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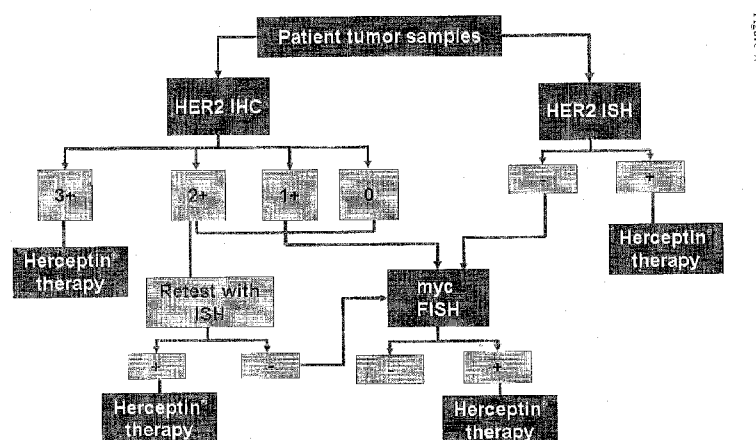
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(54) **Title:** MODULATORS FOR HER2 SIGNALING IN NORMAL HER2 EXPRESSING SETTINGS



(57) **Abstract:** The present invention relates to means and methods for the identification of a patient likely to being sensitive to a modulator of the HER2/neu (ErbB2) signaling pathway. Also described herein are corresponding methods of treatment of a group of patients isolated in accordance with the identification method of the present invention, whereby said group of patients is suspected to suffer from or being prone to suffer from breast cancer.

Modulators for HER2 signaling in normal HER2 expressing settings

The present invention relates to means and methods for the identification of responders for or a patient sensitive to a modulator of the HER2/neu (ErbB2) signaling pathway. Also described herein are corresponding methods of treatment of a group of patients determined and defined in accordance with the identification method of the present invention, whereby said group of patients is known or suspected to suffer from or being prone to suffer from breast cancer.

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, ErbB1, or HER1), HER2 (ErbB2 or p185^{neu}), HER3 (ErbB3) and HER4 (ErbB4). 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- α), 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- α 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 et al. *J. Clin. Invest.* 95:1897-1905 (1995).

The second member of the HER family, p185^{neu}, was originally identified as the product of the transforming gene from neuroblastomas of chemically treated rats. Amplification of the human homolog of *neu* is observed in breast and ovarian cancers and correlates with a poor prognosis (Slamon et al., *Science*, 235:177-182 (1987); Slamon et al., *Science*, 244:707-712 (1989); and US Pat No. 4,968,603). 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); Fukushima 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 proteins 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 WO94/22478. 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 neu-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- α . 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 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- α), 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* 15:1385-1394 (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 ephrenergulin also bind to HER4.

While EGF and TGF α do not bind HER2, EGF stimulates EGFR to form a heterodimer with HER2, which results in transphosphorylation of HER2 by EGFR and vice versa in the heterodimer, 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 of Neuroscience* 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)). 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, US2002/019221A1, US 6,015,567, US 6,333,169, US 4,968,603, US 5,821,337, 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, WO98/17797, US 6,127,526, US 6,333,398, US 6,797,814, US 6,339,142, US 6,417,335, US 6,489,447, WO99/31140, US2003/0147884A1, US2003/0170234A1, US2005/0002928A1, US 6,573,043, US2003/0152987A1, WO99/48527, 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, WO01/89566, 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 412,116 B1, EP 494,135 B1, US 5,824,311, EP 444,181 B1, EP 1,006,194 A2, US 2002/0155527A1, WO 91/02062, US 5,571,894, US 5,939,531, EP 502,812 B1, WO 93/03741, EP 554,441 B1, EP 656,367 A1, 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 711,565, 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 A1, WO 00/78347, WO 01/09187, WO 01/21192, WO 01/32155, WO 01/53354, WO 01/56604, WO 01/76630, WO02/05791, WO 02/11677, US 6,582,919, US2002/0192652A1, US 2003/0211530A1, WO 02/44413, US 2002/0142328, US 6,602,670 B2, WO 02/45653, WO 02/055106, US 2003/0152572, US 2003/0165840, WO 02/087619, 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 B1, US 2003/0103973, US 2003/0108545, US 6,403,630 B1, WO 00/61145, WO 00/61185, US 6,333,348 B1, WO 01/05425, WO 01/64246, US 2003/0022918, US 2002/0051785 A1, US 6,767,541, WO 01/76586, US 2003/0144252, WO 01/87336, US 2002/0031515 A1, 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 are selected for therapy based on HER2 overexpression/amplification. See, for example, WO99/31140 (Paton et al.), US2003/0170234A1 (Hellmann, S.), and US2003/0147884 (Paton et al.); as well as WO01/89566, US2002/0064785, and US2003/0134344 (Mass et al.). See, also, US2003/0152987, 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 HCl. US2004/0209290, Cobleigh et al., concerns gene expression markers for breast cancer prognosis. Patients treated with pertuzumab 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 US2004/0106161 (Bossenmaier et al.).

In another preferred embodiment of the invention, the HER dimerization inhibitor inhibits heterodimerization of HER2 with EGFR or HER3 or Her4. Preferably, the HER dimerization inhibitor is an antibody, preferably the antibody 2C4. Preferred throughout the application is the "antibody 2C4", in particular the humanized variant thereof (WO 01/00245; produced by the hybridoma cell line deposited with the American Type Culture Collection, Manassas, VA, USA under ATCC HB-12697), which binds to a region in the extracellular domain of Her2 (e.g., any one or more residues in the region from about residue 22 to about residue 584 of Her2, inclusive). Examples of humanized 2C4 antibodies are provided in Example 3 of WO 01/00245. The humanized antibody 2C4 is also called or pertuzumab.

Pertuzumab (formerly 2C4) is the first of a new class of agents known as HER dimerization inhibitors (HDIs). Pertuzumab binds to HER2 at its dimerization domain, thereby inhibiting its ability to form active dimer receptor complexes and thus blocking the downstream signal cascade that ultimately results in cell growth and division; see Franklin (2004), Cancer Cell 5, 317 - 328. Pertuzumab is a fully humanized recombinant monoclonal antibody directed against the extracellular domain of HER2. Binding of Pertuzumab to the HER2 on human epithelial cells prevents HER2 from forming complexes with other members of the HER family (including EGFR, HER3, HER4) and probably also HER2 homodimerization. By blocking complex formation, Pertuzumab prevents the growth stimulatory effects and cell survival signals activated by ligands of HER1, HER3 and HER4 (e.g. EGF, TGF α , amphiregulin, and the heregulins). Another name for Pertuzumab is 2C4. Pertuzumab is a fully humanized recombinant monoclonal antibody based on the human IgG1(κ) framework sequences. The structure of Pertuzumab consists of two heavy chains (449 residues) and two light chains (214 residues). Compared to Trastuzumab (Herceptin®), Pertuzumab has 12

amino acid differences in the light chain and 29 amino acid differences in the IgG1 heavy chain. Herceptin is indicated for the treatment of patients with metastatic breast cancer whose tumors overexpress HER2 protein or have HER 2 gene amplification (as defined herein below):

- 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,
- a) 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
- b) In combination with docetaxel for the treatment of those patients who have not received chemotherapy for their metastatic disease.

Herceptin can also be used as Adjuvant Treatment in Early Breast Cancer. Herceptin is also approved for the treatment of patients with HER2-positive early breast cancer following surgery, chemotherapy (neoadjuvant or adjuvant), and radiotherapy (if applicable).

In the art, the treatment of breast cancer patients with Herceptin/Trastuzumab is, for example, recommended and routine for patients having Her2-positive disease. Her2-positive disease is present if a high HER2 (protein) expression level detected by immunohistochemical methods (e.g. HER2 (+++)) or HER2 gene amplification (e.g. a HER2 gene copy number higher than 4 copies of the HER2 gene per tumor cell) 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. Patients with a normal level of HER2 expression (e.g. low or moderate HER2 (protein) expression level) usually do not gain access to treatment with Herceptin. However, clinical studies have shown that in the range of 5 to 10 % of those patients not tested positive for HER2 would also have responded to treatment with a HER2-signalling inhibitor.

Thus, the technical problem underlying the present invention is the provision of means and methods of identification of a patient or a group of patients suffering from or being prone to suffer from breast cancer who may be highly responsive to a treatment of breast cancer with a

modulator of the HER2/neu (ErbB2) signaling pathway, in particular to a treatment with a HER2 antibody such as Trastuzumab/Herceptin.

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

Accordingly, the present invention relates to an in vitro method for the identification of a responder for or a patient sensitive to a modulator of the HER2/neu (ErbB2) signaling pathway, said method comprising the following steps:

- (a) obtaining a sample from a patient suspected to suffer from or being prone to suffer from breast cancer;
- (b) evaluating the HER2 status (protein expression level of HER2 and/or the gene amplification status of the HER2 gene); and
- (c) in case the HER2 status in (b) is normal, evaluating the amplification status of c-myc gene in said sample;

whereby an amplification above normal of the c-myc gene is indicative for a responding patient or is indicative for a sensitivity of said patient to said modulator of the HER2/neu (ErbB2) signaling pathway.

As the skilled artisan knows and appreciates testing for HER2-positive disease may be performed by evaluating the protein expression level of HER2, the mRNA level or the gene amplification status of the HER2 gene individually or by performing two or three types of evaluation after one another or in parallel. HER2-positive disease is present if either protein expression level of HER2, the mRNA level or gene amplification of the HER2 gene, or any of these, are above the corresponding cut-off values.

It is preferred herein that the expression level of HER2 is 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.

The present invention solves the above identified technical problem since, as described herein below, it was surprisingly found that an unexpected group of patients that is characterized by

a normal HER2 status (normal expression level of HER2, e.g. less than HER2 (+++) by IHC, and/or a normal gene amplification status of the HER2 gene) and an amplification of the c-myc gene is highly responsive to a treatment with a modulator of the HER2/neu (erbB2) signaling pathway, in particular with a HER2 antibody, like Herceptin/Trastuzumab.

