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(54) Title: METHODS AND KITS FOR PREDICTING THE RESPONSE TO THERAPY OF CANCER

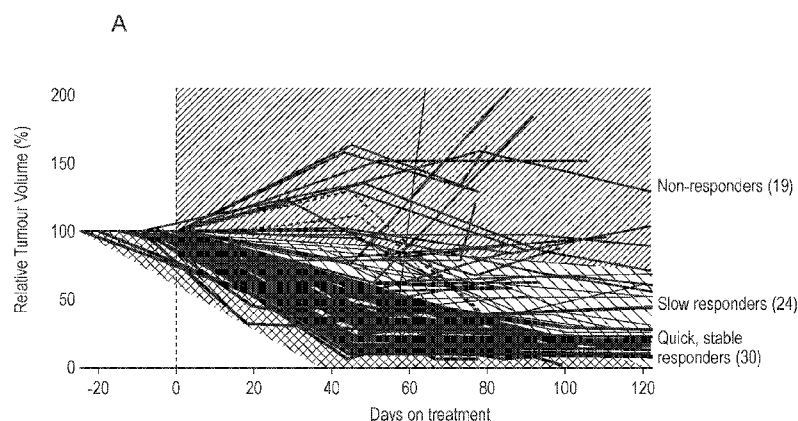


FIG. 1a

(57) Abstract: The present invention relates to methods and kits for predicting an individual's response to cancer therapy, predicting the progression and predicting the prognosis of cancer in an individual, and for selecting a treatment for cancer in an individual.

The present invention relates to methods and kits for predicting an individual's response to cancer therapy, predicting the progression and prognosis of cancer in an individual, and
5 for selecting a treatment for an individual.

Cancer is a broad term for a large number of related diseases which involve excessive and uncontrolled cellular proliferation. This increased cellular proliferation begins in a particular location of the body, but then often spreads to other parts of the body in a
10 process called metastasis. Cancer has been shown to be caused in a number of ways, some of which are environmental and some of which are genetic.

In recent years, research into the field of personalised medicine has increased. Following the sequencing of the human genome, there is a renewed appreciation of how patients
15 differ, and how different treatment strategies and regimes may be more effective in some individuals than in others.

In the emerging field of personalised medicine, the efficient treatment of a disease can be managed by trying to identify early during the course of treatment whether it is effective,
20 and so if the treatment is not effective it can be changed. This is particularly relevant for cancer because cancer treatments are often harmful to the patient, and cancer is often an aggressive disease in which a patient's health can rapidly deteriorate. Therefore, the sooner an ineffective treatment can be identified then the sooner a patient's treatment can be changed, which is likely to improve that patient's prognosis and chances of survival.

25 Additionally, personalised medicine can also be used to predict how cancer will progress in a patient, and what a patient's prognosis is likely to be. That information can be important for the clinician treating the patient because it will allow treatment to be adapted in a manner which will be of most benefit to the patient.

30 Of the known cancers, breast cancer accounts worldwide for 22.9% of all cancers (excluding non-melanoma skin cancers) in women. In 2008, breast cancer caused 458,503 deaths worldwide (13.7% of cancer deaths in women) (World Cancer Report, International Agency for Research on Cancer, 2008).

35 The hormone estrogen is known to drive the growth of the majority (~70%) of breast cancers. Anti-estrogen (endocrine) agents have been demonstrated to be successful in

prevention and treatment of breast cancer. Anti-estrogen treatments include Tamoxifen and a class of drug called Aromatase inhibitors (AIs). These have been shown to be effective in the treatment of estrogen receptor alpha positive (ER+) breast cancer. However, response rates are only 50-70% in the neoadjuvant setting and lower in
5 advanced disease, and therefore there is an urgent need to develop a personalised approach to breast cancer treatment. The level of estrogen receptor is currently the best indicator of which patients will respond to anti-estrogens, but it is clear that not all ER+ patients respond. The histological grade of a tumour (determined by a pathologist) can also be used to predict response, with more aggressive tumours less likely to respond, but
10 this does not accurately predict in which patients treatments (e.g. anti-estrogen treatments) will be effective.

Against this background, the inventors have surprisingly identified a panel of genes that can be used to accurately predict the response to therapy (particularly endocrine therapy)
15 in an individual having cancer. That finding provides effective molecular markers for predicting whether an individual will respond to particular cancer therapies, and allows for the selection of treatment for an individual with cancer, for predicting the prognosis of cancer in an individual, and for predicting the progression of cancer in an individual, as well as kits for undertaking those assays.

20 In a first aspect, the invention provides a method for predicting the response to therapy of cancer in an individual, comprising the steps of:

- (i) providing a first sample comprising one or more cancer cell from the
25 individual before the therapy,
- (ii) determining in the first sample the expression level of the IL6ST gene; and
- (iii) predicting the response to the therapy of cancer in the individual on the basis of the determination in step (ii).

30 It will be appreciated that the invention involves an individual who is known to have cancer. Cancer is a well-known type of disorder, and those skilled in medicine and/or oncology will be familiar with the associated symptoms and be capable of identifying and diagnosing the presence of cancer in an individual.

35 Thus, the present invention includes an individual that has been diagnosed as having cancer; for example, due to the presentation of one or more of the associated symptoms.

The symptoms associated with cancer differ depending on the particular type of cancer. However, in general cancer is associated with one or more of the following symptoms: increased cellular proliferation; swelling of the lymph nodes; weight loss; the presence of cancer markers.

5

The diagnostic techniques used to diagnose a cancer differ depending on the particular type of cancer, and the type of sample used for the diagnosis. However, in general cancer diagnostic techniques include: a blood test; a physical examination; an endoscopy; a CT scan; an X-ray; a genetic test; a urine test.

10

By "cancer", we include a one of more cancer selected from the group comprising: breast cancer; endometrial cancer; ovarian cancer; brain cancer; stomach cancer; prostate cancer; pancreatic cancer; bone cancer; liver cancer; leukemia; melanoma; lymphoma; lung cancer; colon cancer; eye cancer; heart cancer; kidney cancer; thyroid cancer; cervical cancer; an estrogen-dependent cancer. It is particularly preferred in the present invention that the cancer is breast cancer or endometrial cancer or ovarian cancer.

15

The IL6ST gene (Hibi M et al. Cell 1990 Dec;63(6):1149-1157 PMID: 2261637) is also known as gp130, IL6-beta or CD130, and encodes a transmembrane protein that forms one subunit of the type I cytokine receptor within the IL-6 receptor family. All the members of the IL6 cytokine receptor family bind IL6ST, as well as other proteins such as as cardiotrophin 1 (CT-1), leukemia inhibitory factor (LIF), ciliary neurotrophic factor (CNTF), oncostatin M (OSM), and IL-11.

20

By "response to therapy of cancer", we include the likely clinical development and outcome of cancer in the individual in response to therapy, including the severity of the disease and/or the life expectancy or survival of the individual. Thus, by "predicting the response to therapy of cancer" we include the prediction of the likely clinical development of the cancer in response to therapy, and/or the outcome of cancer in the individual in response to therapy, and/or the likely severity of the cancer in response to therapy, and/or the likely life expectancy of the individual, and/or the likely survival of the individual, and/or the likely reduction of the severity of the individual's symptoms, and/or the likely reduction in the number of the individual's symptoms, and/or the likely reduction in the size of the individual's tumour, and/or the likely reduction of the number of tumours in the individual, and/or the likely reduction of cancer metastasis in the individual.

30

35

Preferably, the present invention is undertaken *in vitro* or *ex vivo*. Most preferably, step (i) and/or step (ii) and/or step (iii) of the first aspect of the invention is undertaken *in vitro* or *ex vivo*.

- 5 By “before the therapy”, we include the time before the therapy is administered to the individual to which the individual’s response will be predicted.

It will be appreciated that a sample comprising one or more cancer cell from the individual, and which is suitable for the first aspect of the invention, could be provided in a number of
10 ways, which would be known to those skilled in medicine. Exemplary samples suitable for use present invention are discussed below.

By “cancer cell”, we include a cell which has been identified as being a cancer cell due to the presentation of one or more of the characteristics associated with cancer.

15

It would be clear to those skilled in medicine and/or oncology how to identify a cancer cell from a sample to be used in the invention. It is known that cancer cells display certain characteristics that distinguish them from non-cancer cells, and that those particular characteristics will differ depending on the type of cancer or the type of cell involved. By
20 “characteristics of cancer cells”, we include one or more characteristic selected from the group comprising: increased proliferation; increased nuclear DNA; an increased number of chromosomes; an increase in genetic abnormalities; the presence of genetic cancer markers; the absence of genetic cancer markers; the presence of cancer-related proteins; the absence of cancer-related proteins.

25

It will be appreciated to those skilled in molecular biology that gene expression involves the steps of gene transcription (in which the gene coding sequence is transcribed to mRNA) and translation (in which mRNA is translated to form the encoded protein molecule). Methods for measuring gene expression are known in the art and typically
30 involve detecting the presence and/or activity of the product of transcription (*i.e.* mRNA), and/or the presence and/or activity of the product of translation (*i.e.* protein). Exemplary methods for detecting mRNA or protein associated with a particular gene are discussed herein.

- 35 By “expression level”, we include a measure of the amount of mRNA and/or protein that is produced by gene expression. Expression may be quantified as the total amount of mRNA

and/or protein detectable in a particular sample (such as in a single cell or group of cells), or the amount of mRNA and/or protein produced over a given period.

In one embodiment, step (ii) of the first aspect of the invention comprises determining in
5 the first sample the expression level of only the IL6ST gene.

In one embodiment, step (ii) of the first aspect of the invention further comprises determining in the first sample the expression level of the NGFRAP1 gene.

10 The NGFRAP1 gene encodes the BEX3 protein (Winter EE et al. BMC Evol. Biol. 2005 ;5:54 PMID: 16221301). BEX3 is a p75NTR-associated protein that mediates apoptosis in response to Nerve Growth Factor by interacting with the intracellular death domain of p75-NTR (Mukai J et al. J. Biol. Chem. 2000 Jun;275(23):17566-17570 PMID: 10764727). Besides a small number of studies validating the interaction of BEX3 and p75-NTR *in vivo*,
15 very little is known about the interaction of these proteins (Rapp G et al. DNA Cell Biol. 1990 Sep;9(7):479-485 PMID: 2171551).

Preferably, step (ii) of the first aspect of the invention further comprises determining in the first sample the expression level of the LAX1 gene and/or HPRT1 gene.

20

LAX1, lymphocyte transmembrane adaptor 1, is thought to be involved with immune cell signalling (Zhu M et al. J. Biol. Chem. 2002 Nov;277(48):46151-46158 PMID: 12359715).

The hypoxanthine phosphoribosyltransferase 1 (HPRT1) gene encodes the Hypoxanthine-
25 guanine phosphoribosyltransferase (HGPRT) protein, which is transferase that catalyzes conversion of hypoxanthine to inosine monophosphate and guanine to guanosine monophosphate. The HPRT1 gene product is a phosphoribosylation enzyme in the purine salvage pathway, which will also phosphoribosylate cytotoxic purine analogues such as 6-thioguanine (6-TG) (Finette BA et al. Mutat. Res. 2002 Aug;505(1-2):27-41 PMID:
30 12175903).

Preferably, step (ii) of the first aspect of the invention comprises determining the expression level of at least the IL6ST gene in the first sample, for example: determining the expression level of the IL6ST gene and the NGFRAP1 gene, or determining the
35 expression level of the IL6ST gene and the LAX1 gene, or determining the expression level of the IL6ST gene and the HPRT1 gene, or determining the expression level of the IL6ST gene and the NGFRAP1 gene and the LAX1 gene, or determining the expression

level of the IL6ST gene and the NGFRAP1 gene and the HPRT1 gene, or determining the expression level of the IL6ST gene and the LAX1 gene and the HPRT1 gene, or determining the expression level of the IL6ST gene and the NGFRAP1 gene and the LAX1 gene and the HPRT1 gene in the first sample.

5

Preferably, in the first aspect of the invention:

- step (i) further comprises providing a second sample comprising one or more cancer cell from the individual after the therapy; and
- 10 - step (ii) further comprises determining in the second sample the expression level of the ASPM gene and/or the MCM4 gene and/or the MKI67 gene and/or a gene marker of cellular proliferation.

By "after the therapy", we include the time after the therapy is administered to the individual
15 to which the individual's response will be predicted.

Whilst the inventors have found that a method of determining the expression of the IL6ST gene in a first sample provided before the administration of therapy is very effective for predicting the response to therapy of cancer in an individual, the inventors have also found
20 that determining the expression of an additional panel of genes in a second sample provided after therapy has been administered is particularly effective for predicting the response of that therapy of cancer in an individual.

The ASPM gene encodes abnormal spindle-like microcephaly-associated protein (also
25 known as the abnormal spindle protein homolog or the Asp homolog), which has a role in mitotic spindle function and regulation (Pattison L et al. Am. J. Hum. Genet. 2000 Dec;67(6):1578-1580 PMID: 11078481).

The MCM4 gene encodes a protein that is one of the highly conserved mini-chromosome
30 maintenance proteins (MCM) that are essential for the initiation of eukaryotic genome replication, and is human homologue of the yeast replication protein Cdc21 (Musahl C et al. Eur. J. Biochem. 1995 Jun;230(3):1096-1101 PMID: 7601140).

The MKI67 gene encodes the Ki67 protein (which is also known as antigen Ki67), which
35 is a nuclear protein associated with cellular proliferation (Schlüter C1, Duchrow M, Wohlenberg C, Becker MH, Key G, Flad HD, Gerdes J. J Cell Biol. 1993 Nov;123(3):513-22 PMID: 8227122). The Ki-67 protein is associated with ribosomal RNA transcription in

quiescent and proliferating cells (Bullwinkel J et al. J. Cell. Physiol. 2006 Mar;206(3):624-635 PMID: 16206250, Schonk DM et al. Hum. Genet. 1989 Oct;83(3):297-299 PMID: 2571566, Dowsett, M., T.O. Nielsen, R. A'Hern, J. Bartlett, R.C. Coombes, J. Cuzick, M. Ellis, N.L. Henry, J.C. Hugh, T. Lively, et al. (2011) J Natl Cancer Inst. 103(22): 1656-64. PMID: 21960707).

Genes that are markers of proliferation are well characterised in the literature, and would be well known to those skilled in oncology and/or cell biology. In some cases, the protein products of those genes are required to control cellular proliferation, and when no longer correctly regulated (for example, where the wildtype gene is uncontrollably up-regulated, or is mutated to change its function), then it may have an effect on cell proliferation and/or the cell cycle. In other cases, those genes may not have a direct role in cellular proliferation, but a change in the expression of the gene may be indicative of increased cellular proliferation. Accordingly, by "gene marker of cellular proliferation" we include a gene that negatively regulates the cell cycle, cell proliferation, and/or a gene that positively regulates cell proliferation, and/or a gene whose expression is indicative of cell proliferation, and/or a gene whose change in expression is indicative of cell proliferation, and/or a gene whose regulation is indicative of cell proliferation, and/or a gene whose up-regulation is indicative of cell proliferation, and/or a gene whose down-regulation is indicative of cell proliferation.

