINLTDILKQEKKDETQKVQMKFLVEQMRRPDFFMDALQG
FLSPLNPAHQNLRLLECRIMSSAKRPLWLNWENPDIMSELLFQNNELI
FKNGDDLRRQDMLTLQIIRIMENIWQNQGLDLRLPYGCLSIGDCVGLIEV
VRNSHTIMQIQCKGGLKGALQFNSHTLHQWLKDKNGKEIYDAAIDLFTRS
CAGYCVATFIIIGIGDRHNSNIMVKDDGQLFHIDFGHFLDHKKKKFGYKRE
RVPFVLTQDFLIVISKGAQECTKTREFFERFQEMCYKAYLAIRQHANLFIN
LFSMMLGSGMPELQSFDDIAYIRKTLALDKTEQEALEYFMKQMDAHHGG
WTTKMDWIFHTIKQHALLN

(SEO ID NO: 53)	PI3K (E542K)	cDNA

39/50

FIGURE 7D

(SEQ ID NO: 15) PI3K (E542K) amino acid

MPPRSSGELWGIHLMPPRIILVECLLPNGMIVTLECLREATLTITIKHELF
KEARKYPLHQLQDESSYIFVSVTQEAEREFFDETRRLCDLRLFPFLK
VIEPVGNREEKILNREIGFAIGMPVCEFDVKDPEVQDFRRNINLVCKEA
VDLRDLNSPHSRAMYVYPNVESSELPKHIYNKLDKGQIIIVWIVVSP
NNDKQKYTLKINHDCVPEQVIAEAIKKTRSMLLSSEQKLKCVLEYQCKY
ILKVCGCDEYFLEKYPLSQKYIRSCIMLGRMPNMLMAKESLYSQLPMD
CFTMPSYSRRISTATPYMNGETSTKSLWVINSALRIKILCATYVNVNIRD
IDKIYVRTGIYHGGEPLCDNVNTQVRPCSNPRWNEWLNVDIYIPDLPRAA
RLCISICSVKGRKGAKEEHCPLAWGNINLFDYTDTLVSGKMALNLWPVPH
GLEDLLNPIGVTGSPNPKETPCLELEFDWFSVVVFPDMSVIEEHANWSV
SREAGFSYSHAGLSNRLARDNELRENDKEQLKAISTRDPLSKITEQEKDF
LWSHRHYCVTIPEILPKLLL SVKWN SRDEVAQMYCLVKDWPPIKPEQAME
LLDCNYPDPMPVRGFAVRCLEKYLTDKLSQYLIQLVQLKYEQYLDNLLV
RFLKKALTNQRI GHFFFWHLKSEMHNKTVSQRFGLLLESYCRACGMYLK
HLNRQVEAMEKLIINLTDILKQEKKDETQKVQMKFLVEQMRRPDPMALQG
FLSPLNPAHQLGNLRLIEECRIMSSAKRPLWLNWENPDIMSELLFQNNELI
FKNGDDL RQDMLTLQIRIMENIWQNQGLDLRMLPYGCLSIGDCVGLIEV
VRNSHTIMQIQCKGGLKGALQFNSHTLHQWLKDKNKGEIYDAAIDLFTRS
CAGYCVATFILGIGDRHNSNIMVKDDGQLFHIDFGHFLDHKKKKFGYKRE
RVFFVLTQDFLIVISKGAQECTKTREFFERFQEMCYKAYLAIRQHANLFIN
LFSMMLGSGMPELQSFDDIAYIRKTLALDKTEQEALEYFMKQMNDAHHGG
WTTTKMDWIFHTTIKQHALN