In the present invention it was surprisingly found that the response rate can be increased when patients which have both a normal level of HER2 expression in a biological sample (e.g. biopsies) and at the same time an amplification of the c-myc gene in a biological sample (e.g. biopsies) are treated with such a HER2 antibody. Unexpectedly, the response rate of these patients to a treatment with a modulator of the HER2/neu (erbB2) signaling pathway can increase to at least 15 %. Further testing for c-Myc amplification is warranted if a normal level for HER2 expression either on the nucleic acid level or on the protein level is present.

In some specialized laboratories patients with a normal expression level of HER2 as determined by IHC are routinely tested for HER2 gene amplification as well. The successive testing by HER2 IHC, HER2 ISH and, in case of a normal HER2 ISH result, c-myc amplification, represents a preferred embodiment of the present invention.

The level for HER2 expression can be assessed either on the protein or on the nucleic acid level. The HER2 protein expression level is preferably assessed using immunohistological methods, like "IHC" (immunohistochemistry). The HER2 gene amplification can be assessed by further methods known in the art, which comprise, but are not limited to the determination of the average HER2 gene copy number in cells of a given sample or the determination of the HER2/CEP17 ratio. Details on representative methods are provided herein below.

In accordance with this invention, the (protein) expression level of HER2 is usually and preferably measured by immunohistochemical (IHC) methods employing antibodies against the HER2 protein, whereas the c-myc gene amplification in biological samples is detected by in situ hybridization method, like (and preferably) FISH, CISH or SISH. Such methods, e.g. FISH can also be employed for the assessment of the gene amplification status/level of the HER2 gene in a given biological sample to be assessed in accordance with this invention. In a preferred embodiment of the present invention, HER2 gene amplification status is assessed in addition to HER2 expression level. This assessment of the gene amplification status of HER2

may be performed prior to, in parallel to, or after the HER2 expression level. As described herein, a patient group with a normal expression of HER2 (e.g. less than HER2(+++)) or a normal gene amplification status of HER2 and amplification of c-myc has been identified as being responsive to a modulator of the HER2/neu signaling pathway. A normal expression status of HER2 being for example less than HER2(+++) may be assessed as HER2(++), HER2(+) or HER2(0), as described herein below. As the skilled artisan appreciates the status HER2(0), as determined by IHC, does not necessarily mean that no HER2 is present at all, it rather means that less than 10% of the cells are stained, or that the receptor density on the cancer cells is low and/or that the detection method used did not yield a clearly positive HER2(1+) result. It is also envisaged and preferred herein that a patient with a normal expression of HER2 (e.g. less than HER2(+++)), a normal gene amplification status of HER2 (e.g. an average HER2 gene copy number higher than or equal to 2 but less than or equal to 4 copies of the HER2 gene per tumor cell) and amplification of c-myc is sensitive to said modulator.

The patient group with a normal expression of HER2 (e.g. less than HER2(+++)) and amplification of c-myc genes can easily be separated from patients with high expression of HER2 (e.g. HER2(+++)), since a person skilled in the art is aware of standard tests, in particular of immunohistochemical tests, for such a determination of the expression level of HER2. Therefore, a sub-group of the large patient group showing a normal HER2 expression level which has been thought of not being suitable for an efficient HER2 antibody treatment can be successfully subjected to treatment with a HER2 antibody, i.e. the patients having an amplification of the c-myc gene. As described herein below in more detail, a successful treatment of breast cancer patients having a normal HER2 expression level has neither been described nor proposed in the state of the art. To the contrary, a person skilled in the art would not have believed that certain individuals out of the isolated patient group with a normal HER2 expression level can be successfully treated, even if these patients also have an amplification of c-myc genes, for the following reasons.

It is known in the art that the activity of the cMYC gene is usually tightly controlled because an increased activity of the cMYC gene leads to an enhanced division and proliferation of cells. Thus, an uncontrolled, increased expression of cMYC is known to be one factor which may play a role in the development of breast and other cancers.

However, an increased expression of cMYC does, on its own, not inevitably cause the formation of a breast cancer as a consequence of uncontrolled cell proliferation. While overexpressed or amplified cMYC stimulates cell division, cell death (apoptosis) is triggered at the same time. Thus, cMYC which is amplified or expressed in an unregulated manner may trigger cell death and inhibit the proliferation of cells. This may be considered as a self-protective mechanism of an organism against breast cancer in case of amplified/overexpressed cMYC.

As shown herein in Table 2 (see Figure 5) the amplification status of c-myc alone is not sufficient for selecting patients which are likely to respond to a HER2 antibody therapy. Instead, the results of the study presented herein (see Figures 1 to 6 and in particular Tables 2 and 3 in Figures 5 and 6) clearly show that patients having a very high expression level of HER2 (e.g. HER2 (+++)) appear to have a high response rate to HER2 antibody treatment on average, independent of the c-myc amplification status.

Only patients with HER2-positive disease have been included in the clinical trial the results of which are shown in Figures 5 and 6. HER2 positive disease within this trial had been defined as follows: Overexpression of HER2 by immunohistochemistry IHC3+ and/or HER2 amplification higher than 4 according to fluorescent *in situ* hybridization (FISH), based on central laboratory confirmation. Code "D" referred to in Table 2 refers to a patient group being HER2-positive as described above (i.e. IHC3+ and/or HER2 amplification higher than 4) and subjected to chemotherapy and Herceptin treatment. Code "E" referred to in Table 3 refers to a patient group being HER2-positive and subjected to standard, isolated chemotherapy.

HER2 inhibits the cell death suppressing pathway in a cell and can therefore be considered as an antagonist to cMYC which triggers cell death, in particular when overexpressed. It is evident that a high HER2 expression level is needed to counterbalance the increased activity of cMYC reflected by overexpressed or amplified cMYC. It is apparent that a HER2 antibody, such as Herceptin/Trastuzumab, may exhibit its repressive effect on breast cancer development or growth only in a setting with highly over-expressed HER2 (e.g. HER2 (+++)). A person skilled in the art would not expect that also a low or moderate expression of

HER2 may be sufficient to counterbalance an increased activity of cMYC/cMyc/c-myc. Therefore, a person skilled in the art would not have considered that a treatment of breast cancer patients having a low or moderate expression of HER2 and amplified cMyc with a modulator of the HER2/neu (ErbB2) signaling pathway, such as a HER2 antibody treatment, may be particularly successful. As mentioned above, studies investigating the effect of HER2 antibody treatment in such patients have neither been described nor proposed in the art. The only studies performed on breast cancer patients having a low or moderate level of HER2 expression do not take the c-Myc amplification status/level into account and show a rather disappointing response rate of about 5% to 10% in these patients.

In contrast thereto, the present invention describes for the first time that breast cancer patients having a moderate or low HER2 expression level (e.g. less than HER2(+++), for example HER2(++), HER2(+) or HER2(0) in immunohistochemical detection of HER2 in a biological sample such as a breast cancer cell/tissue) and amplification of the cMYC gene in a biological sample can successfully be treated with a modulator of the HER2/neu (ErbB2) signaling pathway. Particularly useful are therapeutic antibodies, e.g., an HER2 antibody such as Herceptin/Trastuzumab. The group of patients identified by the method of the present application shows an increased response rate to HER2 treatment. As a consequence, a subgroup of patients having a moderate or low expression level of HER2 can be subject to successful treatment with a modulator of the HER2/neu (erbB2) signaling pathway, thus increasing the survival rate of these patients. The finding of the present invention therefore represents a major contribution to the art and provides an important benefit to many breast cancer patients. Furthermore, the present invention relates to a method of treatment of patients having a normal level of expression of HER2 (e.g. HER2(0), HER2(+) or HER2(++)) and amplified c-myc genes and corresponding means and methods.

The terms "responder for a modulator of the HER2/neu (ErbB2) signaling pathway" means in the context of the present invention that a subject/patient suspected to suffer from or being prone to suffer from breast cancer shows a response to a treatment with the modulator. An artisan will readily be in the position to determine whether a person treated with the modulator shows a response. For example, a response to a modulator may be reflected in a decreased suffering from breast cancer, such as a diminished and/or halted growth of a breast cancer tumor and/or a reduction of the size of a tumor, the prevention of the formation of

metastases or a reduction of number or size of metastases. It is preferred that a response is reflected in the prevention of the development of a breast cancer tumor or metastases, for example after resection of a tumor in the prolongation of time to disease progression, or in the reduction of the size of (a) tumor(s) and/or (a) metastases, for example in neoadjuvant therapy.

Similarly, the term “patient sensitive to a modulator of the HER2/neu (ErbB2) signaling pathway” refers in the context of the present invention to a patient which shows in some way a positive reaction when treated with the modulator. This reaction of the patient may be less pronounced when compared to a responder as described herein above. For example, the patient may experience less suffering from breast cancer though no reduction in tumor growth may be measured. The reaction of the patient to the modulator may also be only of a transient nature, i.e. growth of (a) tumor and/or (a) metastasis(es) may only be temporarily reduced or halted. It is preferred that a responder for a modulator will not suffer from breast cancer after treatment with the modulator. Preferably, (a) breast cancer tumor(s) and/or (a) breast cancer metastasis(es) which has been resected will not recur within 1 year after termination of the treatment of the responder with the modulator, more preferably within 2 years, 3 years, 4 years, 5 years, 10 years or, most preferably within 15 year after termination of the treatment.