Preferably, in the present invention the gene marker of cellular proliferation is one of more gene selected from the group comprising: AURKA (Sen et al. Oncogene. 1997 May 8;14(18):2195-200. PMID: 9174055); PCNA (Ogata K, Ogata Y, Nakamura RM, Tan EM J Immunol. 1985 Oct;135(4):2623-7. PMID: 2863307); MYBL2 (Nomura N, Takahashi M, Matsui M, Ishii S, Date T, Sasamoto S, Ishizaki R. Nucleic Acids Res. 1988 Dec 9;16(23):11075-89. PMID: 3060855); BUB1 (Pangilinan F, Li Q, Weaver T, Lewis BC, Dang CV, Spencer F. Genomics. 1997 Dec 15;46(3):379-88. PMID: 9441741); PLK1 (Lake RJ, Jelinek WR. Mol Cell Biol. 1993 Dec;13(12):7793-801. PMID: 7902533); CCNE1 (Lew DJ, Dulić V, Reed SI. Cell. 1991 Sep 20;66(6):1197-206. PMID: 1833066); CCND1 (Rosenberg et al. (1991) Proc. Natl. Acad. Sci. U.S.A. 88:9638-9642. PMID: 1682919 and Motokura et al. (1991) Nature 350 (6318), 512-515 PMID: 1826542); CCNB1 (Pines J and Hunter T (1991) J. Cell Biol. 115 (1), 1-17 PMID:1717476 and Strausfeld et al. (1991) Nature 351:242-245 PMID: 1828290).

Preferably, step (ii) of the first aspect of the invention comprises determining the expression level of at least the ASPM gene or the MCM4 gene or the MKI67 gene or a

gene marker of cellular proliferation in the second sample, for example: determining the expression level of the ASPM gene and the MCM4 gene, or determining the expression level of the ASPM gene and the MKI67 gene, or determining the expression level of the ASPM gene and a gene marker of cellular proliferation, or determining the expression level of the MCM4 gene and the MKI67 gene, or determining the expression level of the MCM4 gene and a gene marker of cellular proliferation, or determining the expression level of the MKI67 gene and a gene marker of cellular proliferation, or determining the expression level of the ASPM gene and the MCM4 gene and the MKI67 gene, or determining the expression level of the ASPM gene and the MCM4 gene and a gene marker of cellular proliferation, or determining the expression level of the MCM4 gene and the MKI67 gene and a gene marker of cellular proliferation, or determining the expression level of the ASPM gene and the MCM4 gene and the MKI67 gene and a gene marker of cellular proliferation in the second sample.

The second sample to be used in the present invention may be the same type of sample as the first sample, or the second sample may be a different type of sample to the first sample. For example, the second sample to be used in the present invention may be provided in the same manner as the first sample, or the second sample may be provided in a different manner to the first sample. Gene expression in the second sample is determined in the same manner as the gene expression in the first sample, or the gene expression in the second sample is determined in a different manner to the gene expression in the first sample. It is preferred that the second sample is the same type of sample and is provided in the same manner, as the first sample.

Preferably, in the first aspect of the invention:

- step (i) further comprises providing a third sample comprising one or more cancer cell from the individual after the therapy, and after the provision of the second sample; and
- step (ii) further comprises determining in the third sample the expression level of the ASPM gene and/or the MCM4 gene and/or the MKI67 gene and/or a gene marker of cellular proliferation.

Preferably, step (ii) of the first aspect of the invention comprises determining the expression level of at least the ASPM gene or the MCM4 gene or the MKI67 gene or a gene marker of cellular proliferation in the third sample, for example: determining the expression level of the ASPM gene and the MCM4 gene, or determining the expression

level of the ASPM gene and the MKI67 gene, or determining the expression level of the ASPM gene and a gene marker of cellular proliferation, or determining the expression level of the MCM4 gene and the MKI67 gene, or determining the expression level of the MCM4 gene and a gene marker of cellular proliferation, or determining the expression level of the MKI67 gene and a gene marker of cellular proliferation, or determining the expression level of the ASPM gene and the MCM4 gene and the MKI67 gene, or determining the expression level of the ASPM gene and the MCM4 gene and a gene marker of cellular proliferation, or determining the expression level of the MCM4 gene and the MKI67 gene and a gene marker of cellular proliferation, or determining the expression level of the ASPM gene and the MCM4 gene and the MKI67 gene and a gene marker of cellular proliferation in the third sample.

The third sample to be used in the present invention may be the same type of sample as the first sample and/or the second sample, or the third sample is a different type of sample to the first sample and/or the second sample. For example, the third sample to be used in the present invention may be provided in the same manner as the first sample and/or the second sample, or the third sample may be provided in a different manner to the first sample and/or the second sample. Gene expression in the third sample is determined in the same manner as the gene expression in the first sample and/or the second sample, or the gene expression in the third sample is determined in a different manner to the gene expression in the first sample and/or second sample. It is preferred that the third sample is the same type of sample and is provided in the same manner, as the first sample and/or the second sample.

Preferably, in the first aspect of the invention:

- step (i) further comprises providing a subsequent sample comprising one or more cancer cell from the individual after the therapy, and after the provision of the third sample; and
- step (ii) further comprises determining in the subsequent sample the expression level of the ASPM gene and/or the MCM4 gene and/or the MKI67 gene and/or a gene marker of proliferation.

Preferably, step (ii) of the first aspect of the invention comprises determining the expression level of at least the ASPM gene or the MCM4 gene or the MKI67 gene or a gene marker of cellular proliferation in the subsequent sample, for example: determining the expression level of the ASPM gene and the MCM4 gene, or determining the expression

level of the ASPM gene and the MKI67 gene, or determining the expression level of the ASPM gene and a gene marker of cellular proliferation, or determining the expression level of the MCM4 gene and the MKI67 gene, or determining the expression level of the MCM4 gene and a gene marker of cellular proliferation, or determining the expression level of the MKI67 gene and a gene marker of cellular proliferation, or determining the expression level of the ASPM gene and the MCM4 gene and the MKI67 gene, or determining the expression level of the ASPM gene and the MCM4 gene and a gene marker of cellular proliferation, or determining the expression level of the MCM4 gene and the MKI67 gene and a gene marker of cellular proliferation, or determining the expression level of the ASPM gene and the MCM4 gene and the MKI67 gene and a gene marker of cellular proliferation in the subsequent sample.

It is included in the present invention that any number of samples comprising one or more cancer cell provided from the individual after the therapy can be used. By "subsequent sample", we include a sample that is provided following the provision of the third sample, which includes a fourth sample, or a fifth sample, or a sixth sample, or a seventh sample, or an eighth sample, or a ninth sample, or a tenth sample, or an eleventh sample, or a twelfth sample, or a thirteenth sample, or a fourteenth sample, or a fifteenth sample, or a sixteenth sample, or a seventeenth sample, or an eighteenth sample, or a nineteenth sample, or a twentieth sample, or more.

Preferably, the first aspect of the invention further comprises the step of continuing to administer the therapy to the individual or ceasing to administer the therapy to the individual.

It would be apparent to those skilled in medicine how a particular treatment could be administered. Preferably, in the first aspect of the invention the therapy could be administered via one or more route selected from the group comprising: oral administration; rectal administration; epidural administration; topical administration; nasal administration; intramuscular administration; intradermal administration; intravenous administration; subcutaneous administration; transdermal administration.

Preferably, in the present invention the therapy is a neoadjuvant therapy.

A neoadjuvant therapy is a term well known in the art to mean a therapy that is administered to a patient before a main treatment, and it would be clear to those skilled in medicine what would be classified as a neoadjuvant therapy. An example of a neoadjuvant

therapy is the administration of a chemotherapeutic drug to a patient with the aim of shrinking a tumour before a surgeon operates to remove it. In that situation, the surgeon would have a better chance of removing the entirety of a small tumour than the entirety of a large tumour, which would be in the best interest of the patient. In that exemplary
5 scenario, the chemotherapeutic agent is a neoadjuvant therapy and the surgery is the main treatment.

By "neoadjuvant therapy", we include a therapy that is administered prior to the main treatment. By "main treatment", we include one or more treatment selected from the group
10 comprising: surgery; radiotherapy; chemotherapy; hormone therapy; bone marrow transplantation; stem cell transplantation. Preferably, in the present invention the main treatment is surgery.

Preferably, in the present invention the therapy is one or more therapy selected from the
15 group comprising: an aromatase inhibitor; an Estrogen Receptor modulator; an Estrogen Receptor down-regulator.

Preferably, in the present invention the neoadjuvant therapy is one or more neoadjuvant therapy selected from the group comprising: an aromatase inhibitor; an Estrogen Receptor
20 modulator; an Estrogen Receptor down-regulator.

Aromatase inhibitors (AIs) are a class of drug that work by inhibiting the action of the enzyme aromatase. Aromatase converts androgens into estrogens by a process called aromatization.
25

Preferably, in the present invention the aromatase inhibitor is one or more aromatase inhibitor selected from the group comprising: anastrozole; letrozole; exemestane; vorozole; formestane; fadrozole.

30 The Estrogen Receptor (ER) is a well characterised receptor that would be known to those skilled in medicine and/or cell biology. ER is a nuclear hormone intracellular receptor that is activated by estrogen. Once activated, the ER can translocate to the nucleus where it binds to DNA and regulates gene expression. There are two forms of ER protein called ER α (Green S et al. Nature 1986 Mar 13-19;320(6058):134-139 PMID: 3754034) and ER β
35 (Mosselman S et al. FEBS Lett. 1996 Aug;392(1):49-53 PMID: 8769313 Europe PMC Pubmed) which are encoded by the ESR1 gene and the ESR2 gene, respectively. Those two forms of ER are often co-expressed. When activated by estrogen, the ER forms

dimers that can either be homodimers of the two ER forms (*i.e.* a homodimer of ER α and ER α , or ER β and ER β) or heterodimers of the two ER forms (*i.e.* ER α and ER β). Most preferably, the ER is ER α .

5 As discussed herein, the aberrant modulation of the ER is associated with a number of cancers, including breast cancer, endometrial cancer and ovarian cancer. Therefore, the ER is a known drug target, and drugs that modulate the function of the ER have been developed.

10 By an "Estrogen Receptor modulator", we include an agent that modulates or changes or alters ER protein function, and/or an agent that modulates or changes or alters cellular signalling downstream of the ER protein, and/or an agent that modulates or changes or alters ER gene expression, and/or an agent that modulates or changes or alters ER transcription, and/or an agent that modulates or changes or alters ER translation.

15

By an "Estrogen Receptor down-regulator", we include an agent that reduces or inhibits ER protein function, and/or an agent that reduces or inhibits cellular signalling downstream of the ER protein, and/or an agent that reduces or inhibits estrogen binding to the ER, and/or an agent that blocks estrogen binding to the ER, and/or an agent that down-regulates or prevents ER gene expression, and/or an agent that down-regulates or prevents ER transcription, and/or an agent that down-regulates or prevents ER translation.

20

Preferably, in the present invention the Estrogen Receptor modulator and/or the Estrogen Receptor down-regulator is one or more selected from the group comprising: tamoxifen; fulvestrant; raloxifene; toremifene; lasofoxifene.

25

It is particularly preferred in the present invention that the cancer is one or more cancer selected from the group comprising: breast cancer; endometrial cancer; ovarian cancer; an estrogen-dependent cancer.

30

Breast cancer is a well-known disorder, and those skilled in medicine and/or oncology would be familiar with the associated symptoms and be capable of identifying and diagnosing the presence of breast cancer in an individual. It will be appreciated that breast cancer is a general term that encompasses a wide range of cancers of the breast. By "breast cancer", we include one or more breast cancer selected from the group comprising: ductal carcinoma; ductal carcinoma *in situ*; lobular carcinoma; lobular carcinoma *in situ*; invasive breast carcinoma; invasive breast cancer; invasive breast cancer of no special

35

type; Luminal A breast cancer; Luminal B breast cancer; metastatic breast cancer; estrogen receptor (ER) positive and/or progesterone receptor positive and/or HER2 positive breast cancer.

- 5 A number of symptoms are associated with breast cancer, and an individual may be diagnosed with breast cancer if they exhibit one or more symptom selected from the group comprising: a noticeable lump in the breast; a lump in the armpit which is associated with a lymph node; thickening different from the other breast tissue; one breast becoming larger or lower; a nipple changing position or shape or becoming inverted; skin puckering or
10 dimpling on the breast, a rash on or around a nipple; discharge from nipple(s), constant pain in part of the breast or armpit; swelling beneath the armpit or around the collarbone; breast pain (known as mastodynia); breast inflammation (such as itching, and/or pain, and/or swelling, and/or nipple inversion, and/or warmth, and/or redness throughout the breast); eczema on the breast (such as redness, and/or discoloration, and/or mild flaking
15 of the nipple skin); tingling of the breast; itching of the breast; increased sensitivity of the breast; a burning sensation of the breast.

There are a number of well-established diagnostic techniques for breast cancer, which include one or more diagnostic technique selected from the group comprising: microscopic
20 examination of a biopsy from the affected part of the breast; physical examination of the breast by a healthcare provider; mammography; ultrasound; magnetic resonance imaging (MRI).

It would be clear to those skilled in medicine and/or oncology how to identify a breast
25 cancer cell from a sample to be used in the invention. It is known that breast cancer cells display certain characteristics that distinguish them from non-cancer cells and/or non-cancer breast cells, which include one or more selected from the group comprising: minimal tubule formation as observed via microscopy; an increase in ER expression; the presence of ER protein; an increase in progesterone receptor expression; the presence of
30 PR protein; an increase in HER2 expression; the presence of HER2 protein.

Endometrial cancer is a well-known disorder, and those skilled in medicine and/or oncology will be familiar with the associated symptoms and be capable of identifying and
35 diagnosing the presence of endometrial cancer in an individual based on the symptoms discussed herein. It will be appreciated that endometrial cancer is a general term that encompasses a wide range of cancers of the uterus, and in particular the lining of the uterus (known as the endometrium).

A number of symptoms are associated with endometrial cancer, and an individual may be diagnosed with endometrial cancer if they exhibit one or more symptom selected from the group comprising: abnormal uterine bleeding; abnormal menstrual periods; bleeding
5 between normal periods in premenopausal women; extremely long, heavy, or frequent episodes of menstrual bleeding; anaemia, caused by chronic loss of blood; lower abdominal pain; pelvic cramping; thin white or clear vaginal discharge in postmenopausal women; unexplained weight gain; swollen glands; swollen lymph nodes in the neck, under chin, back of head and top of clavicles; incontinence.

10

Ovarian cancer is a well-known disorder, and those skilled in medicine and/or oncology will be familiar with the associated symptoms and be capable of identifying and diagnosing the presence of ovarian cancer in an individual based on the symptoms discussed herein.
15 It will be appreciated that ovarian cancer is a general term that encompasses a range of cancers of the ovaries.

A number of symptoms are associated with ovarian cancer, and an individual may be diagnosed with ovarian cancer if they exhibit one or more symptom selected from the
20 group comprising: bloating; pelvic pain; difficulty eating; frequent urination.

It is known that a number of different cancers are dependent on estrogen to allow them to grow and metastasise. Accordingly, by "estrogen-dependent cancer" we include estrogen sensitive cancer, and/or cancer that is dependent on estrogen for growth, and/or cancer
25 that is dependent on estrogen to metastasise.

Preferably, in the present invention the estrogen-dependent cancer is one of more estrogen-dependent cancer selected from the group comprising: estrogen-dependent breast cancer; estrogen-dependent endometrial cancer; estrogen-dependent ovarian
30 cancer.

Preferably, in the present invention the breast cancer is one or more breast cancer selected from the group comprising: an Estrogen Receptor-positive breast cancer; a luminal A breast cancer; a luminal B breast cancer; an invasive breast cancer; a lobular
35 breast cancer; a ductal breast cancer; an Estrogen Receptor-positive lobular breast cancer; an Estrogen Receptor-negative lobular breast cancer; an Estrogen Receptor-positive ductal breast cancer; Estrogen Receptor-negative ductal breast cancer.