(SEQ ID NO: 50)	PI3K (E545A) cDNA

41/50

FIGURE 7F

(SEQ ID NO: 16) PI3K (E545A) amino acid

MPPRPSSGELWGIHLMPPRIILVECLLPNGMIVTLECLREATLITIKHELF
KEARKYPLHQLLQDESSYIFVSVTQEAEREFEFFDETRRLCDLRLFQPFLLK
VIEPVGNREEKIILNREIGFAIGMPVCEFFDMVKDPEVQDFRRNILLNVCKEA
VDLRDLNSPHSRAMYVYPNVESSEPELPHKIYNKLDKGQIIIVVIWIVSP
NNDKQYTLKINHDCVPEQVIAEAIKKTRSMLLSSEQLKL CVLEYQGY
ILKVCGCDEYFLEKYPLSQYKYIRSCIMLGRMPNMLMAKESLYSQLPMD
CFTMPSSYRRISTATPYMNGETSTKSLWVINSALRIKILCATYVNVNIRD
IDKIYVRTGIYHGGEPLCDNVNTQRPVPCSNPRWNEWLNVDIYIPDLPRAA
RLCLSVKGRKGAKKEHCPLAWGNINLFDYTDTLVSGKMAINLWVPVPH
GLEDLLNPIGVTGSNPNETPCLELEFDWFSVVKFPDMSVIEEHANWSV
SREAGFSYSHAGLSNRLARDNELRENDKEQLKALSTRDPLSEITAEKDF
LWTTGKHCVTIPEILPKLLSVKWSRDEVAQMYCLVKDWPPIKPEQAM
ELLDCNYPDEPMVRGFAVRCLEKYLTDKLSQYLIQLVQVLKYEQYLDNLL
VRFLKKALTNQRI GHFFFWHLKSEMHNKTVSQRFGLLLESYCRACGMYL
KHLNRQVEAMEKLINLTDILKQEKKDETKVQMKFLVEQMRRPDEFMDALQ
GFLSPINPAHQLGNLRLFEECRIMSSAKRPLWLNWENPDIMSELLFQNNEL
IFKNGDDLRLQDMLTLQIIRIMENIWQNQGLDLRLMPYGCLSIGDCVGLIE
VVRNSHTIMQIQCKGGLKQALQFNSHTLHQWLKDKNGEIDYDAIDLFTTR
SCAGYCVATFILGIGDRHNSNIMVKDDGQLFHIDFGHFLDHKKKFGYKR
ERVPFVLTQDFLIVISKGAQECTKTREFFERFQEMCYKAYLAIRQHANLFI
NLFSSMMLGSGMPELQSFDDIAYIRKTLALDKTEQEALEYFMKQMNDAHHG
GWTTKMDWIFHTIKQHAIN