As the skilled artisan fully appreciates neither a positive test for HER2 nor a positive test for c-myc-amplification in the HER2 normal subgroup according to this invention translates 1:1 into a successful treatment. By these methods sub-groups of patients are identified that have a higher chance of response (= show a better response rate) to a treatment with a HER2 signaling inhibitor as compared to the sub-groups of patients not showing these positive test results – no more, but most importantly, no less. The response rate of the isolated group of patients identified by the method of the present invention to a modulator of the HER2/neu (ErbB2) signaling pathway is at least 15 %. Also preferred, the response rate is at least, 18 %, or at least 20 %. With other words a positive result indicates (= is indicative for) that the patient has a higher chance (= probability, likelihood) to respond to (= of being susceptible to) treatment with a HER2 signaling inhibitor as compared to a patient being negative, e.g. testing negative in a method according to the present invention. For example, such a patient being tested negative has a moderate or low HER2 expression level (e.g. less than HER2(+++), for example HER2(++), HER2(+) or HER2(0)) and has a c-myc amplification

status which is not above normal (e.g. no amplification of the cMYC gene as described herein below). A patient being tested positive (i.e. identified according to the method of the invention as being sensitive to a modulator of the HER2/neu (ErbB2) signaling pathway) preferably has a normal HER2 expression level (e.g. HER2(++), HER2(+) or HER2(0)) and a c-myc amplification status above normal.

As mentioned above, it is preferred herein that the expression level of HER2 is detected by an immunohistochemical method. Such methods are well known in the art and corresponding commercial kits are easily 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™. Preferably the level of HER2 protein expression is assessed by using the reagents provided with and following the protocol of the HerceptTest™. 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 2005/117553. 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, a moderate expression level and a high expression level. It is preferred in context of this invention that a sample obtained from a patient suspected to suffer from or being prone to suffer from breast cancer shows a low or moderate expression level of HER2 which may be considered as normal expression level of HER2.

Most preferably the protein expression level of HER2 is normal. For example, a protein expression level of HER2 is normal if it is scored less than HER2 (+++) (e.g. HER2(0), HER2(+) or HER2(++)) by immunohistochemical methods. As already stated herein above, in the prior art patients are only eligible for Herceptin/Trastuzumab treatment if they show a strong HER2 overexpression as described by a 3+ score by immunohistochemistry.

The presently 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” with regard to HER2 overexpression according to the table shown herein above), a “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 mentioned above, the term “normal protein expression level” used herein may reflect a “low protein expression level” or “moderate protein expression level”. 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 normal expression level of HER2 protein can be determined by these techniques and a tissue sample of those patients classified as having a normal level of HER2 protein expression further analyzed for c-Myc gene amplification.

The expression level of HER2 may also be determined by the evaluation of mRNA expression by corresponding techniques, such as Northern Blot, real time PCR, RT PCT and the like. All these detection systems are well known in the art and can be deduced from standard text books, such as Lottspeich (Bioanalytik, Spektrum Akademischer Verlag, 1998) or Sambrook and Russell (2001, Molecular Cloning: A Laboratory Manual, CSH Press, Cold Spring Harbor, NY, USA). A normal expression level of HER2 mRNA can be determined by these techniques and a tissue sample of those patients classified as having a normal level of HER2 mRNA expression further analyzed for c-Myc gene amplification.

As pointed out herein, in an alternative preferred embodiment, the "normal" HER2 expressing patients are also identified by assessing the gene amplification status of HER2. In accordance with this assessment, a normal HER2 gene expression may, inter alia, relate to an average HER2 gene copy number equal to 2 or more than 2 but less than or equal to 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 between 1 and 2 per copy (for those test systems using an internal chromosome 17 centromere control probe).

The method for the identification of a responder for or a patient sensitive to a modulator of the HER2/neu (ErbB2) signaling pathway requires that the c-myc gene is at least amplified above normal. It is preferred that the c-myc gene is at least twice (2x; i.e. 4 copies) amplified above normal in a sample obtained from a patient suspected to suffer from or being prone to suffer from breast cancer. Also preferred, the c-myc gene is at least 3 times or at least 4-times amplified above normal or is at least 5-times amplified above normal. A person skilled in the art knows the normal, non-amplified status of the c-myc gene and may therefore easily

determine, for example, a double (2-times), a 3-times, a 4-times or a 5-times amplification status above normal of the c-myc gene.

The c-myc amplification status in accordance with one of the in situ hybridization methods described herein below can be determined as follows: c-myc can be considered as amplified when the ratio between the c-Myc signals and the centromer signals of chromosome 8 is above 2.0, or if the c-Myc signal per nucleus is 4 or above.

In a preferred embodiment of the present invention, the amplification status of c-myc is evaluated by in situ hybridization. Preferably, the in situ hybridization is 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 c-myc amplification status by in situ hybridization are easily available. For example, a FISH test to be used for the determination of the amplification status of c-myc is the Kit "MYC/CEN-8 FISH Probe Mix" in combination with the "Histology FISH Accessory Kit" (both from the company DAKO).

It is also envisaged herein that the expression level of the c-myc gene or protein, respectively, may be measured thus reflecting the amplification status of the c-myc gene. Corresponding means and methods have been described herein above in context of the determination of the expression level of HER2.

The HER2/neu (ErbB2) signaling pathway is well known in the art and a skilled person is readily in the position to identify such modulators based on his general knowledge and the teaching provided herein. Non-limiting examples of modulators to be used in accordance of this invention are antibodies, preferably monoclonal or humanized antibodies, like Herceptin/Trastuzumab or pertuzumab (see, e.g. WO2007/145862). A preferred embodiment according to this invention is the administration of Herceptin/Trastuzumab to the sub-group of breast cancer patients characterized by a normal protein expression level of Her2 (e.g. HER2(0), HER2(+) or HER2(++)) by IHC) and an amplification above normal of the c-myc gene.

In a preferred embodiment of the invention, the modulator of the HER2/neu (ErbB2) signaling pathway is a HER dimerization/signaling inhibitor or an inhibitor of shedding of the HER2 extracellular domain (ECD).

Preferably the HER dimerization/signaling inhibitor is a HER2 dimerization inhibitor. It is also preferred herein that the HER dimerization inhibitor inhibits HER heterodimerization, HER homodimerization, or both.

In a particular preferred embodiment of the present invention the HER dimerization/signaling inhibitor is a HER antibody. The HER antibody may bind to a HER receptor, such as EGFR, HER2 and HER3. Preferably, the antibody binds to HER2. The HER2 antibody may bind to Domain II of HER2 extracellular domain and/or may bind to a junction between domains I, II and III of HER2 extracellular domain.

In a further preferred embodiment, the HER2 antibody to be employed as a modulator of the HER2 signaling pathway by inhibiting receptor dimerization/signaling in accordance with this invention is Pertuzumab.

It is also preferred herein that the HER shedding inhibitor inhibits HER heterodimerization or HER homodimerization.

In a particular preferred embodiment of the present invention the HER shedding inhibitor is a HER antibody. The HER antibody may bind to a HER receptor, such as EGFR, HER2 and HER3. Preferably, the antibody binds to HER2. Also preferred the HER2 antibody binds to sub-domain IV of the HER2 extracellular domain (ECD).

In a further preferred embodiment, the HER2 antibody to be employed as a modulator of the HER2 signaling pathway by inhibiting ECD shedding in accordance with this invention is Herceptin/Trastuzumab.

As pointed out herein below, in particular in the medical uses and methods provided herein Herceptin/Trastuzumab is a preferred modulator of the HER2/neu (ErbB2) signaling pathway for the treatment of breast cancer patients/patient groups as identified by the above recited

method and as described herein. This novel breast cancer patients/patient group is characterized by their biological samples/biopsies which show in in vitro tests, using the two biomarkers HER2 status (protein expression level of HER2 and/or gene amplification status of HER2) and c-myc gene amplification status/expression level, an normal protein expression level of Her2 (e.g. HER2(0), HER2(+) or HER2(++)) and an amplification above normal of the c-myc gene. The terms "normal level of HER2" and "amplification above normal of the c-myc gene" are described herein above and below. Said quantitative assessment of the expression level of HER2 protein and c-myc gene number may be set in correlation to given control samples which may comprise normal tissue samples, i.e. healthy control samples. Such control samples may be obtained from e.g. healthy volunteers or may be a defined, clearly healthy control tissue from the patient to be assessed for its HER2 status/level and its c-myc status/level. The biological sample to be tested and assessed for said HER2 status/level and said c-myc status/level may in particular be a tissue sample obtained through breast tissue biopsy.

The term "antibody" herein is used in the broadest sense and specifically covers intact monoclonal antibodies, polyclonal antibodies, multispecific 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 term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to polyclonal antibody preparations which include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they may be synthesized uncontaminated by other antibodies. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be constructed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler, G. et al., Nature 256 (1975) 495,

or may be made by recombinant DNA methods (see, e.g., U.S. Pat. No. 4,816,567). "Antibody fragments" comprise a portion of an intact antibody. In context of this invention, antibody modulators of the HER2 pathway to be employed in the means and methods provided herein and in particular in the treatment of the newly identified breast cancer group as defined herein are preferably humanized, fully-human or CDR-grafted antibody molecules. A preferred antibody is Herceptin/Trastuzumab.

The term "sample" shall generally mean any biological sample obtained from an individual. The sample preferably is a breast tissue resection, a breast tissue biopsy, a metastatic lesion or a section of a metastatic lesion or a blood sample known or suspected to comprise circulating tumor cells. According to the invention, the biological sample may comprise breast cancer cells and non-breast cancer cells (other cells). The skilled pathologist is able to differentiate cancer cells from normal breast tissue cells. Methods for obtaining tissue biopsies, tissue resections and body fluids from mammals are well known in the art.

In one embodiment of the present invention the novel sub-group of breast cancer patients as defined herein and being characterized by its biological samples/biopsies showing a normal protein expression level of Her2 (e.g. HER2(0), HER2(+) or HER2(++)) or a normal gene amplification of the HER2 gene (e.g. an average gene copy number of equal to or more than 2 and up to or equal to 4 per nucleus) and an amplification above normal of the c-myc gene, may suffer from metastatic breast cancer.