Estrogen Receptor-positive (ER+) breast cancer is a sub-type of breast cancer that is characterised by the presence of the ER protein in a cancer cell. An ER+ breast cancer will depend on the presence of estrogen for its growth. It would be clear to those skilled in medicine and/or oncology whether an individual has an ER+ breast cancer, and it would be clear to that skilled person how an ER+ breast cancer could be diagnosed. By “ER+ breast cancer”, we include a breast cancer whose cells exhibit ER expression; for example, the expression of ESR1 mRNA and/or ESR2 mRNA in cancer cells, and/or the presence of ER α protein and/or ER β protein in cancer cells, and/or the presence of ER protein homodimers (*i.e.* a homodimer of ER α and ER α , and/or of ER β and ER β) in cancer cells, and/or the presence of ER protein heterodimers (*i.e.* a heterodimer of ER α and ER β) in cancer cells.

It would be known to those skilled in molecular biology how ER expression could be measured, and examples of how this could be done (such as PCR and immunohistochemistry) are described herein.

It would be known to those skilled in medicine and/or pathology whether a breast cancer is invasive; for example, non-invasive cancers stay within the milk ducts and/or the breast lobules, and breast cancer that spreads beyond the milk ducts and/or breast lobules is generally invasive. By “invasive breast cancer”, we include a breast cancer that is not restricted to the breast milk ducts, and/or a breast cancer that is not restricted to the breast lobules.

Preferably, in the present invention the breast cancer is one or more breast cancer selected from the group comprising: an invasive Estrogen Receptor-positive breast cancer; an invasive luminal A breast cancer; an invasive luminal B breast cancer; a non-invasive Estrogen Receptor-positive breast cancer.

Preferably, in the present invention the individual is a female. Both male individuals and female individuals have been shown to develop breast cancer. Therefore, the present invention also includes that the individual is a male individual.

Preferably, in the present invention the individual is human or non-human, for example, a non-human mammal (*i.e.* any mammal other than a human), such as one or more non-human mammal selected from the group comprising: a horse; a cow; a goat; a sheep; a pig; a dog; a cat; a rodent; a rabbit; a mouse; a rat.

In relation to breast cancer, the equivalent cancer in a non-human mammal is known as mammary cancer. Accordingly, in the present invention a reference to breast cancer in a non-human mammal be read as mammary cancer.

- 5 Preferably, in the present invention the female individual is one or more female individual selected from the group comprising: a post-menopausal female; a pre-menopausal female; a perimenopausal female.

10 The menopause is the cessation of the reproductive ability of a woman. The menopause is usually defined by the absence of menstrual flow, or the permanent cessation of the ovaries' primary function. Under natural circumstances, a female usually undergoes the menopause during midlife, at around her late-40s or late-50s. However, surgical procedures such as a hysterectomy can also lead to early menopause (*i.e.* surgical menopause). The natural menopause transition period (also known as the
15 perimenopause) can occur over a number of years. During the menopause, the female's body undergoes a number of physical and hormonal changes. Although not investigated extensively, it is known that non-human mammals undergo the menopause. It would be clear to those skilled in medicine whether a female was a post-menopausal female, a pre-menopausal female, or a perimenopausal female.

20

After the menopause, ovaries stop making estrogen, but the body continues to make a small amount of estrogen with an enzyme called aromatase. Aromatase inhibitors are used in post-menopausal women because they have much smaller amounts of estrogen in their bodies and blocking aromatase activity is effective in reducing estrogen levels.
25 Aromatase inhibitors are typically not used in premenopausal women because these drugs have a limited effect on the ovaries, whereas Tamoxifen is used in both premenopausal and post-menopausal women. ER+ breast cancer affects a greater proportion of postmenopausal than pre-menopausal women (Yasui and Potter, Cancer Causes Control. 1999 Oct;10(5):431-7. PMID: 10530614).

30

By "post-menopausal female", we include a female that had a cessation of menstrual flow, and/or a midlife female, and/or a surgical menopausal female.

By "female that had a cessation of menstrual flow", we include a female that has not had
35 menstrual flow for at least two months; for example, a female that has not had menstrual flow for at least three months, or at least four months, or at least five months, or at least six months, or at least seven months, or at least eight months, or at least nine months, or

at least ten months, or at least 11 months, or at least one year, or at least two years, or at least three years, or at least four years, or at least five years.

Preferably, the female is at least 40 years old; for example, a female who is at least 41
5 years, or at least 42 years, or at least 43 years, or at least 44 years, or at least 45 years,
or at least 46 years, or at least 47 years, or at least 48 years, or at least 49 years, or at
least 50 years, or at least 51 years, or at least 52 years, or at least 53 years, or at least 54
years, or at least 55 years, or at least 56 years, or at least 57 years, or at least 58 years,
or at least 59 years, or at least 60 years, or at least 61 years, or at least 62 years, or at
10 least 63 years, or at least 64 years, or at least 65 years, or at least 66 years, or at least 67
years, or at least 68 years, or at least 69 years, or at least 70 years, or at least 71 years,
or at least 72 years, or at least 73 years, or at least 74 years, or at least 75 years old.

By “surgical menopausal female”, we include a female that has had one ovary surgically
15 removed (*i.e.* undergone a unilateral oophorectomy) or a female that has had two ovaries
surgically removed (*i.e.* undergone a bilateral oophorectomy), and/or a female that has
had one fallopian tube surgically removed (*i.e.* undergone a unilateral salpingo-
oophorectomy) or a female that has had two fallopian tubes surgically removed (*i.e.*
undergone a bilateral salpingo-oophorectomy), and/or a female that has had the uterus
20 removed (*i.e.* undergone a hysterectomy).

Preferably, in the present invention the post-menopausal female is a human post-
menopausal female or a non-human mammal post-menopausal female.

25 By “pre-menopausal female”, we include a female who is reproductive and has not
exhibited any of the physical and/or hormonal changes associated with the menopause,
and/or a female who has menstrual flow and has not exhibited any of the physical and/or
hormonal changes associated with the menopause. Preferably, in the present invention
the pre-menopausal female is a human pre-menopausal female or a non-human mammal
30 pre-menopausal female.

By “perimenopausal female”, we include a female who is reproductive and has exhibited
the physical and/or hormonal changes associated with the menopause, and/or a female
who has menstrual flow and has exhibited the physical and/or hormonal changes
35 associated with the menopause, and/or a female that is exhibiting the physical and/or
hormonal changes associated with the menopause. Preferably, in the present invention

the perimenopausal female is a human perimenopausal female or a non-human mammal perimenopausal female.

To identify the genes used in the methods of the present invention, a random forest method (Breiman, L. (2001) Random Forests. Machine Learning. 45(1): 5-32) and CART method (Breiman, Leo; Friedman, J. H.; Olshen, R. A.; Stone, C. J. (1984). Monterey, CA: Wadsworth & Brooks/Cole Advanced Books & Software. ISBN 978-0-412-04841-8) was utilised on an original training dataset to identify the most predictive features. Once those genes had been identified, then this method was not needed to predict patient response.

When predicting patient response based on one or more of the identified genes of the invention, it is necessary to define an optimum cut-off point between 'high' expression and 'low' expression for each of the genes. The optimum cut-off point is that which results in the largest proportion of non-responders to be below the cut-point for a gene whose low level was associated with non-response. Conversely, the optimum cut-off is that which results in the largest proportion of non-responders to be above the cut-off for a gene whose high level was associated with non-response.

In particular, the high/low direction of the genes is more important than the specific cut-off point. For example:

A high IL6ST gene and NGFRAP1 gene expression, and a low ASPM gene and/or MCM4 gene expression is indicative of a response to the therapy and/or a good prognosis, and indicates that individual should stay on the therapy (particularly the anti-estrogen therapy).

A low IL6ST gene and NGFRAP1 gene expression, and a high ASPM gene and/or MCM4 gene expression is indicative of a non-response, and/or a poor prognosis, and indicates that the individual should be moved to alternative therapy and/or treatment.

High LAX1 gene and IL6ST gene expression, and low HPRT1 gene expression is indicative of a response to the therapy and/or a good prognosis (particularly the anti-estrogen therapy).

Low LAX1 gene and IL6ST gene expression, and high HPRT1 gene expression is indicative of a non-response, and/or a poor prognosis, and indicates that the individual should be moved to alternative therapy and/or treatment.

- 5 The order in which the level of more than one gene are considered is determined by a decision tree. A decision tree is a flowchart-like, hierarchical graph or model of decisions and their possible consequences. The level of a gene 'decides' which branch of the tree is followed until a terminal 'node' is reached where a patient is assigned to either the response or non-response groups. Decision trees for the 4 gene and 3 gene models are
10 given in Figure 3A and Figure 3B, respectively.

Assigning each individual unambiguously to 'response' or 'non-response' groups is achieved by considering whether the level of the genes are above or below the optimum cut-points (determined from a training and/or reference set) in the order determined by the
15 decision tree.

For example, when using a 4 gene signature decision tree (Figure 3A, *i.e.* in which the genes used are IL6ST, ASPM, NGFRAP1 and MCM4) to predict the outcome for any given individual; if the IL6ST gene is low (below the technology-specific cut-off) in the before
20 therapy sample and the ASPM gene is high (above the technology-specific cut-point) in the post-therapy sample, the individual will be classed as having a non-response and no other genes need be considered. However, if the IL6ST gene value in the pre-therapy sample and the ASPM gene value in the post-therapy sample are both low (below the technology-specific cut-offs) then the NGFRAP1 gene value in the pre-therapy sample is
25 also used, with a low value (below the technology-specific cut-off) identifying a non-response and a high value (above the technology-specific cut-point) identifying a response. If the IL6ST gene is instead high (above the technology-specific cut-point) in the pre-therapy sample, then a low MCM4 gene value (below the technology-specific cut-off) in the post-therapy sample identifies the individual as having a response. A high level
30 of the IL6ST gene in the pre-therapy sample and a high level of the MCM4 gene in the post-therapy sample would require the consideration of the ASPM gene value in the post-therapy sample, with a low value again identifying the individual as having a response. If not already assigned, a low NGFRAP1 gene value in the pre-therapy sample would identify the individual as having a non-response and a high value as having a response.

35

Preferably, in the first aspect of the invention the gene expression level is determined as a high gene expression or a low gene expression.

Preferably, in the first aspect of the invention the high gene expression or low gene expression is calculated relative to data in a training set and/or to data in a reference set.

5 It would be understood by those skilled in genomics and/or bioinformatics that the value of a high gene expression or low gene expression would depend on the method used to collect the gene expression data, such exemplary methods are described herein. The manner in which the cut-off point between a high gene expression and a low gene expression is preferably calculated using a microarray, such as an Affymetrix GeneChip
10 (Fodor et al. (1993) Nature 364:555-6, PMID: 7687751) or Illumina Beadarray (Oliphant et al. (2002) Biotechniques. Suppl:56-8, 60-1 PMID: 12083399), as described below.

Cut-off point values from the data in a training set and/or data in a reference set can be generated by measuring the gene or protein expression level from a cohort of, ideally, at
15 least 20 patients with known response status (as defined and implemented below), where, ideally, at least a quarter are classified as non-responders. In the case of gene expression, mRNA may be extracted as described below and measured quantitatively using the appropriate technology (e.g. quantitative PCR, microarray, NanoString). Protein expression could be measured by immunohistochemistry, AQUA or similar technique (as
20 described below). The response status of any future sample with unknown response status can be determined by evaluating the level of the measured genes relative to that of the data in a training set and/or data in a reference set.

Preferably, in the first aspect of the invention the data in a training set and/or data in a
25 reference set is obtained from a group of subjects.

By "group of subjects", we include a group comprising two or more subjects, and preferably four or more subjects. Ideally, the group will not exceed 10,000 subjects, and will preferably not exceed 1000 subjects. Preferably, the group comprises or consists of
30 between 4 and 100 subjects.

Preferably, in the present invention the subject is a female subject that has one or more characteristic selected from the group comprising: diagnosed with a cancer or not
35 diagnosed with a cancer; receiving cancer therapy or not receiving cancer therapy; receiving cancer treatment or not receiving cancer treatment; post-menopausal; pre-menopausal; perimenopausal, or the subject is a male subject that has one or more characteristic selected from the group comprising: diagnosed with a cancer or not

diagnosed with a cancer; receiving cancer therapy or not receiving cancer therapy; receiving cancer treatment or not receiving cancer treatment.

Preferably, in the present invention the group of subject consists of male subjects, or
5 female subjects, or male subjects and female subjects.

Preferably, in the present invention the group of subjects consists of subjects that have at least one of the same characteristics, for example at least two, or at least three, or at least four, or at least five, or at least six of the same characteristics.

10

Preferably, in the present invention the group of subjects consists of subjects that have at least one different characteristic, for example at least two, or at least three, or at least four, or at least five, or at least six different characteristics.

15 Preferably, in the present invention the data in a training set and/or data in a reference set is obtained from a sample provided from a subject.

Preferably, in the present invention the sample provided from the subject is the same as the sample provided from the individual, or the sample provided from the subject is
20 different to the sample provided from the individual.

We include that the subject of the invention can be defined in the same manner as the individual of the invention.

25 Preferably, in the present invention the therapy is administered to the individual after the provision of the first sample.

Preferably, in the present invention the therapy is administered to the individual at least one hour after the provision of the first sample; for example, the therapy is administered to
30 the individual at least two hours, or at least three hours, or at least four hours, or at least five hours, or at least six hours, or at least seven hours, or at least eight hours, or at least nine hours, or at least ten hours, or at least 11 hours, or at least 12 hours, or at least 13 hours, or at least 14 hours, or at least 15 hours, or at least 16 hours, or at least 17 hours, or at least 18 hours, or at least 19 hours, or at least 20 hours, or at least 21 hours, or at
35 least 22 hours, or at least 23 hours, or at least one day, or at least two days, or at least three days, or at least four days, or at least five days, or at least six days, or at least seven days, or at least eight days, or at least nine days, or at least ten days, or at least 11 days,

or at least 12 days, or at least 13 days, or at least 14 days, or at least 15 days, or at least 16 days, or at least 17 days, or at least 18 days, or at least 19 days, or at least 20 days, or at least 21 days, or at least 22 days, or at least 23 days, or at least 24 days, or at least 25 days, or at least 26 days, or at least 27 days, or at least 28 days, or at least five weeks, or at least six weeks, or at least seven weeks, or at least eight weeks, or at least nine weeks, or at least ten weeks, or at least 11 weeks, or at least three months, or at least four months, or at least five months, or at least six months, or at least seven months, or at least eight months, or at least nine months, or at least ten months, or at least 11 months, or at least 12 months after the provision of the first sample.

Preferably, in the present invention the therapy is administered at least once per day to the individual, for example: once per day, or once per two days, or once per three days, or once per four days, or once per five days, or once per six days, or once per seven days, or once per eight days, or once per nine days, or once per ten days, or once per eleven days, or once per twelve days, or once per thirteen days, or once per two weeks, or once per three weeks, or once per four weeks, or once per five weeks, or once per six weeks, or once per seven weeks, or once per eight weeks to the individual.

Preferably, in the present invention the second sample is provided from the individual at least one day after the initiation of therapy, for example: at least two days, or at least three days, or at least four days, or at least five days, or at least six days, or at least seven days, or at least eight days, or at least nine days, or at least ten days, or at least 11 days, or at least 12 days, or at least 13 days, or at least 14 days, or at least 15 days, or at least 16 days, or at least 17 days, or at least 18 days, or at least 19 days, or at least 20 days, or at least 21 days, or at least 22 days, or at least 23 days, or at least 24 days, or at least 25 days, or at least 26 days, or at least 27 days, or at least 28 days, or at least five weeks, or at least six weeks, or at least seven weeks, or at least eight weeks, or at least nine weeks, or at least ten weeks, or at least 11 weeks, or at least three months, or at least four months, or at least five months, or at least six months, or at least seven months, or at least eight months, or at least nine months, or at least ten months, or at least 11 months, or at least 12 months after the initiation of therapy. Most preferably in the first aspect of the invention, the second sample is provided from the individual 14 days (*i.e.* two weeks) after the initiation of therapy or three months after the initiation of therapy.

