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(54) Title: IDENTIFICATION OF NON-RESPONDERS TO HER2 INHIBITORS

(57) Abstract: The present invention relates to means and methods for the identification of non-responders to a HER2 inhibitor, whereby one or more mutations in exon 9 of Phosphoinositol-3 kinase (PIK3CA) indicate non-responsiveness.

Identification of non-responders to HER2 inhibitors

The present invention relates to means and methods for the identification of non-responders to a HER2 inhibitor, whereby one or more mutations (mutational SNPs) in exon 9 of Phosphoinositol-3 kinase (PIK3CA) indicate non-responsiveness.

The HER family of receptor tyrosine kinases are important mediators of cell growth, differentiation and survival. The receptor family includes four distinct members including epidermal growth factor receptor (EGFR, ErbBl, or HER1), HER2 (ErbB2 or *pl85"^eu*), HER3 (ErbB3) and HER4 (ErbB4 or tyro2).

EGFR, encoded by the *erbB1* gene, has been causally implicated in human malignancy. In particular, increased expression of EGFR has been observed in breast, bladder, lung, head, neck and stomach cancer as well as glioblastomas. Increased EGFR receptor expression is often associated with increased production of the EGFR ligand, transforming growth factor alpha (TGF-a), by the same tumor cells resulting in receptor activation by an autocrine stimulatory pathway. Baselga and Mendelsohn *Pharmac. Ther.* 64:127-154 (1994). Monoclonal antibodies directed against the EGFR or its ligands, TGF-a and EGF, have been evaluated as therapeutic agents in the treatment of such malignancies. See, *e.g.,* Baselga and Mendelsohn., *supra;* Masui *et al. Cancer Research* 44:1002-1007 (1984); and Wu *el al. J. Clin. Invest.* 95:1897-1905 (1995).

The second member of the HER family, pl85"^e", was originally identified as the product of the transforming gene from neuroblastomas of chemically treated rats. The activated form of the *neu* proto-oncogene results from a point mutation (valine to glutamic acid) in the transmembrane region of the encoded protein. Amplification of the human homolog of *neu* is observed in breast and ovarian cancers and correlates with a poor prognosis (Slamon *el al., Science,* 235:177-182 (1987); Slamon *et al, Science,* 244:707-712 (1989); and US Pat No. 4,968,603). To date, no point mutation analogous to that in the *neu* proto-oncogene has been

reported for human tumors. Overexpression of HER2 (frequently but not uniformly due to gene amplification) has also been observed in other carcinomas including carcinomas of the stomach, endometrium, salivary gland, lung, kidney, colon, thyroid, pancreas and bladder. See, among others, King *et al, Science,* 229:974 (1985); Yokota *et al, Lancet:* 1:765-767 (1986); Fukushige *et al, Mol Cell Biol,* 6:955-958 (1986); Guerin *et al, Oncogene Res.,* 3:21-31 (1988); Cohen *et al, Oncogene,* 4:81-88 (1989); Yonemura *et al, Cancer Res.,* 51:1034 (1991); Borst *et al, Gynecol Oncol,* 38:364 (1990); Weiner *et al, Cancer Res.,* 50:421-425 (1990); Kern *et al, Cancer Res.,* 50:5184 (1990); Park *et al, Cancer Res.,* 49:6605 (1989); Zhau *et al, Mol. Carcinog,* 3:254-257 (1990); Aasland *et al. Br. J. Cancer* 57:358-363 (1988); Williams *et al. Pathobiology* 59:46-52 (1991); and McCann *et al, Cancer,* 65:88-92 (1990). HER2 may be overexpressed in prostate cancer (Gu *et al. Cancer Lett.* 99:185-9 (1996); Ross *et al. Hum. Pathol.* 28:827-33 (1997); Ross *et al. Cancer* 79:2162-70 (1997); and Sadasivan *et al. J. Urol.* 150:126-31 (1993)).

Antibodies directed against the rat p185[neu] and human HER2 protein products have been described. Drebin and colleagues have raised antibodies against the rat *neu* gene product, p185[neu]. See, for example, Drebin *et al, Cell* 41:695-706 (1985); Myers *et al, Meth. Enzym.* 198:277-290 (1991); and W094/22478. Drebin *et al Oncogene* 2:273-277 (1988) report that mixtures of antibodies reactive with two distinct regions of p185[neu] result in synergistic anti-tumor effects on new-transformed NIH-3T3 cells implanted into nude mice. See also U.S. Patent 5,824,311 issued October 20, 1998.

Hudziak *et al, Mol. Cell. Biol.* 9(3): 1165-1172 (1989) describe the generation of a panel of HER2 antibodies which were characterized using the human breast tumor cell line SK-BR-3. Relative cell proliferation of the SK-BR-3 cells following exposure to the antibodies was determined by crystal violet staining of the monolayers after 72 hours. Using this assay, maximum inhibition was obtained with the antibody called 4D5 which inhibited cellular proliferation by 56%. Other antibodies in the panel reduced cellular proliferation to a lesser extent in this assay. The antibody 4D5 was further found to sensitize HER2-overexpressing breast tumor cell lines to the cytotoxic effects of TNF-a. See also U.S. Patent No. 5,677,171 issued October 14, 1997. The HER2 antibodies discussed in Hudziak *et al.* are further characterized in Fendly *et al. Cancer Research* 50:1550-1558 (1990); Kotts *et al In Vitro* 26(3):59A (1990); Sarup *et al. Growth Regulation* 1:72-82 (1991); Shepard *et al. J. Clin.*

Immunol. 11(3): 117-127 (1991); Kumar *et al. Mol. Cell. Biol.* 11(2):979-986 (1991); Lewis *et al. Cancer Immunol. Immunother.* 37:255-263 (1993); Pietras *et al. Oncogene* 9:1829-1838 (1994); Vitetta *et al. Cancer Research* 54:5301-5309 (1994); Sliwkowski *et al. J. Biol. Chem.* 269(20):14661-14665 (1994); Scott *et al. J. Biol. Chem.* 266:14300-5 (1991); D'souza *et al. Proc. Natl. Acad. Sci.* 91:7202-7206 (1994); Lewis *et al. Cancer Research* 56:1457-1465 (1996); and Schaefer *et al. Oncogene* 15:1385-1394 (1997).

A recombinant humanized version of the murine HER2 antibody 4D5 (huMAb4D5-8, rhuMAb HER2, Trastuzumab or Herceptin™ ; U.S. Patent No. 5,821,337) is clinically active in patients with HER2-overexpressing metastatic breast cancers that have received extensive prior anti-cancer therapy (Baselga *et al, J. Clin. Oncol.* 14:737-744 (1996)). Trastuzumab received marketing approval from the Food and Drug Administration September 25, 1998 for the treatment of patients with metastatic breast cancer whose tumors overexpress the HER2 protein.

Other HER2 antibodies with various properties have been described in Tagliabue *et al Int. J. Cancer* 47:933-937 (1991); McKenzie *et al. Oncogene* 4:543-548 (1989); Maier *et al Cancer Res.* 51:5361-5369 (1991); Bacus *et al. Molecular Carcinogenesis* 3:350-362 (1990); Stancovski *et al. PNAS (USA)* 88:8691-8695 (1991); Bacus *et al. Cancer Research* 52:2580-2589 (1992); Xu *et al. Int. J. Cancer* 53:401-408 (1993); WO94/00136; Kasprzyk *et al. Cancer Research* 52:2771-2776 (1992);Hancock *et al Cancer Res.* 51:4575-4580 (1991); Shawver *et al. Cancer Res.* 54:1367-1373 (1994); Arteaga *et al. Cancer Res.* 54:3758-3765 (1994); Harwerth *et al. J. Biol. Chem.* 267:15160-15167 (1992); U.S. Patent No. 5,783,186; and Klapper *et al. Oncogene* 14:2099-2109 (1997).

Homology screening has resulted in the identification of two other HER receptor family members; HER3 (US Pat. Nos. 5,183,884 and 5,480,968 as well as Kraus *et al PNAS (USA)* 86:9193-9197 (1989)) and HER4 (EP Pat. Appln. No 599,274; Plowman *et al, Proc. Natl. Acad. Sci. USA,* 90: 1746-1750 (1993); and Plowman *et al, Nature,* 366:473-475 (1993)). Both of these receptors display increased expression on at least some breast cancer cell lines.

The HER receptors are generally found in various combinations in cells and heterodimerization is thought to increase the diversity of cellular responses to a variety of

HER ligands (Earp *et al. Breast Cancer Research and Treatment* 35: 115-132 (1995)). EGFR is bound by six different ligands; epidermal growth factor (EGF), transforming growth factor alpha (TGF-a), amphiregulin, heparin binding epidermal growth factor (HB-EGF), betacellulin and epiregulin (Groenen *et al. Growth Factors* 11:235-257 (1994)). A family of heregulin proteins resulting from alternative splicing of a single gene are ligands for HER3 and HER4. The heregulin family includes alpha, beta and gamma heregulins (Holmes *et al, Science,* 256:1205-1210 (1992); U.S. Patent No. 5,641,869; and Schaefer *et al. Oncogene* 15:1 385-1 394 (1997)); neu differentiation factors (NDFs), glial growth factors (GGFs); acetylcholine receptor inducing activity (ARIA); and sensory and motor neuron derived factor (SMDF). For a review, see Groenen *et al. Growth Factors* 11:235-257 (1994); Lemke, G. *Molec. & Cell. Neurosci.* 7:247-262 (1996) and Lee *et al. Pharm. Rev.* 47:51-85 (1995). Recently three additional HER ligands were identified; neuregulin-2 (NRG-2) which is reported to bind either HER3 or HER4 (Chang *et al. Nature* 387 509-512 (1997); and Carraway *et al Nature* 387:512-516 (1997)); neuregulin-3 which binds HER4 (Zhang *et al. PNAS* (USA) **94**(**18):9562-7** (1997)); and neuregulin-4 which binds HER4 **(Harari** *et al. Oncogene* 18:2681-89 (1999)) HB-EGF, betacellulin and epiregulin also bind to HER4.

While EGF and TGFa do not bind HER2, EGF stimulates EGFR and HER2 to form a heterodimer, which activates EGFR and results in transphosphorylation of HER2 in the heterodimer. Dimerization and/or transphosphorylation appears to activate the HER2 tyrosine kinase. See Earp *et al, supra.* Likewise, when HER3 is co-expressed with HER2, an active signaling complex is formed and antibodies directed against HER2 are capable of disrupting this complex (Sliwkowski *et al, J. Biol. Chem.,* 269(20): 14661 -14665 (1994)). Additionally, the affinity of HER3 for heregulin (HRG) is increased to a higher affinity state when co-expressed with HER2. See also, Levi *et al, Journal ofNeuroscience* 15: 1329-1340 (1995); Morrissey *et al, Proc. Natl Acad. Sci. USA* 92: 1431-1435 (1995); and Lewis *et al, Cancer Res.,* 56:1457-1465 (1996) with respect to the HER2-HER3 protein complex. HER4, like HER3, forms an active signaling complex with HER2 (Carraway and Cantley. *Cell* 78:5-8 (1994)).

To target the HER signaling pathway, rhuMAb 2C4 (Pertuzumab) was developed as a humanized antibody that inhibits the dimerization of HER2 with other HER receptors, thereby inhibiting ligand-driven phosphorylation and activation, and downstream activation of the

RAS and AKT pathways. In a phase I trial of Pertuzumab as a single agent for treating solid tumors, 3 subjects with advanced ovarian cancer were treated with pertuzumab. One had a durable partial response, and an additional subject had stable disease for 15 weeks. Agus *et al. ProcAm Soc Clin Oncol* 22: 192, Abstract 771 (2003).

Also antibody variant compositions are described in the art. US Patent No. 6,339,142 describes a HER2 antibody composition comprising a mixture of anti-HER2 antibody and one or more acidic variants thereof, wherein the amount of the acidic variant(s) is less than about 25%. Trastuzumab is the exemplified HER2 antibody. Reid *et al.* Poster presented at Well Characterized Biotech Pharmaceuticals conference (January, 2003) "Effects of Cell Culture Process Changes on Humanized Antibody Characteristics" describes an unnamed, humanized IgGl antibody composition with N-terminal heterogeneities due to combinations of VHS signal peptide, N-terminal glutamine, and pyroglutamic acid on the heavy chain thereof. Harris *et al.* "The Ideal Chromatographic Antibody Characterization Method" talk presented at the IBC Antibody Production Conference (February, 2002) reports a VHS extension on the heavy chain of E25, a humanized anti-IgE antibody. Rouse *et al.* Poster presented at WCBP "Glycoprotein Characterization by High Resolution Mass Spectrometry and Its Application to Biopharmaceutical Development" (January 6-9, 2004) describes a monoclonal antibody composition with N-terminal heterogeneity resulting from AHS or HS signal peptide residues on the light chain thereof. In a presentation at IBC Meeting (September, 2000) "Strategic Use of Comparability Studies and Assays for Well Characterized Biologicals," Jill Porter discussed a late-eluting form of ZENAPAX™ with three extra amino acid residues on the heavy chain thereof. US2006/00 18899 describes a composition comprising a main species pertuzumab antibody and an amino-terminal leader extension variant, as well as other variant forms of the pertuzumab antibody.

Patent publications related to HER antibodies include: US 5,677,171, US 5,720,937, US 5,720,954, US 5,725,856, US 5,770,195, US 5,772,997, US 6,165,464, US 6,387,371, US 6,399.063. US2002/019221 1A1 , US 6,01 5,567. US 6.333,169. US 4.968,603. US 5,82 1.337, US 6,054,297, US 6,407,213, US 6,719,971, US 6,800,738, US2004/0236078A1, US 5,648,237, US 6,267,958, US 6,685,940, US 6,821,515, W098/17797, US 6,127,526, US 6,333,398, US 6,797,814, US 6,339,142, US 6,417,335, US 6,489,447, WO99/31 140, US2003/0147884A1, US2003/0170234A1, US2005/0002928A1, US 6,573,043,

US2003/0152987A1, W099/48527, US2002/0141993A1, WO01/00245, US2003/0086924, US2004/0013667A1, WO00/69460, WO01/00238, WO01/15730, US 6,627, 196B1, US6,632,979B1 . WO01/00244, US2002/0090662A1, WOO 1/89566, US2002/0064785, US2003/0134344, WO 04/24866, US2004/0082047, US2003/0175845A1, WO03/087131, US2003/0228663, WO2004/008099A2, US2004/0106161, WO2004/048525, US2004/0258685A1, US 5,985,553, US 5,747,261, US 4,935,341, US 5,401,638, US 5,604,107, WO 87/07646, WO 89/10412, WO 91/05264, EP 412,1 16 Bl, EP 494,135 Bl, US 5,824,31 1, EP 444,181 Bl, EP 1,006,194 A2, US 2002/0 155527A1, WO 91/02062, US 5,571,894, US 5,939,531, EP 502,812 Bl, WO 93/03741, EP 554,441 Bl, EP 656,367 Al, US 5,288,477, US 5,514,554, US 5,587,458, WO 93/12220, WO 93/16185, US 5,877,305, WO 93/21319, WO 93/21232, US 5,856,089, WO 94/22478, US 5,910,486, US 6,028,059, WO 96/07321, US 5,804,396, US 5,846,749, EP 7 11,565, WO 96/16673, US 5,783,404, US 5,977,322, US 6,512,097, WO 97/00271, US 6,270,765, US 6,395,272, US 5,837,243, WO 96/40789, US 5,783,186, US 6,458,356, WO 97/20858, WO 97/38731, US 6,214,388, US 5,925,519, WO 98/02463, US 5.922.845, WO 98/18489, WO 98/33914, US 5,994.071, WO 98/45479, US 6.358,682 B1, US 2003/0059790, WO 99/55367, WO 01/20033, US 2002/0076695 Al, WO 00/78347, WO 01/09187, WO 01/21 192, WO 01/321 55, WO 01/53354, WO 01/56604, WO 01/76630, WO02/05791, WO 02/1 1677, US 6,582.919, US2002/0192652A1, US 2003/021 1530A1, WO 02/44413, US 2002/0142328, US 6,602,670 B2, WO 02/45653, WO 02/055106, US 2003/01 52572, US 2003/0165840, WO 02/08761 9, WO 03/006509, WO03/012072, WO 03/028638, US 2003/0068318, WO 03/041736, EP 1,357,132, US 2003/0202973, US 2004/0138160, US 5,705,157, US 6,123,939, EP 616,812 Bl, US 2003/0103973, US 2003/0108545, US 6,403,630 Bl, WO 00/61 145, WO 00/61 185, US 6,333,348 Bl, WO 01/05425, WO 01/64246, US 2003/0022918, US 2002/0051785 Al, US 6,767,541, WO 01/76586, US 2003/0144252, WO 01/87336, US 2002/0031515 Al, WO 01/87334, WO 02/05791, WO 02/09754, US 2003/0157097, US 2002/0076408, WO 02/055106, WO 02/070008, WO 02/089842 and WO 03/86467.

Patients treated with the HER2 antibody Trastuzumab/Herceptin™ are selected for therapy based on HER2 protein overexpression/ gene amplification; see, for example, WO99/31 140 (Paton et al), US2003/0170234AI (Ilellmann, S.), and US2003/0 147884 (Paton et al); as well as WO01/89566, US2002/0064785, and US2003/01 34344 (Mass et al). See, also, US2003/01 52987, Cohen et al, concerning immunohistochemistry (IHC) and fluorescence in

situ hybridization (FISH) for detecting HER2 overexpression and amplification. WO2004/053497 and US2004/024815A1 (Bacus et al), as well as US 2003/0190689 (Crosby and Smith), refer to determining or predicting response to Trastuzumab therapy. US2004/013297A1 (Bacus et al.) concerns determining or predicting response to ABX0303 EGFR antibody therapy. WO2004/000094 (Bacus et al.) is directed to determining response to GW572016, a small molecule, EGFR-HER2 tyrosine kinase inhibitor. WO2004/063709, Amler et al., refers to biomarkers and methods for determining sensitivity to EGFR inhibitor, erlotinib HC1. US2004/0209290, Cobleigh et al., concerns gene expression markers for breast cancer prognosis.

Patients treated with pertuzumab (a HER2 dimerisation inhibitor described herein below in more detail) can be selected for therapy based on HER activation or dimerization. Patent publications concerning pertuzumab and selection of patients for therapy therewith include: WO01/00245 (Adams et al.); US2003/0086924 (Sliwkowski, M.); US2004/0013667A1 (Sliwkowski, M.); as well as WO2004/008099A2, and US2004/0 106 16 1(Bossenmaier et al.).

Herceptin™/Trastuzumab is indicated in the art for the treatment of patients with metastatic breast cancer whose tumors overexpress HER2 protein or have HER 2 gene amplification:
a) As monotherapy for the treatment of those patients who have received at least two chemotherapy regimens for their metastatic disease. Prior chemotherapy must have included at least an anthracycline and a taxane unless patients are unsuitable for these treatments. Hormone receptor positive patients must also have received hormonal therapy, unless patients are unsuitable for these treatments,
b) In combination with paclitaxel for the treatment of those patients who have not received chemotherapy for their metastatic disease and for whom an anthracycline is not suitable and
c) In combination with docetaxel for the treatment of those patients who have not received chemotherapy for their metastatic disease.

HerceptinTM/Trastuzumab can also be used as adjuvant treatment in early breast cancer. HerceptinTM/ Trastuzumab is also approved for the treatment of patients with HER2-positive early breast cancer following surgery, chemotherapy (neoadjuvant (i.e. before surgery) or adjuvant), and radiotherapy (if applicable). In addition Herceptin in combination with capecitabine or 5-fluorouracil and cisplatin is indicated for the treatment of patients with

HER2 positive locally advance or metastatic adenocarcinoma of the stomach or gastroesophageal junction who have not received prior anti-cancer treatment for their metastatic disease.

In the art, the treatment of breast cancer patients with Herceptin™/Trastuzumab is, for example, recommended and routine for patients having HER2-positive cancer. HER2-positive cancer is present if a high HER2 (protein) expression level detected by immunohistochemical methods (e.g. HER2 (+++)) or HER2 gene amplification detected by in-situ-hybridization (e.g. ISH positive, like a HER2 gene copy number higher than 4 copies of the HER2 gene per tumor cell or ratio of $\geq$ 2.0 for the number of HER2 gene copies to the number of signals for CEP 17.) or both is found in samples obtained from the patients such as breast tissue biopsies or breast tissue resections or in tissue derived from metastatic sites.

The NEOSPHERE study (Neoadjuvant Study of Pertuzumab and Herceptin in an Early Regimen Evaluation) is a randomized multicentre, international Phase II study that was conducted in 78 centres worldwide (except the USA) in 417 women with newly diagnosed HER2-positive early, inflammatory or locally advanced breast cancer who had never received Herceptin. Prior to surgery (neoadjuvant treatment) these women were randomized to four study arms. The primary endpoint was complete tumour disappearance at time of surgery (pathological complete response, pCR) and the results were:

pCR of 29,0 percent for Herceptin and docetaxel

pCR of 45,8 percent for Herceptin, pertuzumab and docetaxel

pCR of 16,8 percent for Herceptin and pertuzumab

pCR of 24,0 percent for pertuzumab and docetaxel

The data shows that the two antibodies plus docetaxel given in the neoadjuvant setting prior to surgery significantly improved the rate of complete tumour disappearance (pathological complete response rate, pCR, of 45.8 percent) in the breast by more than half compared to Herceptin plus docetaxel (pCR of 29. 0 percent), p=0.014. The study is described in detail e.g. in Lancet Oncol. 2012 Jan;13(l):25-32. doi: 10.1016/S1470-2045(11)70336-9. Epub 2011 Dec 6, which is incorporated by reference herein in its entirety. Core biopsies (tumor tissue) from 387 patients were used for biomarkcr analyses.

However, not all patients having HER2-positive cancer or cancer cells respond to treatment with a HER2 inhibitor. Therefore, efforts have been made in the art to identify non-responding patients that may be excluded from treatment. Barbereschi (Clin Cancer Res 2007, 13:6064-6069) investigated the association of phosphoinositide-3 -kinase, catalytic, alpha polypeptide (PIK3CA) mutations on exon 9 and 20 with pathologic features and clinical outcome in breast cancer patients treated with chemotherapy and/or hormone therapy. Berns (Cancer Cell (2007) 12, 395-402) describes that the presence of PIK3CA mutations (inter alia in exon 9 and 20) was associated with poor prognosis after trastuzumab therapy. Also Razis (Breast Cancer Res Treat (DOI 10. 1007/sl 0549-01 1-1572-5) investigates the association of PIK3CA mutations (in exon 9 and 20) with efficacy of trastuzumab therapy and describes that these mutations were associated with shorter median time to progression. Dave (201 1, J Clinical Oncology 29, 166) also find that activating mutations in the PIK3CA conferred resistance to Trastuzumab.

Thus, the technical problem underlying the present invention is the provision of means and methods for identifying a patient or a group of patients with HER2-positive cancer who are non-responsive to a treatment with a HER2 inhibitor, in particular to a treatment with a HHR2 antibody such as Trastuzumab or Pertuzumab.

The technical problem is solved by provision of the embodiments characterized in the claims.