In one embodiment of the present invention, the sample of said patient to be tested in accordance with this invention for its HER2 status as defined herein above and its c-myc gene amplification status/expression level is obtained before neoadjuvant therapy, i.e. before the treatment with a modulator of the HER2/neu (ErbB2) signaling pathway is initiated. However, also adjuvant therapy and corresponding sampling is envisaged.

In a further embodiment of the present invention, a method for the treatment of breast cancer patients (also metastatic breast cancer) is provided, said treatment comprising a step of administering an effective amount of a modulator of the HER2/neu (ErbB2) signaling pathway to a subject identified by the method of any as provided herein above and a subject in need of such a treatment. Said subject is, in accordance with this invention, preferably a

human subject and biological samples, in particular breast tissue biopsies/breast cells of said subject/patient are characterized in having a normal expression level of HER2 and an amplification/amplification level above normal of the c-myc gene. As documented herein, the person skilled in the art is readily in a position to determine said HER2 expression levels in said biological sample, in particular by immunohistochemical methods known in the art. The same applies, *mutatis mutandis*, for the determination of the given c-myc amplification/amplification level. Here, as pointed out above, the preferred (but not limiting) determination method is an in situ hybridization technique, like fluorescent in situ hybridization (FISH), chromogenic in situ hybridization (CISH) or silver in situ hybridization (SISH). The patient to be treated in accordance with this invention is preferably a human patient and said biological sample wherein said expression level of HER2 and said amplification status of the c-myc gene is determined in vitro is a biological sample from a human patient.

Again, the gist of the present invention relates to the fact that surprisingly patients suffering from breast cancer and showing only a normal status of HER2 (protein) expression (and/or a normal HER2 gene amplification) and an elevated amplification of the c-myc gene can successfully be treated by a modulator of the HER2/neu (ErbB2) signaling pathway. Said modulator comprises in particular an antibody molecule directed against the Her2 protein. Preferred antibody molecules in this respect are Herceptin/Trastuzumab as well as Pertuzumab (as, *inter alia*, described in WO 2007/145862).

Other HER2 signaling modulators or HER2 agents to be used in accordance with this invention on patients which show a normal expression level of HER2 protein or a normal HER2 gene amplification and an amplified c-myc status as defined herein, comprise also tyrosine kinase inhibitors, like the oral tyrosine kinase inhibitor Tykerb (lapatinib ditosylate), HKI272 or BIBW229.

As defined herein above in context of the inventive in vitro method for the identification of a responder for or a patient sensitive to a modulator of the HER2/neu (ErbB2) signaling pathway, the person skilled in the art can easily deduce with known methods what "HER2 expression status" is present in a biological sample of said patient. The patient to be treated with the herein defined modulator of the HER2/neu (ErbB2) signaling pathway shows in the

biological samples a “normal protein expression level” of HER2 and an elevated level of c-myc, i.e. a level above normal of the c-myc gene or a c-myc amplification. Again, a “normal protein expression level” of HER2 corresponds to a 0, 1+ or 2+ score (“negative assessment or weak to moderate over-expression”, see the table above) in immunohistological tests as described herein above and an amplified c-myc gene status/level corresponds to an c-myc gene status/level that is amplified above normal in a sample obtained from said patient suspected to suffer from or being prone to suffer from a breast cancer, i.e., the ratio between the c-Myc signals and the centromer signals of chromosome 8 is above 2.0, or the c-Myc signal per nucleus is above 4.

The person skilled in the art can also easily detect and/or verify the gene amplification status of the Her2 gene. This is also routinely done by in situ hybridizations, like fluorescence in situ hybridizations (FISH) or bright field in situ hybridizations. Accordingly, when the gene amplification status of HER2 is tested, FISH-tests are routinely used and the read-out may comprise the determination of the average HER2 gene copy number or the so-called HER2/CEP17 ratio, whereby said HER2/CEP17 ratio sets the HER2 signal in relation to a signal obtained with centromer probe (CEP17). In context of the present invention and in context of the herein defined new patient group accessible for treatment with the herein defined modulators of the HER2 signaling pathway are breast cancer patients that show a normal gene amplification of HER 2 (and an elevated amplification status in the c-myc gene, said c-myc gene status being higher than in a normal biological tissue sample or a normal cell). Such a “normal” HER2 gene expression level may be defined by an average HER2 copy number between 2 and 4 (i.e. a copy number of equal to 2 or higher and less than or equal to 4).

In accordance with this embodiment of the present invention, a method of treating preferably a human breast cancer patient is provided, wherein said patients are evaluated for the expression level of the HER2 protein (or for the HER2 gene amplification level) and the amplification level of c-myc and whereby the correlation of this expression level of the HER2 (protein) or HER2 gene expression level (or the HER2 gene amplification level/status) and the c-myc (gene) amplification is assessed. It is envisaged in accordance with this invention that patients showing a “normal protein expression level” (corresponding to a 0, 1+ or 2+ score or “negative assessment or weak to moderate overexpression”, see the table above) of

HER2 (or showing a “normal” HER2 gene amplification level) and an amplification of the c-myc gene above normal (preferably said c-myc gene is at least twice amplified above normal in a sample obtained from said patient) suspected to suffer from or being prone to suffer from breast cancer, also metastatic breast cancer, show in accordance with this invention a positive survival benefit, a prolongation in time to progression, and/or show less recurrent breast cancers when treated with the herein defined modulator(s) of the HER2/neu (ErbB2) signaling pathway, in particular with Herceptin/Trastuzumab.

The medical uses and methods as described herein relate to the use of the herein described modulator of the HER2/neu (ErbB2) signaling pathway, in particular antibodies against or directed to HER2, like and preferably Herceptin/Trastuzumab, on patients that show the herein determined HER2 “normal (protein) expression status” (versus a “high protein expression level” as defined herein above) or patients that have a “normal HER2 gene amplification status and an amplified c-myc gene amplification status/ expression level as defined herein, i.e. double as high as normal (e.g. 4 copies per cell). In context of this invention, said HER2/neu (ErbB2) signaling pathway, in particular antibodies against or directed to HER2, like and preferably Herceptin/Trastuzumab may be employed in adjuvant as well as in neo-adjuvant breast cancer therapies. Accordingly, said “HER2-modulator” may be administered to a patient in need of such a treatment and having the herein defined biomarker status before, during of 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-signalling pathway modulator (like Herceptin/Trastuzumab) given to the herein defined breast 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 Herceptin/Trastuzumab) are breast cancer patients wherein the two biomarkers, i.e. HER2 protein expression and the c-myc gene amplification status, are assessed and wherein said Her 2 protein expression is a “normal expression status” (HER2(0), HER2(+) or HER2(++)) and said c-myc gene amplification status is above normal (i.e. is at least double as high as the c-myc status in a normal control).

In accordance with the medical as well as the diagnostic (in vitro) methods provided herein the person skilled in the art can, inter alia, determine the status/level of c-myc amplification in a given sample and the expression level/status of HER2 (or the gene amplification status/level

of HER2 gene) by means and methods known in the art. These methods also comprise the comparison of the given sample with a control sample, i.e. with a biological sample which is not cancerous and which, inter alia, be derived from a healthy (control) individual or from non-diseased tissue.

In context of the medical embodiments provided herein, i.e. methods and uses, the modulator of the HER2/neu (ErbB2) signaling pathway to be administered to the herein defined patient group (breast cancer patients/patients with metastatic breast cancer and having a normal (HER2+) expression level of the HER2 protein or an "normal" HER2 gene amplification status and an elevated c-myc gene status/amplified c-myc gene status) may be administered as a single anti-tumor agent. However, also co-therapeutic approaches are envisaged and of use, which comprise, inter alia, the administration of further pharmaceuticals, in particular anti-cancer drugs. Such an additional therapy may be a chemotherapy and may comprise the administration of drugs like, anti-metabolite agents (for example gemcitabine), an anti-hormonal compound, an anti-estrogen, a tyrosine kinase inhibitor, a raf inhibitor, a ras inhibitor, a dual tyrosine kinase inhibitor, taxol, an taxane (like paclitaxel or docetaxel), an anthracycline, like doxorubicin or epirubicin, or adjuvant (anti-) hormonal therapy (i.e. therapy with adjuvant (anti-) hormone drugs, such as tamoxifen or an aromatase inhibitor). Also vinorelbine can be used in the inventive co-therapy approaches. Furthermore co-therapy approaches with in particular Herceptin/Trastuzumab may comprise the administration cyclophosphamide, methotrexate or fluorouracil (which is also known as 5-FU) individual or in form of a combination therapy comprising these three drugs ("CMF therapy"). Also aromatase inhibitors may be used in the herein defined co-therapy approaches for the treatment of the new group of breast cancer patients described in context of this invention. Such aromatase inhibitors comprise, but are not limited to, anastrozole or letrozole.

The preferred therapeutic approach provided herein, i.e. the use of a modulator of HER2 signaling may also be combined with another therapy, Such combination therapy may preferably also rely on the use of chemotherapeutic agent, or it may also comprise hormonal as well as anti-hormonal drugs or anti-angiogenic agents which comprise (but are not limited to) the administration of a VEGF blocker, like, e.g. bevacizumab/Avastin or sunitinib malate-SU-11248).

The person skilled in the art, for example the attending physician, is readily in a position to administer the herein defined modulator of the HER2/neu (ErbB2) signaling pathway to patient/patient group as defined herein. Such an administration may comprise the parenteral route, the oral route, the intravenous route, the subcutaneous route, the intranasal route or the transdermal route. In case of Herceptin/Trastuzumab, the preferred administration route is an intravenous administration. Such an administration of Herceptin/Trastuzumab may, in the novel breast cancer patients (group) /metastatic breast cancer patients (patient group) as defined herein comprises, inter alia, an administration every day, every other day, every third day, every forth day, every fifth day, once a week, once every second week, once every third week, once every month, etc.