Preferably, in the present invention the third sample is provided from the individual at least one day after the initiation of therapy, for example: at least two days, or at least three days, or at least four days, or at least five days, or at least six days, or at least seven days, or at

least eight days, or at least nine days, or at least ten days, or at least 11 days, or at least 12 days, or at least 13 days, or at least 14 days, or at least 15 days, or at least 16 days, or at least 17 days, or at least 18 days, or at least 19 days, or at least 20 days, or at least 21 days, or at least 22 days, or at least 23 days, or at least 24 days, or at least 25 days,
5 or at least 26 days, or at least 27 days, or at least 28 days, or at least five weeks, or at least six weeks, or at least seven weeks, or at least eight weeks, or at least nine weeks, or at least ten weeks, or at least 11 weeks, or at least three months, or at least four months, or at least five months, or at least six months, or at least seven months, or at least eight months, or at least nine months, or at least ten months, or at least 11 months, or at least
10 12 months after the initiation of therapy.

Most preferably in the first aspect of the invention, the second sample is provided from the individual 14 days (*i.e.* two weeks) after the initiation of therapy, and the third sample is provided from the individual three months after the initiation of therapy (*i.e.* two months
15 and two weeks after the second sample has been provided).

Preferably, in the present invention the subsequent sample is provided from the individual at least one day after the initiation of therapy, for example: at least two days, or at least three days, or at least four days, or at least five days, or at least six days, or at least seven
20 days, or at least eight days, or at least nine days, or at least ten days, or at least 11 days, or at least 12 days, or at least 13 days, or at least 14 days, or at least 15 days, or at least 16 days, or at least 17 days, or at least 18 days, or at least 19 days, or at least 20 days, or at least 21 days, or at least 22 days, or at least 23 days, or at least 24 days, or at least 25 days, or at least 26 days, or at least 27 days, or at least 28 days, or at least five weeks,
25 or at least six weeks, or at least seven weeks, or at least eight weeks, or at least nine weeks, or at least ten weeks, or at least 11 weeks, or at least three months, or at least four months, or at least five months, or at least six months, or at least seven months, or at least eight months, or at least nine months, or at least ten months, or at least 11 months, or at least 12 months after the initiation of therapy.

30

Preferably, in the first aspect of the invention the predicted response is one or more predicted response selected from the group comprising: a response; a non-response.

Preferably, in a first aspect of the invention a response or a non-response is selected using
35 a decision tree algorithm.

As outlined above, based on the teaching of the application it would be clear to those skilled in medicine and/or oncology how to predict an individual's response to therapy based on the method of the first aspect of the invention. Based on the determination of the method of the first aspect of the invention, the individual could be categorised as having
5 a response, or non-response. It would be clear to those skilled in medicine how an individual would be categorised in the manner.

By "response", we include that the individual exhibits a reduction in the severity of cancer symptoms, and/or the individual exhibits a reduction in the number of cancer symptoms,
10 and/or the individual exhibits a reduction in tumour size, and/or the individual exhibits a reduction in tumour number, and/or the individual exhibits a reduction in metastasis, and/or the individual exhibits a reduction in cancer affected lymph nodes, and/or the individual exhibits a reduction in tumour volume, and/or the individual exhibits a reduction in ER IHC Allred score, and/or the individual exhibits a reduction in tumour grade, and/or the
15 individual exhibits a reduction of one or more proliferation-associated gene, and/or the individual exhibits a reduction in one or more gene marker of cellular proliferation, and/or the individual exhibits an improvement in prognosis, and/or the individual exhibits a reduction in cancer progression, and/or the individual is classed as being in remission, and/or the individual exhibits no cancer symptoms, and/or the individual has no cancer
20 cells.

By "non-response", we include that the individual exhibits no reduction in the severity of cancer symptoms, and/or the individual exhibits an increase in the severity of cancer symptoms, and/or the individual exhibits no reduction in the number of cancer symptoms,
25 and/or the individual exhibits an increase in the number of cancer symptoms, and/or the individual exhibits no improvement in prognosis, and/or the individual exhibits a worsening in prognosis, and/or the individual exhibits no reduction in cancer progression, and/or the individual exhibits an increase in cancer progression, and/or the individual exhibits no reduction in tumour size, and/or the individual exhibits an increase in tumour size, and/or
30 the individual exhibits no reduction in tumour number, and/or the individual exhibits an increase in tumour number, and/or the individual exhibits no reduction in metastasis, and/or the individual exhibits an increase in metastasis, and/or the individual exhibits no reduction in cancer affected lymph nodes, and/or the individual exhibits an increase in cancer affected lymph nodes, and/or the individual exhibits no reduction in tumour volume, and/or the individual exhibits an increase in tumour volume, and/or the individual exhibits
35 no reduction in tumour grade, and/or the individual exhibits an increase in tumour grade,

and/or the individual exhibits no reduction in ER IHC Allred score, and/or the individual exhibits an increase in ER IHC Allred score.

The tumour grading referred to above is the TNM (Tumour size, lymph Node involvement, and Metastasis) staging system for breast cancer. There are four stages of TNM tumour grade: stage 1 – the cancer (or tumour) is 2cm or smaller and has not spread to the lymph nodes in the armpit; stage 2A – the cancer (or tumour) is smaller than 2cm and has spread to the lymph nodes in the armpit or the cancer (or tumour) is bigger than 2cm and has not spread to the lymph nodes; stage 2B – the cancer is smaller than 5cm and has spread to the lymph nodes in the armpit or is bigger than 5cm but has not spread to the lymph nodes; stage 3A – the cancer (or tumour) is under 5cm and has spread to the lymph nodes in the armpit or it is bigger than 5cm and has spread to the lymph nodes; stage 3B – the cancer (or tumour) has spread to tissue near the breast and there are cancer cells in the lymph nodes in the armpit; stage 3C the cancer (or tumour) has spread to 10 or more lymph nodes in the armpit or the lymph nodes below the breastbone, near the neck or under the collarbone; stage 4 the cancer (or tumour) has metastasised to other parts of the body.

The ER IHC (immunohistochemistry) Allred score referred to above relates to a method of quantifying the amount of ER protein staining in cells; samples are scored on a scale from 0 to 8 (Harvey et al. J Clin Oncol. 1999;17:1474–1481. PMID: 10334533 and Allred et al. Mod Pathol. 1998;11:155–168. PMID: 9504686).

Preferably, in the first aspect of the invention when the expression level of the IL6ST gene and/or the NGFRAP1 gene and/or the ASPM gene and/or the MCM4 gene and/or the LAX1 gene and/or the HPRT1 gene is determined, a response is determined if:

- the IL6ST gene expression is high; and/or
- the NGFRAP1 gene expression is high; and/or
- the LAX1 gene expression is high; and/or
- the HPRT1 gene expression is low; and/or
- the ASPM gene expression is low after the initiation of therapy; and/or
- the MCM4 gene expression is low after the initiation of therapy.

Preferably, when the expression level of the ASPM gene and/or the MCM4 gene is determined, a response is determined if:

- the ASPM gene expression is low two weeks after the initiation of therapy; and/or
- the ASPM gene expression is low three months after the initiation of therapy; and/or
- the MCM4 gene expression is low two weeks after the initiation of therapy; and/or
- the MCM4 gene expression is low three months after the initiation of therapy.

5

Preferably, when the expression level of the IL6ST gene and/or the NGFRAP1 gene and/or the ASPM gene and/or the MCM4 gene is determined, a response is determined if:

- the IL6ST gene expression is high if when measured using a microarray (such as an Affymetrix GeneChip and/or an Illumina Beadarray) it has an approximate Log² mean expression of 8.1 or more, and preferably between 8.1 and 8.3, for example a Log² mean expression of 8.1 or 8.3; and/or
- the NGFRAP1 gene expression is high if when measured using a microarray (such as an Affymetrix GeneChip and/or an Illumina Beadarray) it has an approximate Log² mean expression of 9.4 or more; and/or
- the ASPM gene expression is low if when measured using a microarray (such as an Affymetrix GeneChip and/or an Illumina Beadarray) it has an approximate Log² mean expression of 5.7 or less, and preferably between 5.5 and 5.7, for example a Log² mean expression of 5.5 or 5.55 or 5.7; and/or
- the MCM4 gene expression is low if when measured using a microarray (such as an Affymetrix GeneChip and/or an Illumina Beadarray) it has an approximate Log² mean expression of 6.25 or less, and preferably between 5.7 and 6.25, for example, a Log² mean expression of 5.7 or 6.1 or 6.25.

Preferably, in the first aspect of the invention when the expression level of the IL6ST gene and/or the NGFRAP1 gene and/or the ASPM gene and/or the MCM4 gene and/or the LAX1 gene and/or the HPRT1 gene is determined, a non-response is determined if:

- the IL6ST gene expression is low; and/or
- the NGFRAP1 gene expression is low; and/or
- the LAX1 gene expression is low; and/or
- the HPRT1 gene expression; and/or
- the ASPM gene expression is high after the initiation of therapy; and/or
- the MCM4 gene expression is high after the initiation of therapy.

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Preferably, when the expression level of the ASPM gene and/or the MCM4 gene is determined, a non-response is determined if:

- the ASPM gene expression is high two weeks after the initiation of therapy; and/or
- 5 • the ASPM gene expression is high three months after the initiation of therapy; and/or
- the MCM4 gene expression is high two weeks after the initiation of therapy; and/or
- the MCM4 gene expression is high three months after the initiation of therapy.

10 Preferably, when the expression level of the IL6ST gene and/or the NGFRAP1 gene and/or the ASPM gene and/or the MCM4 gene is determined, a non-response is determined if:

- IL6ST gene expression is low if when measured using a microarray (such as an Affymetrix GeneChip and/or an Illumina Beadarray) it has an approximate Log² mean expression of 7.7 or less; and/or
- 15 • the NGFRAP1 gene expression is low if when measured using a microarray (such as an Affymetrix GeneChip and/or an Illumina Beadarray) it has an approximate Log² mean expression of 8.9 or less; and/or
- the ASPM gene expression is high if when measured using a microarray (such as an Affymetrix GeneChip and/or an Illumina Beadarray) it has an approximate Log² mean expression of 5.4 or more; and/or
- 20 • the MCM4 gene expression is high if when measured using a microarray (such as an Affymetrix GeneChip and/or an Illumina Beadarray) it has an approximate Log² mean expression of 5.6 or more.

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Preferably, in the first aspect of the invention a response is determined if:

- the IL6ST gene expression is high; and
- the NGFRAP1 gene expression is high; and
- 30 • the ASPM gene expression is low after the initiation of therapy; and
- the MCM4 gene expression is low after the initiation of therapy.

Preferably, when the expression level of the ASPM gene and/or the MCM4 gene is determined, a response is determined if:

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- the ASPM gene expression is low two weeks after the initiation of therapy, and/or the ASPM gene expression is low three months after the initiation of therapy; and
- the MCM4 gene expression is low two weeks after the initiation of therapy, and/or the MCM4 gene expression is low three months after the initiation of therapy.

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Preferably, when the expression level of the IL6ST gene and/or the NGFRAP1 gene and/or the ASPM gene and/or the MCM4 gene is determined, a response is determined if:

- the IL6ST gene expression is high if when measured using a microarray (such as an Affymetrix GeneChip and/or an Illumina Beadarray) it has an approximate Log^2 mean expression of 8.1 or more, and preferably between 8.1 and 8.3, for example a Log^2 mean expression of 8.1 or 8.3; and
- the NGFRAP1 gene expression is high if when measured using a microarray (such as an Affymetrix GeneChip and/or an Illumina Beadarray) it has an approximate Log^2 mean expression of 9.4 or more; and
- the ASPM gene expression is low if when measured using a microarray (such as an Affymetrix GeneChip and/or an Illumina Beadarray) it has an approximate Log^2 mean expression of 5.7 or less, and preferably between 5.5 and 5.7, for example a Log^2 mean expression of 5.5 or 5.55 or 5.7; and
- the MCM4 gene expression is low if when measured using a microarray (such as an Affymetrix GeneChip and/or an Illumina Beadarray) it has an approximate Log^2 mean expression of 6.25 or less, and preferably between 5.7 and 6.25, for example, a Log^2 mean expression of 5.7 or 6.1 or 6.25.

Preferably, in the first aspect of the invention a non-response is determined if:

- the IL6ST gene expression is low; and
- the NGFRAP1 gene expression is low; and
- the ASPM gene expression is high after the initiation of therapy; and
- the MCM4 gene expression is high after the initiation of therapy.

Preferably, the expression level of the ASPM gene and/or the MCM4 gene is determined, a non-response is determined if:

- the ASPM gene expression is high two weeks after the initiation of therapy, and/or the ASPM gene expression is high three months after the initiation of therapy; and

- the MCM4 gene expression is high two weeks after the initiation of therapy, and/or the MCM4 gene expression is high three months after the initiation of therapy.

Preferably, when the expression level of the IL6ST gene and/or the NGFRAP1 gene and/or the ASPM gene and/or the MCM4 gene is determined, a non-response is determined if:

- IL6ST gene expression is low if when measured using a microarray (such as an Affymetrix GeneChip and/or an Illumina Beadarray) it has an approximate Log^2 mean expression of 7.7 or less; and
- the NGFRAP1 gene expression is low if when measured using a microarray (such as an Affymetrix GeneChip and/or an Illumina Beadarray) it has an approximate Log^2 mean expression of 8.9 or less; and
- the ASPM gene expression is high if when measured using a microarray (such as an Affymetrix GeneChip and/or an Illumina Beadarray) it has an approximate Log^2 mean expression of 5.4 or more; and
- the MCM4 gene expression is high if when measured using a microarray (such as an Affymetrix GeneChip and/or an Illumina Beadarray) it has an approximate Log^2 mean expression of 5.6 or more.

Preferably, in the first aspect of the invention a response is determined if:

- the IL6ST gene expression is high; and
- the LAX1 gene expression is high; and
- the HPRT1 gene expression is low.

Preferably, when the expression level of the IL6ST gene is determined, a response is determined if:

- the IL6ST gene expression is high if when measured using a microarray (such as an Affymetrix GeneChip and/or an Illumina Beadarray) it has an approximate Log^2 mean expression of 8.1 or more, and preferably between 8.1 and 8.3, for example a Log^2 mean expression of 8.1 or 8.3.

Preferably, in the first aspect of the invention a non-response is determined if:

- the IL6ST gene expression is low; and

- the LAX1 gene expression is low; and
- the HPRT1 gene expression is high.

5 Preferably, when the expression level of the IL6ST gene is determined, a non-response is determined if:

- IL6ST gene expression is low if when measured using a microarray (such as an Affymetrix GeneChip and/or an Illumina Beadarray) it has an approximate Log^2 mean expression of 7.7 or less.

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It would be understood by those skilled in oncology and/or medicine how to calculate the Log^2 mean expression of a gene based on the method of the first aspect of the invention. By “ Log^2 mean expression”, we include that the value is calculated using the formula: $x = \log^2(y)$, wherein x is the $\log^2$ value where y is the unlogged value.

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Preferably, in the first aspect of the invention there is a further determination of a response or a non-response by measuring one or more selected from the group comprising: the expression level of one or more proliferation-associated gene (such as a cyclin); the expression level of one or more mini-chromosome maintenance gene; the expression level
20 of one or more mitotic spindle associated gene; the expression level of one or more glycolysis and oxidative phosphorylation gene; the expression level of one or more immune/inflammatory response gene; the expression level of one or more ECM stromal remodelling gene; tumour volume.