Accordingly, the present invention relates to a method for identifying a non-responder to a HER2-inhibitor, said method comprising evaluating the presence of one or more mutations in exon 9 of the catalytic subunit of Phosphoinositol-3 kinase (PIK3CA or p i 10a) in a sample from a patient with HER2-positive cancer; and whereby the presence of one or more mutations in exon 9 indicates non-responsiveness of said patient to said HER2 inhibitor.

In the present invention, it was surprisingly found that mutations in exon 9 of Phosphoinositol-3 kinase (PIK3CA) are indicative for non-responsiveness of a patient with HER2-positive cancer to a HER2 inhibitor, such as Trastuzumab and, in particular Pertuzumab. In contrast, as found herein, mutations in exon 20 were not predictive for non-responsiveness. In other words, it was unexpectedly found that the evaluation only of mutations in exon 9 of Phosphoinositol-3 kinase (PIK3CA) is sufficient for a highly reliable

determination of non-responders to treatment with HER2 inhibitors, in particular anti-HER2 antibodies. Therefore, exon 9 mutations are, preferably, the only PIK3CA mutations evaluated in the methods of the present invention. In accordance with the invention, solely the presence of mutations in exon 9 of PIK3CA is determined/evaluated/measured, i.e. the presence of mutations in other parts (e.g. like exon 20) of the PIK3CA gene or coding sequence are not evaluated or determined. The invention is, thus, based on the surprising finding that the determination/evaluation of solely (only) mutations (like mutational SNPs) in exon 9 of PIK3CA is enough for a reliable read-out whether a patient will or will not respond to treatment with a HER2 inhibitor. The evaluation of such mutations and/or SNPs is described herein below in more detail and exemplified in the examples. None of the documents discussed above discloses or proposes the use of only (solely) PIK3CA mutations (or mutational SNPs) in exon 9 for identifying non-responders to therapy with HER2 inhibitors.

Exemplary mutations/SNPs in exon 9 of PIK3CA that can be determined/evaluated in **the** herein provided method for identification of non-responders to HER2-inhibitors are those where the mutation results in a change in the amino acid sequence at position 542 and 545 of the full length protein sequence of PIK3CA as shown in SEQ ID NO: 2. In the wild-type protein sequence of PIK3CA the amino acid at position 542 and 545 is "E". In the mutant forms of PIK3CA to be determined herein, the wild type "E" at these positions is replaced by the amino acid "K" ("E542K" or "E545K"), amino acid "A" ("E545A") or amino acid "G" ("E545G"). These changes at amino acid level are also reflected in mutations at the nucleic acid level (like mutational SNPs) and corresponding mutated triplets (codons) to be determined/detected/evaluated are given herein further below.

The non-responders identified by the herein provided means and methods may be subject to other treatments than treatment with a HER2 inhibitor; for example, they may advantageously be treated with compounds other than HER2 inhibitors. The term "non-responder" as used herein can refer to an individual/patient/subject that is less likely to respond to a treatment using a HER2 inhibitor (like pertuzumab or trastuzumab). "Less likely to respond" as used herein refers to a decreased likeliness that a pathological complete response (pcR) will occur in a patient treated with a HER2 inhibitor. In cases where (with the methods of the present invention) it was assessed that the subject is a "non-responder" or is "less likely to respond",

said subject is to receive phosphoinositol-3 kinase-targeted agents. Such agents are known in the art and comprise, but are not limited to fused pyrimidine derivatives as disclosed in US 8,022,205 B2 or fused pyrrolopyrimidine derivatives as disclosed in WO2009/099163.

The sample to be evaluated can be obtained from a patient with HER2-positive cancer. The HER2-positive cancer may assessed be breast cancer, such as early-stage breast cancer. However, the method of identifying non-responders provided herein can be applied to a wide range of HER2-positive cancers, like gastric cancer, colon cancer, lung cancer and the like. In a preferred embodiment, the HER2 inhibitor is an anti-HER2 antibody, like pertuzumab or trastuzumab. Preferably, the patient is a human.

Accordingly, this invention relates to a method for identifying a non-responder to a HER2-inhibitor, said method comprising detecting/measuring the presence of one or more mutations in exon 9 of the catalytic subunit of Phosphoinositol-3 kinase (PIK3CA or p110a) in a sample from a patient with HER2-positive cancer; and whereby the presence of one or more mutations in exon 9 indicates non-responsiveness of said patient to said HER2 inhibitor.

The present invention relates to a method for identifying a non-responder to a HER2-inhibitor, said method comprising the steps

(a) obtaining a sample from a patient with HER2-positive cancer;

(b) evaluating the presence of one or more mutations in exon 9 of the catalytic subunit of Phosphoinositol-3 kinase (PIK3CA or p110a) in said sample;

whereby the presence of one or more mutations in exon 9 of the catalytic subunit of Phosphoinositol-3 kinase (PIK3CA or p110a) indicates non-responsiveness of said patient to said HER2 inhibitor.

As mentioned, it has been found herein that the presence of one or more mutations in exon 9 of the catalytic subunit of PI3K i.e. PIK3CA indicates non-responsiveness to a HER2 inhibitor. The following provides some background information on PIK3CA and the family of Phosphatidylinositol 3-kinase to which it belongs; the mutations in exon 9 of the catalytic subunit of PI3K are explained in more detail further below. The mutation may be the replacement or exchange (substitution) of one or more amino acids as compared to the wild-type sequence of exon 9 of Phosphoinositol-3 kinase catalytic subunit (PIK3CA).

Corresponding nucleic acid sequences and amino acid sequences of wild-type PIK3CA are shown in SEQ ID NO. 1 and SEQ ID NO: 2, respectively, and in Figure 7. As used herein, the term "PIK3CA" refers to the catalytic subunit of Phosphoinositol-3 kinase (PI3K), isoform alpha, also refered to as p1 lOalpha. The terms "PIK3CA", "catalytic subunit of Phosphoinositol-3 kinase isoform alpha" or, short, "pl lOalpha'V'pl 10a" are used interchangeably herein. "PIK3CA" is the term recommended and commonly used in the art; however, the entire protein is also known as PI3K. Phosphatidylinositol 3-kinase (PI3K) is composed of an 85 kDa regulatory subunit and a 110 kDa catalytic subunit. The protein encoded by the PIK3CA gene represents the catalytic subunit of PI3K, which uses ATP to phosphorylate Ptdlns, PtdIns4P and PtdIns(4,5)P2 (i.e. this catalytic subunit is „PIK3CA" as defined and used herein). This gene has been found to be oncogenic and has been implicated in a variety of cancers.

Phosphoinositol-3 kinase belongs to the family of Phosphatidylinositol 3-kinases (PI 3-kinascs or "PI3Ks"). This is a family of enzymes involved in cellular functions such as cell growth, proliferation, differentiation, motility, survival and intracellular trafficking, which in turn are involved in cancer. In response to lipopolysaccharide, PI3Ks phosphorylate p65, inducing anandamide synthesis to inhibit NF-κB activation. This is under the control of FAAH limiting the ability of LPS to increase AEA levels and is also inhibited by wortmannin and cannabidiol, one of the only natural compounds to inhibit FAAH. The phosphoinositoI-3-kinase family is divided into three different classes: Class I, Class II, and Class III. The classifications are based on primary structure, regulation, and *in vitro* lipid substrate specificity.

The following table provides an overview of the human genes/proteins of Phosphatidylinositol 3-kinases family members. PIK3CA is highlighted in bold.

group	gene	protein	synonyms
class 2	PIK3C2A	PI3K, class 2, alpha polypeptide	PI3K-C2α
	PIK3C2B	PI3K, class 2, beta polypeptide	PI3K-C2p
	PIK3C2G	PI3K, class 2, gamma polypeptide	PI3K-C2y

class 3	PIK3C3	PI3K, class 3	Vps34
class 1 catalytic	**PIK3CA**	**PI3K, catalytic, alpha polypeptide**	**p110-α**
	PIK3CB	PI3K, catalytic, beta polypeptide	p110-β
	PIK3CG	PI3K, catalytic, gamma polypeptide	p110-γ
	PIK3CD	PI3K, catalytic, delta polypeptide	p110-δ
class 1 regulatory	PIK3R1	PI3K, regulatory subunit 1 (alpha)	p85-α
	PIK3R2	PI3K, regulatory subunit 2 (beta)	p85-β
	PIK3R3	PI3K, regulatory subunit 3 (gamma)	p55-γ
	PIK3R4	PI3K, regulatory subunit 4	p150
	PIK3R5	PI3K, regulatory subunit 5	p101
	PIK3R6	PI3K, regulatory subunit 6	p87

PIK3CA and its genetic variants to be used in the herein provided methods for identifying non-responders to HER2-inhibitors and their use in screening methods for responsiveness to treatment with a compound are described, for example, in WO 2011/031861, WO 2005/091849 and WO 2011/060380.

In context of the present invention, the mutation (mutational SNP) to be determined/assessed in accordance with the present invention may be a mutation in the codon encoding an amino acid at position 542 and/or 545 of the full-length amino acid sequence of Phosphatidylinositol-3 kinase (PIK3CA) (see e.g. SEQ ID NO. 2 of Figure 7). The mutation may comprise one or more of the mutations E542K, E545K, E545A and E545G (i.e. mutations/SNP in exon 9 encoding the amino acid K, A or G at position 542 or 545 of the amino acid sequence of Phosphatidylinositol-3 kinase (PIK3CA) instead of wildtype E. The term E542K, E545K, E545A and E545G as used herein refer to amino acid substitutions at a given position of the amino acid sequence of wild type PIK3CA. Corresponding nucleic acid sequences (codons/triplets) encoding the amino acid at positions 542 and 545 in wild type and mutant PIK3CA genes/coding sequences are described below. In accordance with internationally accepted nomenclature, the term "E542K" refers to a substitution/replacement of amino acid "E" at position 542 of the the amino acid sequence of wild type PIK3CA by

amino acid "K". The same explanation applies, mutatis mutandis, to "E545K", "E545A" and "E545G". These mutations are well known in the art and corresponding mutated sequences can be retrieved from the respective databases like Uniprot. Based on the herein provided teaching, the presence of these mutations can readily be determined by a person skilled in the art. Preferably, the mutation is deteremined on a nucleic acid level as described below and exemplified in the examples. The mutations E542K E545K, E545A and E545G are also illustrated in the herein described sequences. For example, nucleic acid and amino acid sequences of mutation (mutational SNP) E542K are shown in SEQ ID NO. 17 and SEQ ID NO. 18, respectively; nucleic acid and amino acid sequences of mutation E545K are shown in SEQ ID NO: 19 and SEQ ID NO. 20, respectively; nucleic acid and amino acid sequences of mutation E545A are shown in SEQ ID NO: 21 and SEQ ID NO. 22, respectively, and nucleic acid and amino acid sequences of mutation E545G are shown in SEQ ID NO: 23 and SEQ ID NO. 24, respectively.

Methods for the determining/evaluation assessed/measured of the presence of the mutations are described herein and provided in the examples. Exemplary, non-limiting methods to be used are methods for sequencing of nucleic acids (e.g. Sanger di-deoxy sequencing), "next generation" sequencing methods, single molecule sequencing, methods enabling detection of variant alleles/mutations, such as Real-time PGR, PCR-RFLP assay (see Cancer Research 59 (1999), 5169-5175), mass-spectrometric genotyping (e.g. MALDI-TOF), HPLC, enzymatic methods and SSPC (single strand conformation polymorphism analysis; sec Pathol Int (1996) 46, 801-804).

Such methods may include enzymatic amplification of DNA or cDNA fragments using oligonucleotides specifically hybridizing to exon 9 (or parts thereof) of the PIK3CA gene by PGR. Given that mutations in exon 9 of the PIK3CA gene are to be evaluated, such amplifications may be carried out in one or two reactions when employing RNA or genomic DNA. The resulting PGR products may be subjected to either conventional Sanger-based dideoxy nucleotide sequencing methods or employing parallel sequencing methods ("next generation sequencing") such as those marketed by Roche (454 technology), Illumina (Solexa technology) or ABI (Solid technology). Mutations may be identified from sequence reads by comparison with publicly available gene sequence data bases. Alternatively, mutations may be identified by incorporation of allele-specific probes that can either be detected using

enzymatic detection reactions, fluorescence, mass spectrometry or others; see Vogeser (2007) Dtsch Arztebl 104 (31-32), A2 194-200.

Paraffin-embedded clinical material as well as fresh frozen tissue may be used in the detection of these mutations. Detection may comprise a histolopathology review of the sample to be tested to see whether tumour tissue is present. The following table shows exemplary nucleic acid sequences of the mutations (mutational SNPs) to be determined in accordance with the present invention; any other point mutation(s) that result in an amino acid change at position 542 and/or 545 (or position 546, like the E545D mutation having the sequence "gat'V'T" mutation") of full-length the amino acid sequence of PIK3CA can be included in the assessment in accordance with the present invention.

gaa	codon/triplet encoding wild type E542
gag	codon/triplet encoding wild type E545
aaa	codon/triplet encoding mutant E542K ((E»K)
aag	codon/triplet encoding mutant E545K (E»K),
gcg	codon/triplet encoding mutant E545A (E»A),
ggg	codon/triplet encoding mutant E545G(E»G),

Accordingly, the term "mutation E542K in exon 9 of Phosphoinositol-3 kinase (PIK3CA)" as used herein may refer to a codon/triplet (like aaa) encoding amino acid K at position 542 of the full-length amino acid sequence of PIK3CA (the wild-type sequence thereof is shown in Figure 7 and SEQ ID NO: 2). The term "mutation E545K in exon 9 of Phosphoinositol-3 kinase (PIK3CA)" as used herein may refer to a codon/triplet (like aag) encoding amino acid K at position 545 of the full-length amino acid sequence of PIK3CA (the wild-type sequence thereof is shown in Figure 7 and SEQ ID NO: 2). The term "mutation E545A in exon 9 of Phosphoinositol-3 kinase (PIK3CA)" as used herein may refer to a codon/triplet (like gcg) encoding amino acid A at position 545 of the full-length amino acid sequence of PIK3CA (the wild-type sequence thereof is shown in Figure 7 and SEQ ID NO: 2). The term "mutation E545G in exon 9 of Phosphoinositol-3 kinase (PIK3CA)" as used herein may refer to a codon/triplet (like ggg) encoding amino acid G at position 545 of the full-length amino acid sequence of PIK3CA (the wild-type sequence thereof is shown in Figure 7 and SEQ ID NO: 2).

The following exemplary test may be used.

The PGR amplification of isolated DNA and mutation detection procedures for the PIK3CA mutation detection test are summarized below.

Each standard 50-µL amplification reaction targeting one of the Exons 7, 9 or 20 included 100 ng genomic DNA, dNTPs (including dUTP), 0.05 Ʊ/µï¸ Z05, DNA polymerase, 0.04 U/µL uracil-DNA glycosylase (UNG), and 200-400 nM forward and reverse primer (Table 1), 75-200 nM mutant and wild-type specific probes (Table 2). Amplification was performed in the cobas® 4800 analyzer using the following temperature profile; 5 min at 50°C followed by 55 cycles of 95°C for 10 sec and 63°C for 50 sec, followed by a single round of 40°C for 2 min and 25°C for 10 sec (melting curve analysis). Fluorescence data was collected during each amplification cycle and during the final melting curve analysis.

The following exemplary primers/primer pair may be used in the method for identifying a non-rcspondcr to a HER2-inhibitor provided herein, wherein the non-responder has a mutation in exon 9 of P1K3CA.

Table 1: Primer Sequences for the PIK3CA Mutation Detection Test

Primer	Sequence 5′ to 3′
542/545 Forward primer PIK3CA-9F13	UAAAAUUUAUUGAGAAUGUAUUUGCTTTTTC (SEQ ID NO: 25)
542/545 Reverse primer PIK3CA-9R01	TCCATTTTAGCACTTACCTGTGAC (SEQ ID NO: 26)

Key: U = 5-propynyl dU

The following exemplary probes may be used in the method for identifying a non-responder to a HER2-inhibitor provided herein, wherein the non-responder has a mutation in exon 9 of PIK3CA.

Table!: Probe Sequences for the PIK3CA Mutation Detection Test

Probe	Sequence 5'to 3'
542 WT Probe	FTTTCAQAGAGAGGAUEUEGUGUAGAAAUUGEP (SEQ ID NO: 27)
542 542K Mutation Probe	LATTTTQGAGAGAGGAUEUEGUGUAGAAAUUGEUUP (SEQ ID NO: 28)
545 WT Probe	OCTGCTCAGTQAUUUIAGAGAGAGGATCTCGTGTP (SEQ ID NO: 29)

Probe	Sequence 5' to 3'
545 545K Mutation Probe	JAATCACTAAGQAGGAGAAAGAUUUUEUAUGGAGUEP (SEQ ID NO: 30)
545 545A Mutation Probe	FCTGCGCQGGAGAAAGAUUUUEUAUGGAGUEAP (SEQ ID NO: 31)
545 545G Mutation Probe	LCCTGCCCQGTGAUUUIAGAGAGAGGATCTCGP (SEQ ID NO: 32)

Key: F = FAM Reporter Dye , J = JA270 Reporter Dye, O = CY5.5 Reporter Dye, L = HEX Reporter Dye, U = 5-propynyl dU, E = 5-methyl dC, , I = deoxyinosine, Z = 7-deaza dG, Q = BHQ2 Quencher Dye, P = 3 'Phosphate

In accordance with the present invention, the presence of one or more mutations in exon 9 of PIK3CA may be evaluated/determined/measured in the herein provided method for identifying a non-responder to HER2 inhibitors (the terms "evaluating", "determining" and "measuring" can be used interchangeably in context of the present invention). In one embodiment, the presence of only one of the mutations is evaluated/determined. Accordingly, the presence of only one of the E542K, E545K, E545A and E545G mutations of PIK3CA (i.e. the nucleic acids in exon 9 of PIK3CA encoding the amino acid at these positions in the full length amino acid sequence of PIK3CA) may be evaluated, i.e. only E542K, only E545K, only E545A or only E545G. The methods of the present invention may also comprise the subsequent evaluation of the presence of two or more of these mutations in any order. For example, the evaluation of the presence of E542K may be followed by the evaluation of E545K (or vice versa) which may be followed by the evaluation of the presence of E545A, which may be followed by the evaluation of the presence of E545G. Other possible orders of evaluation are easily conceivable by a person skilled in the art and contemplated herein.

The mutations may be evaluated in combination/simultaneously. Again, any combination is envisaged. For example, the presence of E542K and E545K is evaluated; or the presence of E542K and E545A; or the presence of E542K and E545G is evaluated. Other combinations are easily conceivable. The evaluation of a combination of these mutations may be followed or preceded by the evaluation of the presence of one other mutation or a combination of other mutations.

As mentioned, the present invention provides for means to determine whether an individual/patient with HER2-positive cancer (i.e. suffering from, suspected to suffer from or being prone to suffer from HER2-positive cancer) will respond to treatment with a HER2

inhibitor or will not respond to treatment to a HER2 inhibitor. This assessment may be advantageously done before the start of treatment with the HER2 inhibitor. Even if a patient has been treated with a HER2 inhibitor, a person skilled in the art can determine whether a person showed no response after the treatment with the HER2 inhibitor. For example, a non-response to an inhibitor may be reflected in an increased suffering from cancer, such as an increased growth of a cancer/tumor and/or increase in the size of a tumor, the (increase in) the formation of metastases or a increase in the number or size of metastases. A non-response may also be the development of a tumor or metastases, for example after resection of a tumor, in the shortening of time to disease progression, or in the increase in the size of (a) tumor(s) and/or (a) metastases, for example in neoadjuvant therapy.

In accordance with the methods provided in the present invention a patient group can be identified that does not respond to treatment with HER2 inhibitors, like Pertuzumab or Trastuzumab. It has been found herein that some individuals with HER2 positive cancer or cancer cells do not adequately respond to treatment with a HER2 inhibitor, if the patients have mutations in exon 9 of PIK3CA. In one embodiment of the present invention, at least 80 %, 90 %, 95% or more of the patient group identified by the herein provided method do not respond to treatment with a HER2 inhibitor. That means that at least 80 % of the identified individuals having the herein described mutation(s) in exon 9 of PIK3CA will not respond to the treatment with the herein defined HER2 inhibitors, like Pertuzumab or Trastuzumab.

As the skilled artisan fully appreciates a positive test for one or more mutations in exon 9 of PIK3CA in a sample of a patient with HER2-positive cancer does not indicate that the patient will not respond to treatment with absolute certainty. However, by the herein provided methods sub-groups of patients are identified that have a lower chance of response (= show a lower response rate) to a treatment with a HER2 inhibitor like pertuzumab or trastuzumab as compared to the sub-groups of patients not having these mutations in exon 9 of PIK3CA. With other words the determination of a presence of one or more mutations in exon 9 of PIK3CA indicates (= is indicative for) that the patient has a lower chance (= probability, likelihood) to respond to treatment with a HER2 inhibitor, as compared to a patient having no mutation in exon 9 of PIK3CA (wild type PIK3CA). Preferably, the response is pathologic complete response (pCR). The term "pCR" as used herein refers to the absence of invasive cancer cells in tissue like breast tissue or absence of invasive tumor cells in tissue like breast

tissue and/or lymph nodes. pCR is commonly used as an endpoint in neoadjuvant treatment such as in breast cancer treatment.

The term "HER2-positive cancer" as used herein refers to a cancer/tumorous tissue etc. which comprises cancer cells which have higher than normal levels of HER2. Examples of HER2-positive cancer include HER2-positive breast cancer and HER2-positive gastric cancer. For the purpose of the present invention, "HER2-positive cancer" has an immunohistochemistry (IHC) score of at least 2+ and/or an *in situ* hybridization (ISH) amplification ratio >2.0 (i.e. is ISH-positive). Accordingly, HER2-positive cancer is present if a high HER2 (protein) expression level detected e.g. by immunohistochemical methods and/or HER2 gene amplification detected by in-situ-hybridization (ISH positive, like a HER2 gene copy number higher than 4 copies of the HER2 gene per tumor cell or ratio of $\geq$ 2.0 for the number of HER2 gene copies to the number of signals for CEP17.) is found in samples obtained from the patients such as breast tissue biopsies or breast tissue resections or in tissue derived from metastatic sites. In one embodiment "HER2-positive cancer" has an immunohistochemistry (IHC) score of HER2(3+) and/or is ISH positive.

The expression level of HER2 may be detected by an immunohistochemical method, whereas said HER2 gene amplification status can be measured with in situ hybridization methods, like fluorescence in situ hybridization techniques (FISH). Corresponding assays and kits are well known in the art, for protein expression assays as well as for the detection of gene amplifications. Alternatively other methods like qRT-PCR might be used to detect levels of HER2 gene expression.

The expression level of HER2 can, inter alia, be detected by an immunohistochemical method. Such methods are well known in the art and corresponding commercial kits are available. Exemplary kits which may be used in accordance with the present invention are, inter alia, HerceptTest™ produced and distributed by the company Dako or the test called Ventana Pathway™. The level of HER2 protein expression may be assessed by using the reagents provided with and following the protocol of the HercepTest™. A skilled person will be aware of further means and methods for determining the expression level of HER2 by immunohistochemical methods; see for example WO 2005/1 17553. Therefore, the expression level of HER2 can be easily and reproducibly determined by a person skilled in the art

without undue burden. However, to ensure accurate and reproducible results, the testing must be performed in a specialized laboratory, which can ensure validation of the testing procedures.