Again, the attending physician may modify, change or amend the administration schemes for modulator of the HER2/neu (ErbB2) signaling pathway in accordance with his/her professional experience. In a particular preferred embodiment of the present invention, a method for the treatment of breast cancer patient or patient group is provided, said method comprising the administration of Herceptin/Trastuzumab to said patient/patient group, whereby said patient/patient group is characterized in the assessment of a biological sample (in particular a biopsy, most preferably breast tissue resections), said sample showing an normal protein expression level of Her2 (HER2(0), HER2(+) or HER2(++)) (or a "normal HER2 gene amplification status") and an amplification above normal of the c-myc gene, in particular a c-myc amplification level/status which is at least 3 times higher than the c-myc gene level/status in a healthy control sample. Therefore, the present invention also provides for the use of Herceptin/Trastuzumab in the preparation of pharmaceutical compositions for the treatment of breast cancer patients which are characterized by the herein disclosed biomarker status (a normal protein expression level of Her2 (HER2(0), HER2(+) or Her2(++)) or a "normal HER2 gene amplification status" as defined herein above and an amplification above normal of the c-myc gene) or which have been identified by the herein described in vitro method for the identification of a responder for or a patient sensitive to a modulator of the HER2/neu (ErbB2) signaling pathway. Said breast cancer patient/patient group may also suffer from a metastatic breast cancer.

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

New data show that the addition of Herceptin (Trastuzumab) to chemotherapy prior to breast cancer surgery (neoadjuvant therapy) completely eradicates tumors in nearly three times as many women with inflammatory HER2-positive breast cancer compared to chemotherapy alone. Inflammatory breast cancer is a rare, but highly aggressive form of the disease - the tumors spread quickly, often leading to the need for total mastectomies, and it has a worse outlook than other breast cancers. These results are particularly significant as treatment with Herceptin in this setting may actually lead to more breast conserving surgery and most importantly to potentially improved survival.

The results from the NeOAdjuvant Herceptin (NOAH) study demonstrate that Herceptin plus chemotherapy led to the complete disappearance of the tumor in the breast (a pathological complete response to treatment) in nearly three times as many patients with inflammatory breast cancer (55% vs. 19%, $p=0.004$) compared to chemotherapy alone. Furthermore, the combination led to complete disappearance of the tumors from both the breast and the lymph nodes (a total pathological complete response to treatment) in 48% of patients, compared to only 13% of those who received chemotherapy alone ($p=0.002$). The treatment was well tolerated with acceptable cardiac safety. NOAH is a phase III trial assessing neoadjuvant Herceptin in combination with chemotherapy in patients with HER2-positive locally advanced breast cancer (LABC). Patients were assigned to one of two cohorts depending on HER2 status. All patients received neoadjuvant chemotherapy before surgery consisting of three cycles of doxorubicin-paclitaxel (AT), four cycles of paclitaxel (T) and three cycles of cyclophosphamide / methotrexate / 5-fluorouracil (CMF). Patients with HER2-positive disease were randomized to receive concomitant Herceptin for one year or chemotherapy only. Out of 228 fully documented patients with HER2-positive breast cancer that were included in the study, 61 had inflammatory breast cancer (IBC). Of the 99 fully documented patients with HER2-negative breast cancer (e.g. no HER2(3+) status, no HER2 amplification), 14 had IBC. 31 patients with HER2-positive IBC received Herceptin in addition to chemotherapy.

The full term and explanation of abbreviations used in the figures is given herein below:

pCR (pathological Complete Response), RD (Residual Disease). The evaluation “pCR” or “RD” is made by the pathologist who investigates the tissue after resection upon neoadjuvant chemotherapy. The status “pCR” is given if no tumor tissue is found. The status “RD” is given, if tumor is still found.

ER means Estrogen Receptor, PR means Progesterone Receptor (which are receptors for the hormones estrogen and progesterone). The ER and PR status of breast tumors is routinely checked. Patients with positive hormone receptors are treated correspondingly, e.g. by standard, isolated chemotherapy, such as Tamoxifen or Anastrozol chemotherapy.

In Figure 7 a possible HER2/myc testing algorithm in accordance with the present invention is shown. A HER2 protein expression level assessed as “normal” (HER2(0), HER2(+)) or “HER2(++)) by immunohistochemical methods may be followed by a retest of HER2 gene amplification status by e.g. in situ hybridization. For example, a sample with a protein expression level scored as HER2(2+) followed by a retest wherein the HER2 gene amplification status is evaluated “normal” (equal to 2 or higher and less than or equal to 4 copies) may be subjected to evaluation of the c-myc status. However, said additional retest is not compulsory and, accordingly, samples having a normal protein expression level of HER2 (HER2(0), HER2(+)) or HER2(++)) by IHC) may be subjected to evaluation of the c-myc amplification status without being tested for HER2 amplification status prior thereto. Generally, a sample which is evaluated “normal” in respect of its HER2 status (protein expression level and/or gene amplification level), may be subjected to evaluation of c-myc status (in particular gene amplification status). As described herein above, a sample with a normal HER2 status and amplification above normal of the c-myc gene is indicative for a responding patient.

The Figures show:

Figure 1.

Figure 1 shows the whole data (with biomarker info) used in the study. In Figure 1A the univariate logistic regression (# of cases included in analysis = 247) and in particular the variables in the equation are given. Figure 1 B shows the Multivariate logistic regression (# of cases included in analysis = 192) and the corresponding Model Summary (wherein “a” means

“Estimation terminated at iteration number 6 because parameter estimates changed by less than .001.”), the classification table and the variables in the Equation (wherein “a” means Variable(s) entered on step 1: ER, PGR, tcode, TCLIN, age, NCLIN, pten.cyt.IRS, pten.memb.IRS, pten.nucl.IRS, igfr.cyt.IRS, igfr.memb.IRS, igfr.nucl.IRS, myc.status, her3.cyt.IRS, her3.memb.IRS, her3.nucl.IRS.)

Figure 2.

Figure 2 shows data for treatment code = D. Figure 2A shows the univariate logistic regression ((# of cases included in analysis = 98) and corresponding variables in the Equation. Figure 2 B shows the multivariate logistic regression (# of cases included in analysis = 84). Due to the smaller sample size, a model with all the independent variables may be overfitting. So the multivariate regression model was build by using variables whose p-values are less than 0.25 in the univariate analysis. Also a model summary (wherein “a” means “Estimation terminated at iteration number 5 because parameter estimates changed by less than .001.”), a classification table and Variables in the Equation (wherein “a” means “Variable(s) entered on step 1: age, ER, PGR, myc.status, igfr.memb.IRS, igfr.nucl.IRS, igfr.total.IRS, her3.memb.IRS, her3.total.IRS”) are given.

Figure 3.

Figure 3 shows data for treatment code = E. Figure 3A shows the univariate logistic regression ((# of cases included in analysis = 86) and corresponding variables in the Equation. Figure 3B shows the Multivariate logistic regression (# of cases included in analysis = 74). Due to the smaller sample size, a model with all the independent variables may be overfitting. So the multivariate regression model was build by using variables whose p-values are less than 0.25 in the univariate analysis. Also a Model Summary (wherein “a” means “Estimation terminated at iteration number 6 because parameter estimates changed by less than .001.”), a classification table and Variables in the Equation (wherein “a” means “Variable(s) entered on step 1: PGR, NCLIN, myc.status, pten.memb.IRS, pten.nucl.IRS, pten.total.IRS, igfr.nucl.IRS, her3.nucl.IRS”) are given.

Figure 4.

Figure 4 shows Table 1 with Clinical information and demographics of the 327 patients included in the study. The column percentages are listed. P-values were from chi-square test

except the number of cases was less than 5 in any category, for which Fisher's exact test was used. * indicates p-value got from two-sample t test with unequal variances.

Figure 5.

Figure 5 shows Table 2 with data in respect of Treatment code = D (# of patients = 98). The row percentages are listed. P-values were from chi-square test except the number of cases was less than 5 in any category, for which Fisher's exact test was used. * indicates p-value got from two-sample t test.

Figure 6.

Figure 6 shows Table 3 with data in respect of Treatment code = E (# of patients = 86). The row percentages are listed. P-values were from chi-square test except the number of cases was less than 5 in any category, for which Fisher's exact test was used. * indicates p-value got from two-sample t test.

Figure 7.

Figure 7 shows how testing for c-myc amplification status can be integrated into a workflow for identification of patients likely to be responsive/sensitive to a treatment with a modulator of the HER2/neu (ErbB2) signaling pathway, like Herceptin.

"3+", "2+", "1+" and "0" refer to the protein expression level "HER2 (3+)", "HER2(2+)", "HER2(1+)" and "HER2(0)", respectively, as described herein above, in particular in the table herein above. "HER2(2+)", "HER2(1+)" and "HER2(0)" are considered as "normal" HER2 protein expression level. "-" used in the assessment of the HER2 gene amplification status ("Retest with ISH" and "HER2 ISH", respectively) refers to a "normal" gene amplification status of the HER2 gene (equal to or higher than 2 and lower than or equal to 4 copies), whereas "+" refers in this context to a high gene amplification status of the HER2 gene (higher than 4 copies). "+" in the assessment of the amplification status of the c-myc gene ("myc FISH") refers to an amplification above normal, whereas "-" refers to a normal amplification of the c-myc gene.