FIGURE 7G

(SEQ ID NO: 54) PI3K (H1047L) cDNA

ATGCCCTCAAAGACCATCATCAGGTGAACCTGTGGGGCATCCACTTGATGCCCCCCAAGAAATCCTAGTAGAATGTTTACTACCAAAATGGAATGATAGTGACTT
 TAGAATGCCCTCCGTGAGGTACATTAATAACCATAAAGCATGAACATAATTAAAGAAAGCAAGAAATACCCCTCCATCAAACTTCTTCAAGATGAATCTTC
 TTACATTTTTCGTAAGTGTACCCAAAGAGCAGAAAGGAAAGAAATTTTGTGATGAAACAAGACGACATTTGTGACCTTCGGCTTTTCAACCCCTTTTAAAAA
 GTAATTGAACCATGAGGCAACCGTGAAGAAAGATCCCTCAATCGAGAAATTGGTTTTCGCTATCGGCATGCCAGTGTGTGAATTTGATATGGTTAAAGATC
 CAGAAGTACAGGACTTCCGAAGAAATATCTGAAACGTTTGTAAAGAGCTGTGGAATCTTAGGGACCTCAATTACCTCATAGTAGAGCAATGTATGTCTTA
 TCCTCCAAATGTAGAAATCTTCCACAGAAATTCACCAAGCACATATATAATAATTAAGATAAAGGGCAAAATAATAGTGGTGATCTGGGTAATAAGTTTCTCCA
 AATAATGACAAGCAGAAGTATATCTGTGAAAATCAACCATGACTGTGTACAGAACAGTAATTTTAAAGTGTGGATGTGAATACCTTCCCTAGAAAAATATCCTCT
 TATCCTCTGAAACAACATAAATCTCTGTGTTTGAATAATCAGGGCAAGTATATTTTAAAGTGTGGATGTGAATACCTTCTATTTCTCAACTGCCAATGGAC
 GAGTCAGTATAAGTATATAAGAAAGCTGTATAATGCTTGGGAGGATGCCCAATTTGATGTTGATGGCTAAAGAAAGCTCTATTTCTCAACTGCCAATGGAC
 TGTTTTACAAATGCCATCTTATTTCCAGACGCTATCCACAGCTACACCATATATGAATGGAGAAACATCTACAAAATCCCTTTGGGTTATAAATAGTGCAC
 TCAGAAATAAAAATTTCTTGTGCAACCTACGTGAATGTAAATATTCGAGACATTTGATAAGATCTATGTTTCGAACAGGTATCTACCATGGAGAGAACCTTT
 ATGTGACAAATGTGAACACTCAAAGAGTACCTTGTTCCTCAATCCAGGTGGAATGAATGGCTGAATPATGATATATACATTCCTCGATCTTCCCTCGTGTGCT
 CGACTTTGCTTTCCTATTTGCTCTGTGTTAAAGCCGGAAGGCTGCTAAAGAGGAACACTGTCCATTTGGCATGGGAAATATAAACTTTGTTTGTATACACAG
 ACACCTCTAGTATCTGGAATAAATGGCTTTGAAATCTTTGGCCAGTACCTCATGGACTAGAAAGATTTGCTGTAAGATTTGAGAGCATGCCCAATTTGGTCTGTA
 TAAAGAAACTCCTATGCTTAGAGTTGGAGTTTGACTGCTTCAAGCTAGCTAGAGACAAATGAATTAAGGGAAATAGACAAAAGCAAGCTCAAAGCAA
 TCCCGAGAAAGCAGGATTTAGCTATTTCCACGAGGACTGAGTAACAGACTAGCTAGAGACAAATGATTTGTGTAACATACTCCCGAAAATTTCTACCCAA
 TTTCTACACGAGATCTCTCTGTGAAATCACTGAGCAGGAGAAAGATTTTCTATGGAGTCAACAGACACTATTTGTGTAACATACTCCCGAAAATTTCTACCCAA
 ATTGCTTCTGCTGTGTTAAATGGAAATCTTAGAGATGAAGTAGCCAGATGTAATGCTTGTGTAAGAAATGGCTTCAATCAACCTGAACAGGCTATGGAA
 CTTCTGGACTGTAAATTACCCAGATCCCTATGGTTTCGAGGTTTGTGCTGCTTGGAAAATAATTAACAGATGACAAAATTTCTCAGTATTTAATTC
 AGCTAGTACAGGTCCTAAAATATGAACAAATATTTGGATAAATTTGCTTGTGAGATTTTACTGAAGAAAGCATTTGACTAATCAAGGATTTGGCACTTTT
 CTTTGGCATTTAAATCTGAGATGCAACAATAAAGCTCATTAACCTAAGCTGACATTTCTCAACAGGAGAAAGGATGAACACAAAGGTACAGATGAAGT
 CACTGAATAGGCAAGTCGAGGCAATGGAAAGCTCATTAACCTAAGCTGACATTTCTCAACAGGAGAAAGGATGAACACAAAGGTACAGATGAAGT
 TTTTAGTTGAGCAAAATGAGGCGACCAAGATTTCTATGGATGCTCTACAGGGCTTTCTGTCTCTAAACCTGCTCATCAACTAGGAAACCTCAGGCTTGA
 AGAGTGTCCGAATATGTCTCTGCAAAAAGGCCACTGTGTTGAATTTGGAGAACCCAGACATCATGTCCAGAGTTACTGTTTTCAGAAACAATGAGATCATC
 TTTTAAATAATGGGATGATTTACGGCAAGATATGCTAACACTTCAAAATTAATTCGTATTTAGGAAATACTGTGCAAAATCAAGGTCTTGATCTTCGAATGT
 TACCTTATGGTTGTCTGTCAATCGGTGACTGTGTGGACTTATTTGAGGTGGTGGGAAATTTCTCACACTATTTATGCAAAATTCAGTCAAGGCGCTTGAA
 AGGTGCACTGCAAGTTCAACAGCCACACACTACATCAGTGGCTCAAAAGACAAGAAATATATGATGTCAGCCCATTTGACCTGTTTACACGTTCA
 TGTGCTGGATACCTGTAGCTACCTTCAATTTTGGAAATTTGGAGATCTGTCAATAGTAACATCATGGTGAAGACGATGGACAGCTGTTTTCATATAGATT
 TTGGACACTTTTGGATCACAGAGAAAGAAATTTGGTTATAAACGAGAACGTTGTGCCATTTGTTTGTGACACAGGATTTCTTAATAGTGAATGAAGG
 AGCCCAAGAAATGCACAAAGACAAGAGAAATTTGAGAGGTTTTCAGGAGATGTGTACAGGCTTATCTAGCTATTTGACAGCATGCCAATCTCTTCATATAAT
 CTTTCTCAATGATGCTTGGCTCTGGAAATGCCAGAACTACAATCTTTTGTATGACATTCGAAAGACCTTAGCCTTAGATAAAACTGAGCAAG
 AGGCTTTGGAGTATTTCAATGAACAAATGAATGATGCACCTTCAATGGCTGGACAAACAAAATGGATTGGATCTTCCACACAAATTAACACAGCATGCATT
 GAACCTGA