The expression level of HER2 can be classified in a low expression level, an intermediate expression level and a high expression level. It is preferred in context of this invention that HER2-positive disease is defined by a strong expression level of HER2 (e.g. HER2(3+) by IHC), for example determined in a sample of a cancer patient.

The recommended scoring system to evaluate the IHC staining patterns which reflect the expression levels of HER2 designated herein HER2(0), HER2(+), HER2(++) and HER2(+++), is as follows:

Staining Intensity Score	Staining Pattern	HER2 overexpression assessment
0	No staining is observed or membrane staining is observed in < 10 % of the tumor cells	negative
1+	A faint/barely perceptible membrane staining is detected in > 10 % of the tumor cells. The cells are only stained in part of their membrane.	negative
2+	A weak to moderate complete staining is detected in > 10 % of the tumor cells.	weak to moderate overexpression.
3+	A strong complete membrane staining is detected in > 10 % of the tumor cells.	strong overexpression.

The terms HER2(+), HER2(++) and HER2(+++) used herein are equivalent to the terms HER2(1+), HER2(2+) and HER2(3+). A "low protein expression level" used in context of this invention corresponds to a 0 or 1+ score ("negative assessment" according to the table shown herein above), an “weak to moderate protein expression level" corresponds to a *2+* score ("weak to moderate overexpression", see the table above) and a "high protein expression level" corresponds to a 3+ score ("strong overexpression", see the table above). As described herein above in detail, the evaluation of the protein expression level (i.e. the scoring

system as shown in the table) is based on results obtained by immunohistochemical methods. As a standard or routinely, the HER-2 status is, accordingly, performed by immunohistochemistry with one of two FDA-approved commercial kits available; namely the Dako Herceptest™ and the Ventana Pathway™. These are semi-quantitative assays which stratify expression levels into 0 (<20,000 receptors per cell, no expression visible by IHC staining), 1+ (-100,000 receptors per cell, partial membrane staining, < 10% of cells overexpressing HER-2), 2+ (-500,000 receptors per cell, light to moderate complete membrane staining, > 10% of cells overexpressing HER-2), and 3+ (-2,000,000 receptors per cell, strong complete membrane staining, > 10% of cells overexpressing HER-2).

Alternatively, further methods for the evaluation of the protein expression level of HER2 may be used, e.g. Western Blots, ELISA-based detection systems and so on.

A HER2-positive cancer may also be diagnosed by assessing the gene amplification status of HER2. HER2-positive cancer is, accordingly, diagnosed if this assessment by ISH is positive. In accordance with this assessment, a HER2-positive cancer may, inter alia, relate to an average HER2 gene copy number higher than 4 copies of the HER2 gene per tumor cell (for those **test** systems without **an** internal centromere control probe) or to **a** HER2/CEP17 ratio of >=2.0 (for those test systems using an internal chromosome 17 centromere control probe). In other words, the HER2-positive cancer may, inter alia, relate to a HER2 gene copy number greater than 4. The amplification level of the HER2 gene may easily be identified by in situ hybridization (ISH) like fluorescent in situ hybridization (FISH), chromogenic in situ hybridization (CISH) and silver in situ hybridization (SISH). These methods are known to the skilled artisan. The principles of these methods can be deduced from standard text books. Commercial kits for the determination of the HER2 gene amplification status by in situ hybridization are available.

The HER2-positive cancer may be breast cancer or gastric cancer. Further, the HER2-positive cancer may be ovarian cancer, lung cancer, colorectal cancer, kidney cancer, bone cancer, bone marrow cancer, bladder cancer, skin cancer, prostate cancer, esophagus cancer, salivary gland cancer, pancreas cancer, liver cancer, head and neck cancer, CNS (especially brain) cancer, cervix cancer, cartilage cancer, colon cancer, genitourinary cancer, gastrointestinal tract cancer, pancreas cancer, synovium cancer, testis cancer, thymus cancer, thyroid cancer

and uterine cancer. In one embodiment the breast cancer is early-stage breast cancer, as also assessed in the appended example.

The sample to be assessed in accordance with the herein provided methods for identification a non-responder to a HER2 inhbitor may comprise non-diseased cells and/or diseased cells, i.e. non-cancerous cells and/or cancerous cells however the content of cancerous cells among non canerous cells should be higher than 50%. The sample may also (or even solely) comprise cancer/tumor cell(s), such as breast cancer/tumor cell(s). The term "sample" shall generally mean any biological sample obtained from a patient's tumor. The sample may be a tissue resection or a tissue biopsy. The sample may also be a metastatic lesion or a section of a metastatic lesion or a blood sample known or suspected to comprise circulating tumor cells. In accordance with the above, the biological sample may comprise cancer cells and to a certain extent i.e. less than 50% non-cancer cells (other cells). The skilled pathologist is able to differentiate cancer cells from normal tissue cells. Methods for obtaining tissue biopsies, tissue resections and body fluids and the like from mammals, such as humans, are well known in the art.

As mentioned, the sample is obtained from a patient with HER2-positive cancer as defined above. For example, the sample may be obtained from a tumorous tissue, (a) tumor(s) and, accordingly, is (a) tumor cell(s) or (a) tumor tissue(s) suspected of being HER2-positive tumour, like a breast tumor and the like. A person skilled in the art is in the position to identify such tumors and/or individuals/patients suffering from corresponding cancer using standard techniques known in the art and methods disclosed herein. Generally, said tumor cell or cancer cell may be obtained from any biological source/organism, particularly any biological source/organism, suffering from the above-mentioned cancer. In context of this invention particular useful cells are, preferably, human cells. These cells can be obtained from e.g. biopsies or from biological samples. The tumor/cancer/tumor cell/cancer cell is a solid tumor/ cancer/tumor cell/cancer cell. In accordance with the above, the cancer/tumor cell may be a breast cancer/tumor cell or said sample comprises a cancer/tumor cell, such as a breast cancer/tumor cell. In line with the above, said tumor/cancer may be a breast tumor/cancer.

The method for identifying a non-responder to a HER2-inhibitor provided herein may further comprise obtaining a sample of tissue from a patient with HER2-positive cancer prior to said

step of identifying. The tissue may be cancerous tissue. The method may further comprise adjusting the treatment of the patient in response to the presence of said one or more mutations in exon 9.

The identification of non-responders allows for the treatment of patients that do not have the mutations in exon 9 of PIK3CA, as these patients respond well to treatment with HER2 inhibitor(s), such as Pertuzumab. Accordingly, the present invention relates in one embodiment to an inhibitor of HER2 for use in treating a patient with HER2-positive cancer, whereby the the cancer has been found to be PIK3CA mutation-negative in Exon 9 of P1K3CA. Also the use of an inhibitor of HER2 for the preparation of a pharmaceutical composition for the treatment of a HER2-positive cancer patient is envisaged, whereby the the cancer has been found to be PIK3CA mutation-negative.

Further, the present invention relates to the use of a HER2 inhibitor to treat a HER2-positive cancer patient by administering the HER2 dimerization inhibitor in an amount effective to treat the cancer, provided the cancer has been found to be PIK3CA mutation-negative. Accordingly, a method for the treatment of a HER2-positive cancer patient is provided which comprises administering the HER2 dimerization inhibitor in an amount effective to treat the cancer, provided the cancer has been found to be P1K3CA mutation-negative. The term "PIK3CA mutation-negative" as used herein means that the mutations are not present (absent). As mentioned above, the PIK3CA mutation comprises or consists of preferably one or more mutations in exon 9 of Phosphoatidylinositol-3 kinase (PIK3CA) as defined herein above. Preferably, the patient is a human.

In one embodiment of the present invention, the HER2 inhibitor is to be administered as a single anti-tumor agent. In a further embodiment, the inhibitor may be administered in form of a combination therapy, such as chemotherapy, an anti-hormonal therapy and/or another HER2 targeted agent/another HER2 targeted therapy in addition.

The chemotherapy may be docetaxel, anthracycline/taxane chemotherapy, therapy with an anti-metabolite agents, therapy with an anti-hormonal compound, therapy with an anti-estrogen, therapy with a tyrosine kinase inhibitor, therapy with a raf inhibitor, therapy with a ras inhibitor, therapy with a dual tyrosine kinase inhibitor, therapy with taxol, therapy with taxane, therapy with doxorubicin, therapy with adjuvant (anti-) hormone drugs and/or therapy with cisplatin and the like. In accordance with the present invention, the HER2 inhibitor may

be administered by any one of a parenteral route, oral route, intravenous route, subcutaneous route, intranasal route or transdermal route. Further, the HER2 inhibitor may be employed in a neoadjuvant or adjuvant setting. Accordingly, said HER2 inhibitor may be administered to a patient in need of such a treatment and having the herein defined biomarker status before, during or after a surgical intervention/resection of the cancerous tissue. Therefore, the present invention is useful in neoadjuvant therapy, i.e. the treatment with the herein defined HER2 inhibitor (like Pertuzumab or Trastuzumab) given to the herein defined cancer patient group prior to surgery, as well as in adjuvant therapy. Again, the patient group of the present invention to be treated by the means and methods provided herein (in particular with Pertuzumab) are cancer patients without one or more mutations in exon 9 of PIK3CA. The attending physician may modify, change or amend the administration schemes for the HER2 inhibitor in accordance with his/her professional experience.

In one embodiment, the HER2 inhibitor is a HER dimerization/signaiing inhibitor or an inhibitor of HER2 shedding. The HER dimerization inhibitor may be a HER2 dimerization inhibitor. HER dimerization inhibitor inhibits HER heterodimerization or HER homodimerization. The HER dimerization inhibitor may be an anti-HER antibody.

The term "antibody" herein is used in the broadest sense and specifically covers intact monoclonal antibodies, polyclonal antibodies, muitispecific antibodies (e.g., bispecific antibodies) formed from at least two intact antibodies, and antibody fragments, so long as they exhibit the desired biological activity. Also human and humanized as well as CDR-grafted antibodies are comprised.

The HER antibody may bind to a HER receptor selected from the group consisting of EGFR, HER2 and HER3. Preferably, the antibody binds to HER2. In one embodiment, the anti HER2 antibody may bind to domain II of HER2 extracellular domain. In another embodiment, the antibody may bind to a junction between domains I, II and III of IIER2 extracellular domain. Most preferably, the anti HER2 antibody is Pertuzumab.

For the purposes herein, "Pertuzumab" and "rhuMAb 2C4", which are used interchangeably, refer to an antibody comprising the variable light and variable heavy domains (amino acid sequences thereof shown in SEQ ID Nos. 5 and 6, respectively, as depicted in Figure 2). The

variable light and variable heavy domains of variant 574/Pertuzumab are also shown in Figure 2 (amino acid sequences thereof shown in SEQ ID Nos. 7 and 8, respectively, as depicted in Figure 2). Where Pertuzumab is an intact antibody, it preferably comprises an IgGl antibody; in one embodiment comprising the light chain amino acid sequence in it preferably comprises the light chain and heavy chain amino acid sequences in SEQ ID Nos. 11 and 12, respectively, as shown in Figure 3A/3B and 5A/5B (Fig. 5A/5B show the light chain and heavy chain amino acid sequences of a variant Pertuzumab, SEQ ID NO:s 15 and 16, respectively). The heavy chain amino acid sequences of Pertuzumab as shown in SEQ ID NO: 12 (Fig. 3B) may optionally comprise an additional amino acid "K" at position 449 at the C-terminus. The antibody is optionally produced by recombinant Chinese Hamster Ovary (CHO) cells. The terms "Pertuzumab" and "rhuMAb 2C4" herein cover biosimilar versions of the drug with the United States Adopted Name (USAN) or International Nonproprietary Name (INN): Pertuzumab. Again, corresponding sequences are shown in Figures 2 to 5.

In a further embodiment, the inhibitor of HER shedding is a HER2 shedding inhibitor. The inhibitor of HER shedding may inhibit HER heterodimerization or HER homodimerization. Said inhibitor of HER shedding may be an anti-HER antibody. The anti-HER antibody may bind to a HER receptor selected from the group consisting of EGFR, HER2 and HER3. Preferably, the antibody binds to HER2. In one embodiment, the HER2 antibody binds to sub-domain IV of the HER2 extracellular domain. Preferably, the HER2 antibody is Herceptin™ /Trastuzumab.

For the purposes herein, "Trastuzumab" and "rhuMAb4D5-8", which are used interchangeably, refer to an antibody comprising the variable light domains and variable heavy domains (amino acid sequences thereof are shown in Fig 4 in SEQ ID NO: 13 and 14, respectively; the domain is indicated by arrows). Where Trastuzumab is an intact antibody, it preferably comprises an IgGl antibody; in one embodiment comprising the light chain amino acid sequence of SEQ ID NO: 13 and the heavy chain amino acid sequence of SEQ ID NO: 14 as shown in Figure 4. The antibody is optionally produced by Chinese Hamster Ovary (CHO) cells. The terms "Trastuzumab" and "rhuMAb4D5-8" herein cover biosimilar versions of the drug with the United States Adopted Name (USAN) or International Nonproprietary Name (INN): Trastuzumab.

The HER2 positive cancer to be treated may be breast cancer, such early stage breast cancer. The term "early-stage breast cancer" as used herein refers to breast cancer that has not spread beyond the breast or the axilliary lymph nodes. Such cancer is generally treated with neoadjuvant or adjuvant therapy. "Neoadjuvant therapy" refers to systemic therapy given prior to surgery. "Adjuvant therapy" refers to systemic therapy given after surgery. Also other HER2 positive cancer types like gastric cancer can be treated in accordance with the present invention. In one embodiment, the treatment is neoadjuvant or adjuvant therapy of the early-stage breast cancer.

The pharmaceutical composition to be employed in the medical uses of the present invention, will be formulated and dosed in a fashion consistent with good medical practice, taking into account the clinical condition of the individual patient, the site of delivery of the pharmaceutical composition, the method of administration, the scheduling of administration, and other factors known to practitioners. The "effective amount" of the pharmaceutical composition for purposes herein is thus determined by such considerations.

The skilled person knows that the effective amount of pharmaceutical composition administered to an individual will, inter alia, depend on the nature of the compound. For example, if said compound is a (poly)peptide or protein the total pharmaceutically effective amount of pharmaceutical composition administered parenterally per dose will be in the range of about 1 µg protein /kg/day to 10 mg protein /kg/day of patient body weight, although, as noted above, this will be subject to therapeutic discretion. More preferably, this dose is at least 0.01 mg protein /kg/day, and most preferably for humans between about 0.01 and 1 mg protein /kg/day.

The following administration may be employed in respect of Pertuzumab:

A dosing regimen of pertuzumab administered every 3 weeks to patients in Phase II studies (TOC2689g, BO16934) using a fixed 840 mg loading dose (equivalent to 12 mg/kg for a 70 kg patient) for treatment cycle 1 followed by a fixed 420 mg "maintenance" dose (equivalent to 6 mg/kg) for subsequent treatment cycles resulted in steady-state serum trough concentrations of approximately 60 µg/mL by the second treatment cycle. A dose based on body-surface area or weight was not superior to a fixed dose, supporting the continued use of a fixed dose of pertuzumab in female patients with locally advanced, inflammatory or early stage HER2-positive breast cancer, metastatic breast cancer and ovarian cancer.

If given continuously, the pharmaceutical composition is typically administered at a dose rate of about 1 μg/kg/hour to about 50 μg/kg/hour, either by 1-4 injections per day or by continuous subcutaneous infusions, for example, using a mini-pump. An intravenous bag solution may also be employed. The length of treatment needed to observe changes and the interval following treatment for responses to occur appears to vary depending on the desired effect. The particular amounts may be determined by conventional tests which are well known to the person skilled in the art. Pharmaceutical compositions of the invention may be administered orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments, drops or transdermal patch), bucally, or as an oral or nasal spray.

Pharmaceutical compositions of the invention may comprise a pharmaceutically acceptable carrier. By "pharmaceutically acceptable earner" is meant a non-toxïc solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

The pharmaceutical composition is also suitably administered by sustained release systems. Suitable examples of sustained-release compositions include semi-permeable polymer matrices in the form of shaped articles, e.g., f lms, or mirocapsules. Sustained-release matrices include polylactides (U.S. Pat. No. 3,773,919. EP 58,48 1), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate (Sidman, U. et al., Biopolymers 22:547-556 (1983)), poly (2-hydroxyethyl methacrylate) (R. Langer et al., J. Biomed. Mater. Res. 15:167-277 (1981), and R. Langer, Chem. Tech. 12:98-105 (1982)), ethylene vinyl acetate (R. Langer et al., Id.) or poly-D-(-)-3-hydroxybutyric acid (EP 133,988). Sustained release pharmaceutical composition also include liposomally entrapped compound. Liposomes containing the pharmaceutical composition are prepared by methods known per se: DE 3,218,121; Epstein et al., Proc. Natl. Acad. Sci. (USA) 82:3688-3692 (1985); Hwang et al., Proc. Natl. Acad. Sci. (USA) 77:4030-4034 (1980); EP 52,322; EP 36,676; EP 88,046; EP 143,949; EP 142,641; Japanese Pat. **Appl.** 83-1 18008; U.S. Pat. Nos. 4,485,045 and 4,544,545; and EP 102,324. Ordinarily, the liposomes are of the small (about 200-800 Angstroms) unilamellar type in which the lipid content is greater than about 30 mol. percent cholesterol, the selected proportion being adjusted for the optimal therapy.

For parenteral administration, the pharmaceutical composition is formulated generally by mixing it at the desired degree of purity, in a unit dosage injectable form (solution, suspension, or emulsion), with a pharmaceutically acceptable carrier, i.e., one that is non-toxic to recipients at the dosages and concentrations employed and is compatible with other ingredients of the formulation.

Generally, the formulations are prepared by contacting the components of the pharmaceutical composition uniformly and intimately with liquid carriers or finely divided solid carriers or both. Then, if necessary, the product is shaped into the desired formulation. The carrier may be a parenteral carrier, such as a solution that is isotonic with the blood of the recipient. Examples of such carrier vehicles include water, saline, Ringer's solution, and dextrose solution. Non aqueous vehicles such as fixed oils and ethyl oleate are also useful herein, as well as liposomes. The carrier suitably contains minor amounts of additives such as substances that enhance isotonicity and chemical stability. Such materials are non-toxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, succinate, acetic acid, and other organic acids or their salts; antioxidants such as ascorbic acid; low molecular weight (less than about ten residues) (poly)peptides, e.g., polyarginine or tripeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids, such as glycine, glutamic acid, aspartic acid, or arginine; monosaccharides, disaccharides, and other carbohydrates including cellulose or its derivatives, glucose, manose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; counterions such as sodium; and/or nonionic surfactants such as polysorbates, poloxamers, or PEG.

The components of the pharmaceutical composition to be used for therapeutic administration must be sterile. Sterility is readily accomplished by filtration through sterile filtration membranes (e.g., 0.2 micron membranes). Therapeutic components of the pharmaceutical composition generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper picrceable by a hypodermic injection needle.

The components of the pharmaceutical composition ordinarily will be stored in unit or multi-dose containers, for example, sealed ampoules or vials, as an aqueous solution or as a lyophilized formulation for rcconstitution. As an example of a lyophilized formulation, 10-ml vials are filled with 5 ml of sterile-filtered 1% (w/v) aqueous solution, and the resulting

mixture is lyophilized. The infusion solution is prepared by reconstituting the lyophilized compound(s) using bacteriostatic Water-for-Injection.

Another embodiment of the present invention relates to the use of a nucleic acid or antibody capable of detecting a mutation in exon 9 of PIK3CA for identifying a non-responder to a HER2-inhibitor in accordance with the herein provided methods. The oligonucleotide(s) may be about 15 to 100 nucleotides in length.

Accordingly, the present invention relates in one embodiment to a forward primer having the sequence 5'-UAAAAUUUAUUGAGAAUGUAUUUGCTTTTTC-3' (SEQ ID NO: 25). This forward primer can be used in amplification of exon 9 or a part thereof encoding the mutant triplet which encodes position 542 and/or 545 of the herein described mutant exon 9 of PIKC3A. In a further embodiment, the present invention relates to a reverse primer having the sequence 5'-TCCATTTTAGC ACTTACCTGTGAC-3' (SEQ ID NO: 26). This reverse primer can also be used in amplification of exon 9 or a part thereof encoding the mutant triplet which encodes position 542 and/or 545 of the herein described mutant exon 9 of PIKC3A. The present invention provides a primer pair of the forward primer having the sequence S'-UAAAAUUUAUuGAGAAUGUAUUUGCTTTTTC-S' (SEQ ID NO: 25) and the reverse primer having the sequence 5'-TCCATTTTAGC ACTTACCTGTGAC-3' (SEQ ID NO: 26).

In a further embodiment, the present invention relates to probe(s)/probe sequence(s) for evaluating/determining the presence of one or more mutations in exon 9 of PIK3CA. In one embodiment, the present invention relates to a probe having the sequence 5'-FTTTCAQAGAGAGGAUEUEGUGUAGAAAUUGEP-3' ("542 WT Probe") (SEQ ID NO: 27). In one embodiment, the present invention relates to a probe having the sequence 5'-LATTTTQGAGAGAGGAUEUEGUGUAGAAAUUGEUUP-3' (542K Mutation Probe) (SEQ ID NO: 28). In one embodiment, the present invention relates to a probe having the sequence 5'- OCTGCTCAGTQAUUUIAGAGAGAGGATCTCGTGTP-3' (545 WT Probe) (SEQ ID NO: 29). In one embodiment, the present invention relates to a probe having the sequence 5'- JAATCACTAAGQAGGAGAAAGAUUUUEUAUGGAGUEP-3' (545K Mutation Probe) (SEQ ID NO: 30). In one embodiment, the present invention relates to a probe having the sequence 5'- FCTGCGCQGGAGAAAGAUUUUEUAUGGAGUEAP-3'

(545A Mutation Probe) (SEQ ID NO: 31). In one embodiment, the present invention relates to a probe having the sequence 5'-LCCTGCCCQGTGAUUUI AGAGAG AGGATCTCGP-3' (545G Mutation Probe) (SEQ ID NO: 32). In this context, the following abbreviations used: F= FAM Reporter Dye , J = JA270 Reporter Dye, O = CY5.5 Reporter Dye, L = HEX Reporter Dye, U = 5-propynyl dU, E = 5-methyl dC, , I = deoxyinosine, Z = 7-deaza dG, Q = BHQ2 Quencher Dye, P = 3' Phosphate

A person skilled in the art is, based on his general knowledge and the teaching provided herein, in the position to identify and/or prepare further oligo- or polynucleotide(s) for use in the present methods. In particular these oligo- or polynucleotides may be used as probe(s) in the detection methods described herein. A skilled person will know, for example, computer programs which may be useful for the identification of corresponding probes to be used herein. For example, the PIK3CA coding sequence (SEQ ID NO: 1) may be used in this context. Exemplary nucleic acid sequences are available on corresponding databases, such as the NCBI database (www.ncbi.nlm.nih.gov/sites/entrez).