25 Preferably, in the first aspect of the invention the further determination of a response or non-response occurs at least one day after the initiation of therapy; for example: at least two days, or at least three days, or at least four days, or at least five days, or at least six days, or at least seven days, or at least eight days, or at least nine days, or at least ten days, or at least 11 days, or at least 12 days, or at least 13 days, or at least 14 days, or at
30 least 15 days, or at least 16 days, or at least 17 days, or at least 18 days, or at least 19 days, or at least 20 days, or at least 21 days, or at least 22 days, or at least 23 days, or at least 24 days, or at least 25 days, or at least 26 days, or at least 27 days, or at least 28 days, or at least five weeks, or at least six weeks, or at least seven weeks, or at least eight weeks, or at least nine weeks, or at least ten weeks, or at least 11 weeks, or at least three
35 months, or at least four months, or at least five months, or at least six months, or at least seven months, or at least eight months, or at least nine months, or at least ten months, or at least 11 months, or at least 12 months after the initiation of therapy. Most preferably in

the first aspect of the invention, the further determination of a response or non-response occurs 14 days (*i.e.* two weeks) after the initiation of therapy or three months after the initiation of therapy.

5 Preferably, in the first aspect of the invention a response is determined if:

- the expression level of one or more proliferation-associated gene (such as a cyclin) is significantly down-regulated or down-regulated, and/or
- 10 • the expression level of one or more mini-chromosome maintenance gene is significantly down-regulated or down-regulated, and/or
- the expression level of one or more mitotic spindle associated gene is significantly down-regulated or down-regulated, and/or
- the expression level of one or more glycolysis and oxidative phosphorylation gene is significantly down-regulated or down-regulated, and/or
- 15 • the expression level of one or more immune/inflammatory response gene is up-regulated or significantly up-regulated, and/or
- the expression level of one or more ECM stromal remodelling gene is up-regulated or significantly up-regulated, and/or
- the one or more immune/inflammatory response gene and one or more ECM
20 stromal remodelling gene are co-expressed; most preferably, the expression level of one or more proliferation-associated gene (such as a cyclin) is significantly down-regulated 14 days (*i.e.* two weeks) after the initiation of therapy, and/or
- the expression level of one or more proliferation-associated gene (such as a cyclin) is significantly down-regulated three months after the initiation of therapy, and/or
- 25 • the expression level of one or more mini-chromosome maintenance gene is significantly down-regulated 14 days (*i.e.* two weeks) after the initiation of therapy, and/or
- the expression level of one or more mini-chromosome maintenance gene is significantly down-regulated three months after the initiation of therapy, and/or
- 30 • the expression level of one or more mitotic spindle associated gene is significantly down-regulated 14 days (*i.e.* two weeks) after the initiation of therapy, and/or
- the expression level of one or more mitotic spindle associated gene is significantly down-regulated three months after the initiation of therapy, and/or
- the expression level of one or more glycolysis and oxidative phosphorylation gene
35 is significantly down-regulated three months after the initiation of therapy, and/or

- the expression level of one or more immune/inflammatory response gene is up-regulated three months after the initiation of therapy, and/or
- the expression level of one or more ECM stromal remodelling gene is up-regulated three months after the initiation of therapy.

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Preferably, in the first aspect of the invention a non-response is determined if:

- the expression level of one or more proliferation-associated gene (such as a cyclin) is down-regulated or unchanged, and/or
- 10 • the expression level of one or more mini-chromosome maintenance gene is down-regulated or unchanged, and/or
- the expression level of one or more mitotic spindle associated gene is down-regulated or unchanged, and/or
- 15 • the expression level of one or more glycolysis and oxidative phosphorylation gene is unchanged, and/or
- the expression level of one or more immune/inflammatory response gene is unchanged, and/or
- the expression level of one or more ECM stromal remodelling gene is unchanged; most preferably, the expression level of one or more proliferation-associated gene (such as a cyclin) is down-regulated or unchanged 14 days (*i.e.* two weeks) after the initiation of therapy, and/or
- 20 • the expression level of one or more proliferation-associated gene (such as a cyclin) is down-regulated or unchanged three months after the initiation of therapy, and/or
- the expression level of one or more mini-chromosome maintenance gene is down-regulated or unchanged 14 days (*i.e.* two weeks) after the initiation of therapy, and/or
- 25 • the expression level of one or more mini-chromosome maintenance gene is down-regulated or unchanged three months after the initiation of therapy, and/or
- the expression level of one or more mitotic spindle associated gene is down-regulated or unchanged 14 days (*i.e.* two weeks) after the initiation of therapy, and/or
- 30 • the expression level of one or more mitotic spindle associated gene is down-regulated or unchanged three months after the initiation of therapy, and/or
- the expression level of one or more glycolysis and oxidative phosphorylation gene is unchanged three months after the initiation of therapy, and/or
- 35

- the expression level of one or more immune/inflammatory response gene is unchanged three months after the initiation of therapy, and/or
- the expression level of one or more ECM stromal remodelling gene is unchanged after the initiation of therapy.

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Preferably, in the present invention the proliferation-associated gene is one or more gene selected from the group comprising: CCNA2 (Hillier et al. Nature. 2005 Apr 7;434(7034):724-31.PMID: 15815621 and Tsang et al. J Cell Biol. 2007 Aug 13;178(4):621-33. PMID: 17698606); CCNB1; CCND1.

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Preferably, in the present the invention the mini chromosome maintenance gene is one or more gene selected from the group comprising: MCM2 (Mincheva et al. Cytogenet Cell Genet. 1994;65(4):276-7.PMID: 8258304 and Nakatsuru et al. Cytogenet Cell Genet. 1995;68(3-4):226-30. PMID: 7842741); MCM4; MCM6 (Harvey et al. FEBS Lett. 1996 Dec 2;398(2-3):135-40.PMID: 8977093).

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Preferably, in the present invention the mitotic spindle associated gene is one or more gene selected from the group comprising: ASPM; AURKA.

20 As discussed above, it is important that the application contains sequence information for the genes it discloses.

It would be known to those skilled in molecular biology how the expression of the abovementioned genes could be measured, and how their relative expression levels could be calculated.

25

Preferably, in the present invention the expression level is determined by measuring the presence and/or amount of one or more product of the gene, for example: protein or mRNA.

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Assaying protein levels in a biological sample can be performed using any method known in the art. Preferred for assaying protein levels in a biological sample are antibody-based techniques. Such techniques may involve a primary antibody (which specifically recognises the target protein) and a secondary antibody (which specifically recognises the primary antibody) which comprises a detectable moiety.

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Other antibody-based methods useful for detecting protein levels include immunoassays,

such as an enzyme linked immunosorbent assay (ELISA) and a radioimmunoassay (RIA). For example, a protein-specific monoclonal antibody can be used both as an immune-adsorbent and as an enzyme-labelled probe to detect and quantify the protein. The amount of protein present in a sample can be calculated by reference to the amount present in a standard preparation using a linear regression computer algorithm. Such an ELISA for detecting a tumour antigen is described in Iacobelli et al., Breast Cancer Research and Treatment 11: 19-30 (1988). In another ELISA assay, two distinct specific monoclonal antibodies can be used to detect protein in a body fluid. In this assay, one of the antibodies is used as the immune-adsorbent and the other as the enzyme-labelled probe.

The above techniques may be conducted essentially as a "one-step" or "two-step" assay. The "one-step" assay involves contacting protein with immobilized antibody and, without washing, contacting the mixture with the labelled antibody. The "two-step" assay involves washing before contacting the mixture with the labelled antibody. Other conventional methods may also be employed as suitable. It is usually desirable to immobilize one component of the assay system on a support, thereby allowing other components of the system to be brought into contact with the component and readily removed from the sample.

Suitable enzyme labels include, for example, those from the oxidase group, which catalyse the production of hydrogen peroxide by reacting with substrate. Glucose oxidase is particularly preferred as it has good stability and its substrate (glucose) is readily available. Activity of an oxidase label may be assayed by measuring the concentration of hydrogen peroxide formed by the enzyme-labelled antibody/substrate reaction. Besides enzymes, other suitable labels include radioisotopes, such as iodine (^{125}I , ^{121}I), carbon (^{14}C), sulphur ^{35}S , tritium (^3H), indium (^{112}In), and technetium ($^{99\text{m}}\text{Tc}$), and fluorescent labels, such as fluorescein and rhodamine, and biotin.

It will be appreciated that protein-specific antibodies for use in the present invention can be raised against the intact protein or an antigenic polypeptide fragment thereof, which may be presented together with a carrier protein, such as an albumin, to an animal system (such as rabbit or mouse) or, if it is long enough (at least about 25 amino acids), without a carrier.

As used herein, the term "antibody" (Ab) or "monoclonal antibody" (Mab) includes intact molecules as well as antibody fragments (such as, for example, Fab and F(ab')₂

fragments) which are capable of specifically binding to the target protein. Fab and F(ab')₂ fragments lack the Fc fragment of intact antibody, clear more rapidly from the circulation, and may have less non-specific tissue binding of an intact antibody (Wahl et al., J. Nucl. Med. 24:316-325 (1983)). Thus, these fragments are preferred.

5

Further suitable labels for protein-specific antibodies are provided below. Examples of suitable enzyme labels include malate dehydrogenase, staphylococcal nuclease, delta-5-steroid isomerase, yeast-alcohol dehydrogenase, alpha-glycerol phosphate dehydrogenase, triose phosphate isomerase, peroxidase, alkaline phosphatase, asparaginase, glucose oxidase, beta-galactosidase, ribonuclease, urease, catalase, glucose-6-phosphate dehydrogenase, glucoamylase, and acetylcholine esterase.

10

Examples of suitable radio-isotopic labels include ³H, ¹¹¹In, ¹²⁵I, ¹³¹I, ³²P, ³⁵S, ¹⁴C, ⁵¹Cr, ⁵⁷Co, ⁵⁸Co, ⁵⁹Fe, ⁷⁵Se, ¹⁵²Eu, ⁹⁰Y, ⁶⁷Cu, ²¹⁷Pb, ²¹¹At, ²¹²Pb, ⁴⁷Sc, and ¹⁰⁹Pd. Examples of suitable non-radioactive isotopic labels include ¹⁵⁷Gd, ⁵⁵Mn, ¹⁶²Dy, ⁵²Tr, and ⁵⁶Fe.

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Examples of suitable fluorescent labels include an ¹⁵²Eu label, a fluorescein label, an isothiocyanate label, a rhodamine label, a phycoerythrin label, a phycocyanin label, an allophycocyanin label, an o-phthaldehyde label, and a fluorescamine label. Examples of suitable toxin labels include diphtheria toxin, ricin, and cholera toxin. Examples of chemiluminescent labels include a luminal label, an isoluminal label, an aromatic acridinium ester label, an imidazole label, an acridinium salt label, an oxalate ester label, a luciferin label, a luciferase label, and an aequorin label. Examples of nuclear magnetic resonance contrasting agents include heavy metal nuclei such as Gd, Mn, and iron.

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Typical techniques for binding the above-described labels to antibodies are provided by Kennedy et al., Clin. Chim. Acta 70:1-31 (1976), and Schurs et al, Clin. Chim. Acta 81:1-40 (1977). Coupling techniques mentioned in the latter are the glutaraldehyde method, the periodate method, the dimaleimide method, the m-maleimidobenzyl-N-hydroxy-succinimide ester method, all of which methods are incorporated by reference herein.

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Preferably, in the present invention the protein is measured using one or more method selected from the group comprising: Raman spectroscopy; Acoustic Membrane MicroParticle technology; immunohistochemistry; an antibody-based detection method, for example, RPPA or AQUA.

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Acoustic Membrane MicroParticle (AMMP) technology is a non-optical detection technology for determining protein concentration. In brief, micro-particles are used to capture a protein analyte in a sample, and rapidly transport it to a sensor surface, thus resulting in a measurable signal that is tracked by observing the sensor response.

5

Preferably, in the present invention mRNA is measured using one or more method selected from the group comprising: a PCR-based approach (such as RT-PCR); microarray technology; NanoString.

10 The RT-PCR method is described in Makino et al, Technique 2:295-301 (1990), and involves the radio-activities of the “amplicons” in the polyacrylamide gel bands being linearly related to the initial concentration of the target mRNA. Briefly, that method involves adding total RNA isolated from a biological sample in a reaction mixture containing a RT primer and appropriate buffer. After incubating for primer annealing, the mixture can be
15 supplemented with a RT buffer, dNTPs, DTT, RNase inhibitor and reverse transcriptase. After incubation to achieve reverse transcription of the RNA, the RT products are subjected to PCR using labelled primers. Alternatively, rather than labelling the primers, a labelled dNTP can be included in the PCR reaction mixture. PCR amplification can be performed in a DNA thermal cycler according to conventional techniques. After a suitable number of
20 rounds to achieve amplification, the PCR reaction mixture is electrophoresed on a polyacrylamide gel. After drying the gel, the radioactivity of the appropriate bands (corresponding to the mRNA) is quantified using an imaging analyser. RT and PCR reaction ingredients and conditions, reagent and gel concentrations, and labelling methods are well known in the art. Variations on the RT-PCR method will be apparent to those
25 skilled in the art. Any set of oligonucleotide primers which will amplify reverse transcribed target mRNA can be used and those skilled in the art will be aware of how to design, manufacture and use such primers.

There are numerous commercially available microarray platforms that can be used to
30 quantify mRNA, such as those available from Affymetrix and Illumina. Those arrays deploy different types of technology which provide a surface on which mRNA with specific sequences can anneal, then, often during subsequent amplification steps, those specific sequences can be quantified.

35 NanoString is a commercially available alternative to microarray technology, which allows for high-throughput analysis of gene expression. NanoString technology uses direct multiplexed measurement of gene expression with color-coded probe pairs (Geiss GK1,

Bumgarner RE, Birditt B, Dahl T, Dowidar N, Dunaway DL, Fell HP, Ferree S, George RD, Grogan T, James JJ, Maysuria M, Mitton JD, Oliveri P, Osborn JL, Peng T, Ratcliffe AL, Webster PJ, Davidson EH, Hood L, Dimitrov K. Nat Biotechnol. 2008 Mar;26(3):317-25. PMID: 18278033 and www.nanostring.com/).

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Preferably, in the present invention the sample is one or more sample selected from the group comprising: a tumour biopsy; surgical tumour resection; blood; serum; plasma; lymphatic fluid; urine; nipple aspirate fluid.

10 By "tumour biopsy", we include: a core biopsy; a vacuum-assisted breast biopsy; an excisional biopsy; a lymph node biopsy.

Preferably, in the first aspect of the invention if a response is determined the method further comprises the step of continuing to administer the therapy to the individual.

15

Preferably, in the first aspect of the invention if a non-response is determined the method further comprises administering a different therapy and/or a treatment to the individual.

20 Preferably, in the first aspect of the invention the treatment is one or more treatment selected from the group comprising: surgery; radiotherapy; chemotherapy; an mTOR inhibitor; an anti-HER2 therapy; an aromatase inhibitor; an Estrogen Receptor modulator; an Estrogen Receptor down-regulator.

25 It would be apparent to those skilled in medicine how a particular therapy and/or a particular treatment could be administered. For example, appropriate treatment and/or therapy could be administered via one or more route selected from the group comprising: oral administration; rectal administration; epidural administration; topical administration; nasal administration; intramuscular administration; intradermal administration; intravenous administration; subcutaneous administration; transdermal administration.

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In a second aspect, the invention provides a method for selecting a treatment for an individual with cancer, comprising the steps of:

- 35
- a) providing a first sample comprising one or more cancer cell from the individual;
 - b) determining in the first sample the expression level of the IL6ST gene; and

- c) selecting a treatment for the individual on the basis of the determination in step (b).

By "selecting a treatment for an individual with cancer", we include the selection of a treatment that is likely to be effective in treating cancer in the individual, including being able to effect a response, as defined above in the first aspect of the invention. Thus by "method of selecting a treatment for an individual" we include the prediction of a treatment that is likely to be effective in treating cancer in the individual, including being able to effect a response, as defined above.

For the second aspect of the invention, by "individual", "cancer", "first sample", "cancer cell", "expression", "treatment" and "the IL6ST gene" we include the definition of those terms as outlined for the first aspect of the invention.