CLAIMS

1. An in vitro method for the identification of a responder for or a patient sensitive to a modulator of the HER2/neu (ErbB2) signaling pathway, said method comprising the following steps:
 - (a) obtaining a sample from a patient suspected to suffer from or being prone to suffer from breast cancer;
 - (b) evaluating the HER2 status (protein expression level of HER2 and/or the gene amplification status of the HER2 gene); and
 - (c) in case the HER2 status is (b) is normal, evaluating the amplification status of the c-myc gene in said sample;whereby an amplification above normal of the c-myc gene is indicative for a responding patient or is indicative for a sensitivity of said patient to said modulator of the HER2/neu (ErbB2) signaling pathway.
2. The method of claim 1, wherein said protein expression level of HER2 is detected by an immunohistochemical (IHC) method or wherein said gene amplification status of the HER2 gene is detected by an in situ hybridization method.
3. The method of claim 2, wherein said protein expression level of HER2 is normal if it is scored less than HER2 (+++) in IHC.
4. The method of any one of claims 1 to 3, wherein the response rate of a group of patients identified by the method of claim 1 to a modulator of the HER2/neu (ErbB2) signaling pathway is at least 15 %.
5. The method of any one of claims 1 to 4, wherein said sample is selected from the group consisting of breast tissue resection, breast tissue biopsy, metastatic lesion and circulating tumor cells.
6. The method of anyone of claims 2 to 5, wherein said immunohistochemical method is performed with the HerceptTest™ assay

7. The method of any one of claims 1 to 6, wherein said c-myc gene is at least twice amplified.
8. The method of any one of claims 1 to 7, wherein said amplification status of c-myc is evaluated by in situ hybridization.
9. The method of claim 8, wherein said in situ hybridization is selected from the group consisting of fluorescent in situ hybridization (FISH), chromogenic in situ hybridization (CISH) and silver in situ hybridization (SISH).
10. The method of claim 9, wherein said FISH test is selected from the group consisting of the "MYC/CEN-8 FISH Probe Mix" in combination with the "Histology FISH Accessory Kit".
11. The method of any one of claims 1 to 10, wherein said sample is obtained before neoadjuvant therapy.
12. The method of any one of claims 1 to 10, wherein said sample is obtained before adjuvant therapy.
13. A modulator of the HER2/neu (ErbB2) signaling pathway for use in treating breast cancer in a patient identified by the method of any one of claims 1 to 12.
14. Use of a modulator of the HER2/neu (ErbB2) signaling pathway for the preparation of a pharmaceutical composition for the treatment of breast cancer in a patient identified by the method of any one of claims 1 to 12.
15. A method for the treatment of breast cancer comprising administering an effective amount of a modulator of the HER2/neu (ErbB2) signaling pathway to a subject identified by the method of any one of claims 1 to 12 in need of such a treatment.
16. The method of claim 15, wherein said subject is a human.

17. The method of claim 15 or 16, the modulator of claim 13 or the use of claim 14, wherein said modulator of the HER2/neu (ErbB2) signaling pathway is administered as a single anti-tumor agent.
18. The method of claim 15 or 16, the modulator of claim 13 or the use of claim 14, wherein said modulator of the HER2/neu (ErbB2) signaling pathway is administered in form of a combination therapy .
19. The method of claim 18, the modulator of claim 18 or the use of claim 18, wherein the therapy used in said combination therapy is chemotherapy or an anti-hormonal therapy.
20. The method of claim 19, the modulator of claim 19, or the use of claim 19, wherein said chemotherapy is selected from the group consisting of 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.
21. The method of any one of claims 15 to 20, the modulator of any one of claims 13 and 17 to 20 or the use of any one of claims 14 and 17 to 20, wherein said modulator of the HER2/neu (ErbB2) signaling pathway is administered by any one of a parenteral route, oral route, intravenous route, subcutaneous route, intranasal route or transdermal route.
22. The method of any one of claims 15 to 20, the modulator of any one of claims 13 and 17 to 20 or the use of any one of claims 14 and 17 to 20, wherein said modulator of the HER2/neu (ErbB2) signaling pathway is administered in a neoadjuvant or adjuvant setting.
23. The method of any one of claims 1 to 12 and 15 to 22, the modulator of any one of

claims 13 and 17 to 22 or the use of any one of claims 14 and 17 to 22, wherein said modulator of the HER2/neu (ErbB2) signaling pathway is a HER dimerization/signaling inhibitor or an inhibitor of HER shedding.

24. The method of claim 23, the modulator of claim 23 or the use of claim 23, wherein said HER dimerization inhibitor is a HER2 dimerization inhibitor.
25. The method of claim 23 or 24, the modulator of claim 23 or 24 or the use of claim 23 or 24, wherein said HER dimerization inhibitor inhibits HER heterodimerization or HER homodimerization.
26. The method of any one of claims 23 to 25, the modulator of any one of claims 23 to 25 or the use of any one of claims 23 to 25, wherein said HER dimerization inhibitor is a HER antibody.
27. The method of claim 26, the modulator of claim 26 or the use of claim 26, wherein said HER antibody binds to a HER receptor selected from the group consisting of EGFR, HER2 and HER3.
28. The method of claim 27, the modulator of claim 27 or the use of claim 27, wherein said antibody binds to HER2 .
29. The method of claim 28, the modulator of claim 28 or the use of claim 28, wherein said HER2 antibody binds to domain II of HER2 extracellular domain.
30. The method of claim 29, the modulator of claim 29 or the use of claim 29, wherein said antibody binds to a junction between domains I, II and III of HER2 extracellular domain.
31. The method of any one of claims 27 to 30, the modulator of any one of claims 27 to 30 or the use of any one of claims 27 to 30, wherein said HER2 antibody is Pertuzumab.

32. The method of claim 23, the modulator of claim 23 or the use of claim 23, wherein said inhibitor of HER shedding is a HER2 shedding inhibitor.
33. The method of claim 23 or 32, the modulator of claim 23 or 32 or the use of claim 23 or 32, wherein said inhibitor of HER shedding inhibits HER heterodimerization or HER homodimerization.
34. The method of any one of claims 23, 32 and 33, the modulator of any one of claims 23, 32 and 33, or the use of any one of claims 23, 32 and 33, wherein said inhibitor of HER shedding is a HER antibody.
35. The method of claim 34, the modulator of claim 34, or the use of claim 34, wherein said HER antibody binds to a HER receptor selected from the group consisting of EGFR, HER2 and HER3.
36. The method of claim 35, the modulator of claim 35 or the use of claim 35, wherein said antibody binds to HER2.
37. The method of claim 36, the modulator of claim 36 or the use of claim 36, wherein said HER2 antibody binds to sub-domain IV of the HER2 extracellular domain.
38. The method of any one of claims 35 to 37, the modulator of any one of claims 35 to 37 or the use of any one of claims 35 to 37, wherein said HER2 antibody is Herceptin/Trastuzumab.
39. The method of any one of claims 1 to 12 and 15 to 38, the modulator of any one of claims 13 and 17 to 38 or the use of any one of claims 14 and 17 to 38, wherein said breast cancer is metastatic breast cancer.

Figure 1.

A.

	B	S.E.	Wald	df	Sig.	Exp(B)	R square	Correct prediction (%)
age	.003	.014	.049	1	.824	1.003	0.001	71
ER	-1.414	.352	16.168	1	.000	0.243	0.075	71
PR	-1.674	.456	13.462	1	.000	0.188	0.072	71
Tcode (C)	-.405	.448	.818	1	.366	.667	0.075	71
Tcode (D)	1.083	.334	10.500	1	.001	2.954	0.075	71
Tumor size	-.028	.143	.038	1	.845	.972	0.010	71
Node status	.088	.175	.255	1	.613	1.092	0.001	71
myc.status (deleted)	.379	.641	.350	1	.554	1.461	0.007	72
myc.status (amplified)	.409	.353	1.342	1	.247	1.505	0.007	72
pten.cyt.IRS	.000	.003	.002	1	.968	1.000	0.001	73
pten.memb.IRS	-.001	.003	.110	1	.740	.999	0.001	73
pten.nucl.IRS	.000	.002	.007	1	.932	1.000	0.001	73
pten.total.IRS	.000	.001	.035	1	.852	1.000	0.001	73
igfr.cyt.IRS	-.007	.003	4.160	1	.041	.993	0.019	73
igfr.memb.IRS	-.006	.003	5.453	1	.020	.994	0.027	72
igfr.nucl.IRS	-.001	.002	.107	1	.744	.999	0	73
igfr.total.IRS	-.003	.001	4.581	1	.032	.997	0.023	73
Her3.cyt.IRS	.001	.002	.086	1	.769	1.001	0.001	71
Her3.memb.IRS	-.004	.002	3.387	1	.066	.996	0.015	71
Her3.nucl.IRS	-.006	.009	.372	1	.542	.994	0.002	71
Her3.total.IRS	-.003	.002	3.131	1	.077	.997	0.013	71

Figure 1 (cont.).

B.

Step	-2 Log likelihood	Cox & Snell R Square	Nagelkerke R Square
1	181.170(a)	.209	.302

Classification Table^a

Observed		Predicted		
		overall.pcr		Percentage Correct
		.00	1.00	
Step 1	overall.pcr	.00	1.00	
		131	8	94.2
		31	22	41.5
	Overall Percentage			79.7

a. The cut value is .500

		B	S.E.	Wald	df	Sig.	Exp(B)	95.0% C.I. for EXP(B)	
		Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper
Step 1(a)	ER(1)	-1.125	.633	3.159	1	.076	.325	.094	1.123
	PGR(1)	-1.599	.653	5.992	1	.014	.202	.056	.727
	tcode			16.473	2	.000			
	tcode(C)	-.670	.829	.654	1	.419	.512	.101	2.596
	tcode(D)	1.476	.424	12.099	1	.001	4.377	1.905	10.056
	TCLIN	-.454	.229	3.926	1	.048	.635	.406	.995
	age	.012	.019	.399	1	.527	1.012	.975	1.052
	NCLIN	-.404	.290	1.940	1	.164	.668	.379	1.179
	pten.cyt.IRS	.009	.005	2.556	1	.110	1.009	.998	1.019
	pten.memb.IRS	.003	.005	.479	1	.489	1.003	.994	1.012
	pten.nucl.IRS	-.003	.003	.593	1	.441	.997	.991	1.004
	igfr.cyt.IRS	-.007	.005	1.576	1	.209	.993	.983	1.004
	igfr.memb.IRS	.003	.004	.498	1	.481	1.003	.995	1.010
	igfr.nucl.IRS	.001	.004	.106	1	.745	1.001	.993	1.010
	myc.status			2.772	2	.250			
	myc.status(1)	.260	.747	.121	1	.728	1.296	.300	5.603
	myc.status(2)	.742	.446	2.769	1	.096	2.099	.876	5.029
	her3.cyt.IRS	-.005	.003	2.007	1	.157	.995	.988	1.002
	her3.memb.IRS	-.001	.004	.027	1	.870	.999	.991	1.007
	her3.nucl.IRS	.003	.010	.071	1	.790	1.003	.984	1.021
	Constant	.651	1.802	.130	1	.718	1.917		

Figure 2.