The present invention also relates to a kit useful for carrying out the herein provided methods, the kit comprising a nucleic acid or an antibody capable of detecting a mutation in exon 9 of PIK3CA. Also envisaged herein is the use of the herein described kit for carrying out the herein provided methods.

For example, said kit may comprise (a) compound(s) required for specifically determining the one or more mutations in exon 9 of PIK3CA. Moreover, the present invention also relates to the use of (a) compound(s) required for specifically determining the presence of one or more mutations in exon 9 of PIK3CA for the preparation of a kit for carrying out the methods or uses of this invention.

On the basis of the teaching of this invention, the skilled person knows which compound(s) is (are) required for specifically determining the presence of one or more mutations in exon 9 of PIK3CA. Particularly, such compound(s) may be (a) (nucleotide) probe(s), (a) primer(s) (pair(s)), (an) antibody(ies) and/or (an) aptamer(s) specific to the mutation described herein. The kit (to be prepared in context) of this invention may be a diagnostic kit.

The kit (to be prepared in context) of this invention or the methods and uses of the invention may further comprise or be provided with (an) instruction manual(s). For example, said instruction manual(s) may guide the skilled person (how) to determine one or more mutations in exon 9 of PIK3CA i.e. (how) to diagnose non-responsiveness to a HER2 inhibitor. Particularly, said instruction manual(s) may comprise guidance to use or apply the herein provided methods or uses.

The kit (to be prepared in context) of this invention may further comprise substances/chemicals and/or equipment suitable/required for carrying out the methods and uses of this invention. For example, such substances/chemicals and/or equipment are solvents, diluents and/or buffers for stabilizing and/or storing (a) compound(s) required for specifically determining the presence of a mutation in exon 9 of PIK3CA.

The present invention relates to a method of detecting mutations in the human PI3KCA nucleic acid in a sample comprising:

(a) contacting the nucleic acid in the sample with at least one mutation-specific oligonucleotide from Table 2;

(b) incubating the sample under conditions allowing hybridization of the oligonucleotide to the target sequence within the PI3KCA nucleic acid;

(c) detecting the hybridization thereby detecting the presence of the mutation in the PI3KCA nucleic acid.

Table2: Probe Sequences for the PIK3CA Mutation Detection Test

Probe	Sequence 5′ to 3′
542 WT Probe	FTTTCAQAGAGAGGAUEUEGUGUAGAAAUUGEP (SEQ ID NO: 27)
542 542K Mutation Probe	LATTTTQGAGAGAGGAUEUEGUGUAGAAAUUGEUUP (SEQ ID NO: 28)
545 WT Probe	OCTGCTCAGTQAUUUIAGAGAGAGGATCTCGTGTP (SEQ ID NO: 29)
545 545K Mutation Probe	JAATCACTAAGQAGGAGAAAGAUUUUEUAUGGAGUEP (SEQ ID NO: 30)
545 545A Mutation Probe	FCTGCGCQGGAGAAAGAUUUUEUAUGGAGUEAP (SEQ ID NO: 31)
545 545G Mutation Probe	LCCTGCCCQGTGAUUUIAGAGAGAGGATCTCGP (SEQ ID NO: 32)

Key: F = FAM Reporter Dye , J = JA270 Reporter Dye, O = CY5.5 Reporter Dye, L = HEX Reporter Dye, U = 5-propynyl dU, E = 5-methyl dC, , I = deoxyinosine, Z = 7-deaza dG, Q = BHQ2 Quencher Dye, P = 3 'Phosphate

The present invention relates to a method of detecting mutations in the human PI3KCA nucleic acid in a sample comprising:

(a) contacting the nucleic acid in the sample with one or more of the following mutation-specific oligonucleotides:

LATTTTQGAGAGAGGAUEUEGUGUAGAAAUUGEUUP (SEQ ID NO: 28) (542 542K Mutation Probe);

JAATCACTAAGQAGGAGAAAGAUUUUEUAUGGAGUEP (SEQ ID NO: 30) (545 545K Mutation Probe);

FCTGCGCQGGAGAAAGAUUUUEUAUGGAGUEAP (SEQ ID NO: 31) (545 545A Mutation Probe); and/or

LCCTGCCCQGTGAUUUIAGAGAGAGGATCTCGP (SEQ ID NO: 32) (545 545G Mutation Probe);

(b) incubating the sample under conditions allowing hybridization of the one or more oligonucleotide to the target sequence within the PI3KCA nucleic acid;

(c) detecting the hybridization thereby detecting the presence of the mutation in the PI3KCA nucleic acid.

The method may further comprise, prior to detection in step (c), contacting the nucleic acid in the sample with at least one oligonucleotide from Table I and generating an amplification product containing the target sequence within the PI3KCA nucleic acid. Table 1: Primer Sequences for the PIK3CA Mutation Detection Test

Primer	Sequence 5′ to 3′
542/545 Forward primer PIK3CA-9F13	UAAAAUUUAUUGAGAAUGUAUUUGCTTTTTC (SEQ ID NO: 25)
542/545 Reverse primer PIK3CA-9R01	TCCATTTTAGCACTTACCTGTGAC (SEQ ID NO: 26)

Key: U = 5-propynyl dU

The present invention relates to a method of detecting mutations in the human PI3KCA nucleic acid in a sample comprising:

(a) contacting the nucleic acid in the sample with at least one mutation-specific oligonucleotide from Table 2;

(b) (i) incubating the sample under conditions allowing hybridization of the oligonucleotide to the target sequence within the PI3KCA nucleic acid;

(ii) contacting the nucleic acid in the sample with at least one oligonucleotide from Table 1

(iii) generating an amplification product containing the target sequence within the PI3KCA nucleic acid;

(c) detecting the hybridization thereby detecting the presence of the mutation in the PI3KCA nucleic acid.

In accordance with the above, the present invention provides a method of detecting mutations in the human PI3KCA nucleic acid in a sample comprising:

(a) contacting the nucleic acid in the sample with with one or more of the following mutation-specific oligonucleotides:

LATTTTQGAGAGAGGAUEUEGUGUAGAAAUUGEUUP (SEQ ID NO: 28) (542 542K Mutation Probe);

JAATCACTAAGQAGGAGAAAGAUUUUEUAUGGAGUEP (SEQ ID NO: 30) (545 545K Mutation Probe);

FCTGCGCQGGAGAAAGAUUUUEUAUGGAGUEAP (SEQ ID NO: 31) (545 545A Mutation Probe); and/or

LCCTGCCCQGTGAUUUIAGAGAGAGGATCTCGP (SEQ ID NO: 32) (545 545G Mutation Probe):

(b) (i) incubating the sample under conditions allowing hybridization of the oligonucleotide to the target sequence within the PI3KCA nucleic acid;

(ii) contacting the nucleic acid in the sample with one or both of the following oligonucleotides:

UAAAAUUUAUUGAGAAUGUAUUUGCTTTTTC (SEQ ID NO: 25) (542/545 Forward primer PIK3CA-9F 13) and/or TCCATTTTAGCACTTACCTGTGAC (SEQ ID NO: 26) (542/545 Reverse primer PIK3CA-9R01);

(iii) generating an amplification product containing the target sequence within the PI3KCA nucleic acid;

(c) detecting the hybridization thereby detecting the presence of the mutation in the PI3KCA nucleic acid.

The present invention provides a method of determining whether a patient having a malignant tumor is likely to respond to a HER2-inhibitor, comprising:

(a) contacting the nucleic acid in the sample from the patient with the oligonucleotide from Table 2;

(b) incubating the sample under conditions allowing hybridization of the oligonucleotide to the target sequence within the PI3KCA nucleic acid;

(c) detecting the hybridization thereby detecting the presence of the mutation in the PI3KCA nucleic acid, wherein the presence of the mutation indicates that the patient is likely to respond to the HER2 inhibitor.

Table2: Probe Sequences for the PIK3CA Mutation Detection Test

Probe	Sequence 5′ to 3′
542 WT Probe	FTTTCAQAGAGAGGAUEUEGUGUAGAAAUUGEP (SEQ ID NO: 27)
542 542K Mutation Probe	LATTTTQGAGAGAGGAUEUEGUGUAGAAAUUGEUUP (SEQ ID NO: 28)
545 WT Probe	OCTGCTCAGTQAUUUIAGAGAGAGGATCTCGTGTP (SEQ ID NO: 29)
545 545K Mutation Probe	JAATCACTAAGQAGGAGAAAGAUUUUEUAUGGAGUEP (SEQ ID NO: 30)
545 545A Mutation Probe	FCTGCGCQGGAGAAAGAUUUUEUAUGGAGUEAP (SEQ ID NO: 31)
545 545G Mutation Probe	LCCTGCCCQGTGAUUUIAGAGAGAGGATCTCGP (SEQ ID NO: 32)

Key: F-- FAM Reporter Dye , J = JA270 Reporter Dye, O = CY5.5 Reporter Dye, L = HEX Reporter Dye, U = 5-propynyl dU, E = 5-methyl dC, , I = deoxyinosine, Z = 7-deaza dG, Q = BH02 Quencher Dye, P = 3 'Phosphate

The method may further comprise, prior to detection in step (c), contacting the nucleic acid in the sample with at least one oligonucleotide from Table 1 and generating an amplification product containing the target sequence within the PI3KCA nucleic acid.

Table 1: Primer Sequences for the PIK3CA Mutation Detection Test

Primer	Sequence 5′ to 3′
542/545 Forward primer PIK3CA-9F13	UAAAAUUUAUUGAGAAUGUAUUUGCTTTTTC (SEQ ID NO: 25)
542/545 Reverse primer PIK3CA-9R01	TCCATTTTAGCACTTACCTGTGAC (SEQ ID NO: 26)

Key: U = 5-propynyl dU

The present invention provides a method of determining whether a patient having a malignant tumor is less likely to respond to a HER2-inhibitor, comprising:

(a) contacting the nucleic acid in the sample from the patient with with one or more of the following mutation-specific oligonucleotides:

LATTTTQGAGAGAGGAUEUEGUGUAGAAAUUGEUUP (SEQ ID NO: 28) (542 542K Mutation Probe);

JAATCACTAAGQAGGAGAAAGAUUUUEUAUGGAGUEP (SEQ ID NO: 30) (545 545K Mutation Probe);

FCTGCGCQGGAGAAAGAUUUUEUAUGGAGUEAP (SEQ ID NO: 31) (545 545A Mutation Probe); and/or

LCCTGCCCQGTGAUUUIAGAGAGAGGATCTCGP (SEQ ID NO: 32) (545 545G Mutation Probe);

(b) incubating the sample under conditions allowing hybridization of the oligonucleotide to the target sequence within the PI3KCA nucleic acid;

(c) detecting the hybridization thereby detecting the presence of the mutation in the PI3KCA nucleic acid, wherein the presence of the mutation indicates that the patient is less likely to respond to the HER2 inhibitor.

The present invention relates to a method of determining whether a patient having a malignant tumor is less likely to respond to a HER2-inhibitor, comprising:

(a) contacting the nucleic acid in the sample from the patient with with one or more of the following mutation-specific oligonucleotides:

LATTTTQGAGAGAGGAUEUEGUGUAGAAAUUGEUUP (SEQ ID NO: 28) (542 542K Mutation Probe);

JAATCACTAAGQAGGAGAAAGAUUUUEUAUGGAGUEP (SEQ ID NO: 30) (545 545K Mutation Probe);

FCTGCGCQGGAGAAAGAUUUUEUAUGGAGUEAP (SEQ ID NO: 31) (545 545A Mutation Probe); and/or

LCCTGCCCQGTGAUUUIAGAGAGAGGATCTCGP (SEQ ID NO: 32) (545 545G Mutation Probe):

(b) (i) incubating the sample under conditions allowing hybridization of the oligonucleotide to the target sequence within the PI3KCA nucleic acid;

(ii) contacting the nucleic acid in the sample with one or both of the following oligonucleotides:

UAAAAUUUAUUGAGAAUGUAUUUGCTTTTTC (SEQ ID NO: 25) (542/545 Forward primer PIK3CA-9F 13) and/or TCCATTTTAGCACTTACCTGTGAC (SEQ ID NO: 26) (542/545 Reverse primer PIK3CA-9R01);

(iii) generating an amplification product containing the target sequence within the PI3KCA nucleic acid;

(c) detecting the hybridization thereby detecting the presence of the mutation in the PI3KCA nucleic acid, wherein the presence of the mutation indicates that the patient is less likely to respond to the HER2 inhibitor.

The present invention provides a method for identifying a non-responder to a HER2-inhibitor, said method comprising

a) detecting the presence of one or more mutations in exon 9 of the catalytic subunit of Phosphoinositol-3 kinase (PIK3CA or p i 10a) nucleic acid in a sample from an individual,

(b)) identifying the patient as less likely to respond to a HER.2 inhibitor if the presence of one or more mutations in exon 9 of the catalytic subunit of Phosphoinositol-3 kinase (PIK3CA or p1 10a) nucleic acid is detected.

The present invention provides a method for identifying a non-responder to a HER2-inhibitor, said method comprising detecting the presence of one or more mutations in exon 9 of the catalytic subunit of Phosphoinositol-3 kinase (PIK3CA or p i 10a) nucleic acid by

(a) contacting the nucleic acid in the sample from a patient with HER2-positive cancer with a oligonucleotide comprising a sequence from Table 2;

(b) incubating the sample under conditions allowing hybridization of the oligonucleotide to the target sequence within the P1K3CA nucleic acid;

(c) detecting hybridization

(d) identifying the patient as less likely to respond to a HER2 inhibitor if the presence of one or more mutations in exon 9 of the catalytic subunit of Phosphoinositol-3 kinase (PIK3CA or p i 10a) nucleic acid is detected.

Table2: Probe Sequences for the PIK3C A Mutation Detection Test

Probe	Sequence 5'to 3'
542 WT Probe	FTTTCAQAGAGAGGAUEUEGUGUAGAAAUUGEP (SEQ ID NO: 27)
542 542K Mutation Probe	LATTTTQGAGAGAGGAUEUEGUGUAGAAAUUGEUUP (SEQ ID NO: 28)

Probe	Sequence 5' to 3'
545 WT Probe	OCTGCTCAGTQAUUUIAGAGAGAGGATCTCGTGTP (SEQ ID NO: 29)
545 545K Mutation Probe	JAATCACTAAGQAGGAGAAAGAUUUUEUAUGGAGUEP (SEQ ID NO: 30)
545 545A Mutation Probe	FCTGCGCQGGAGAAAGAUUUUEUAUGGAGUEAP (SEQ ID NO: 31)
545 545G Mutation Probe	LCCTGCCCQGTGAUUUIAGAGAGAGGATCTCGP (SEQ ID NO: 32)

Key: F = FAM Reporter Dye , J = JA270 Reporter Dye, O = CY5.5 Reporter Dye, L = HEX Reporter Dye, U = 5-propynyl dU, E = 5-methyl dC, , I = deoxyinosine, Z = 7-deaza dG, Q = BHQ2 Quencher Dye, P = 3' Phosphate

The term "non-responder" as used herein can refer to a "patient who is less likely to respond" "Less likely to respond" as used herein refers to a decreased likeliness that a pathological complete response (pcR) will occur in a patient treated with a HER2 inhibitor..

The present invention relates to a method for identifying a non-responder to a HER2-inhibitor, said method comprising detecting the presence of one or more mutations in exon 9 of the catalytic subunit of Phosphoinositol-3 kinase (PIK3CA or pi 10a) nucleic acid by

(a) contacting the nucleic acid in the sample from a patient with HER2-positive cancer with one or more of the following mutation-specific oligonucleotides:
LATTTTQGAGAGAGGAUEUEGUGUAGAAAUUGEUUP (SEQ ID NO: 28) (542 542K Mutation Probe);
JAATCACTAAGQAGGAGAAAGAUUUUEUAUGGAGUEP (SEQ ID NO: 30) (545 545K Mutation Probe);
FCTGCGCQGGAGAAAGAUUUUEUAUGGAGUEAP (SEQ ID NO: 31) (545 545A Mutation Probe); and/or
LCCTGCCCQGTGAUUUIAGAGAGAGGATCTCGP (SEQ ID NO: 32) (545 545G Mutation Probe);

(b) incubating the sample under conditions allowing hybridization of the oligonucleotide to the target sequence within the PIK3CA nucleic acid;

(c) detecting hybridization

(d) identifying the patient as less likely to respond to a HER2 inhibitor if the presence of one or more mutations in exon 9 of the catalytic subunit of Phosphoinositol-3 kinase (PIK3CA or pi 10a) nucleic acid is detected.

The method can further comprise, prior to detection in step (c), contacting the nucleic acid in the sample with at least one oligonucleotide from Table 1 and generating an amplification product containing the target sequence within the PI3KCA nucleic acid.

Table 1: Primer Sequences for the PIK3CA Mutation Detection Test

Primer	Sequence 5′ to 3′
542/545 Forward primer PIK3CA-9F13	UAAAAUUUAUUGAGAAUGUAUUUGCTTTTTC (SEQ ID NO: 25)
542/545 Reverse primer PIK3CA-9R01	TCCATTTTAGCACTTACCTGTGAC (SEQ ID NO: 26)

Key: U = 5-propynyl dU

The present invention relates to a method for identifying a non-responder to a HER2-inhibitor, said method comprising detecting the presence of one or more mutations in exon 9 of the catalytic subunit of Phosphoinositol-3 kinase (PIK3CA or p110a) nucleic acid by

(a) contacting the nucleic acid in the sample from a patient with HER2-positive cancer with one or more of the following mutation-specific oligonucleotides:

LATTTTQGAGAGAGGAUEUEGUGUAGAAAUUGEUUP (SEQ ID NO: 28) (542 542K Mutation Probe);

JAATCACTAAGQAGGAGAAAGAUUUUEUAUGGAGUEP (SEQ ID NO: 30) (545 545K Mutation Probe);

FCTGCGCQGGAGAAAGAUUUUEUAUGGAGUEAP (SEQ ID NO: 31) (545 545A Mutation Probe); and/or

LCCTGCCCQGTGAUUUIAGAGAGAGGATCTCGP (SEQ ID NO: 32) (545 545G Mutation Probe);

(b) (i) incubating the sample under conditions allowing hybridization of the oligonucleotide to the target sequence within the PI3KCA nucleic acid;

(ii) contacting the nucleic acid in the sample with one or both of the following oligonucleotides:

UAAAAUUUAUUGAGAAUGUAUUUGCTTTTTC (SEQ ID NO: 25) (542/545 Forward primer PIK3CA-9F13) and/or TCCATTTTAGCACTTACCTGTGAC (SEQ ID NO: 26) (542/545 Reverse primer PIK3CA-9R01);

(iii) generating an amplification product containing the target sequence within the PI3KCA nucleic acid;

(c) detecting hybridization

(d) identifying the patient as less likely to respond to a HER2 inhibitor if the presence of one or more mutations in exon 9 of the catalytic subunit of Phosphoinositol-3 kinase (PIK3CA or p i 10a) nucleic acid is detected.

The present invention is further illustrated by reference to the following non-limiting figures and examples.

The Figures show:

Figure 1.

Figure 1 provides a schematic of the HER2 protein structure, and amino acid sequences for Domains i-IV (SEQ ID Nos. 1-4, respectively) of the extracellular domain thereof.

Figure 2,

Figures 2A and 2B depict alignments of the amino acid sequences of the variable light (VL) (Fig. 2A) and variable heavy (V_H) (Fig. 2B) domains of murine monoclonal antibody 2C4 (SEQ ID Nos. 5 and 6, respectively); V_L and V H domains of variant 574/Pertuzumab (SEQ ID Nos. 7 and 8, respectively), and human V L and V H consensus frameworks (hum κï, light kappa subgroup I; humlll, heavy subgroup III) (SEQ ID Nos. 9 and 10, respectively). Asterisks identify differences between variable domains of Pertuzumab and murine monoclonal antibody 2C4 or between variable domains of Pertuzumab and the human framework. Complementarity Determining Regions (CDRs) are in brackets.

Figure 3.

Figures 3A and 3B show the amino acid sequences of Pertuzumab light chain (Fig. 3A; SEQ ID NO. 11) and heavy chain (Fig. 3B; SEQ ID No. 12). CDRs are shown in bold. Calculated molecular mass of the light chain and heavy chain are 23,526.22 Da and 49,216.56 Da (cysteines in reduced form). The carbohydrate moiety is attached to Asn 299 of the heavy chain.

Figure 4.

Figures 4A and 4B show the amino acid sequences of Trastuzumab light chain (Fig. 4A; SEQ ID NO. 13) and heavy chain (Fig. 4B; SEQ ID NO. 14), respectively. Boundaries of the variable light and variable heavy domains are indicated by arrows.

Figure 5.

Figures 5A and 5B depict a variant Pertuzumab light chain sequence (Fig. 5A; SEQ ID NO. 15) and a variant Pertuzumab heavy chain sequence (Fig. 5B; SEQ ID NO. 16), respectively.

Figure 6.

Figure 6 shows Results of PIK3CA mutational analyses. PIK3CA mutations were in general associated with decreased sensitivity to HER2-targeted therapy in NeoSphere (The NeoSphere study is described in detail e.g. in Lancet Oncol. 2012 Jan;13(l):25-32. doi: 10.1016/S1470-2045(11)70336-9. Epub 2011 Dec 6). Analyses per Exon i.e. Exons 7, 9 and 20 was carried out to explore in more detail the impact of specific mutations. Exon 9 mutations: Out of 28 mutations detected across the 4 arms, 26 were found to be in the non-responder group. Exon 20 mutations had little impact on pCR. Too few exon 7 mutations to draw conclusions. TH = Patients treated with docetaxel (75-»100 mg/m^2) and trastuzumab (8—»6 mg/kg), THP = Patients treated with docetaxel (75—»100 mg/m^2), trastuzumab (8-»6 mg/kg) and pertuzumab (840—>420 mg), HP = Patients treated with trastuzumab (8—>6 mg/kg) and pertuzumab (840—>420 mg), TP= Patients treated with docetaxel (75—→100 mg/m^2) and pertuzumab (840→420 mg).

Figure 7.

Figure 7 shows the PIK3CA gene and the protein sequence aligned. Exon9 is annotated with ***. The wild-type triplets encoding positions E542 and E545 of the wild-type amino acid sequence are indicated in bold letters.

Example. Identification of non-responders to HER2 inhibitors by determining the presence of mutations in exon 9 of PIK3CA.

Each standard 50-µL amplification reaction targeting one of the Exons 7, 9 or 20 included 100 ng genomic DNA, dNTPs (including dUTP), 0.05 U/µL Z05, DNA polymerase, 0.04 U/µL uracil-DNA glycosylase (UNG), and 200-400 nM forward and reverse primer (Table 1), 75-200 nM mutant and wild-type specific probes (Table 2). Amplification was performed in the cobas® 4800 analyzer using the following temperature profile: 5 min at 50°C followed by 55 cycles of 95°C for 10 sec and 63°C for 50 sec, followed by a single round of 40°C for 2 min and 25°C for 10 sec (melting curve analysis). Fluorescence data was collected during each amplification cycle and during the final melting curve analysis.