FIGURE 7H

(SEQ ID NO: 17) PI3K (H1047L) amino acid

MPPRSSGELWGIHLMPPRIILVECLLPNGMIVTLECLREATLTITIKHELF
KEARKYPLHQLLODESSYIFVSVTQEAEREFEFFDETRRLCDLRLFPFLK
VIEPVGNREEKILNREIGFAIGMPVCEFDVKDPEVQDFRRNINLVCKEA
VDLRDLNSPHSRAMYVYPPNVESSPELPHIYNKLDKGQIIIVVIWIVSP
NNDKQYTLKINHDCVPEQVIAEAIKKKTRSMLLSSEQKLKLVLEYQGY
ILKVCGCDEYFLEKYPLSQYKIRSCIMLGRMPNMLMAKESLYSQLPMD
CFTMPYSRRISTATPYMNGETSTKSLWVINSALRIKILCATYVNVNIRD
IDKIYVRTGIYHGGEPLCDNVNTQVRPCSNPRWNEWLNIDIYIPDLPRAA
RLCLISICSVKGRKGAKEEHCPLAWGNINLFDYTDTLVSGKMALNLWVPVPH
GLEDLLNPIGVTGSPNPKETPCLELEFDWESSVVKFPDMSVIEEHANWSV
SREAGFSYSHAGLSNRLARDNELRENDKEQLKAISTRDPLSEITEQEKDF
LWSHRHYCVTIPEILPKLLLSVKWNSRDEVAQMYCLVKDWPPPIKPEQAME
LLDCNYPDPMPVRGFAVRCLEKYLTDKLSQYLIQLVQVLKYEQYLDNLLV
RFLKKALTNQRI GHFFFWHLKSEMHNKTVSQRFGLLLESYCRACGMYLK
HLNRQVEAMEKLINLTDILKQEKDETQVKQMKFLVEQMRPDPDFMDALQG
FLSPLNPAHQLGNLRLKECRIMSSAKRPLWLNWENPDIMSELLFQNNELI
FKNGDDL RQDMLTLQIRIMENIWQNQGLDLRLPYGCLSIGDCVGLIEV
VRNSHTIMQIQCKGGLKGALQFNSHTLHQWLKDKNKG EYDAAIDLFTRS
CAGYCVATFILGIGDRHNSNIMVKDDGQLFHIDFGHFLDHKKKFGYKRE
RVFFVLTQDFLIVISKGAQECTKTREFFERFQEMCYKAYLAIRQHANLFIN
LFSMMLGSGMPELQSFDDIAYIRKTLALDKTEQEALEYFMKQMNDA LHGG
WTTKMDWIFHTIKQHALLN

(SEQ ID NO: 60) Wild-type b-RAF cDNA

[illegible]

FIGURE 8B

(SEQ ID NO: 18) Wildtype b-RAF amino acid

MAALSGGGGGAEPGQALFNGDMEPEAGAGAGAAAASSAADPAIPEEVWNI
KQMIKLTQEHIEALLDKFGGEHNPPSIYLEAYEYTSKLDALQOREQQLL
ESLNGTDFSVSSASMDTVTSSSSSLSVLPSSLVFNPTDVARSNPK
SPQKPIVRVFLPNKQRTVVPARCGVTVRDSLKKALMMRGLIPECCAVYRI
QDGEKKPIGWDTDISWLTGEELHVEVLENVPLTTHNFVRKTFFTLAFCDF
CRKLLFQGFRCQTCGYKFHQRCSTEVPLMCVNYDQLDLLFVSKFFEHHPI
PQEEASLAETALTSGSSPSAPASDSIGPQILTSPPSPKSIPIPPFRPAD
EDHRNQFGQDRSSSAPNVHINTIEPVNIDDLIRDQGFGRDGGSTTGLSA
TPPASLPGSLTNVKALQKSPGPQREKSSSSSEDRNRMKTLGRRDSSDDW
EIPDGQITVQQRIGSGSFGTVYKGKWHGDVAVKMLNVTAFTPQQLQAFKN
EVGVLKTRHVNILLFMGYSTKPQLAIVTQWCEGSSLYHHLHIETKFEM
IKLIDIAHQTAQGM DY LHAKSIIHRDLKSNNIFLHEDLTVKIGDFGLATV
KSRWSGSHQFEQLSGSILWMAPEVIRMQDNKPYSFQSDVYAFGLVYELM
TGQLPYSNINNRRDQIIFMVGRGYLSPDLKVRSNCPKAMKRLMAECLKKK
RDERPLFPQILASIELLARSLPKIHRSASEPSLNRAFQTEDFSLYACAS
PKTPIQAGGYGAFPVH