A.

	B	S.E.	Wald	df	Sig.	Exp(B)	R square	Correct prediction (%)
age	.028	.023	1.585	1	.208	1.029	0.016	56
ER	-1.337	.520	6.606	1	.010	.263	0.073	59
PR	-1.786	.666	7.192	1	.007	.168	0.091	58
Tumor size	.018	.198	.008	1	.928	1.018	0.000	56
Node status	.169	.258	.431	1	.511	1.184	0.004	53
myc.status (deleted)	.114	.952	.014	1	.904	1.121	0.022	60
myc.status (amplified)	.674	.477	1.999	1	.157	1.962	0.022	60
pten.cyt.IRS	-.001	.004	.059	1	.808	.999	0.001	58
pten.memb.IRS	-.004	.005	.538	1	.463	.996	0.006	58
pten.nucl.IRS	-.002	.003	.431	1	.512	.998	0.005	58
pten.total.IRS	-.001	.002	.546	1	.460	.999	0.006	58
igfr.cyt.IRS	-.004	.005	.606	1	.436	.996	0.007	56
igfr.memb.IRS	-.014	.005	7.494	1	.006	.986	0.104	63
igfr.nucl.IRS	.007	.006	1.649	1	.199	1.007	0.019	62
igfr.total.IRS	-.005	.003	3.190	1	.074	.995	0.037	62
Her3.cyt.IRS	-.002	.003	.386	1	.535	.998	0.004	56
Her3.memb.IRS	-.007	.004	3.331	1	.068	.993	0.037	53
Her3.nucl.IRS	-.739	456.697	.000	1	.999	.477	0.037	56
Her3.total.IRS	-.007	.003	5.635	1	.018	.993	0.063	69

Figure 2 (cont.)

B.

Step	-2 Log likelihood	Cox & Snell R Square	Nagelkerke R Square
1	88.501(a)	.268	.360

Classification Table^a

Observed		Predicted		
		overall.pcr		Percentage Correct
		.00	1.00	
Step 1	overall.pcr	.00		
		36	12	75.0
		11	25	69.4
	Overall Percentage			72.6

a. The cut value is .500

		B	S.E.	Wald	df	Sig.	Exp(B)	95.0% C.I. for EXP(B)	
		Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper
Step	age								
1(a)		.056	.029	3.642	1	.056	1.058	.998	1.121
	ER(1)	-1.594	.975	2.674	1	.102	.203	.030	1.372
	PGR(1)	-1.410	.840	2.818	1	.093	.244	.047	1.266
	myc.status			5.562	2	.062			
	myc.status(1)	-.209	1.101	.036	1	.849	.811	.094	7.015
	myc.status(2)	1.573	.691	5.186	1	.023	4.821	1.245	18.665
	igfr.memb.IRS	.002	.013	.029	1	.865	1.002	.977	1.028
	igfr.nucl.IRS	.005	.014	.130	1	.718	1.005	.978	1.033
	igfr.total.IRS	-.003	.008	.128	1	.720	.997	.981	1.014
	her3.memb.IRS	.008	.006	1.674	1	.196	1.008	.996	1.021
	her3.total.IRS	-.011	.005	4.637	1	.031	.989	.980	.999
	Constant	-1.058	1.667	.403	1	.526	.347		

Figure 3.

A.

	B	S.E.	Wald	df	Sig.	Exp(B)	R square	Correct prediction (%)
age	.013	.025	.286	1	.593	1.013	0.003	79
ER	-.515	.623	.683	1	.409	.597	0.008	79
PR	-1.483	1.072	1.915	1	.166	.227	0.031	79
Tumor size	.024	.280	.007	1	.933	1.024	0.000	79
Node status	-.549	.353	2.419	1	.120	.578	0.029	79
myc.status (deleted)	.549	.921	.354	1	.552	1.731	0.033	79
myc.status (amplified)	-1.323	1.084	1.489	1	.222	.266	0.033	79
pten.cyt.IRS	.005	.005	.914	1	.339	1.005	0.011	80
pten.memb.IRS	.009	.005	2.683	1	.101	1.009	0.031	80
pten.nucl.IRS	.007	.004	2.773	1	.096	1.007	0.034	80
pten.total.IRS	.004	.002	3.446	1	.063	1.004	0.043	79
igfr.cyt.IRS	-.007	.007	1.251	1	.263	.993	0.016	79
igfr.memb.IRS	.002	.004	.331	1	.565	1.002	0.004	79
igfr.nucl.IRS	-.019	.016	1.477	1	.224	.981	0.027	79
igfr.total.IRS	-.001	.003	.255	1	.613	.999	0.003	79
Her3.cyt.IRS	.004	.005	.624	1	.430	1.004	0.008	80
Her3.memb.IRS	.004	.004	.890	1	.346	1.004	0.010	79
Her3.nucl.IRS	.014	.012	1.364	1	.243	1.014	0.017	80
Her3.total.IRS	.004	.003	1.162	1	.281	1.004	0.014	78

Figure 3 (cont.).

B.

Step	-2 Log likelihood	Cox & Snell R Square	Nagelkerke R Square
1	54.188(a)	.212	.341

Classification Table^a

Observed		Predicted		
		overall.pcr		Percentage Correct
		.00	1.00	
Step 1	overall.pcr	.00		
		56	4	93.3
		11	3	21.4
	Overall Percentage			79.7

a. The cut value is .500

		B	S.E.	Wald	df	Sig.	Exp(B)	95.0% C.I. for EXP(B)	
		Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper
Step	PGR(1)	-1.300	1.361	.912	1	.340	.273	.019	3.926
1(a)	NCLIN	-.364	.475	.586	1	.444	.695	.274	1.764
	myc.status			.248	2	.883			
	myc.status(1)	.109	1.060	.011	1	.918	1.115	.140	8.916
	myc.status(2)	-.551	1.167	.223	1	.636	.576	.059	5.671
	pten.memb.IRS	.041	.019	4.466	1	.035	1.041	1.003	1.081
	pten.nucl.IRS	.030	.016	3.467	1	.063	1.031	.998	1.064
	pten.total.IRS	-.020	.012	2.829	1	.093	.980	.957	1.003
	igfr.nucl.IRS	-.041	.031	1.761	1	.185	.960	.903	1.020
	her3.nucl.IRS	.038	.030	1.635	1	.201	1.039	.980	1.101
	Constant	-1.080	.962	1.260	1	.262	.340		

Figure 4.

	Cases without biomarker data (N=80)	Cases in biomarker study (N=247)	p-value ¹
Mean Age (SD)	49.1 (10.90)	52.3 (10.17)	*0.016
Median Age (Min-Max)	48 (31-73)	52 (26-80)	
Race			1.000
Caucasian	80 (100%)	245 (99.2%)	
Black	0	1 (0.4%)	
Oriental	0	1 (0.4%)	
Treatment Arm			<0.001
C	38 (48%)	61 (25%)	
D	16 (20%)	99 (40%)	
E	26 (32%)	87 (35%)	
ER Status			0.217
Positive	37 (46%)	95 (39%)	
Negative	43 (54%)	152 (61%)	
PR Status			0.001
Positive	37 (46%)	64 (26%)	
Negative	43 (54%)	183 (74%)	
Baseline Tumor Size			0.184
1	3 (4%)	2 (1%)	
2	9 (11%)	31 (12%)	
3	20 (25%)	46 (19%)	
4	34 (42%)	106 (43%)	
5 (inflammatory)	14 (18%)	62 (25%)	
Baseline Node Stage			0.457
0	9 (11%)	41 (17%)	
1	38 (47%)	94 (38%)	
2	30 (38%)	99 (40%)	
3	3 (4%)	13 (5%)	
Histology			
No residual	12 (22%)	56 (26%)	
Only in situ	4 (8%)	21 (10%)	
IDC	27 (51%)	105 (49%)	0.638
ILC	6 (11%)	11 (5%)	
IDC+ILC	0	1 (0.5%)	
Invasive NOS	3 (6%)	12 (5.5%)	
Other	1 (2%)	7 (3%)	
Response Outcome			0.183
pCR	14 (18%)	70 (28%)	
RD	54 (67%)	174 (71%)	
Unknown	12 (15%)	3 (1%)	

Figure 5.