Table A: /'rimer Sequences for the PIK3CA Mutation Detection Test

Primer	Sequence 5′ to 3′
Codon 420 Forward Primer PIK3CA-7F03	UUUUGGGGAAGAAAAGUGUUUUGAA (SEQ ID NO: 33)
Codon 420 Reverse primer PIK3CA-7R04	GATTCAAAGCCATTTTTCCAGATACTAGA (SEQ ID NO: 34)
Codon 542/545 Forward primer PIK3CA-9F13	UAAAAUUUAUUGAGAAUGUAUUUGCTTTTTC (SEQ ID NO: 25)
Codon 542/545 Reverse primer PIK3CA-9R01	TCCATTTTAGCACTTACCTGTGAC (SEQ ID NO: 26)
Codon 1047 Forward primer PIK3CA-20F01	GAGGCTTTGGAGTATTTCATGAA (SEQ ID NO: 35)
Codon 1047 Reverse primer PIK3CA-20R01	CCAATCCATTTTTGTTGTCCA (SEQ ID NO: 36)

Key: U = 5-propynyl d U

Table B: Probe Sequences for the PIK3CA Mutation Detection Test

Probe	Sequence 5' to 3'
Codon 420 WT Probe	JCAATGGACAGQGUUEEUUAAAAAAEAAAGAAAAAUAUUP (SEQ ID NO: 37)
Codon 420 420R Mutation Probe	OGAACACCQTCCAUUGGEAUGGGGAAAUAUAAAP (SEQ ID NO: 38)
Codon 542 WT Probe	FTTTCAQAGAGAGGAUEUEGUGUAGAAAUUGEP (SEQ ID NO: 27)
Codon 542 542K Mutation Probe	I_**ATTTTQ**GAGAGAGGAUEUEGUGUAGAAAUUGEUUP (SEQ ID NO: 28)
Codon 545 WT Probe	OCTGCTCAGTQAUUUIAGAGAGAGGATCTCGiGiP (SEQ ID NO: 29)
Codon 545 545K Mutation Probe	JAATCACTAAGQAGGAGAAAGAUUUUEUAUGGAGUEP (SEQ ID NO: 30)
Codon 545 545A Mutation Probe	FCTGCGCQGGAGAAAGAUUUUEUAUGGAGUEAP (SEQ ID NO: 31)

Probe	Sequence 5' to 3'
Codon 545 545G Mutation Probe	LCCTGCCCQGTGAUUUIAGAGAGAGGATCTCGP (SEQ ID NO: 32)
Codon 1047 WT Probe	FTZCACATCQTZZTZZCTZZACAACAAP (SEQ ID NO: 39)
Codon 1047 1047R Mutation Probe	LGACGTQCAUEAUUEAUUUGUUUEAUGP (SEQ ID NO: 40)
Codon 1047 1047L Mutation Probe	JGCACTTCATGQTGGCTGGACAACAAAAAP (SEQ ID NO: 41)
Codon 1047 1047Y Mutation Probe	OACCATGATATQCAUEAUUEAUUUGUUUEP (SEQ ID NO: 42)

Key: F = FAM Reporter Dye , J = JA270 Reporter Dye, O = CY5.5 Reporter Dye, L = HEX Reporter Dye, U = 5-propynyl dU, E = 5-methyl dC, , I = deoxyinosine, Z = 7-deaza dG, Q = BHQ2 Quencher Dye, P = 3 'Phosphate

Figure 6 shows Results of PIK3CA mutational analyses. PIK3CA mutations were in general associated with decreased sensitivity to HER2-targeted therapy in NeoSphere. Analyses per exon i.e. exons 7, 9 and 20 was carried out to explore in more detail the impact of specific mutations. For exon 9 mutations, out of 28 mutations detected across the 4 arms, 26 were found to be in the non-responder group. Exon 20 mutations had little impact on pCR. There were too few exon 7 mutations to draw conclusions.

While the invention has been described in detail with reference to specific examples, it will be apparent to one skilled in the art that various modifications can be made within the scope of this invention. Thus the scope of the invention should not be limited by the examples described herein, but by the claims presented below.

The present invention refers to the following nucleotide and amino acid sequences:
The sequences provided herein are available in the NCBI database and can be retrieved from www.ncbi.nlm.nih.go v/sites/entrez?db=gene ; Theses sequences also relate to annotated and modified sequences. The present invention also provides techniques and methods wherein homologous sequences, and variants of the concise sequences provided herein are used. Preferably, such "variants' ' are genetic variants.

SEQ ID No. 1:
Nucleotide sequence encoding homo sapiens phosphoinositide-3 -kinase, catalytic, alpha (PIK3CA), (NCBI accession number: NG_0121 13.1 GT237858742).

SEQ ID No. 2:

Amino acid sequence of homo sapiens phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit. The sequence can be retrieved from the NCBI database under accession number NP_006209.2 GL54792082 or from Uniprot database under accession number >sp|P42336|.

Positions 542 and 545 are indicated in bold letters.

MPPRPSSGELWGIHLMPPRILVECLLPNGMIVTLECLREATLITIKHELFKEAPvKYPLH
Q
LLQDESSYIFVSVTQEAEPvEEFFDETRRLCDLRLFQPFLKVIEPVGNREEKILNREIGFA
IGMPVCEFDMVKDPEVQDFRRNILNVCKEAVDLRDLNSPHSRAMYVYPPNVESSPEL
PKH
lYNKLDKGQIIVVIWVIVSPNNDKQKYTLKINHDCVPEQVIAEAIRKKTRSMLLSSEQL
K
LCVLEYQGKYILKVCGCDEYFLEKYPLSQYKYIRSCIMLGRMPNLMLMAKESLYSQL
PMD
CFTMPSYSRRISTATPYMNGETSTKSLWVINSALRIKILCATYVNVNIRDIDKIYVRTGI
YHGGEPLCDNW,TQRVPCSNPRWEWLNYDIYIPDLPRAARLCLSICSVKGRKGAKE
EHC
PLAWGNINLFDYTDTLVSGKMALNLWPVPHGLEDLLNPIGVTGSNPNKETPCLELEF
DWF
SSVVKFPDMSVIEEHANWSVSREAGFSYSHAGLSNRLARDNELRENDKEQLKAISTR
DPL
SEITEQEKDFLWSHRHYCVTIPEILPKLLLSVKWNSRDEVAQMYCLVKDWPPIKPEQ
AME
LLDCNYPDPMVRGFAVRCLEKYLTDDKLSQYLIQLVQVLKYEQYLDNLLVRFLLKK
ALTN
QRIGHFFFWHLKSEMHNKTVSQRFGLLLESYCRACGMYLKHLNRQVEAMEKLINLT
DILK
QEKKDETQKVQMKFLVEQMRRPDFMDALQGFLSPLNPAHQLGNLRLEECRIMSSAK
RPLW
LNWENPDIMSELLFQNNEIIFKNGDDLRQDMLTLQIIRIMENIWQNQGLDLRMLPYGC
LS

IGDCVGLIEVVRNSHTIMQIQCKGGLKGALQFNSHTLHQWLKDKNKGEIYDAAIDLF
TRS
CAGYCVATFILGIGDRHNSNIMVKDDGQLFHIDFGHFLDHKKKKFGYKRERVPFVLT
QDF
LIVISKGAQECTKTREFERFQEMCYKAYLAIRQHANLFINLFSMMLGSGMPELQSFDD
IA
YIRKTLALDKTEQEALEYFMKQMNDAHHGGWTTKMDWIFHTIKQHALN

SEQ ID No. 3:

Nucleotide sequence encoding exon 9 of homo sapiens phosphoinositide-3-kinase, catalytic, alpha (PIK3CA).

AGTAACAGACTAGCTAGAGACAATGAATTAAGGGAAAATGACAAAGAACAGCTC
AAAGCAATTTCTACACGAGATCCTCTCTCTGAAATCACTGAGCAGGAGAAAGATT
TTCTATGGAGTCACAG

SEQ ID No. 4:

Amino acid sequence encoded by exon 9 of homo sapiens phosphoinositide-3-kinase, catalytic, alpha (PIK3CA).

SEQ ID No. 5:

Amino acid sequence of the variable light (VL) (Fig. 2A) domain of murine monoclonal antibody 2C4 (SEQ ID Nos. 5 and 6, respectively) as shown in Figure 2.

SEQ ID No. 6:

Amino acid sequence of the variable heavy (VH) (Fig. 2B) domain of murine monoclonal antibody 2C4 as shown in Figure 2.

SEQ ID No. 7:

Amino acid sequence of the variable light (V_L) (Fig. 2A) domain of variant 574/Pertuzumab as shown in Figure 2.

SEQ ID No. 8:

Amino acid sequence of the variable heavy (V_H) (Fig. 2B) domain of variant 574/Pertuzumab as shown in Figure 2.

SEQ ID No. 9:

human V_L consensus frameworks (hum κï, light kappa subgroup I; humlll, heavy subgroup III) as shown in Figure 2.

SEQ ID No. 10:

human VH consensus frameworks (hum κï, light kappa subgroup I; humlll, heavy subgroup III) as shown in Figure 2.

SEQ ID No. 11:

Amino acid sequences of Pertuzumab light chain as shown in Figure 3A.

SEQ ID No. 12:

Amino acid sequences of Pertuzumab heavy chain as shown in Figure 3B.

SEQ ID No. 13:

Amino acid sequence of Trastuzumab light chain domain as shown in Fig. 4A. Boundaries of the variable light domain are indicated by arrows.

SEQ ID No. 14:

Amino acid sequence of Trastuzumab heavy chain as shown in Fig. 4B. Boundaries of the variable heavy domain are indicated by arrows.

SEQ ID No. 15:

Amino acid sequence of variant Pertuzumab light chain sequence (Fig. 5A).

SEQ ID No. 16:

Amino acid sequence of variant Pertuzumab heavy chain sequence (Fig. 5B).

SEQ ID No. 17:

Nucleotide sequence encoding exon 9 of homo sapiens E542K mutant of phosphoinositide-3-kinase, catalytic, alpha (PIK3CA). The triplet (codon) encoding the mutant amino acid "K" at position 542 of the full-length amino acid sequence of PIKC3CA (see SEQ ID NO: 2 and Figure 7) is highlighted in bold letters.

AGTAACAGACTAGCTAGAGACAATGAATTAAGGGAAAATGACAAA
GAACAGCTCAAAGCAATTTCTACACGAGATCCTCTCTCTAAAATCACTGAG
CAGGAGAAAGATTTTCTATGGAGTCACAG

SEQ ID No. 18:

Amino acid sequence of homo sapiens E542K mutant of phosphoinositide-3-kinase, catalytic, alpha (PIK3CA). Position 542 is highlighted in bold. The mutant shows an increase in lipid kinase activity; oncogenic in vivo; occurs in the interface between the PIK3CA helical domain and the nSH2 (N-terminal SH2) region of the p85 regulatory subunit and may reduce the inhibitory effect of p85; requires interaction with RAS to induce cellular transformation.

```
MPPRPSSGEL WGIHLMPPRI LVECLLPNGM IVTLECLREA TLITIKHELF KEARKYPLHQ

70         80         90        100        110        120

LLQDESSYIF VSVTQEAERE EFFDETRRLC DLRLFQPFLK VIEPVGNREE KILNREIGFA

130        140        150        160        170        180

IGMPVCEFDM VKDPEVQDFR RNILNVCKEA VDLRDLNSPH SRAMYVYPPN VESSPELPKH

190        200        210        220        230        240

IYNKLDKGQI IVVIWVIVSP NNDKQKYTLK INHDCVPEQV IAEAIRKKTR SMLLSSEQLK

250        260        270        280        290        300

LCVLEYQGKY ILKVCGCDEY FLEKYPLSQY KYIRSCIMLG RMPNLMLMAK ESLYSQLPMD

310        320        330        340        350        360

CFTMPSYSRR ISTATPYMNG ETSTKSLWVI NSALRIKILC ATYVNVNIRD IDKIYVRTGI

370        380        390        400        410        420

YHGGEPLCDN VNTQRVPCSN PRWNEWLNYD IYIPDLPRAA RLCLSICSVK GRKGAKEEHC
```

430 440 450 460 470 480

PLAWGNINLF DYTDTLVSGK MALNLWPVPH GLEDLLNPIG VTGSNPNKET PCLELEFDWF

490 500 510 520 530 540

SSVVKFPDMS VIEEHANWSV SREAGFSYSH AGLSNRLARD NELRENDKEQ LKAI STRDPL

550 560 570 580 590 600

SKI TEQEKDF LWSHRHYCVT I PEILPKLLL SVKWNSRDEV AQMYCLVKDW PPIKPEQAME

610 620 630 640 650 660

LLDCNYPDPM VRGFAVRCLE KYLTDDKLSQ YL IQLVQVLK YEQYLDNLLV RFLLKKALTN

670 680 690 700 710 720

QRIGHFFFWH LKSEMHNKTV SQR.FGLLLES YCPACGMYLK HLNR.QVEAME KLINLTDILK

730 740 750 760 770 780

QEKKDETQKV QMKFLVEQMR RPDFMDALQG FLS PLNPAHQ LGNLRLEECR IMS SAKRPLW

790 800 810 820 830 840

LNWENPDIMS ELLFQNNEI I FKNGDDLRQD MLTLQI I RIM ENIWQMQGLD LRMLPYGCLS

850 860 870 880 890 900

I GDCVGLI EV VRNS HT IMQI QCKGGLKGAL QFNSHTLHQW LKDKNKGEI Y DAAI DLFTRS

910 920 930 940 950 960

CAGYCVATFI LGIGDRHNSN IMVKDDGQLF HI DFGHFLDH KKKKFGYKRE RVPFVLTQDF

970 980 990 1000 1010 1020

LIVI SKGAQE CTKTREFERF QEMCYKAYLA IRQHANLFIN LFSMMLGSGM PELQS FDDIA

1030 1040 1050 1060

YI RKTLALDK TEQEALEYFM KQMNDAHHGG WTTKMDWI FH TI KQHALN

SEQ ID No. 19 :

Nucleotide sequence encoding exon 9 of homo sapiens E545K mutant of phosphoinositide-3-kinase, catalytic, alpha (PIK3CA). The triplet (codon) encoding the mutant amino acid "K" at position 545 of the full-length amino acid sequence of PIKC3CA (see SEQ ID NO: 2 and Figure 7) is highlighted in bold letters.

AGTAACAGACTAGCTAGAGACAATGAATTAAGGGAAAATGACAAA
GAACAGCTCAAAGCAATTTCTACACGAGATCCTCTCTCTGAAATCACTAAG
CAGGAGAAAGATTTTCTATGGAGTCACAG

SEQ ID No. 20:

Amino acid sequence of homo sapiens E545K mutant of phosphoinositide-3 -kinase, catalytic, alpha (PIK3CA). Position 545 is highlighted in bold. The mutant shows an increase in lipid kinase activity; oncogenic in vivo; occurs in the interface between the PIK3CA helical domain and the nSH2 (N-terminal SH2) region of the p85 regulatory subunit and may reduce the inhibitory effect of p85; requires interaction with RAS to induce cellular transformation; enhances invadopodia-mediated extracellular matrix degradation and invasion in breast cancer cells.

```
MPPRPSSGEL  WGIHLMPPRI  LVECLLPNGM  IVTLECLREA  TLITIKHELF  KEARKYPLHQ
  70          80          90          100         110         120

LLQDESSYIF  VSVTQEAERE  EFFDETRRLC  DLRLFQPFLK  VIEPVGNREE  KILNREIGFA
  130         140         150         160         170         180

IGMPVCEFDM  VKDPEVQDFR  RNILNVCKEA  VDLRDLNSPH  SRAMYVYPPN  VESSPELPKH
  190         200         210         220         230         240

IYNKLDKGQI  IWIWVIVSP   NNDKQKYTLK  INHDCVPEQV  IAEAIRKKTR  SMLLSSEQLK
  250         260         270         280         290         300

LCVLEYQGKY  ILKVCGCDEY  FLEKYPLSQY  KYIRSCIMLG  RMPNLMLMAK  ESLYSQLPMD
  310         320         330         340         350         360

CFTMPSYSRR  ISTATPYMNG  ETSTKSLWVI  NSALRIKILC  ATYVNVNIRD  IDKIYVRTGI
  370         380         390         400         410         420

YHGGEPLCDN  VNTQRVPCSN  PRWNEWLNYD  IYIPDLPRAA  RLCLSICSVK  GRKGAKEEHC
  430         440         450         460         470         480

PLAWGNINLF  DYTDTLVSGK  MALNLWPVPH  GLEDLLNPIG  VTGSNPNKET  PCLELEFDWF
  490         500         510         520         530         540
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SSVVKFPDMS VIEEHANWSV SREAGFSYSH AGLSNRLARD NELRENDKEQ LKAISTRDPL
  550         560         570         580         590         600

SEITKQEKDF LWSHRHYCVT IPEILPKLLL SVKWNSRDEV AQMYCLVKDW PPIKPEQAME
  610         620         630         640         650         660

LLDCNYPDPM VRGFAVRCLE KYLTDDKLSQ YLIQLVQVLK YEQYLDNLLV RFLLKKALTN
  670         680         690         700         710         720

QRIGHFFFWH LKSEMHNKTV SQRFGLLLES YCRACGMYLK HLNRQVEAME KLINLTDILK
  730         740         750         760         770         780

QEKKDETQKV QMKFLVEQMR RPDFMDALQG FLSPLNPAHQ LGNLRLEECR IMSSAKRPLW
  790         800         810         820         830         840

LNWENPDIMS ELLFQNNEI I FKNGDDLRQD MLTLQI IRIM ENIWQNQGLD LRMLPYGCLS
  850         860         870         880         890         900

IGDCVGLIEV VRNSHTIMQI QCKGGLKGAL QFNSHTLHQW LKDKNKGEIY DAAIDLFTRS
  910         920         930         940         950         960

CAGYCVATFI LGIGDRHNSN IMVKDDGQLF HIDFGHFLDH KKKKFGYKRE RVPFVLTQDF
  970         980         990        1000        1010        1020

LIVISKGAQE CTKTREFERF QEMCYKAYLA IRQHANLFIN LFSMMLGSGM PELQSFDDIA
 1030        1040        1050        1060

YIRKTLALDK TEQEALEYFM KQMNDAHHGG WTTKMDWIFH TIKQHALN
```

SEQ ID No.21 :

Nucleotide sequence encoding encoding exon 9 of homo sapiens E545A mutant of phosphoinositide-3 -kinase, catalytic, alpha (PIK3CA). The triplet (codon) encoding the mutant amino acid "A" at position 545 of the full-length amino acid sequence of PIKC3CA (see SEQ ID NO: 2 and Figure 7) is highlighted in bold letters.

AGTAACAGACTAGCTAGAGACAATGAATTAAGGGAAAATGACAAA
GAACAGCTCAAAGCAATTTCTACACGAGATCCTCTCTCTGAAATCACTGCG
CAGGAGAAAGATTTTCTATGGAGTCACAG

SEQ ID No. 22:

Amino acid sequence of homo sapiens E545A mutant of phosphoinositide-3 -kinase, catalytic, alpha (PIK3CA). Position 545 is highlighted in bold.

```
MPPRPSSGEL WGIHLMPPRI LVECLLPNGM IVTLECLREA TLITIKHELF KEARKYPLHQ
  70          80          90         100         110         120

LLQDESSYIF VSVTQEAERE EFFDETRRLC DLRLFQPFLK VIEPVGNREE KILNREIGFA
 130         140         150         160         170         180
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IGMPVCEFDM VKDPEVQDFR RNILNVCKEA VDLRDLNSPH SRAMYVYPPN VESSPELPKH
  190        200        210        220        230        240

IYNKLDKGQI IVVIWVIVSP NNDKQKYTLK INHDCVPEQV IAEAIRKKTR SMLLSSEQLK
  250        260        270        280        290        300

LCVLEYQGKY ILKVCGCDEY FLEKYPLSQY KYIRSCIMLG RMPNLMLMAK ESLYSQLPMD
  310        320        330        340        350        360

CFTMPSYSRR ISTATPYMNG ETSTKSLWVI NSALRIKILC ATYVNVNIRD IDKIYVRTGI
  370        380        390        400        410        420

YHGGEPLCDN VNTQRVPCSN PRWNEWLNYD IYIPDLPRAA RLCLSICSVK GRKGAKEEHC
  430        440        450        460        470        480

PLAWGNINLF DYTDTLVSGK MALNLWPVPH GLEDLLNPIG VTGSNPNKET PCLELEFDWF
  490        500        510        520        530        540

SSVVKFPDMS VIEEHANWSV SREAGFSYSH AGLSNRLARD NELRENDKEQ LKAISTRDPL
  550        560        570        580        590        600

SEITAQEKDF LWSHRHYCVT IPEILPKLLL SVKWNSRDEV AQMYCLVKDW PPIKPEQAME
  610        620        630        640        650        660

LLDCNYPDPM VRGFAVRCLE KYLTDDKLSQ YLIQLVQVLK YEQYLDNLLV RFLLKKALTN
  670        680        690        700        710        720

QRIGHFFFWH LKSEMHNKTV SQRFGLLLES YCRACGMYLK HLNRQVEAME KLINLTDILK
  730        740        750        760        770        780

QEKKDETQKV QMKFLVEQMR RPDFMDALQG FLSPLNPAHQ LGNLRLEECR IMSSAKRPLW
  790        800        810        820        830        840

LNWENPDIMS ELLFQNNEII FKNGDDLRQD MLTLQIIRIM ENIWQNQGLD LRMLPYGCLS
  850        860        870        880        890        900

IGDCVGLIEV VRNSHTIMQI QCKGGLKGAL QFNSHTLHQW LKDKNKGEIY DAAIDLFTRS
  910        920        930        940        950        960

CAGYCVATFI LGIGDRHNSN IMVKDDGQLF HIDFGHFLDH KKKKFGYKRE RVPFVLTQDF
  970        980        990        1000       1010       1020

LIVISKGAQE CTKTREFERF QEMCYKAYLA IRQHANLFIN LFSMMLGSGM PELQSFDDIA
  1030       1040       1050       1060

YIRKTLALDK TEQEALEYFM KQMNDAHHGG WTTKMDWIFH TIKQHALN
```

SEQ ID No. 23:

Nucleotide sequence encoding exon 9 of homo sapiens E545G mutant of phosphoinositide-3-kinase, catalytic, alpha (PIK3CA). The triplet (codon) encoding the mutant amino acid "G" at position 545 of the full-length amino acid sequence of PIKC3CA (see SEQ ID NO: 2 and Figure 7) is highlighted in bold letters.