[illegible]

FIGURE 8D

(SEQ ID NO: 19) b-RAF (V600E) amino acid

MAALSGGGGGAEPGQALFNGDMEPEAGAGAGAAASSAADPAIPEEVWNI
KOMIKLTQEHIEALLDKFGGEHNPPSIYLEAYEYTSKLDALQOREQQLL
ESLNGTDFSVSSASMDTVTSSSSSLSVLPSSLVFNPTDVARSNPK
SPQKPIVRVFLPNKQRTVVPARCGVTVRDSLKKALMMRGLIPECCAVYRI
QDGEKKPIGWDTDISWLTGEELHVEVLENVPLTTHNFVRKTFFTLAFCDF
CRKLLFQGFRCQTCGYKFHQRCSTEVPLMCVNYDQLDLLFVSKFFEHHPI
PQEEASLAETALTSGSSPSAPASDSIGPQILTSPPSKSIPQPFRPAD
EDHRNQFGQDRSSSAPNVHINTIEPVNIDDLIRDQGFGRDGGSTTGLSA
TTPASLPGSLTNVKALQKSPGPQREKSSSSSEDRNRMTLGRDSSDDW
EIPDGQITVGQRIGSGSFGTVYKKGWHDVAVKMLNVTAPTPOQLQAFKN
EVGLRKTRHVNIILFMGYSTKPQLAIVTQWCEGSSLYHHLHIIETKFFEM
IKLIDIAROT AQMDYLHAKSIIHRDLKSNIFLHEDLTVKIGDFGLATE
KSRWSGSHQFEQLSGSILWMAPEVIRMQKNPYSFQSDVYAFGIVLYELM
TGQLPYSNINNRDQIIFMVGRGYLSPDL SKVRSNCPKAMKRLMAECLKKK
RDERPLFPQILASIELLARSLPKIHRSA SEPSLN RAGFQTEDFS LYACAS
PKTPIQAGGYGAFPVH

(SEQ ID NO: 57)	b-RAF (K601E)	cDNA

[illegible]

FIGURE 8F

(SEQ ID NO: 20) b-RAF (K601E) amino acid

MAALSGGGGGAEPGQALFNGDMEPEAGAGAGAAAASSAADPAIPEEVWNI
KQMIKLTQEHIEALLDKFGGEHNPPSIYLEAYEETSKLDALQREQQLL
ESLNGTDFSVSSASMDTVTSSSSSLSVLPSSLVFNPTDVARSNPK
SPQKPIVRVFLPNKQRTVVPARCGVTVRDSLKKALMMRGLIPECCAVYRI
QDGEKKPIGWDTDISWLTGEELHVEVLENVPLTTHNFVRKTFFTLAFCD
CRKLLFQGFRCQTCGYKFHQRCSTEVPLMCVNYDQLDLLFVSKFFEHHPI
PQEEASIAETALTSGSSPAPASDSIGPQILTSPPSKSIPQPFRPAD
EDHRNQFGQDRSSAPNVHINTIEPVNIDDLIRDQGFGRDGGSTGLSA
TPPASLPGSLTNVKALQKSPGPQREKSSSSSEDRNRMTLGRDSSDDW
EIPDGQITVGQIRIGSGSFGTVYKKGWHDVAVKMLNVTAPTQQLQAFKN
EVGVLKTRHVNILLFMGYSTKPQLAIVTQWCEGSSLYHHLHIETKFEM
IKLIDIAARQTAQMDYLAHAKSIIHRDLKSNNIFLHEDLTVKIGDFGLATV
ESRWSGSHQFEQLSGSILWMAPEVIRMQDNKPYSFQSDVYAFGIVLYELM
TGQLPYSNINNRRDQIIIFMVGRGYLSPDLKVRSNCPKAMKRLMAECLKKK
RDERPLFPQILASIELLARSLPKIHRSASEPSLNRAGFQTEDFSLYACAS
PKTPIQAGGYGAFPVH