In one embodiment, step (b) of the second aspect of the invention comprises determining in the first sample the expression level of only the IL6ST gene.

Preferably, the second aspect of the invention further comprises determining in the first sample the expression level of the NGFRAP1 gene. For the second aspect of the invention, by "the NGFRAP1 gene" we include the definition of that term as outlined for the first aspect of the invention.

Preferably, in the second aspect of the invention step (b) further comprises determining in the first sample the expression level of the LAX1 gene and/or HPRT1 gene. For the second aspect of the invention, by "the LAX1 gene" and "the HPRT1 gene" we include the definition of those terms as outlined for the first aspect of the invention.

Preferably, in the second aspect of the invention step (b) further comprises determining in the first sample the expression level the ASPM gene and/or the MCM4 gene and/or the MKI67 gene and/or a gene marker of cellular proliferation. For the second aspect of the invention, by "the ASPM gene", "the MCM4 gene", "the MKI67 gene" and "a gene marker of cellular proliferation" we include the definition of those terms as outlined for the first aspect of the invention.

Preferably, step (b) of the second aspect of the invention comprises determining the expression level of at least the IL6ST gene in the first sample, for example:

- determining the expression level of the IL6ST gene and the NGFRAP1 gene, or
- determining the expression level of the IL6ST gene and the LAX1 gene, or
- determining the expression level of the IL6ST gene and the HPRT1 gene, or
- determining the expression level of the IL6ST gene and the ASPM gene, or
- 5 • determining the expression level of the IL6ST gene and the MCM4 gene, or
- determining the expression level of the IL6ST gene and the MKI67 gene, or
- determining the expression level of the IL6ST gene and a gene marker of cellular proliferation, or
- determining the expression level of the IL6ST gene and the NGFRAP1 gene and
- 10 the LAX1 gene, or
- determining the expression level of the IL6ST gene and the NGFRAP1 gene and the HPRT1 gene, or
- determining the expression level of the IL6ST gene and the NGFRAP1 gene and the ASPM gene, or
- 15 • determining the expression level of the IL6ST gene and the NGFRAP1 gene and the MCM4 gene, or
- determining the expression level of the IL6ST gene and the NGFRAP1 gene and the MKI67 gene, or
- determining the expression level of the IL6ST gene and the NGFRAP1 gene and
- 20 a gene marker of cellular proliferation, or
- determining the expression level of the IL6ST gene and the LAX1 gene and the HPRT1 gene, or
- determining the expression level of the IL6ST gene and the LAX1 gene and the ASPM gene, or
- 25 • determining the expression level of the IL6ST gene and the LAX1 gene and the MCM4 gene, or
- determining the expression level of the IL6ST gene and the LAX1 gene and the MKI67 gene, or
- determining the expression level of the IL6ST gene and the LAX1 gene and a gene
- 30 marker of cellular proliferation, or
- determining the expression level of the IL6ST gene and the NGFRAP1 gene and the LAX1 gene and the HPRT1 gene, or
- determining the expression level of the IL6ST gene and the NGFRAP1 gene and the LAX1 gene and the HPRT1 gene and the ASPM gene, or
- 35 • determining the expression level of the IL6ST gene and the NGFRAP1 gene and the LAX1 gene and the HPRT1 gene and the MCM4 gene, or

- determining the expression level of the IL6ST gene and the NGFRAP1 gene and the LAX1 gene and the HPRT1 gene and the MKI67 gene, or
- determining the expression level of the IL6ST gene and the NGFRAP1 gene and the LAX1 gene and the HPRT1 gene and a gene marker of cellular proliferation, or
- 5 • determining the expression level of the IL6ST gene and the NGFRAP1 gene and the LAX1 gene and the HPRT1 gene and the ASPM gene and the MCM4 gene, or
- determining the expression level of the IL6ST gene and the NGFRAP1 gene and the LAX1 gene and the HPRT1 gene and the ASPM gene and the MKI67 gene, or
- 10 • determining the expression level of the IL6ST gene and the NGFRAP1 gene and the LAX1 gene and the HPRT1 gene and the ASPM gene and a gene marker of cellular proliferation, or
- determining the expression level of the IL6ST gene and the NGFRAP1 gene and the LAX1 gene and the HPRT1 gene and the ASPM gene and the MCM4 gene and the MKI67 gene, or
- 15 • determining the expression level of the IL6ST gene and the NGFRAP1 gene and the LAX1 gene and the HPRT1 gene and the ASPM gene and the MCM4 gene and a gene marker of cellular proliferation, or
- determining the expression level of the IL6ST gene and the NGFRAP1 gene and the LAX1 gene and the HPRT1 gene and the ASPM gene and the MCM4 gene and the MKI67 gene and a gene marker of cellular proliferation in the first sample.
- 20

Preferably, step (a) and/or step (b) and/or step (c) of the second aspect of the invention is undertaken *in vitro* or *ex vivo*.

- 25 The exemplary high gene expression determinations and the low gene expression determinations outlined in the first aspect of the invention for the IL6ST gene, the NGFRAP1 gene, the ASPM gene, the MCM4 gene, the LAX1 gene and the HPRT1 gene are also included in the second aspect of the invention. Also included in the second aspect of the invention are Log² mean expression values measured using a microarray (such as
- 30 an Affymetrix GeneChip and/or an Illumina Beadarray) for the IL6ST gene, the NGFRAP1 gene, the ASPM gene and the MCM4 gene, as outlined in the first aspect of the invention. Those skilled in the art of medicine would know how to use the high gene expression determinations and/or the low gene expression determinations and/or the Log² mean expression values for selecting a treatment for an individual with cancer.

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Preferably, in the second aspect of the invention step (a) further comprises providing the first sample before a therapy and/or a treatment.

For the second aspect of the invention, by “therapy” and “Log² mean expression” we
5 include the definition of that term as outlined for the first aspect of the invention.

Preferably, the second aspect of the invention further comprises the step of administering the selected treatment to the individual.

10 It would be apparent to those skilled in medicine how a particular treatment could be administered. Preferably, in the second aspect of the invention the selected treatment could be administered via one or more route selected from the group comprising: oral administration; rectal administration; epidural administration; topical administration; nasal administration; intramuscular administration; intradermal administration; intravenous
15 administration; subcutaneous administration; transdermal administration.

Preferably, in the second aspect of the invention there is a further determination for selecting a treatment for an individual with cancer by measuring parameter one or more selected from the group comprising: the expression level of one or more proliferation-associated gene (such as a cyclin); the expression level of one or more mini-chromosome
20 maintenance gene; the expression level of one or more mitotic spindle associated gene; the expression level of one or more glycolysis and oxidative phosphorylation gene; the expression level of one or more immune/inflammatory response gene; the expression level of one or more ECM stromal remodelling gene; tumour volume.

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In a third aspect, the invention provides a method for predicting the prognosis of cancer in an individual, comprising the steps of:

- 1) providing a first sample comprising one or more cancer cell from the
30 individual;
- 2) determining in the first sample the expression level of the IL6ST gene; and
- 3) predicting the prognosis of cancer on the basis of the determination in step (2).

35 By “prognosis of cancer in an individual”, we include the likely clinical development and outcome of cancer in the individual, including the severity of the disease and/or the life expectancy or survival of the individual. Thus by “predicting the prognosis of cancer in an

individual" we include the prediction of the likely clinical development and outcome of cancer in the individual, including the likely severity of the disease and/or the likely life expectancy or survival of the individual.

- 5 For the third aspect of the invention, by "individual", "cancer", "first sample", "cancer cell", "expression" and "the IL6ST gene" we include the definition of those terms as outlined for the first aspect of the invention.

10 In one embodiment, step (2) of the third aspect of the invention comprises determining in the first sample the expression level of only the IL6ST gene.

Preferably, the third aspect of the invention further comprises determining in the first sample the expression level of the NGFRAP1 gene. For the third aspect of the invention, by "the NGFRAP1 gene" we include the definition of that term as outlined for the first aspect
15 of the invention.

Preferably, in the third aspect of the invention step (2) further comprises determining in the first sample the expression level of the LAX1 gene and/or HPRT1 gene. For the second aspect of the invention, by "the LAX1 gene" and "the HPRT1 gene" we include the definition
20 of those terms as outlined for the first aspect of the invention.

Preferably, in the third aspect of the invention step (2) further comprises determining in the first sample the expression level the ASPM gene and/or the MCM4 gene and/or the MKI67 gene and/or a gene marker of cellular proliferation. For the third aspect of the invention,
25 by "the ASPM gene", "the MCM4 gene", "the MKI67 gene" and "a gene marker of cellular proliferation" we include the definition of those terms as outlined for the first aspect of the invention.

Preferably, step (2) of the third aspect of the invention comprises determining the
30 expression level of at least the IL6ST gene in the first sample, for example:

- determining the expression level of the IL6ST gene and the NGFRAP1 gene, or
- determining the expression level of the IL6ST gene and the LAX1 gene, or
- determining the expression level of the IL6ST gene and the HPRT1 gene, or
- 35 • determining the expression level of the IL6ST gene and the ASPM gene, or
- determining the expression level of the IL6ST gene and the MCM4 gene, or
- determining the expression level of the IL6ST gene and the MKI67 gene, or

- determining the expression level of the IL6ST gene and a gene marker of cellular proliferation, or
- determining the expression level of the IL6ST gene and the NGFRAP1 gene and the LAX1 gene, or
- 5 • determining the expression level of the IL6ST gene and the NGFRAP1 gene and the HPRT1 gene, or
- determining the expression level of the IL6ST gene and the NGFRAP1 gene and the ASPM gene, or
- 10 • determining the expression level of the IL6ST gene and the NGFRAP1 gene and the MCM4 gene, or
- determining the expression level of the IL6ST gene and the NGFRAP1 gene and the MKI67 gene, or
- determining the expression level of the IL6ST gene and the NGFRAP1 gene and a gene marker of cellular proliferation, or
- 15 • determining the expression level of the IL6ST gene and the LAX1 gene and the HPRT1 gene, or
- determining the expression level of the IL6ST gene and the LAX1 gene and the ASPM gene, or
- determining the expression level of the IL6ST gene and the LAX1 gene and the MCM4 gene, or
- 20 • determining the expression level of the IL6ST gene and the LAX1 gene and the MKI67 gene, or
- determining the expression level of the IL6ST gene and the LAX1 gene and a gene marker of cellular proliferation, or
- 25 • determining the expression level of the IL6ST gene and the NGFRAP1 gene and the LAX1 gene and the HPRT1 gene, or
- determining the expression level of the IL6ST gene and the NGFRAP1 gene and the LAX1 gene and the HPRT1 gene and the ASPM gene, or
- determining the expression level of the IL6ST gene and the NGFRAP1 gene and the LAX1 gene and the HPRT1 gene and the MCM4 gene, or
- 30 • determining the expression level of the IL6ST gene and the NGFRAP1 gene and the LAX1 gene and the HPRT1 gene and the MKI67 gene, or
- determining the expression level of the IL6ST gene and the NGFRAP1 gene and the LAX1 gene and the HPRT1 gene and a gene marker of cellular proliferation, or
- 35 • determining the expression level of the IL6ST gene and the NGFRAP1 gene and the LAX1 gene and the HPRT1 gene and the ASPM gene and the MCM4 gene, or

- determining the expression level of the IL6ST gene and the NGFRAP1 gene and the LAX1 gene and the HPRT1 gene and the ASPM gene and the MKI67 gene, or
- determining the expression level of the IL6ST gene and the NGFRAP1 gene and the LAX1 gene and the HPRT1 gene and the ASPM gene and a gene marker of cellular proliferation, or
- determining the expression level of the IL6ST gene and the NGFRAP1 gene and the LAX1 gene and the HPRT1 gene and the ASPM gene and the MCM4 gene and the MKI67 gene, or
- determining the expression level of the IL6ST gene and the NGFRAP1 gene and the LAX1 gene and the HPRT1 gene and the ASPM gene and the MCM4 gene and a gene marker of cellular proliferation, or
- determining the expression level of the IL6ST gene and the NGFRAP1 gene and the LAX1 gene and the HPRT1 gene and the ASPM gene and the MCM4 gene and the MKI67 gene and a gene marker of cellular proliferation in the first sample.

Preferably, step (1) and/or step (2) and/or step (3) of the third aspect of the invention is undertaken *in vitro* or *ex vivo*.

Preferably, in the third aspect of the invention:

step (1) further comprises providing the first sample before a therapy.

For the third aspect of the invention, by “therapy” we include the definition of that term as outlined for the first aspect of the invention.

Preferably, in the third aspect of the invention:

step (1) further comprises providing the first sample before a therapy and providing a second sample after the therapy, and

step (2) further comprises determining in the first sample at least the expression of the IL6ST gene, for example: the expression the IL6ST gene; and the NGFRAP1 gene and/or the LAX1 gene and/or the HPRT1 gene, and determining in the second sample the expression level of the ASPM gene and/or the MCM4 gene and/or the MKI67 gene and/or a gene marker of cellular proliferation.

Preferably, step (2) of the third aspect of the invention comprises determining the expression level of at least the ASPM gene or the MCM4 gene or the MKI67 gene or a

gene marker of cellular proliferation in the second sample, for example: determining the expression level of the ASPM gene and the MCM4 gene, or determining the expression level of the ASPM gene and the MKI67 gene, or determining the expression level of the ASPM gene and a gene marker of cellular proliferation, or determining the expression level of the MCM4 gene and the MKI67 gene, or determining the expression level of the MCM4 gene and a gene marker of cellular proliferation, or determining the expression level of the MKI67 gene and a gene marker of cellular proliferation, or determining the expression level of the ASPM gene and the MCM4 gene and the MKI67 gene, or determining the expression level of the ASPM gene and the MCM4 gene and a gene marker of cellular proliferation, or determining the expression level of the MCM4 gene and the MKI67 gene and a gene marker of cellular proliferation, or determining the expression level of the ASPM gene and the MCM4 gene and the MKI67 gene and a gene marker of cellular proliferation in the second sample.

Preferably, in the third aspect of the invention the second sample is provided from the individual at least one day after the initiation of therapy, for example: at least two days, or at least three days, or at least four days, or at least five days, or at least six days, or at least seven days, or at least eight days, or at least nine days, or at least ten days, or at least 11 days, or at least 12 days, or at least 13 days, or at least 14 days, or at least 15 days, or at least 16 days, or at least 17 days, or at least 18 days, or at least 19 days, or at least 20 days, or at least 21 days, or at least 22 days, or at least 23 days, or at least 24 days, or at least 25 days, or at least 26 days, or at least 27 days, or at least 28 days, or at least five weeks, or at least six weeks, or at least seven weeks, or at least eight weeks, or at least nine weeks, or at least ten weeks, or at least 11 weeks, or at least three months, or at least four months, or at least five months, or at least six months, or at least seven months, or at least eight months, or at least nine months, or at least ten months, or at least 11 months, or at least 12 months after the initiation of therapy. Most preferably in the third aspect of the invention, the second sample is provided from the individual 14 days (*i.e.* two weeks) after the initiation of therapy or three months after the initiation of therapy.

Preferably, in the third aspect of the invention the step of predicting the prognosis of cancer in the individual is one or more parameter selected from the group comprising: percentage response of the individual to cancer treatment; overall survival of the individual; disease-specific survival of the individual; progression-free survival of the individual.

Preferably, in the present invention the percentage response of the individual to cancer treatment could be at least 10 percent; for example, at least 15 percent, or at least 20

percent, or at least 25 percent, or at least 30 percent, or at least 35 percent, or at least 40 percent, or at least 45 percent, or at least 50 percent, or at least 55 percent, or at least 60 percent, or at least 65 percent, or at least 70 percent, or at least 80 percent, or at least 85 percent, or at least 90 percent, or at least 95 percent or 100 percent.