AGTAACAGACTAGCTAGAGACAATGAATTAAGGGAAAATGACAAA
GAACAGCTCAAAGCAATTTCTACACGAGATCCTCTCTCTGAAATCACTGGG
CAGGAGAAAGATTTTCTATGGAGTCACAG

SEQ ID No. 24:

Amino acid sequence of homo sapiens E545G mutant of phosphoinositide-3-kinase, catalytic, alpha (PIK3CA). Position 545 is highlighted in bold.

```
MPPRPSSGEL WGIHLMPPRI LVECLLPNGM IVTLECLREA TLITIKHELF KEARKYPLHQ
  70         80         90         100        110        120

LLQDESSYIF VSVTQEAERE EFFDETRRLC DLRLFQPFLK VIEPVGNREE KILNREIGFA
  130        140        150        160        170        180

IGMPVCEFDM VKDPEVQDFR RNILNVCKEA VDLRDLNSPH SRAMYVYPPN VESSPELPKH
  190        200        210        220        230        240

lYNKLDKGQI IVVIWVIVSP NNDKQKYTLK INHDCVPEQV IAEAIRKKTR SMLLSSEQLK
  250        260        270        280        290        300

LCVLEYQGKY ILKVCGCDEY FLEKYPLSQY KYIRSCIMLG RMPNLMLMAK ESLYSQLPMD
  310        320        330        340        350        360

CFTMPSYSRR ISTATPYMNG ETSTKSLWVI NSALRIKILC ATYVNVNIRD IDKIYVRTGI
  370        380        390        400        410        420

YHGGEPLCDN VNTQRVPCSN PRWNEWLNYD IYIPDLPRAA RLCLSICSVK GRKGAKEEHC
  430        440        450        460        470        480

PLAWGNINLF DYTDTLVSGK MALNLWPVPH GLEDLLNPIG VTGSNPNKET PCLELEFDWF
  490        500        510        520        530        540

SSVVKFPDMS VIEEHANWSV SREAGFSYSH AGLSNRLARD NELRENDKEQ LKAISTRDPL
  550        560        570        580        590        600

SEITGQEKDF LWSHRHYCVT IPEILPKLLL SVKWNSRDEV AQMYCLVKDW PPIKPEQAME
  610        620        630        640        650        660

LLDCNYPDPM VRGFAVRCLE KYLTDDKLSQ YLIQLVQVLK YEQYLDNLLV RFLLKKALTN
  670        680        690        700        710        720

QRIGHFFFWH LKSEMHNKTV SQRFGLLLES YCRACGMYLK HLNRQVEAME KLINLTDILK
  730        740        750        760        770        780

QEKKDETQKV QMKFLVEQMR RPDFMDALQG FLSPLNPAHQ LGNLRLEECR IMSSAKRPLW
  790        800        810        820        830        840

LNWENPDIMS ELLFQNNEII FKNGDDLRQD MLTLQIIRIM ENIWQNQGLD LRMLPYGCLS
  850        860        870        880        890        900

IGDCVGLIEV VRNSHTIMQI QCKGGLKGAL QFNSHTLHQW LKDKNKGEIY DAAIDLFTRS
  910        920        930        940        950        960
```

```
CAGYCVATFI  LGIGDRHNSN  IMVKDDGQLF  HIDFGHFLDH  KKKKFGYKRE  RVPFVLTQDF
  970         980         990         1000        1010        1020

LIVISKGAQE  CTKTREFERF  QEMCYKAYLA  IRQHANLFIN  LFSMMLGSGM  PELQSFDDIA
  1030        1040        1050        1060

YIRKTLALDK  TEQEALEYFM  KQMNDAHHGG  WTTKMDWIFH  TIKQHALN
```

All references cited herein are fully incorporated by reference. Having now fully described the invention, it will be understood by a person skilled in the art that the invention may be practiced within a wide and equivalent range of conditions, parameters and the like, without affecting the spirit or scope of the invention or any embodiment thereof.

CLAIMS

1. A method for identifying a non-responder to a HER2-inhibitor, said method comprising evaluating the presence of one or more mutations in exon 9 of the catalytic subunit of Phosphoinositol-3 kinase (PIK3CA or p110a) in a sample from a patient with HER2-positive cancer; and
whereby the presence of one or more mutations in exon 9 indicates non-responsiveness of said patient to said IIER2 inhibitor.

2. The method of claim 1, wherein the HER2-positive cancer is breast cancer.

3. The method of claim 2, wherein the breast cancer is early-stage breast cancer.

4. The method of any one of claims 1 to 3, wherein the HER2 inhibitor is an anti-HER2 antibody.

5. The method of claim 4, wherein said anti-HER2 antibody is pertuzumab.

6. The method of any one of claims 1 to 4, wherein exon 9 mutations are the only PIK3CA mutations evaluated.

7. The method of any one of claims 1 to 6, wherein the mutation is the replacement or exchange of an amino acid as compared to the wild-type sequence of exon 9 of Phosphoinositol-3 kinase (PIK3CA).

8. The method of any one of claims 1 to 7, wherein the mutation is at position 542 and/or 545 of exon 9 of Phosphoinositol-3 kinase (PIK3CA).

9. The method of claim any one of claims 1 to 8, wherein the mutation is one or more of the mutations E542K E545K, E545A and E545G in exon 9 of Phosphoinositol-3 kinase (PIK3CA).

10. The method of claim 9, wherein said mutation E542K is shown in SEQ ID NO. 17, wherein said mutation E545K is shown in SEQ ID NO: 19, wherein said mutation E545A is shown in SEQ ID NO: 21 and/or wherein said mutation E545G is shown in SEQ ID NO: 23.

11. The method of any one of claims 1 to 10, wherein said sample comprises a breast cancer cancer/tumor cell.

12. The method of any one of claims 1 to 11, wherein at least 80 % of the patient group identified by the method of any of claims 1 tol l do not respond to treatment with a modulator of the HER2/neu (ErbB2) signaling pathway.

13. The method of claim 12, wherein the response is pathologic complete response (pCR).

14. The method of any one of claims 1 to 13, wherein said sample is selected from the group consisting of breast tissue resection, breast tissue biopsy, metastatic lesion and circulating tumor cells.

15. The method of any one of claims 1 to 14, further comprising detecting the presence of one or more mutations in exon 9 of the catalytic subunit of Phosphoinositol-3 kinase (PIK3CA or pi 10a) nucleic acid by

(a) contacting the nucleic acid in the sample from a patient with HER2-positive cancer with one or more of the following mutation-specific oligonucleotides:
LATTTTQGAGAGAGGAUEUEGUGUAGAAAUUGEUUP (SEQ ID NO: 28) (542 542K Mutation Probe);
JAATCACTAAGQAGGAGAAAGAUUUUEUAUGGAGUEP (SEQ ID NO: 30) (545 545K Mutation Probe):
FCTGCGCQGGAGAAAGAUUUUEUAUGGAGUEAP (SEQ ID NO: 31) (545 545A Mutation Probe); and/or

LCCTGCCCQGTGAUUUIAGAGAGAGGATCTCGP (SEQ ID NO: 32) (545 545G Mutation Probe);

(b) incubating the sample under conditions allowing hybridization of the oligonucleotide to the target sequence within the P1K3CA nucleic acid;

(c) detecting hybridization; and

(d) identifying the patient as less likely to respond to a HER2 inhibitor if the presence of one or more mutations in exon 9 of the catalytic subunit of Phosphoinositol-3 kinase (PIK3CA or p i 10a) nucleic acid is detected.

16. The method of claim 15, wherein step (b) further comprises the steps (i) contacting the nucleic acid in the sample with one or both of the following oligonucleotides:
UAAAAUUUAUUGAGAAUGUAUUUGCTTTTTC (SEQ ID NO: 25) (542/545 Forward primer PIK3CA-9F13) and/or TCCATTTTAGCACTTACCTGTGAC (SEQ ID NO: 26) (542/545 Reverse primer PIK3CA-9R01);
(ii) generating an amplification product containing the target sequence within the PI3KCA nucleic acid.

17.. An inhibitor of HER2 for use in treating a patient with HER2-positive cancer, whereby the the cancer has been found to be P1K3CA mutation-negative in Exon 9of PIK3CA

18.. Use of an inhibitor of HER2 for the preparation of a pharmaceutical composition for the treatment of a HER2-positive cancer patient, whereby the the cancer has been found to be PIK3CA mutation-negative.

19.. Use of HER2 inhibitor to treat a HER2-positive cancer patient by administering the HER2 dimerization inhibitor in an amount effective to treat the cancer, provided the cancer has been found to be PIK3CA mutation-negative.

20.. A method for the treatment of a HER2-positive cancer patient comprising administering the HER2 dimerization inhibitor in an amount effective to treat the cancer, provided the cancer has been found to be PIK3CA mutation-negative.

21.. The inhibitor of claim 17, or the use of claim 18 or 19 or , or the method of claim 20, wherein the PIK3CA mutation comprises one or more mutations in exon 9 of Phosphoinositol-3 kinase (PIK3CA) as defined in claims 7 to 10.

22. The inhibitor of claim 17 or 21, or the use of any one of claims 18, 19 and 21, or the method of any one of claims 1 to 16, 20 and 21 , wherein said patient is a human.

23. The method of any one of claims 1 to 16, and 20 to 22, The inhibitor of any one of claims 17, 21 and 22, or the use of any one of claims 18, 19, 21 and 22 , wherein said HER2 inhibitor is to be administered as a single anti-tumor agent.

24. The method of any one of claims 1 to 16, and 20 to 22, the inhibitor of any one of claims 17, 21 and 22, or the use of any one of claims 18, 19, 21 and 22, wherein said HER2 inhibitor is to be administered in form of a combination therapy.

25. The method of claim 24, or the inhibitor of claim 24, or the use of claim 24, wherein the therapy used in said combination therapy is chemotherapy or an anti-hormonal therapy or another HER2 targeted therapy in addition.

26. The method of claim 25, or the inhibitor of claim 25, or the use of claim 25, wherein said chemotherapy is selected from the group consisting of docetaxel, anthracycline/'taxane chemotherapy, therapy with an anti-metabolite agents, therapy with an anti-hormonal compound, therapy with an anti-estrogen, therapy with a tyrosine kinase inhibitor, therapy with a raf inhibitor, therapy with a ras inhibitor, therapy with a dual tyrosine kinase inhibitor, therapy with taxol, therapy with taxane, therapy with doxorubicin, therapy with adjuvant (anti-) hormone drugs, therapy with cisplatin and the like.

27. The method of any one of claims 1 to 16, and 20 to 26, the inhibitor of any one of claims 17, 21 and 22 to 26, or the use of any one of claims 18, 19, and 21 to 26, wherein said HER2 inhibitor is to be administered by any one of a parenteral route, oral route, intravenous route, subcutaneous route, intranasal route or transdermal route.

28. The method of any one of claims 1 to 16, and 20 to 27, the inhibitor of any one of claims 17, 21 and 22 to 27, or the use of any one of claims 18, 19, and 21 to 27, wherein said HER2 inhibitor is to be administered in a neoadjuvant or adjuvant setting.

29. The method of any one of claims 1 to 16, and 20 to 28, the inhibitor of any one of claims 17, 21 and 22 to 28, or the use of any one of claims 18, 19, and 21 to 28, wherein said HER2 inhibitor is a HER dimerization/signaling inhibitor or an inhibitor of HER2 shedding.

30. The method of claim 29, or the inhibitor of claim 29, or the use of claim 29, wherein said HER dimerization inhibitor is a HER2 dimerization inhibitor.

31. The method of claim 29 or 30, or the inhibitor of claim 29 or 30, or the use of claim 29 or 30, wherein said HER dimerization inhibitor inhibits HER heterodimcrization or HER homodimerization.

32. The method of any one of claim 29 to 31, or the inhibitor of any one of claims 29 to 31, or the use of any one of claim 29 to 31, wherein said HER dimerization inhibitor is a anti HER antibody.

33. The method of claim 32, or the inhibitor of claim 32, or the use of claim 32, wherein said HER antibody binds to a HER receptor selected from the group consisting of EGFR, HER2 and HER3.

34. The method of claim 33, or the inhibitor of claim 33, or the use of claim 33, wherein said antibody binds to HER2.

35. The method of claim 34, or the inhibitor of claim 34, or the use of claim 34, wherein said anti HER2 antibody binds to domain II of HER2 extracellular domain.

36. The method of claim 35, or the inhibitor of claim 35, or the use of claim 35, wherein said antibody binds to a junction between domains I, II and III of HER2 extracellular

domain.

37. The method of any one of claims 32 to 36, or the inhibitor of claim 32 to 36, or the use of any one of claims 32 to 36, wherein said anti HER2 antibody is Pertuzumab.

38. The method of any one of claims 1 to 16, and 20 to 28, the inhibitor of any one of claims 17, 21 and 22 to 28, or the use of any one of claims 18, 19, and 21 to 28, wherein said inhibitor of HER shedding is a HER2 shedding inhibitor.

39. The method of claim 38, or the inhibitor of claim 38, or the use of claim 38, wherein said inhibitor of HER shedding inhibits HER heterodimerization or HER homodimerization.

40. The method of claim 38 or 39, or the inhibitor of claim 38 or 39, or the use of claim 38 or 39, wherein said inhibitor of HER shedding is a anti HER antibody.

41. The method of claim 40, or the inhibitor of claim 40, or the use of claim 40, wherein said HER antibody binds to a HER receptor selected from the group consisting of EGFR, HER2 and HER3.

42. The method of claim 41, or the inhibitor of claim 41, or the use of claim 41, wherein said antibody binds to HER2.

43. The method of claim 42, or the inhibitor of claim 42, or the use of claim 42, wherein said HER2 antibody binds to sub-domain IV of the HER2 extracellular domain.

44. The method of any one of claims 38 to 43, or the inhibitor of claim 38 to 43, or the use of any one of claims 38 to 43, wherein said HER2 antibody is Herceptin™ /Trastuzumab.

45. The method of any one of claims 1 to 16, and 20 to 244, the inhibitor of any one of claims 17, 21 and 22 to 28, or the use of any one of claims 18, 19, and 21 to 28, wherein the HER2-positive cancer is breast cancer.

46. The method of claim 45, or the inhibitor of claim 45, or the use of claim 45, wherein said breast cancer is early stage breast cancer.

47. The method of claim 46, or the inhibitor of claim 46, or the use of claim 46, wherein the treatment is neoadjuvant or adjuvant therapy of the early-stage breast cancer.

Figure 1.

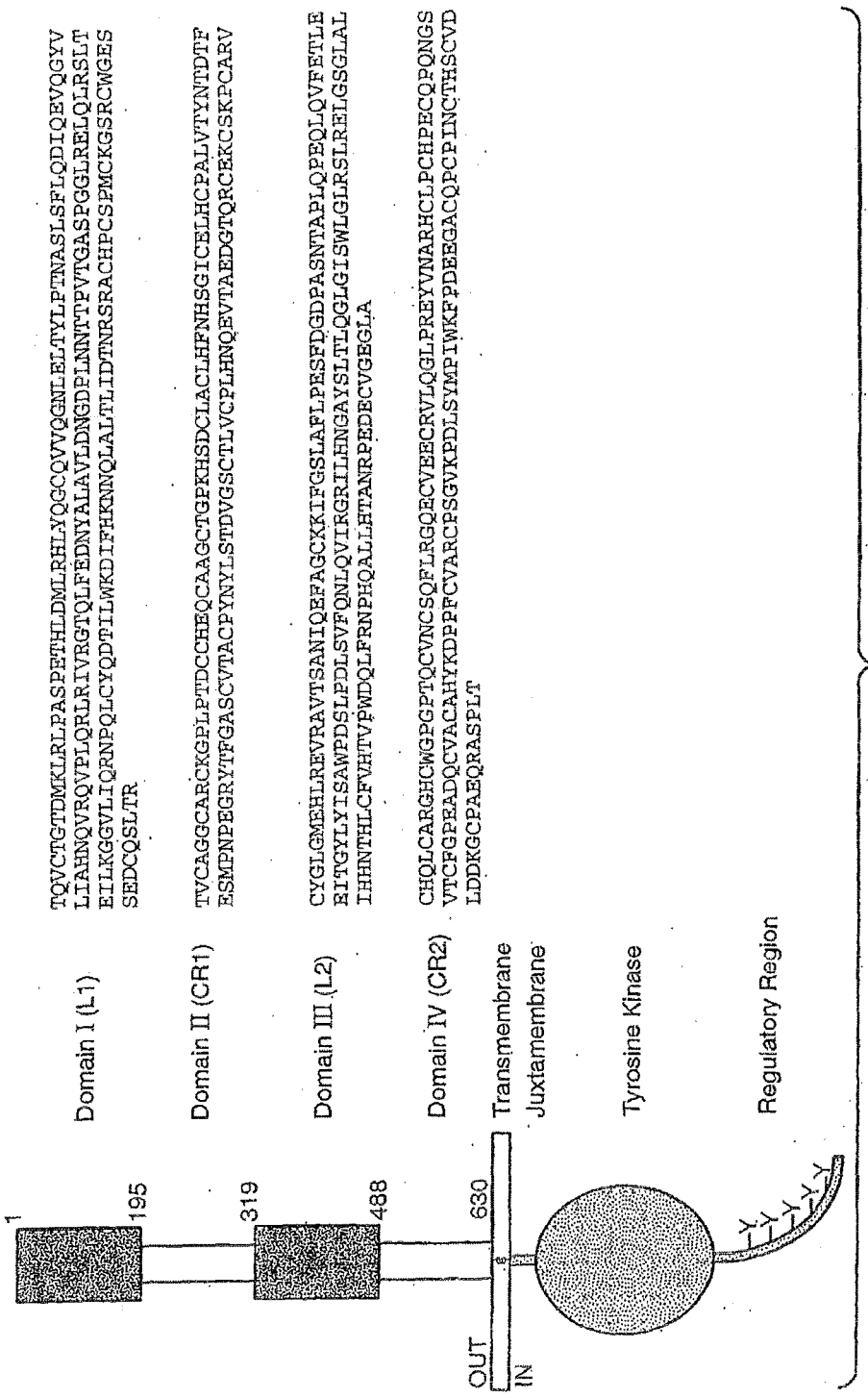

1
195
319
488
630
Domain I (L1)
Domain II (CR1)
Domain III (L2)
Domain IV (CR2)
Transmembrane
Juxtamembrane
Tyrosine Kinase
Regulatory Region
OUT
IN
TQVCTGTDMKLRLPASPETHLDMLRHLYQGCQVVQGNLELTYLPTNASLSFLQDIQEVQGYV
LIAHNQVRQVPLQRLRIVRGTQLFEDNYALAVLDNGDPLNNTTPVTGASPGGLRELQLRSLT
EILKGGVLIQRNPQLCYQDTILWKDIFHKNNQLALTLIDTNRSRACHPCSPMCKGSRCWGES
SEDCQSLTR
TVCAGGCARCKGPLPTDCCHEQCAAGCTGPKHSDCLACLHFNHSGICELHCPALVTYNTDTF
ESMPNPEGRYTFGASCVTACPYNYLSTDVGSCTLVCPLHNQEVTAEDGTQRCEKCSKPCARV
CYGLGMEHLREVRAVTSANIQEFAGCKKIFGSLAFLPESFDGDPASNTAPLQPEQLQVFETLE
EITGYLYISAWPDSLPDLSVFQNLQVIRGRILHNGAYSLTLQGLGISWLGLRSLRELGSGLAL
IHHNTHLCFVHTVPWDQLFRNPHQALLHTANRPEDECVGEGLA
CHQLCARGHCWGPGPTQCVNCSQFLRGQECVEECRVLQGLPREYVNARHCLPCHPECQPQNGS
VTCFGPEADQCVACAHYKDPPFCVARCPSGVKPDLSYMPIWKFPDEEGACQPCPINCTHSCVD
LDDKGCPAEQRASPLT
FIG. 1

Figure 2.

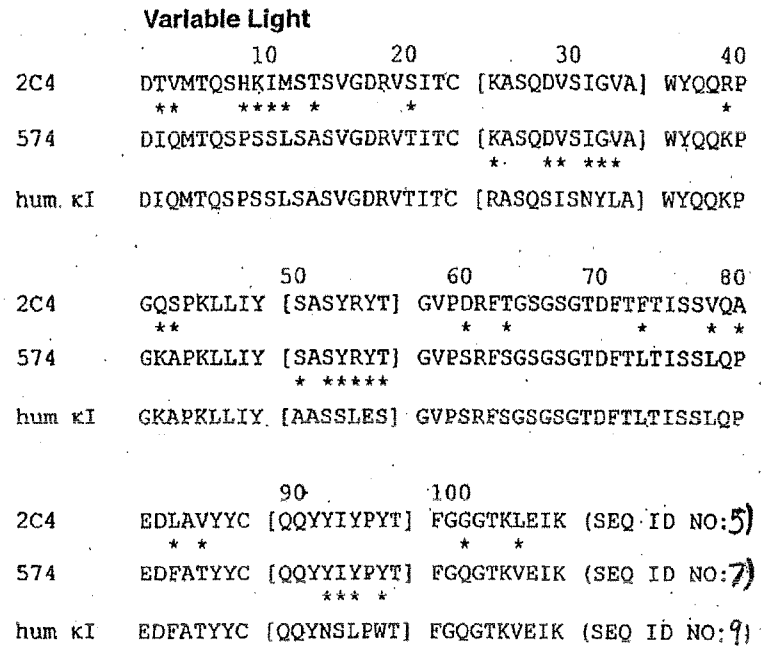


FIG. 2A

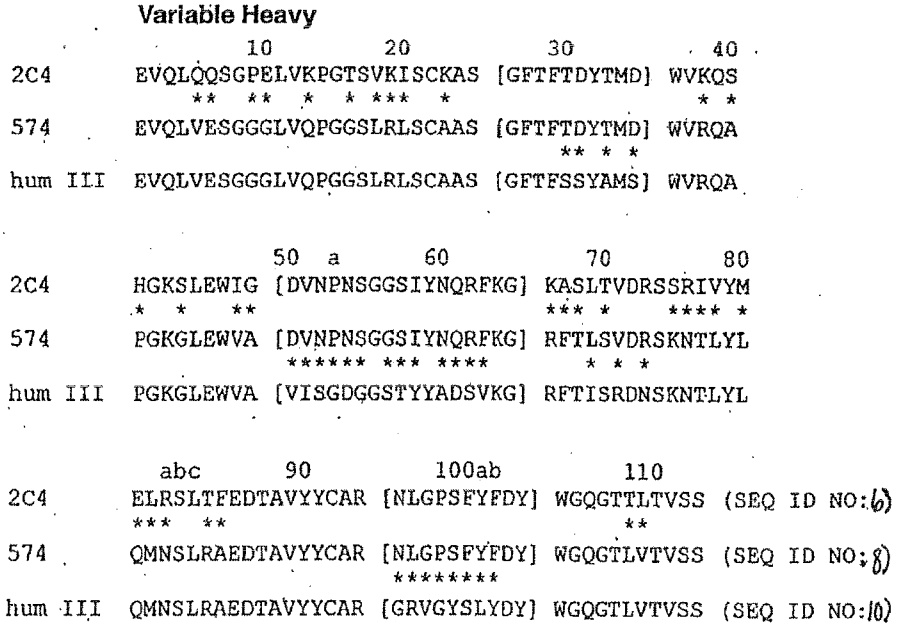


FIG. 2B

Figure 3.

Amino Acid Sequence for Pertuzumab Light Chain

```
1         10        20        30        40        50        60
DIQMTQSPSSLSASVGDRVTITC KASQDVSIGVA WYQQKPGKAPKLLIY SASYRYT GVPS
          70        80        90        100       110       120
RFSGSGSGTDFTLTISSLQPEDFATYYC QQYYIYPYT FGQGTKVEIKRTVAAPSVFIFPP
          130       140       150       160       170       180
SDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLT
          190       200       210
LSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
```

FIG. 3A

Amino Acid Sequence for Pertuzumab Heavy Chain

```
1         10        20        30        40        50        60
EVQLVESGGGLVQPGGSLRLSCAAS GFTFTDYTMD WVRQAPGKGLEWVA DVNPNSGGSIY
          70        80        90        100       110       120
NQRFK GRFTLSVDRSKNTLYLQMNSLRAEDTAVYYCARN LGPSFYFDY WGQGTLVTVSSA
          130       140       150       160       170       180
STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
          190       200       210       220       230       240
LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGP
          250       260       270       280       290       300
SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS
          310       320       330       340       350       360
TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEM
          370       380       390       400       410       420
TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ
          430       440       448
QGNVFSCSVMHEALHNHYTQKSLSLSPG
```

FIG. 3B

Figure 4.