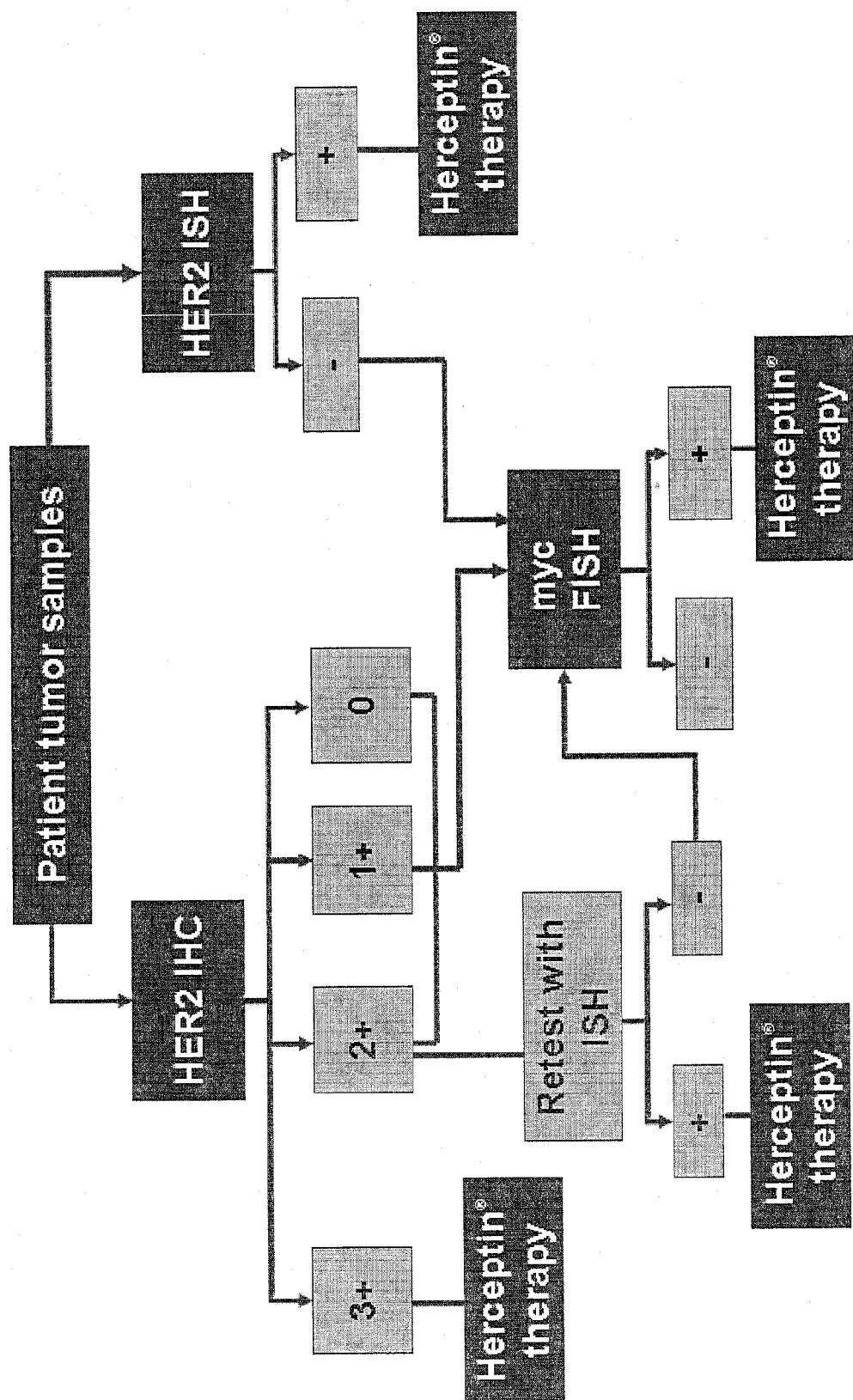
	RD (N=55)	pCR (N=43)	p-value	Spearman Correlation
Mean Age (SD)	49.55 (8.56)	51.93 (10.06)	*0.208	0.128
Median Age (Min-Max)	48 (35-73)	54 (26-76)		
Race			1.000	-0.128
Caucasian	53 (55%)	43 (45%)		
Black	1 (100%)	0		
Oriental	1 (100%)	0		
ER Status			0.008	0.269
Positive	21 (78%)	6 (22%)		
Negative	34 (48%)	37 (52%)		
PR Status			0.005	0.295
Positive	17 (85%)	3 (15%)		
Negative	38 (49%)	40 (51%)		
Baseline Tumor Size			0.338	0.055
1	0	2 (100%)		
2	6 (55%)	5 (45%)		
3	11 (65%)	6 (35%)		
4	25 (63%)	15 (37%)		
5 (inflammatory)	13 (46%)	15 (54%)		
Baseline Node Stage			0.497	0.079
0	8 (67%)	4 (33%)		
1	23 (59%)	16 (41%)		
2	19 (48%)	21 (52%)		
3	5 (71%)	2 (29%)		
Histology			NA	NA
IDC	33 (100%)	0		
ILC	0	0		
Invasive NOS	4 (100%)	0		
Other	1 (100%)	0		
MYC Status			0.381	0.146
Deleted	3 (60%)	2 (40%)		
Normal	37 (63%)	22 (37%)		
Amplified	12 (46%)	14 (54%)		
Mean PTEN cyt IRS (SD)	93.98 (44.41)	91.49 (55.71)	*0.811	-0.025
Mean PTEN memb IRS (SD)	38.24 (43.42)	31.77 (40.06)	*0.466	-0.113
Mean PTEN nuc IRS (SD)	112.41 (66.77)	102.72 (75.89)	*0.516	-0.059
Mean PTEN total IRS (SD)	244.63 (108.53)	225.97 (136.08)	*0.481	-0.058
Mean IGFR cyt IRS (SD)	61.73 (42.03)	55.13 (38.10)	*0.441	-0.092
Mean IGFR memb IRS (SD)	58.14 (68.37)	21.38 (36.57)	*0.002	-0.338
Mean IGFR nuc IRS (SD)	14.73 (35.73)	25.30 (40.86)	*0.199	0.080
Mean IGFR total IRS (SD)	134.59 (91.71)	101.80 (74.39)	*0.070	-0.158
Mean HER3 cyt IRS (SD)	154.98 (61.78)	146.83 (67.19)	*0.539	-0.085
Mean HER3 memb IRS (SD)	63.09 (67.78)	39.40 (51.74)	*0.055	-0.173
Mean HER3 nuc IRS (SD)	3.30 (15.53)	0.00 (0.00)	*0.128	-0.160
Mean HER3 total IRS (SD)	222.57 (70.92)	183.46 (81.19)	*0.015	-0.266

Figure 6.

	RD (N=68)	pCR (N=18)	p-value ¹	Spearman Correlation
Mean Age (SD)	53.03 (10.78)	54.56 (11.16)	*0.608	0.058
Median Age (Min-Max)	53.5 (32-75)	52 (35-80)		
Race			NA	NA
Caucasian	68 (79%)	18 (21%)		
Black	0	0		
Oriental	0	0		
ER Status			0.566	0.090
Positive	22 (85%)	4 (15%)		
Negative	46 (77%)	14 (23%)		
PR Status			0.177	0.161
Positive	14 (93%)	1 (7%)		
Negative	54 (76%)	17 (24%)		
Baseline Tumor Size			0.760	-0.014
1	0	0		
2	9 (90%)	1 (10%)		
3	11 (73%)	4 (27%)		
4	28 (76%)	9 (24%)		
5 (inflammatory)	20 (83%)	4 (17%)		
Baseline Node Stage			0.087	-0.143
0	7 (54%)	6 (46%)		
1	32 (87%)	5 (13%)		
2	25 (78%)	7 (22%)		
3	4 (100%)	0		
Histology			NA	NA
IDC	42 (100%)	0		
ILC	0	0		
Invasive NOS	6 (100%)	0		
Other	3 (100%)	0		
MYC Status			0.297	-0.100
Deleted	4 (67%)	2 (33%)		
Normal	45 (78%)	13 (22%)		
Amplified	13 (93%)	1 (7%)		
Mean PTEN cyt IRS (SD)	94.69 (53.34)	108.82 (58.75)	*0.342	0.104
Mean PTEN memb IRS (SD)	40.94 (44.23)	62.06 (52.17)	*0.139	0.175
Mean PTEN nuc IRS (SD)	106.99 (66.31)	137.12 (59.27)	*0.092	0.199
Mean PTEN total IRS (SD)	242.61 (128.84)	308.00 (105.63)	*0.057	0.266
Mean IGFR cyt IRS (SD)	59.00 (46.53)	45.29 (37.18)	*0.265	-0.122
Mean IGFR memb IRS (SD)	52.95 (61.37)	63.29 (83.60)	*0.569	0.053
Mean IGFR nuc IRS (SD)	17.26 (31.95)	6.76 (15.30)	*0.058	-0.147
Mean IGFR total IRS (SD)	129.22 (104.81)	115.35 (87.66)	*0.618	-0.041
Mean HER3 cyt IRS (SD)	147.47 (63.77)	160.35 (42.95)	*0.330	0.075
Mean HER3 memb IRS (SD)	62.77 (62.45)	78.56 (64.38)	*0.348	0.109
Mean HER3 nuc IRS (SD)	1.46 (11.78)	8.33 (35.36)	*0.427	0.110
Mean HER3 total IRS (SD)	214.48 (81.78)	238.33 (86.93)	*0.283	0.112

10/10

Figure 7.



INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2009/064568

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12Q1/68 G01N33/574

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 G01N

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 practical, search terms used)

EPO-Internal, WPI Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	PAIK SOONMYUNG ET AL: "HER2 status and benefit from adjuvant trastuzumab in breast cancer." THE NEW ENGLAND JOURNAL OF MEDICINE 27 MAR 2008, vol. 358, no. 13, 27 March 2008 (2008-03-27), pages 1409-1411, XP002570557 ISSN: 1533-4406 the whole document	1-39
Y	WO 2006/065940 A2 (NSABP FOUNDATION INC [US]; PAIK SOONMYUNG [US]; KIM CHUNGYEUL [US]) 22 June 2006 (2006-06-22) page 16, last paragraph; claims 13-14 ----- -/--	1-39

☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

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Date of the actual completion of the international search

2 March 2010

Date of mailing of the international search report

19/03/2010

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INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2009/064568

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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A	CHEN YINGHUA ET AL: "MYC in breast tumor progression" EXPERT REVIEW OF ANTICANCER THERAPY, FUTURE DRUGS LTD, UK, vol. 8, no. 10, 1 October 2008 (2008-10-01), pages 1689-1698, XP009130012 ISSN: 1744-8328 the whole document	1-39
A	CARLSON ROBERT W ET AL: "HER2 testing in breast cancer: NCCN Task Force report and recommendations" JOURNAL OF THE NATIONAL COMPREHENSIVE CANCER NETWORK, JONES AND BARTLETT PUBLISHERS, SUDBURY, MA, US, vol. 4, no. Suppl 3, 1 July 2006 (2006-07-01), pages S1-S22, XP002544691 ISSN: 1540-1405 the whole document	1-39

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/EP2009/064568

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