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Preferably, in step (3) of the third aspect of the invention, and on the basis of the determination in step (2), the prognosis of the cancer is predicted to be good, or the prognosis of the cancer is predicted to be poor.

10 Those skilled in the art of medicine would appreciate the definition of such prognoses.

By “the prognosis of the cancer is predicted as being good”, we include that the individual is predicted to exhibit an increase in the severity of cancer symptoms, and/or the individual is predicted to exhibit an increase in the number of cancer symptoms, and/or the individual is predicted to exhibit a reduction in tumour size, and/or the individual is predicted to exhibit a reduction in tumour number, and/or the individual is predicted to exhibit a reduction in metastasis, and/or the individual is predicted to exhibit a reduction in cancer affected lymph nodes, and/or the individual is predicted to exhibit a reduction in tumour volume, and/or the individual is predicted to exhibit a reduction in ER IHC Allred score, and/or the individual is predicted to exhibit a reduction in tumour grade, and/or a reduction of one or more proliferation-associated gene, and/or a reduction in one or more gene marker of cellular proliferation, and/or the individual is predicted to exhibit a reduction in cancer progression, and/or the individual is predicted to be classed as being in remission, and/or the individual is predicted to exhibit no cancer symptoms, and/or the individual is predicted to exhibit have no cancer cells, and/or the individual is predicted not to die as a result of the cancer, and/or the individual is predicted not to have a reoccurrence of the cancer.

By “the prognosis of the cancer is predicted to be poor”, we include that the individual is predicted to exhibit no increase in the severity of cancer symptoms, and/or the individual is predicted to exhibit a reduction in the severity of cancer symptoms, and/or the individual is predicted to exhibit no reduction in the number of cancer symptoms, and/or the individual is predicted to exhibit an increase in the number of cancer symptoms, and/or the individual is predicted to exhibit no reduction in cancer progression, and/or the individual is predicted to exhibit an increase in cancer progression, and/or the individual is predicted to exhibit no reduction in tumour size, and/or the individual is predicted to exhibit an increase in tumour size, and/or the individual is predicted to exhibit no reduction in tumour number, and/or the individual is predicted to exhibit an increase in tumour number, and/or the individual is

predicted to exhibit no reduction in metastasis, and/or the individual is predicted to exhibit an increase in metastasis, and/or the individual is predicted to exhibit no reduction in cancer affected lymph nodes, and/or the individual is predicted to exhibit an increase in cancer affected lymph nodes, and/or the individual is predicted to exhibit no reduction in tumour volume, and/or the individual is predicted to exhibit an increase in tumour volume, and/or the individual is predicted to exhibit no reduction in tumour grade, and/or the individual is predicted to exhibit an increase in tumour grade, and/or the individual is predicted to exhibit no reduction in ER IHC Allred score, and/or the individual is predicted to exhibit an increase in ER IHC Allred score, and/or the individual is predicted to die as a result of the cancer, and/or the individual is predicted have a reoccurrence of the cancer.

Preferably, in the third aspect of the invention when the expression level of the IL6ST gene and/or the NGFRAP1 gene and/or the ASPM gene and/or the MCM4 gene and/or the LAX1 gene and/or the HPRT1 gene is determined, it is determined that the prognosis of the cancer is predicted to be good if:

- the IL6ST gene expression is high; and/or
- the NGFRAP1 gene expression is high; and/or
- the LAX1 gene expression is high; and/or
- the HPRT1 gene expression is low; and/or
- the ASPM gene expression is low after the initiation of therapy; and/or
- the MCM4 gene expression is low after the initiation of therapy.

Preferably, when the expression level of the ASPM gene and/or the MCM4 gene is determined, the prognosis of the cancer is predicted to be good if:

- the ASPM gene expression is low two weeks after the initiation of therapy; and/or
- the ASPM gene expression is low three months after the initiation of therapy; and/or
- the MCM4 gene expression is low two weeks after the initiation of therapy; and/or
- the MCM4 gene expression is low three months after the initiation of therapy.

Preferably, when the expression level of the IL6ST gene and/or the NGFRAP1 gene and/or the ASPM gene and/or the MCM4 gene is determined, the prognosis of the cancer is predicted to be good if:

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- the IL6ST gene expression is high if when measured using a microarray (such as an Affymetrix GeneChip and/or an Illumina Beadarray) it has an approximate Log^2 mean expression of 8.1 or more, and preferably between 8.1 and 8.3, for example a Log^2 mean expression of 8.1 or 8.3; and/or
- 5 • the NGFRAP1 gene expression is high if when measured using a microarray (such as an Affymetrix GeneChip and/or an Illumina Beadarray) it has an approximate Log^2 mean expression of 9.4 or more; and/or
- the ASPM gene expression is low if when measured using a microarray (such as an Affymetrix GeneChip and/or an Illumina Beadarray) it has an approximate Log^2 mean expression of 5.7 or less, and preferably between 5.5 and 5.7, for example
- 10 a Log^2 mean expression of 5.5 or 5.55 or 5.7; and/or
- the MCM4 gene expression is low if when measured using a microarray (such as an Affymetrix GeneChip and/or an Illumina Beadarray) it has an approximate Log^2 mean expression of 6.25 or less, and preferably between 5.7 and 6.25, for
- 15 example, a Log^2 mean expression of 5.7 or 6.1 or 6.25.

Preferably, in the third aspect of the invention when the expression level of the IL6ST gene and/or the NGFRAP1 gene and/or the ASPM gene and/or the MCM4 gene and/or the LAX1 gene and/or the HPRT1 gene is determined, it is determined that the prognosis of

20 the cancer is predicted to be poor if:

- the IL6ST gene expression is low; and/or
- the NGFRAP1 gene expression is low; and/or
- the LAX1 gene expression is low; and/or
- 25 • the HPRT1 gene expression is high; and/or
- the ASPM gene expression is high after the initiation of therapy; and/or
- the MCM4 gene expression is high after the initiation of therapy.

Preferably, when the expression level of the ASPM gene and/or the MCM4 gene is

30 determined, the prognosis of the cancer is predicted to be poor if:

- the ASPM gene expression is high two weeks after the initiation of therapy; and/or
- the ASPM gene expression is high three months after the initiation of therapy; and/or
- 35 • the MCM4 gene expression is high two weeks after the initiation of therapy; and/or
- the MCM4 gene expression is high three months after the initiation of therapy.

Preferably, when the expression level of the IL6ST gene and/or the NGFRAP1 gene and/or the ASPM gene and/or the MCM4 gene is determined, the prognosis of the cancer is predicted to be poor if:

5

- IL6ST gene expression is low if when measured using a microarray (such as an Affymetrix GeneChip and/or an Illumina Beadarray) it has an approximate Log^2 mean expression of 7.7 or less; and/or
- the NGFRAP1 gene expression is low if when measured using a microarray (such as an Affymetrix GeneChip and/or an Illumina Beadarray) it has an approximate Log^2 mean expression of 8.9 or less; and/or
- the ASPM gene expression is high if when measured using a microarray (such as an Affymetrix GeneChip and/or an Illumina Beadarray) it has an approximate Log^2 mean expression of 5.4 or more; and/or
- the MCM4 gene expression is high if when measured using a microarray (such as an Affymetrix GeneChip and/or an Illumina Beadarray) it has an approximate Log^2 mean expression of 5.6 or more.

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Preferably, in the third aspect of the invention it is determined that the prognosis of the cancer is predicted to be good if:

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- the IL6ST gene expression is high; and
- the NGFRAP1 gene expression is high; and
- the ASPM gene expression is low after the initiation of therapy; and
- the MCM4 gene expression is low after the initiation of therapy.

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Preferably, when the expression level of the ASPM gene and/or the MCM4 gene is determined, the prognosis of the cancer is predicted to be good if:

30

- the ASPM gene expression is low two weeks after the initiation of therapy, and/or the ASPM gene expression is low three months after the initiation of therapy; and
- the MCM4 gene expression is low two weeks after the initiation of therapy, and/or the MCM4 gene expression is low three months after the initiation of therapy.

Preferably, when the expression level of the IL6ST gene and/or the NGFRAP1 gene and/or the ASPM gene and/or the MCM4 gene is determined, the prognosis of the cancer is predicted to be good if:

- 5 • the IL6ST gene expression is high if when measured using a microarray (such as an Affymetrix GeneChip and/or an Illumina Beadarray) it has an approximate Log^2 mean expression of 8.1 or more, and preferably between 8.1 and 8.3, for example a Log^2 mean expression of 8.1 or 8.3; and
- 10 • the NGFRAP1 gene expression is high if when measured using a microarray (such as an Affymetrix GeneChip and/or an Illumina Beadarray) it has an approximate Log^2 mean expression of 9.4 or more; and
- 15 • the ASPM gene expression is low if when measured using a microarray (such as an Affymetrix GeneChip and/or an Illumina Beadarray) it has an approximate Log^2 mean expression of 5.7 or less, and preferably between 5.5 and 5.7, for example a Log^2 mean expression of 5.5 or 5.55 or 5.7; and
- 20 • the MCM4 gene expression is low if when measured using a microarray (such as an Affymetrix GeneChip and/or an Illumina Beadarray) it has an approximate Log^2 mean expression of 6.25 or less, and preferably between 5.7 and 6.25, for example, a Log^2 mean expression of 5.7 or 6.1 or 6.25.

Preferably, in the third aspect of the invention it is determined that the prognosis of the cancer is predicted to be poor if:

- 25 • the IL6ST gene expression is low; and
- the NGFRAP1 gene expression is low; and
- the ASPM gene expression is high after the initiation of therapy; and
- the MCM4 gene expression is high after the initiation of therapy.

Preferably, when the expression level of the ASPM gene and/or the MCM4 gene is determined, the prognosis of the cancer is predicted to be poor if:

- 35 • the ASPM gene expression is high two weeks after the initiation of therapy, and/or the ASPM gene expression is high three months after the initiation of therapy; and
- the MCM4 gene expression is high two weeks after the initiation of therapy, and/or the MCM4 gene expression is high three months after the initiation of therapy.

Preferably, when the expression level of the IL6ST gene and/or the NGFRAP1 gene and/or the ASPM gene and/or the MCM4 gene is determined, the prognosis of the cancer is predicted to be poor if:

- 5 • IL6ST gene expression is low if when measured using a microarray (such as an Affymetrix GeneChip and/or an Illumina Beadarray) it has an approximate Log² mean expression of 7.7 or less; and
- the NGFRAP1 gene expression is low if when measured using a microarray (such as an Affymetrix GeneChip and/or an Illumina Beadarray) it has an approximate
- 10 Log² mean expression of 8.9 or less; and
- the ASPM gene expression is high if when measured using a microarray (such as an Affymetrix GeneChip and/or an Illumina Beadarray) it has an approximate Log² mean expression of 5.4 or more; and
- the MCM4 gene expression is high if when measured using a microarray (such as
- 15 an Affymetrix GeneChip and/or an Illumina Beadarray) it has an approximate Log² mean expression of 5.6 or more.

Preferably, in the third aspect of the invention it is determined that the prognosis of the cancer is predicted to be good if:

- 20 • the IL6ST gene expression is high; and
- the LAX1 gene expression is high; and
- the HPRT1 gene expression is low.

25 Preferably, when the expression level of the IL6ST gene is determined, the prognosis of the cancer is predicted to be good if:

- the IL6ST gene expression is high if when measured using a microarray (such as an Affymetrix GeneChip and/or an Illumina Beadarray) it has an approximate Log²
- 30 mean expression of 8.1 or more, and preferably between 8.1 and 8.3, for example a Log² mean expression of 8.1 or 8.3.

Preferably, in the third aspect of the invention it is determined that the prognosis of the cancer is predicted to be poor if:

- 35 • the IL6ST gene expression is low; and

- the LAX1 gene expression is low; and
- the HPRT1 gene expression is high.

Preferably, when the expression level of the IL6ST gene is determined, the prognosis of
5 the cancer is predicted to be poor if:

- IL6ST gene expression is low if when measured using a microarray (such as an Affymetrix GeneChip and/or an Illumina Beadarray) it has an approximate Log² mean expression of 7.7 or less.

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For the third aspect of the invention, by “high gene expression”, “low gene expression” and “Log² mean expression” we include the definition of those terms as outlined for the first aspect of the invention.

15 Preferably, in the third aspect of the invention there is a further determination for predicting the prognosis of cancer by measuring one or more selected from the group comprising: the expression level of one or more proliferation-associated gene (such as a cyclin); the expression level of one or more mini-chromosome maintenance gene; the expression level of one or more mitotic spindle associated gene; the expression level of one or more
20 glycolysis and oxidative phosphorylation gene; the expression level of one or more immune/inflammatory response gene; the expression level of one or more ECM stromal remodelling gene; tumour volume.

Preferably, in the third aspect of the invention when a second sample is provided after the
25 therapy the further determination occurs at least one day after the initiation of therapy and/or treatment; for example: at least two days, or at least three days, or at least four days, or at least five days, or at least six days, or at least seven days, or at least eight days, or at least nine days, or at least ten days, or at least 11 days, or at least 12 days, or at least 13 days, or at least 14 days, or at least 15 days, or at least 16 days, or at least 17
30 days, or at least 18 days, or at least 19 days, or at least 20 days, or at least 21 days, or at least 22 days, or at least 23 days, or at least 24 days, or at least 25 days, or at least 26 days, or at least 27 days, or at least 28 days, or at least five weeks, or at least six weeks, or at least seven weeks, or at least eight weeks, or at least nine weeks, or at least ten weeks, or at least 11 weeks, or at least three months, or at least four months, or at least
35 five months, or at least six months, or at least seven months, or at least eight months, or at least nine months, or at least ten months, or at least 11 months, or at least 12 months after the initiation of therapy. Most preferably, in the third aspect of the invention when a

second sample is provided after the therapy the further determination occurs 14 days (*i.e.* two weeks) after the initiation of therapy, or three months after the initiation of therapy.

5 In a fourth aspect, the invention provides a method for predicting the progression of cancer in an individual, comprising the steps of:

- A) providing a first sample comprising one or more cancer cell from the individual;
- B) determining in the first sample the expression level of the IL6ST gene; and
- 10 C) predicting the progression of cancer on the basis of the determination in step (B).

By "progression of cancer in an individual", we include the likely physical, cellular and/or molecular development of cancer in the individual, including the progression between
15 stages and grades of the disease, which are discussed above. Thus by "predicting the progression of cancer in an individual" we include the prediction of the likely physical, cellular and/or molecular development of cancer in the individual, including the likelihood of progression between stages and grades of the disease.

20 For the fourth aspect of the invention, by "individual", "cancer", "first sample", "cancer cell", "expression" and "the IL6ST gene" we include the definition of those terms as outlined for the first aspect of the invention.

In one embodiment, step (B) of the fourth aspect of the invention comprises determining
25 in the first sample the expression level of only the IL6ST gene.

Preferably, the fourth aspect of the invention further comprises determining in the first sample the expression level of the NGFRAP1 gene. For the fourth aspect of the invention, by "the NGFRAP1 gene" we include the definition of that term as outlined for the first aspect
30 of the invention.

Preferably, in the fourth aspect of the invention step (B) further comprises determining in the first sample the expression level of the LAX1 gene and/or HPRT1 gene. For the fourth aspect of the invention, by "the LAX1 gene" and "the HPRT1 gene" we include the definition
35 of those terms as outlined for the first aspect of the invention.

Preferably, in the fourth aspect of the invention step (B) further comprises determining in the first sample the expression level the ASPM gene and/or the MCM4 gene and/or the MKI67 gene and/or a gene marker of cellular proliferation. For the fourth aspect of the invention, by "the ASPM gene", "the MCM4 gene", "the MKI67 gene" and "a gene marker of cellular proliferation" we include the definition of those terms as outlined for the first aspect of the invention.