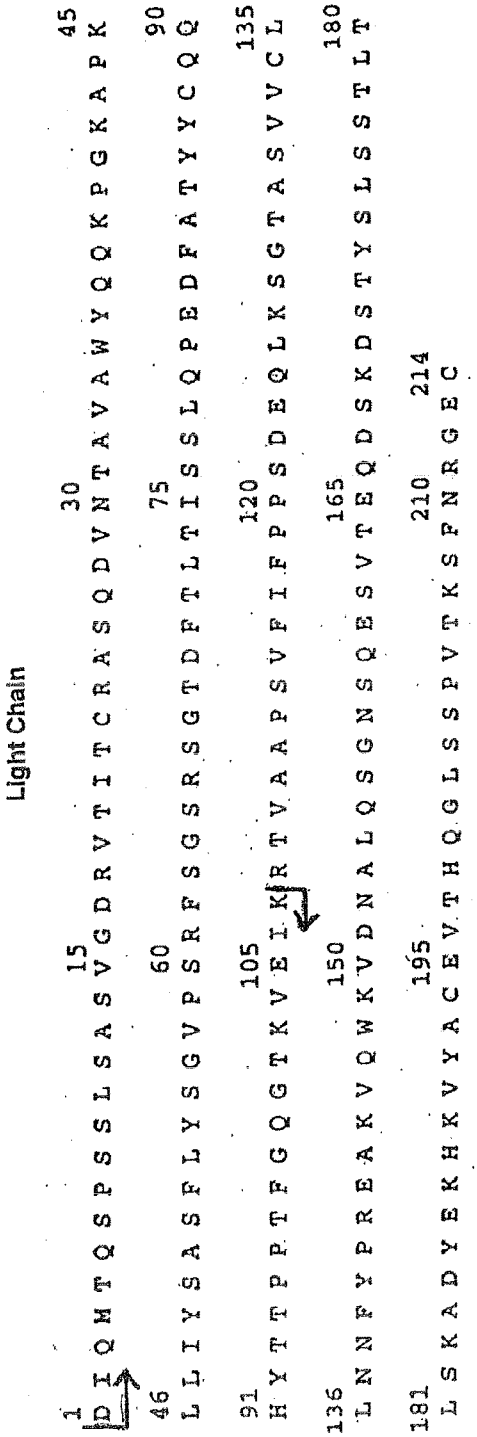


FIG. 4A

Figure 4 (cont.).

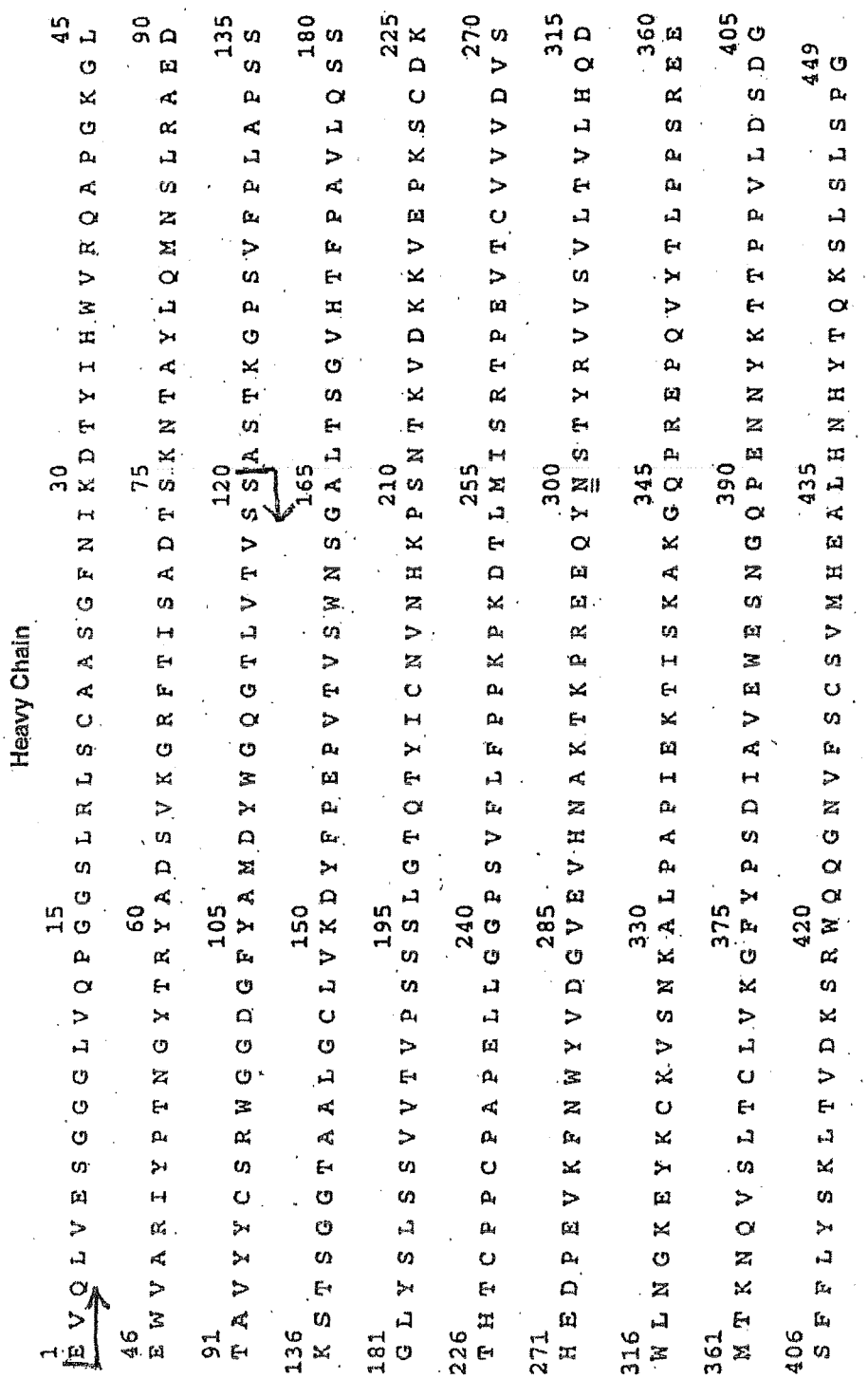


FIG. 4B

Figure 5.

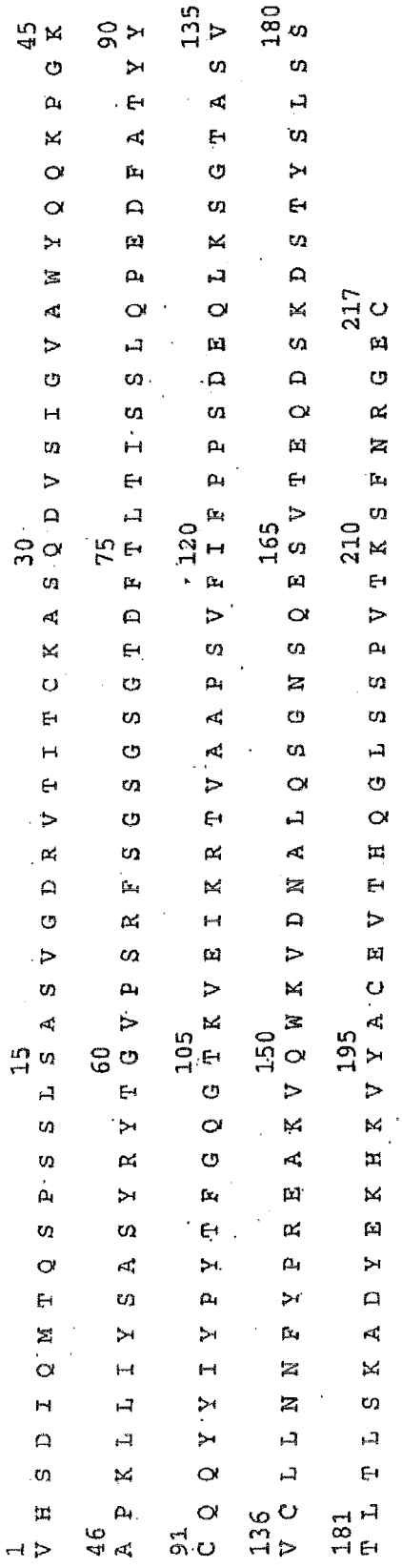


FIG. 5A

Figure 5 (cont.).

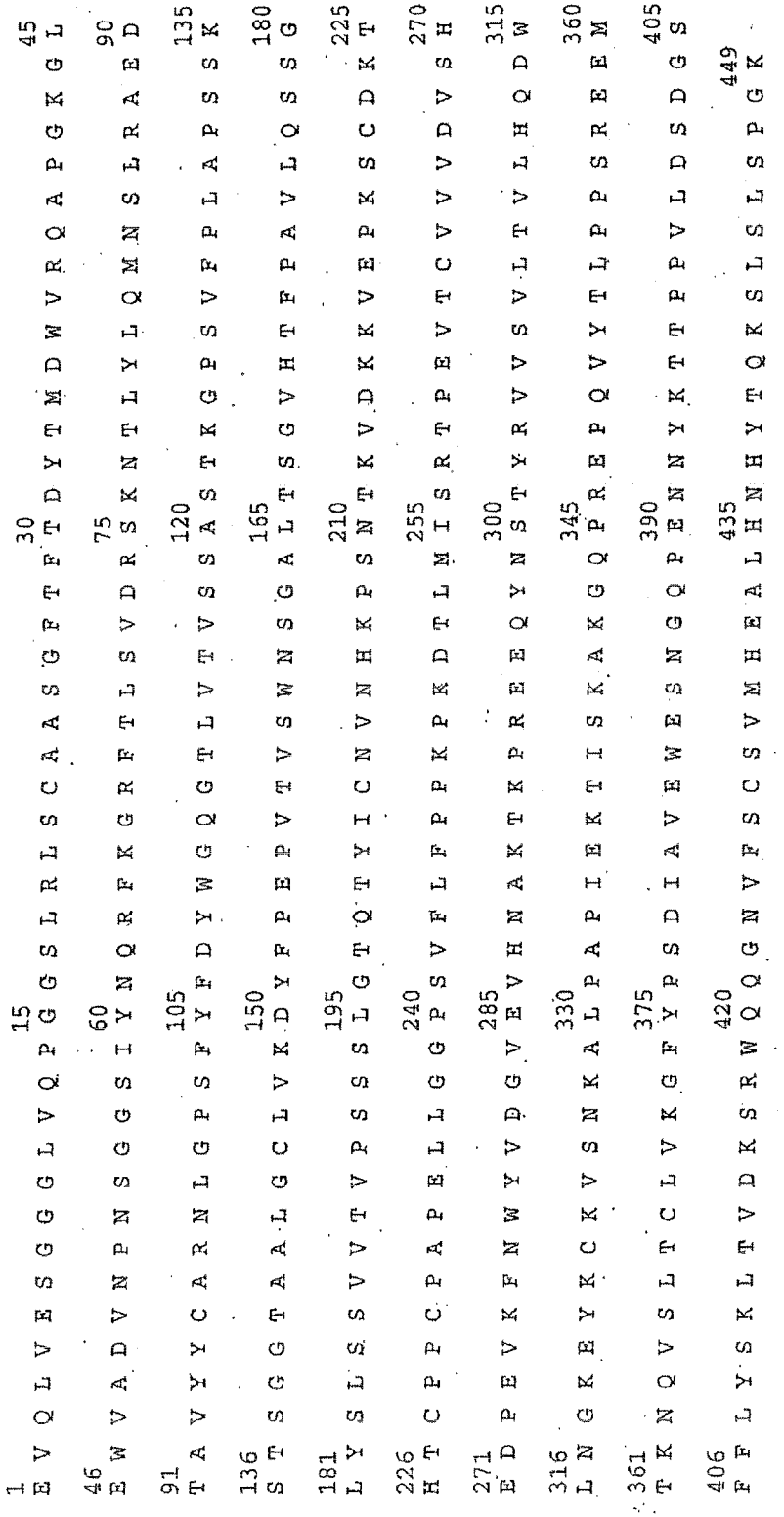
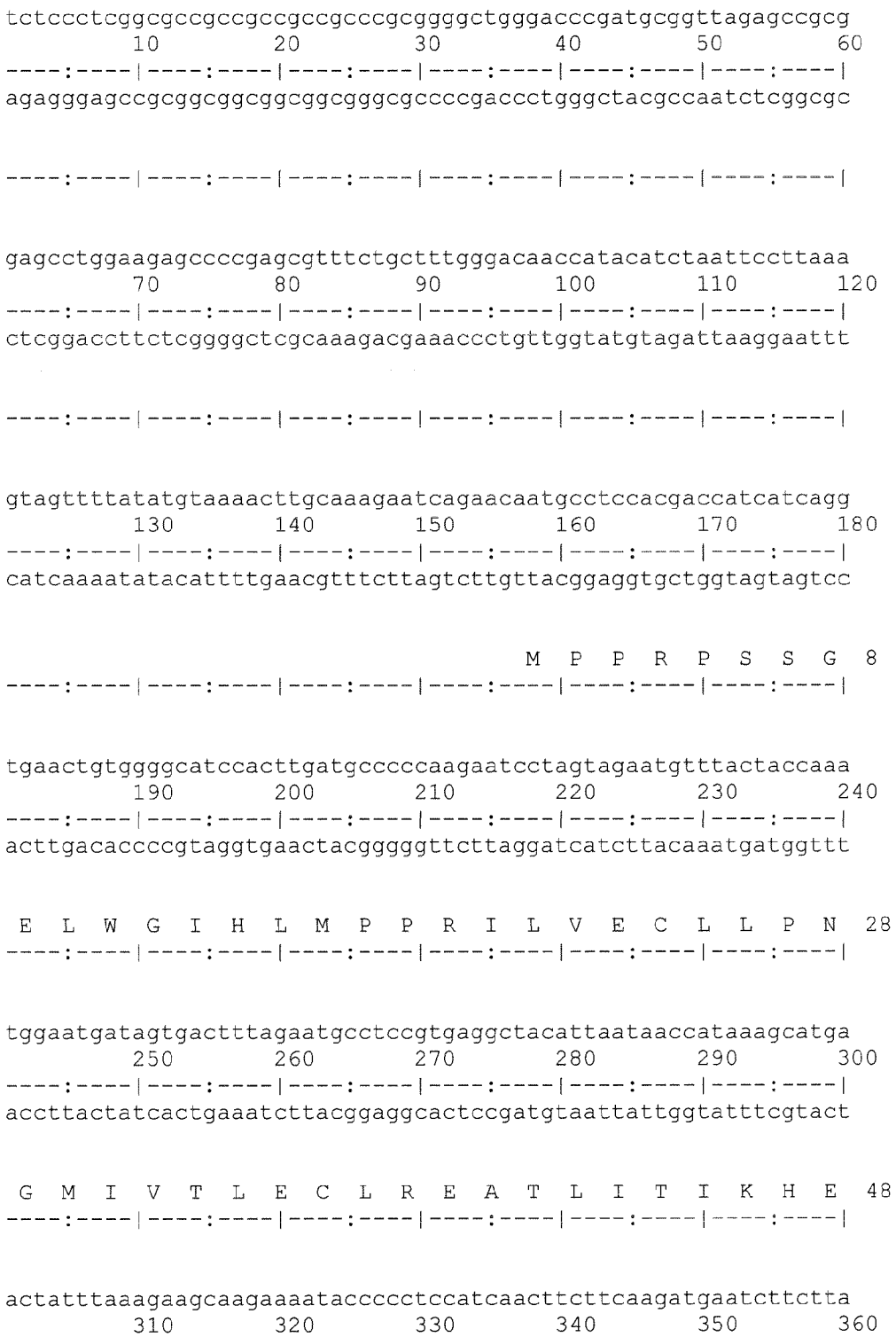


FIG. 5B

Figure 6.

Mutation	TH		THP		HP		THP	
	non-pCR	pCR	non-pCR	pCR	non-pCR	pCR	non-pCR	pCR
Exon 7	2	0	1	0	1	0	0	0
Exon 9*	8	0	4	1	5	0	9	1
Exon 20	13	7	8	7	15	2	11	3
	23/67 (34.3%)	7/29 (24.1%)	13/51 (25.4%)	8/41 (19.5%)	21/82 (25.6%)	2/18 (11%)	20/62 (32.2%)	4/23 (17%)

Figure 7.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70 80 90 100 110 120
ctcggaccttctcggggctcgcaaagacgaaaccctgttggtatgtagattaaggaattt
gtagttttatatgtaaaacttgcaaagaatcagaacaatgcctccacgaccatcatcagg
130 140 150 160 170 180
catcaaaatatacattttgaacgtttcttagtcttgttacggaggtgctggtagtagtcc
M P P R P S S G 8
tgaactgtggggcatccacttgatgcccccaagaatcctagtagaatgtttactaccaaa
190 200 210 220 230 240
acttgacaccccgtaggtgaactacgggggttcttaggatcatcttacaaatgatggttt
E L W G I H L M P P R I L V E C L L P N 28
tggaatgatagtgactttagaatgcctccgtgaggctacattaataaccataaagcatga
250 260 270 280 290 300
accttactatcactgaaatcttacggaggcactccgatgtaattattggtatttcgtact
G M I V T L E C L R E A T L I T I K H E 48
actatttaaagaagcaagaaaatacccccctccatcaacttcttcaagatgaatcttctta
310 320 330 340 350 360

```
----:----|----:----|----:----|----:----|----:----|----:----|----:----|
tgataaatttcttcgttcttttatgggggaggtagttgaagaagttctacttagaagaat

 L  F  K  E  A  R  K  Y  P  L  H  Q  L  L  Q  D  E  S  S  Y  68
----:----|----:----|----:----|----:----|----:----|----:----|----:----|

catttttcgtaagtgttactcaagaagcagaaagggaagaatttttttgatgaaacaagacg
         370       380       390       400       410       420
----:----|----:----|----:----|----:----|----:----|----:----|----:----|
gtaaaagcattcacaatgagttcttcgtctttcccttcttaaaaaactactttgttctgc

 I  F  V  S  V  T  Q  E  A  E  R  E  E  F  F  D  E  T  R  R  88
----:----|----:----|----:----|----:----|----:----|----:----|----:----|

actttgtgaccttcggctttttcaacccttttttaaaagtaattgaaccagtaggcaaccg
         430       440       450       460       470       480
----:----|----:----|----:----|----:----|----:----|----:----|----:----|
tgaaacactggaagccgaaaaagttgggaaaaattttcattaacttggtcatccgttggc

 L  C  D  L  R  L  F  Q  P  F  L  K  V  I  E  P  V  G  N  R  108
----:----|----:----|----:----|----:----|----:----|----:----|----:----|

tgaagaaaagatcctcaatcgagaaattggttttgctatcggcatgccagtgtgtgaatt
         490       500       510       520       530       540
----:----|----:----|----:----|----:----|----:----|----:----|----:----|
acttcttttctaggagttagctctttaaccaaaacgatagccgtacggtcacacacttaa

 E  E  K  I  L  N  R  E  I  G  F  A  I  G  M  P  V  C  E  F  128
----:----|----:----|----:----|----:----|----:----|----:----|----:----|

tgatatggttaaagatccagaagtacaggacttccgaagaaatattctgaacgtttgtaa
         550       560       570       580       590       600
----:----|----:----|----:----|----:----|----:----|----:----|----:----|
actataccaatttctaggtcttcatgtcctgaaggcttctttataagacttgcaaacatt

 D  M  V  K  D  P  E  V  Q  D  F  R  R  N  I  L  N  V  C  K  148
----:----|----:----|----:----|----:----|----:----|----:----|----:----|

agaagctgtggatcttagggacctcaattcacctcatagtagagcaatgtatgtctatcc
         610       620       630       640       650       660
----:----|----:----|----:----|----:----|----:----|----:----|----:----|
tcttcgacacctagaatccctggagttaagtggagtatcatctcgttacatacagatagg

 E  A  V  D  L  R  D  L  N  S  P  H  S  R  A  M  Y  V  Y  P  168
----:----|----:----|----:----|----:----|----:----|----:----|----:----|

tccaaatgtagaatcttcaccagaattgccaaagcacatatataataaattagataaagg
         670       680       690       700       710       720
```

```
----:----|----:----|----:----|----:----|----:----|----:----|----:----|
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 P  N  V  E  S  S  P  E  L  P  K  H  I  Y  N  K  L  D  K  G  188
----:----|----:----|----:----|----:----|----:----|----:----|----:----|

gcaaataatagtggtgatctgggtaatagtttctccaaataatgacaagcagaagtatac
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----:----|----:----|----:----|----:----|----:----|----:----|----:----|
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 Q  I  I  V  V  I  W  V  I  V  S  P  N  N  D  K  Q  K  Y  T  208
----:----|----:----|----:----|----:----|----:----|----:----|----:----|

tctgaaaatcaaccatgactgtgtaccagaacaagtaattgctgaagcaatcaggaaaaa
         790       800       810       820       830       840
----:----|----:----|----:----|----:----|----:----|----:----|----:----|
agacttttagttggtactgacacatggtcttgttcattaacgacttcgttagtccttttt

 L  K  I  N  H  D  C  V  P  E  Q  V  I  A  E  A  I  R  K  K  228
----:----|----:----|----:----|----:----|----:----|----:----|----:----|

aactcgaagtatgttgctatcctctgaacaactaaaactctgtgttttagaatatcaggg
         850       860       870       880       890       900
----:----|----:----|----:----|----:----|----:----|----:----|----:----|
ttgagcttcatacaacgataggagacttgttgattttgagacacaaaatcttatagtccc

 T  R  S  M  L  L  S  S  E  Q  L  K  L  C  V  L  E  Y  Q  G  248
----:----|----:----|----:----|----:----|----:----|----:----|----:----|

caagtatattttaaaagtgtgtggatgtgatgaatacttcctagaaaaatatcctctgag
         910       920       930       940       950       960
----:----|----:----|----:----|----:----|----:----|----:----|----:----|
gttcatataaaattttcacacacctacactacttatgaaggatctttttataggagactc

 K  Y  I  L  K  V  C  G  C  D  E  Y  F  L  E  K  Y  P  L  S  268
----:----|----:----|----:----|----:----|----:----|----:----|----:----|

tcagtataagtatataagaagctgtataatgcttgggaggatgcccaatttgatgttgat
         970       980       990       1000      1010      1020
----:----|----:----|----:----|----:----|----:----|----:----|----:----|
agtcatattcatatattcttcgacatattacgaaccctcctacgggttaaactacaacta

 Q  Y  K  Y  I  R  S  C  I  M  L  G  R  M  P  N  L  M  L  M  288
----:----|----:----|----:----|----:----|----:----|----:----|----:----|

ggctaaagaaagcctttattctcaactgccaatggactgttttacaatgccatcttattc
         1030      1040      1050      1060      1070      1080
```

```
----:----|----:----|----:----|----:----|----:----|----:----|----:----|
ccgatttctttcggaaataagagttgacggttacctgacaaaatgttacggtagaataag

 A  K  E  S  L  Y  S  Q  L  P  M  D  C  F  T  M  P  S  Y  S  308
----:----|----:----|----:----|----:----|----:----|----:----|----:----|

cagacgcatttccacagctacaccatatatgaatggagaaacatctacaaaatccctttg
         1090      1100      1110      1120      1130      1140
----:----|----:----|----:----|----:----|----:----|----:----|----:----|
gtctgcgtaaaggtgtcgatgtggtatatacttacctctttgtagatgttttagggaaac

 R  R  I  S  T  A  T  P  Y  M  N  G  E  T  S  T  K  S  L  W  328
----:----|----:----|----:----|----:----|----:----|----:----|----:----|

ggttataaatagtgcactcagaataaaaattctttgtgcaacctacgtgaatgtaaatat
         1150      1160      1170      1180      1190      1200
----:----|----:----|----:----|----:----|----:----|----:----|----:----|
ccaatatttatcacgtgagtcttatttttaagaaacacgttggatgcacttacatttata

 V  I  N  S  A  L  R  I  K  I  L  C  A  T  Y  V  N  V  N  I  348
----:----|----:----|----:----|----:----|----:----|----:----|----:----|

tcgagacattgataagatctatgttcgaacaggtatctaccatggaggagaaacccttatg
         1210      1220      1230      1240      1250      1260
----:----|----:----|----:----|----:----|----:----|----:----|----:----|
agctctgtaactattctagatacaagcttgtccatagatggtacctcctcttgggaatac

 R  D  I  D  K  I  Y  V  R  T  G  I  Y  H  G  G  E  P  L  C  268
----:----|----:----|----:----|----:----|----:----|----:----|----:----|

tgacaatgtgaacactcaaagagtaccttgttccaatcccaggtggaatgaatggctgaa
         1270      1280      1290      1300      1310      1320
----:----|----:----|----:----|----:----|----:----|----:----|----:----|
actgttacacttgtgagtttctcatggaacaaggttagggtccaccttacttaccgactt

 D  N  V  N  T  Q  R  V  P  C  S  N  P  R  W  N  E  W  L  N  388
----:----|----:----|----:----|----:----|----:----|----:----|----:----|

ttatgatatatacattcctgatcttcctcgtgctgctcgactttgcctttccatttgctc
         1330      1340      1350      1360      1370      1380
----:----|----:----|----:----|----:----|----:----|----:----|----:----|
aatactatatatgtaaggactagaaggagcacgacgagctgaaacggaaaggtaaacgag

 Y  D  I  Y  I  P  D  L  P  R  A  A  R  L  C  L  S  I  C  S  408
----:----|----:----|----:----|----:----|----:----|----:----|----:----|

tgttaaaggccgaaagggtgctaaagaggaacactgtccattggcatggggaaatataaa
         1390      1400      1410      1420      1430      1440
```

```
----:----|----:----|----:----|----:----|----:----|----:----|
acaatttccggctttcccacgatttctccttgtgacaggtaaccgtacccctttatattt

 V  K  G  R  K  G  A  K  E  E  H  C  P  L  A  W  G  N  I  N  428
----:----|----:----|----:----|----:----|----:----|----:----|

cttgtttgattacacagacactctagtatctggaaaaatggctttgaatctttggccagt
         1450      1460      1470      1480      1490      1500
----:----|----:----|----:----|----:----|----:----|----:----|
gaacaaactaatgtgtctgtgagatcatagacctttttaccgaaacttagaaaccggtca

 L  F  D  Y  T  D  T  L  V  S  G  K  M  A  L  N  L  W  P  V  448
----:----|----:----|----:----|----:----|----:----|----:----|

acctcatggattagaagatttgctgaaccctattggtgttactggatcaaatccaaataa
         1510      1520      1530      1540      1550      1560
----:----|----:----|----:----|----:----|----:----|----:----|
tggagtacctaatcttctaaacgacttgggataaccacaatgacctagtttaggtttatt

 P  H  G  L  E  D  L  L  N  P  I  G  V  T  G  S  N  P  N  K  468
----:----|----:----|----:----|----:----|----:----|----:----|

agaaactccatgcttagagttggagtttgactggttcagcagtgtggtaaagttcccaga
         1570      1580      1590      1600      1610      1620
----:----|----:----|----:----|----:----|----:----|----:----|
tctttgaggtacgaatctcaacctcaaactgaccaagtcgtcacaccatttcaagggtct

 E  T  P  C  L  E  L  E  F  D  W  F  S  S  V  V  K  F  P  D  488
----:----|----:----|----:----|----:----|----:----|----:----|

tatgtcagtgattgaagagcatgccaattggtctgtatcccgagaagcaggatttagcta
         1630      1640      1650      1660      1670      1680
----:----|----:----|----:----|----:----|----:----|----:----|
atacagtcactaacttctcgtacggttaaccagacatagggctcttcgtcctaaatcgat

 M  S  V  I  E  E  H  A  N  W  S  V  S  R  E  A  G  F  S  Y  508
----:----|----:----|----:----|----:----|----:----|----:----|

                  ******************************************
exon9
ttcccacgcaggactgagtaacagactagctagagacaatgaattaagggaaaatgacaa
         1690      1700      1710      1720      1730      1740
----:----|----:----|----:----|----:----|----:----|----:----|
aagggtgcgtcctgactcattgtctgatcgatctctgttacttaattccctttttactgtt

 S  H  A  G  L  S  N  R  L  A  R  D  N  E  L  R  E  N  D  K  528
----:----|----:----|----:----|----:----|----:----|----:----|

************************************************************
exon9
```

agaacagctcaaagcaatttctacacgagatcctctctct**gaa**atcact**gag**caggagaa
1750 1760 1770 1780 1790 1800
tcttgtcgagtttcgttaaagatgtgctctaggagagagactttagtgactcgtcctctt

E Q L K A I S T R D P L S **E** I T **E** Q E K 548

****
agattttctatggagtcacagacactattgtgtaactatccccgaaattctacccaaatt
1810 1820 1830 1840 1850 1860
tctaaaagatacctcagtgtctgtgataacacattgatagggggctttaagatgggtttaa

D F L W S H R H Y C V T I P E I L P K L 568

gcttctgtctgttaaatggaattctagagatgaagtagcccagatgtattgcttggtaaa
1870 1880 1890 1900 1910 1920
cgaagacagacaatttaccttaagatctctacttcatcgggtctacataacgaaccattt

L L S V K W N S R D E V A Q M Y C L V K 588

agattggcctccaatcaaacctgaacaggctatggaacttctggactgtaattacccaga
1930 1940 1950 1960 1970 1980
tctaaccggaggttagtttggacttgtccgataccttgaagacctgacattaatgggtct

D W P P I K P E Q A M E L L D C N Y P D 608

tcctatggttcgaggttttgctgttcggtgcttggaaaaatatttaacagatgacaaact
1990 2000 2010 2020 2030 2040
aggataccaagctccaaaacgacaagccacgaacctttttataaattgtctactgtttga

P M V R G F A V R C L E K Y L T D D K L 628

ttctcagtatttaattcagctagtacaggtcctaaaatatgaacaatatttggataactt
2050 2060 2070 2080 2090 2100
aagagtcataaattaagtcgatcatgtccaggattttatacttgttataaacctattgaa

S Q Y L I Q L V Q V L K Y E Q Y L D N L 648

```
gcttgtgagatttttactgaagaaagcattgactaatcaaaggattgggcacttttttctt
         2110      2120      2130      2140      2150      2160
----:----|----:----|----:----|----:----|----:----|----:----|
cgaacactctaaaaatgacttctttcgtaactgattagtttcctaacccgtgaaaaagaa

 L  V  R  F  L  L  K  K  A  L  T  N  Q  R  I  G  H  F  F  F  668
----:----|----:----|----:----|----:----|----:----|----:----|----:----|

ttggcatttaaaatctgagatgcacaataaaacagttagccagaggtttggcctgctttt
         2170      2180      2190      2200      2210      2220
----:----|----:----|----:----|----:----|----:----|----:----|
aaccgtaaattttagactctacgtgttattttgtcaatcggtctccaaaccggacgaaaa

 W  H  L  K  S  E  M  H  N  K  T  V  S  Q  R  F  G  L  L  L  688
----:----|----:----|----:----|----:----|----:----|----:----|----:----|

ggagtcctattgtcgtgcatgtgggatgtatttgaagcacctgaataggcaagtcgaggc
         2230      2240      2250      2260      2270      2280
----:----|----:----|----:----|----:----|----:----|----:----|
cctcaggataacagcacgtacaccctacataaacttcgtggacttatccgttcagctccg

 E  S  Y  C  R  A  C  G  M  Y  L  K  H  L  N  R  Q  V  E  A  708
----:----|----:----|----:----|----:----|----:----|----:----|----:----|

aatggaaaagctcattaacttaactgacattctcaaacaggagaagaaggatgaaacaca
         2290      2300      2310      2320      2330      2340
----:----|----:----|----:----|----:----|----:----|----:----|
ttacctttcgagtaattgaattgactgtaagagtttgtcctcttcttcctactttgtgt

 M  E  K  L  I  N  L  T  D  I  L  K  Q  E  K  K  D  E  T  Q  728
----:----|----:----|----:----|----:----|----:----|----:----|----:----|

aaaggtacagatgaagtttttagttgagcaaatgaggcgaccagatttcatggatgctct
         2350      2360      2370      2380      2390      2400
----:----|----:----|----:----|----:----|----:----|----:----|
tttccatgtctacttcaaaaatcaactcgtttactccgctggtctaaagtacctacgaga

 K  V  Q  M  K  F  L  V  E  Q  M  R  R  P  D  F  M  D  A  L  748
----:----|----:----|----:----|----:----|----:----|----:----|----:----|

acagggctttctgtctcctctaaaccctgctcatcaactaggaaacctcaggcttgaaga
         2410      2420      2430      2440      2450      2460
----:----|----:----|----:----|----:----|----:----|----:----|
tgtcccgaaagacagaggagatttgggacgagtagttgatcctttggagtccgaacttct

 Q  G  F  L  S  P  L  N  P  A  H  Q  L  G  N  L  R  L  E  E  768
----:----|----:----|----:----|----:----|----:----|----:----|----:----|
```

```
gtgtcgaattatgtcctctgcaaaaaggccactgtggttgaattgggagaacccagacat
         2470      2480      2490      2500      2510      2520
----:----|----:----|----:----|----:----|----:----|----:----|
cacagcttaatacaggagacgtttttccggtgacaccaacttaaccctcttgggtctgta

 C  R  I  M  S  S  A  K  R  P  L  W  L  N  W  E  N  P  D  I  788
----:----|----:----|----:----|----:----|----:----|----:----|

catgtcagagttactgtttcagaacaatgagatcatctttaaaaatggggatgatttacg
         2530      2540      2550      2560      2570      2580
----:----|----:----|----:----|----:----|----:----|----:----|
gtacagtctcaatgacaaagtcttgttactctagtagaaatttttacccctactaaatgc

 M  S  E  L  L  F  Q  N  N  E  I  I  F  K  N  G  D  D  L  R  808
----:----|----:----|----:----|----:----|----:----|----:----|

gcaagatatgctaacacttcaaattattcgtattatggaaaatatctggcaaaatcaagg
         2590      2600      2610      2620      2630      2640
----:----|----:----|----:----|----:----|----:----|----:----|
cgttctatacgattgtgaagtttaataagcataataccttttatagaccgttttagttcc

 Q  D  M  L  T  L  Q  I  I  R  I  M  E  N  I  W  Q  N  Q  G  828
----:----|----:----|----:----|----:----|----:----|----:----|

tcttgatcttcgaatgttaccttatggttgtctgtcaatcggtgactgtgtgggacttat
         2650      2660      2670      2680      2690      2700
----:----|----:----|----:----|----:----|----:----|----:----|
agaactagaagcttacaatggaataccaacagacagttagccactgacacaccctgaata

 L  D  L  R  M  L  P  Y  G  C  L  S  I  G  D  C  V  G  L  I  848
----:----|----:----|----:----|----:----|----:----|----:----|

tgaggtggtgcgaaattctcacactattatgcaaattcagtgcaaaggcggcttgaaagg
         2710      2720      2730      2740      2750      2760
----:----|----:----|----:----|----:----|----:----|----:----|
actccaccacgctttaagagtgtgataatacgtttaagtcacgtttccgccgaactttcc

 E  V  V  R  N  S  H  T  I  M  Q  I  Q  C  K  G  G  L  K  G  868
----:----|----:----|----:----|----:----|----:----|----:----|

tgcactgcagttcaacagccacacactacatcagtggctcaaagacaagaacaaaggaga
         2770      2780      2790      2800      2810      2820
----:----|----:----|----:----|----:----|----:----|----:----|
acgtgacgtcaagttgtcggtgtgtgatgtagtcaccgagtttctgttcttgtttcctct

 A  L  Q  F  N  S  H  T  L  H  Q  W  L  K  D  K  N  K  G  E  888
----:----|----:----|----:----|----:----|----:----|----:----|
```

```
aatatatgatgcagccattgacctgtttacacgttcatgtgctggatactgtgtagctac
         2830      2840      2850      2860      2870      2880
----:----|----:----|----:----|----:----|----:----|----:----|
ttatatactacgtcggtaactggacaaatgtgcaagtacacgacctatgacacatcgatg

 I  Y  D  A  A  I  D  L  F  T  R  S  C  A  G  Y  C  V  A  T  908
----:----|----:----|----:----|----:----|----:----|----:----|----:----|

cttcattttgggaattggagatcgtcacaatagtaacatcatggtgaaagacgatggaca
         2890      2900      2910      2920      2930      2940
----:----|----:----|----:----|----:----|----:----|----:----|
gaagtaaaacccttaacctctagcagtgttatcattgtagtaccactttctgctacctgt

 F  I  L  G  I  G  D  R  H  N  S  N  I  M  V  K  D  D  G  Q  928
----:----|----:----|----:----|----:----|----:----|----:----|----:----|

actgtttcatatagattttggacacttttttggatcacaagaagaaaaaatttggttataa
         2950      2960      2970      2980      2990      3000
----:----|----:----|----:----|----:----|----:----|----:----|
tgacaaagtatatctaaaacctgtgaaaaacctagtgttcttctttttttaaaccaatatt

 L  F  H  I  D  F  G  H  F  L  D  H  K  K  K  K  F  G  Y  K  948
----:----|----:----|----:----|----:----|----:----|----:----|----:----|

acgagaacgtgtgccatttgttttgacacaggatttcttaatagtgattagtaaaggagc
         3010      3020      3030      3040      3050      3060
----:----|----:----|----:----|----:----|----:----|----:----|
tgctcttgcacacggtaaacaaaactgtgtcctaaagaattatcactaatcatttcctcg

 R  E  R  V  P  F  V  L  T  Q  D  F  L  I  V  I  S  K  G  A  968
----:----|----:----|----:----|----:----|----:----|----:----|----:----|

ccaagaatgcacaaagacaagagaatttgagaggtttcaggagatgtgttacaaggctta
         3070      3080      3090      3100      3110      3120
----:----|----:----|----:----|----:----|----:----|----:----|
ggttcttacgtgtttctgttctcttaaactctccaaagtcctctacacaatgttccgaat

 Q  E  C  T  K  T  R  E  F  E  R  F  Q  E  M  C  Y  K  A  Y  988
----:----|----:----|----:----|----:----|----:----|----:----|----:----|

tctagctattcgacagcatgccaatctcttcataaatcttttctcaatgatgcttggctc
         3130      3140      3150      3160      3170      3180
----:----|----:----|----:----|----:----|----:----|----:----|
agatcgataagctgtcgtacggttagagaagtatttagaaaagagttactacgaaccgag

 L  A  I  R  Q  H  A  N  L  F  I  N  L  F  S  M  M  L  G  S
1008
----:----|----:----|----:----|----:----|----:----|----:----|----:----|
```

```
          tggaatgccagaactacaatcttttgatgacattgcatacattcgaaagaccctagcctt
                   3190      3200      3210      3220      3230      3240
          ----:----|----:----|----:----|----:----|----:----|----:----|
          accttacggtcttgatgttagaaaactactgtaacgtatgtaagctttctgggatcggaa

           G  M  P  E  L  Q  S  F  D  D  I  A  Y  I  R  K  T  L  A  L
1028
          ----:----|----:----|----:----|----:----|----:----|----:----|

          agataaaactgagcaagaggctttggagtatttcatgaaacaaatgaatgatgcacatca
                   3250      3260      3270      3280      3290      3300
          ----:----|----:----|----:----|----:----|----:----|----:----|
          tctattttgactcgttctccgaaacctcataaagtactttgtttacttactacgtgtagt

           D  K  T  E  Q  E  A  L  E  Y  F  M  K  Q  M  N  D  A  H  H
1048
          ----:----|----:----|----:----|----:----|----:----|----:----|

          tggtggctggacaacaaaaatggattggatcttccacacaattaaacagcatgcattgaa
                   3310      3320      3330      3340      3350      3360
          ----:----|----:----|----:----|----:----|----:----|----:----|
          accaccgacctgttgtttttacctaacctagaaggtgtgttaatttgtcgtacgtaactt

           G  G  W  T  T  K  M  D  W  I  F  H  T  I  K  Q  H  A  L  N
1068
          ----:----|----:----|----:----|----:----|----:----|----:----|

          ctgaaaagataactgagaaaatgaaagctcactctggattccacactgcactgttaataa
                   3370      3380      3390      3400      3410      3420
          ----:----|----:----|----:----|----:----|----:----|----:----|
          gacttttctattgactcttttactttcgagtgagacctaaggtgtgacgtgacaattatt

           *
          ----:----|----:----|----:----|----:----|----:----|----:----|

          ctctcagcaggcaaagaccgattgcataggaattgcacaatccatgaacagcattagaat
                   3430      3440      3450      3460      3470      3480
          ----:----|----:----|----:----|----:----|----:----|----:----|
          gagagtcgtccgtttctggctaacgtatccttaacgtgttaggtacttgtcgtaatctta

          ----:----|----:----|----:----|----:----|----:----|----:----|

          ttacagcaagaacagaaataaaatactatataatttaaataatgtaaacgcaaacagggt
                   3490      3500      3510      3520      3530      3540
          ----:----|----:----|----:----|----:----|----:----|----:----|
          aatgtcgttcttgtctttattttatgatatattaaatttattacatttgcgtttgtccca

          ----:----|----:----|----:----|----:----|----:----|----:----|
```

```
ttgatagcacttaaactagttcatttcaaaattaagctttagaataatgcgcaatttcat
         3550      3560      3570      3580      3590      3600
----:----|----:----|----:----|----:----|----:----|----:----|
aactatcgtgaatttgatcaagtaaagttttaattcgaaatcttattacgcgttaaagta


 ----:----|----:----|----:----|----:----|----:----|----:----|


gttatgccttaagtccaaaaaggtaaactttgaagattgtttgtatctttttttaaaaaa
         3610      3620      3630      3640      3650      3660
----:----|----:----|----:----|----:----|----:----|----:----|
caatacggaattcaggtttttccatttgaaacttctaacaaacatagaaaaaaatttttt


 ----:----|----:----|----:----|----:----|----:----|----:----|


caaaacaaaacaaaaatccccaaaatatatagaaatgatggagaaggaaaaaaaaaaaaa
         3670      3680      3690      3700      3710      3720
----:----|----:----|----:----|----:----|----:----|----:----|
gttttgttttgtttttaggggttttatatatctttactacctcttccttttttttttttt


 ----:----|----:----|----:----|----:----|----:----|----:----|


aaaa

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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2012/074857

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12Q1/68
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12Q

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal , BIOSIS, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 1 980 626 Al (HET NL KANKER I [NL]) 15 October 2008 (2008-10-15)	1-14, 17-47
Y	claims 1,7-11 paragraphs [0012] , [0054] - [0056] , [0071] , [0072] , [0079] - [0082]	15 , 16

X	WO 2011/070499 Al (WYETH LLC [US] ; BERKENBLIT ANNA [US] ; COUGHLIN CHRISTINA MARIE [US] ; F) 16 June 2011 (2011-06-16)	1-14, 17-47
Y	claims 1,8,9 , 12 page 7, paragraph 3 page 9, last paragraph - page 11, paragraph 1 page 17, last paragraph - page 20, paragraph 1	15 , 16

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
6 February 2013	20/02/2013

Name and mailing address of the ISA/	Authorized officer
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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	VORKAS P A ET AL: "PI3K Pathway Activity and Response to First-Line Chemotherapy in Combination with Trastuzumab in Patients with HER2-Positive Metastatic Breast Cancer" , CANCER RESEARCH; 32ND ANNUAL SAN ANTONIO BREAST CANCER SYMPOSIUM, AMERICAN ASSOCIATION FOR CANCER RESEARCH, US; SAN ANTONIO, TX, USA, vol. 69, no. 24, Suppl. 3, 15 December 2009 (2009-12-15) , page 599S, XP008159913 , ISSN: 0008-5472 , DOI: 10.1158/0008-5472 .SABCS-09-2022	1-14, 17-47
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X	REXER B N ET AL: "Exon 9 and exon 20 mutations in PIK3CA confer resistance to HER2 inhibitors in HER2-overexpressing breast cancer cells", CANCER RESEARCH; 31ST ANNUAL SAN ANTONIO BREAST CANCER SYMPOSIUM, AMERICAN ASSOCIATION FOR CANCER RESEARCH, US; SAN ANTONIO, TX, USA, vol. 69, no. 2, Suppl. S, 15 January 2009 (2009-01-15) , pages 266S-267S, XP008159912 , ISSN: 0008-5472 , DOI: 10.1158/0008-5472 .SABCS-4054	1-14, 17-47
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X	V. SERRA ET AL: "NVP-BEZ235, a Dual PI3K/mTOR Inhibitor, Prevents PI3K Signaling and Inhibits the Growth of Cancer Cells with Activating PI3K Mutations" , CANCER RESEARCH, vol. 68, no. 19, 1 October 2008 (2008-10-01) , pages 8022-8030, XP055018423 , ISSN: 0008-5472 , DOI: 10.1158/0008-5472 .CAN-08-1385	1-14, 17-47
Y	the whole document	15, 16

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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X	BERNS KATRIEN ET AL: "A functional genetic approach identifies the PI3K pathway as a major determinant of trastuzumab resistance in breast cancer", CANCER CELL, CELL PRESS, US, vol. 12, no. 4, 16 October 2007 (2007-10-16), pages 395-402, XP002592491, ISSN: 1535-6108, DOI: 10.1016/J.CCR.2007.08.030 [retrieved on 2007-10-15]	1-14, 17-47
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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Y	W0 2010/014920 Al (UNIV JOHNS HOPKINS [US] ; DI EHL FRANK [DE] ; DIAZ LUIS [US] ; KINZLER KEN) 4 February 2010 (2010-02-04) sequences 24,25 ,274 paragraph [0071]	15 , 16

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INTERNATIONAL SEARCH REPORT

Information on patent family members

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