Preferably, step (B) of the fourth aspect of the invention comprises determining the expression level of at least the IL6ST gene in the first sample, for example:

- determining the expression level of the IL6ST gene and the NGFRAP1 gene, or
- determining the expression level of the IL6ST gene and the LAX1 gene, or
- determining the expression level of the IL6ST gene and the HPRT1 gene, or
- determining the expression level of the IL6ST gene and the ASPM gene, or
- determining the expression level of the IL6ST gene and the MCM4 gene, or
- determining the expression level of the IL6ST gene and the MKI67 gene, or
- determining the expression level of the IL6ST gene and a gene marker of cellular proliferation, or
- determining the expression level of the IL6ST gene and the NGFRAP1 gene and the LAX1 gene, or
- determining the expression level of the IL6ST gene and the NGFRAP1 gene and the HPRT1 gene, or
- determining the expression level of the IL6ST gene and the NGFRAP1 gene and the ASPM gene, or
- determining the expression level of the IL6ST gene and the NGFRAP1 gene and the MCM4 gene, or
- determining the expression level of the IL6ST gene and the NGFRAP1 gene and the MKI67 gene, or
- determining the expression level of the IL6ST gene and the NGFRAP1 gene and a gene marker of cellular proliferation, or
- determining the expression level of the IL6ST gene and the LAX1 gene and the HPRT1 gene, or
- determining the expression level of the IL6ST gene and the LAX1 gene and the ASPM gene, or
- determining the expression level of the IL6ST gene and the LAX1 gene and the MCM4 gene, or

- determining the expression level of the IL6ST gene and the LAX1 gene and the MKI67 gene, or
- determining the expression level of the IL6ST gene and the LAX1 gene and a gene marker of cellular proliferation, or
- 5 • determining the expression level of the IL6ST gene and the NGFRAP1 gene and the LAX1 gene and the HPRT1 gene, or
- determining the expression level of the IL6ST gene and the NGFRAP1 gene and the LAX1 gene and the HPRT1 gene and the ASPM gene, or
- 10 • determining the expression level of the IL6ST gene and the NGFRAP1 gene and the LAX1 gene and the HPRT1 gene and the MCM4 gene, or
- determining the expression level of the IL6ST gene and the NGFRAP1 gene and the LAX1 gene and the HPRT1 gene and the MKI67 gene, or
- determining the expression level of the IL6ST gene and the NGFRAP1 gene and the LAX1 gene and the HPRT1 gene and a gene marker of cellular proliferation, or
- 15 • determining the expression level of the IL6ST gene and the NGFRAP1 gene and the LAX1 gene and the HPRT1 gene and the ASPM gene and the MCM4 gene, or
- determining the expression level of the IL6ST gene and the NGFRAP1 gene and the LAX1 gene and the HPRT1 gene and the ASPM gene and the MKI67 gene, or
- determining the expression level of the IL6ST gene and the NGFRAP1 gene and the LAX1 gene and the HPRT1 gene and the ASPM gene and a gene marker of cellular proliferation, or
- 20 • determining the expression level of the IL6ST gene and the NGFRAP1 gene and the LAX1 gene and the HPRT1 gene and the ASPM gene and the MCM4 gene and the MKI67 gene, or
- 25 • determining the expression level of the IL6ST gene and the NGFRAP1 gene and the LAX1 gene and the HPRT1 gene and the ASPM gene and the MCM4 gene and a gene marker of cellular proliferation, or
- determining the expression level of the IL6ST gene and the NGFRAP1 gene and the LAX1 gene and the HPRT1 gene and the ASPM gene and the MCM4 gene and the MKI67 gene and a gene marker of cellular proliferation in the first sample.
- 30

Preferably, step (A) and/or step (B) and/or step (C) of the fourth aspect of the invention is undertaken *in vitro* or *ex vivo*.

35 Preferably, in the fourth aspect of the invention:

step (A) further comprises providing the first sample before a therapy.

For the fourth aspect of the invention, by "therapy" we include the definition of that term as outlined for the first aspect of the invention.

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Preferably, in the fourth aspect of the invention:

step (A) further comprises providing the first sample before a therapy and providing a second sample after the therapy, and

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step (B) further comprises determining in the first sample at least the expression of the IL6ST gene, for example: the expression the IL6ST gene; and the NGFRAP1 gene and/or the LAX1 gene and/or the HPRT1 gene, and determining in the second sample the expression level of the ASPM gene and/or the MCM4 gene and/or the MKI67 gene and/or a gene marker of cellular proliferation.

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Preferably, step (B) of the fourth aspect of the invention comprises determining the expression level of at least the ASPM gene or the MCM4 gene or the MKI67 gene or a gene marker of cellular proliferation in the second sample, for example: determining the expression level of the ASPM gene and the MCM4 gene, or determining the expression level of the ASPM gene and the MKI67 gene, or determining the expression level of the ASPM gene and a gene marker of cellular proliferation, or determining the expression level of the MCM4 gene and the MKI67 gene, or determining the expression level of the MCM4 gene and a gene marker of cellular proliferation, or determining the expression level of the MKI67 gene and a gene marker of cellular proliferation, or determining the expression level of the ASPM gene and the MCM4 gene and the MKI67 gene, or determining the expression level of the ASPM gene and the MCM4 gene and a gene marker of cellular proliferation, or determining the expression level of the MCM4 gene and the MKI67 gene and a gene marker of cellular proliferation, or determining the expression level of the ASPM gene and the MCM4 gene and the MKI67 gene and a gene marker of cellular proliferation in the second sample.

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Preferably, in the fourth aspect of the invention the second sample is provided from the individual at least one day after the initiation of therapy, for example: at least two days, or at least three days, or at least four days, or at least five days, or at least six days, or at least seven days, or at least eight days, or at least nine days, or at least ten days, or at least 11 days, or at least 12 days, or at least 13 days, or at least 14 days, or at least 15 days, or at least 16 days, or at least 17 days, or at least 18 days, or at least 19 days, or at

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least 20 days, or at least 21 days, or at least 22 days, or at least 23 days, or at least 24 days, or at least 25 days, or at least 26 days, or at least 27 days, or at least 28 days, or at least five weeks, or at least six weeks, or at least seven weeks, or at least eight weeks, or at least nine weeks, or at least ten weeks, or at least 11 weeks, or at least three months, or at least four months, or at least five months, or at least six months, or at least seven months, or at least eight months, or at least nine months, or at least ten months, or at least 11 months, or at least 12 months after the initiation of therapy. Most preferably in the fourth aspect of the invention, the second sample is provided from the individual 14 days (*i.e.* two weeks) after the initiation of therapy or three months after the initiation of therapy.

Preferably, in the fourth aspect of the invention the step of predicting the progression of cancer in the individual is one or more selected parameter selected from the group comprising: percentage response of the individual to cancer treatment; overall survival of the individual; disease-specific survival of the individual; progression-free survival of the individual.

Preferably, in step (C) of the fourth aspect of the invention the cancer is predicted to progress, or the cancer is predicted not to progress.

It would be known to those skilled in medicine what is meant by “the cancer is predicted to progress” and “the cancer is predicted not to progress”.

By “the cancer is predicted to progress”, we include that the individual is predicted to exhibit a reduction in the severity of cancer symptoms, and/or the individual is predicted to exhibit a reduction in the number of cancer symptoms, and/or the individual is predicted to exhibit a reduction in tumour size, and/or the individual is predicted to exhibit a reduction in tumour number, and/or the individual is predicted to exhibit a reduction in metastasis, and/or the individual is predicted to exhibit a reduction in cancer affected lymph nodes, and/or the individual is predicted to exhibit a reduction in tumour volume, and/or the individual is predicted to exhibit a reduction in ER IHC Allred score, and/or the individual is predicted to exhibit a reduction in tumour grade, and/or a reduction of one or more proliferation-associated gene, and/or a reduction in one or more gene marker of cellular proliferation, and/or the individual is predicted to exhibit a reduction in cancer progression, and/or the individual is predicted to be classed as being in remission, and/or the individual is predicted to exhibit no cancer symptoms, and/or the individual is predicted to exhibit have no cancer cells.

By “cancer is predicted not to progress”, we include that the individual is predicted to exhibit no reduction in the severity of cancer symptoms, and/or the individual is predicted to exhibit an increase in the severity of cancer symptoms, and/or the individual is predicted to exhibit no reduction in the number of cancer symptoms, and/or the individual is predicted to exhibit an increase in the number of cancer symptoms, and/or the individual is predicted to exhibit no reduction in cancer progression, and/or the individual is predicted to exhibit an increase in cancer progression, and/or the individual is predicted to exhibit no reduction in tumour size, and/or the individual is predicted to exhibit an increase in tumour size, and/or the individual is predicted to exhibit no reduction in tumour number, and/or the individual is predicted to exhibit an increase in tumour number, and/or the individual is predicted to exhibit no reduction in metastasis, and/or the individual is predicted to exhibit an increase in metastasis, and/or the individual is predicted to exhibit no reduction in cancer affected lymph nodes, and/or the individual is predicted to exhibit an increase in cancer affected lymph nodes, and/or the individual is predicted to exhibit no reduction in tumour volume, and/or the individual is predicted to exhibit an increase in tumour volume, and/or the individual is predicted to exhibit no reduction in tumour grade, and/or the individual is predicted to exhibit an increase in tumour grade, and/or the individual is predicted to exhibit no reduction in ER IHC Allred score, and/or the individual is predicted to exhibit an increase in ER IHC Allred score.

Preferably, in the fourth aspect of the invention when the expression level of the IL6ST gene and/or the NGFRAP1 gene and/or the ASPM gene and/or the MCM4 gene and/or the LAX1 gene and/or the HPRT1 gene is determined, it is determined that the cancer is predicted not to progress if:

- the IL6ST gene expression is high; and/or
- the NGFRAP1 gene expression is high; and/or
- the LAX1 gene expression is high; and/or
- the HPRT1 gene expression is low; and/or
- the ASPM gene expression is low after the initiation of therapy; and/or
- the MCM4 gene expression is low after the initiation of therapy.

Preferably, when the expression level of the ASPM gene and/or the MCM4 gene is determined, the cancer is predicted not to progress if:

- the ASPM gene expression is low two weeks after the initiation of therapy; and/or

- the ASPM gene expression is low three months after the initiation of therapy; and/or
- the MCM4 gene expression is low two weeks after the initiation of therapy; and/or
- the MCM4 gene expression is low three months after the initiation of therapy.

5 Preferably, when the expression level of the IL6ST gene and/or the NGFRAP1 gene and/or the ASPM gene and/or the MCM4 gene is determined, the cancer is predicted not to progress if:

- 10 • the IL6ST gene expression is high if when measured using a microarray (such as an Affymetrix GeneChip and/or an Illumina Beadarray) it has an approximate Log^2 mean expression of 8.1 or more, and preferably between 8.1 and 8.3, for example a Log^2 mean expression of 8.1 or 8.3; and/or
- 15 • the NGFRAP1 gene expression is high if when measured using a microarray (such as an Affymetrix GeneChip and/or an Illumina Beadarray) it has an approximate Log^2 mean expression of 9.4 or more; and/or
- the ASPM gene expression is low if when measured using a microarray (such as an Affymetrix GeneChip and/or an Illumina Beadarray) it has an approximate Log^2 mean expression of 5.7 or less, and preferably between 5.5 and 5.7, for example a Log^2 mean expression of 5.5 or 5.55 or 5.7; and/or
- 20 • the MCM4 gene expression is low if when measured using a microarray (such as an Affymetrix GeneChip and/or an Illumina Beadarray) it has an approximate Log^2 mean expression of 6.25 or less, and preferably between 5.7 and 6.25, for example, a Log^2 mean expression of 5.7 or 6.1 or 6.25.

25 Preferably, in the fourth aspect of the invention when the expression level of the IL6ST gene and/or the NGFRAP1 gene and/or the ASPM gene and/or the MCM4 gene and/or the LAX1 gene and/or the HPRT1 gene is determined, it is determined that the cancer is predicted to progress if:

- 30 • the IL6ST gene expression is low; and/or
- the NGFRAP1 gene expression is low; and/or
- the LAX1 gene expression is low; and/or
- the HPRT1 gene expression is high; and/or
- the ASPM gene expression is high after the initiation of therapy; and/or
- 35 • the MCM4 gene expression is high after the initiation of therapy.

Preferably, when the expression level of the ASPM gene and/or the MCM4 gene is determined, the cancer is predicted to progress if:

- the ASPM gene expression is high two weeks after the initiation of therapy; and/or
- 5 • the ASPM gene expression is high three months after the initiation of therapy; and/or
- the MCM4 gene expression is high two weeks after the initiation of therapy; and/or
- the MCM4 gene expression is high three months after the initiation of therapy.

10 Preferably, when the expression level of the IL6ST gene and/or the NGFRAP1 gene and/or the ASPM gene and/or the MCM4 gene is determined, the cancer is predicted to progress if:

- IL6ST gene expression is low if when measured using a microarray (such as an
- 15 Affymetrix GeneChip and/or an Illumina Beadarray) it has an approximate Log^2 mean expression of 7.7 or less; and/or
- the NGFRAP1 gene expression is low if when measured using a microarray (such as an Affymetrix GeneChip and/or an Illumina Beadarray) it has an approximate Log^2 mean expression of 8.9 or less; and/or
- 20 • the ASPM gene expression is high if when measured using a microarray (such as an Affymetrix GeneChip and/or an Illumina Beadarray) it has an approximate Log^2 mean expression of 5.4 or more; and/or
- the MCM4 gene expression is high if when measured using a microarray (such as an Affymetrix GeneChip and/or an Illumina Beadarray) it has an approximate Log^2
- 25 mean expression of 5.6 or more.

Preferably, in the fourth aspect of the invention it is determined that the cancer is predicted not to progress if:

- 30 • the IL6ST gene expression is high; and
- the NGFRAP1 gene expression is high; and
- the ASPM gene expression is low after the initiation of therapy; and
- the MCM4 gene expression is low after the initiation of therapy.

35 Preferably, when the expression level of the ASPM gene and/or the MCM4 gene is determined, the cancer is predicted not to progress if:

- the ASPM gene expression is low two weeks after the initiation of therapy, and/or the ASPM gene expression is low three months after the initiation of therapy; and
- the MCM4 gene expression is low two weeks after the initiation of therapy, and/or the MCM4 gene expression is low three months after the initiation of therapy.

Preferably, when the expression level of the IL6ST gene and/or the NGFRAP1 gene and/or the ASPM gene and/or the MCM4 gene is determined, the cancer is predicted not to progress if:

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- the IL6ST gene expression is high if when measured using a microarray (such as an Affymetrix GeneChip and/or an Illumina Beadarray) it has an approximate Log^2 mean expression of 8.1 or more, and preferably between 8.1 and 8.3, for example a Log^2 mean expression of 8.1 or 8.3; and
- the NGFRAP1 gene expression is high if when measured using a microarray (such as an Affymetrix GeneChip and/or an Illumina Beadarray) it has an approximate Log^2 mean expression of 9.4 or more; and
- the ASPM gene expression is low if when measured using a microarray (such as an Affymetrix GeneChip and/or an Illumina Beadarray) it has an approximate Log^2 mean expression of 5.7 or less, and preferably between 5.5 and 5.7, for example a Log^2 mean expression of 5.5 or 5.55 or 5.7; and
- the MCM4 gene expression is low if when measured using a microarray (such as an Affymetrix GeneChip and/or an Illumina Beadarray) it has an approximate Log^2 mean expression of 6.25 or less, and preferably between 5.7 and 6.25, for example, a Log^2 mean expression of 5.7 or 6.1 or 6.25.

Preferably, in the fourth aspect of the invention it is determined that the cancer is predicted to progress if:

- the IL6ST gene expression is low; and
- the NGFRAP1 gene expression is low; and
- the ASPM gene expression is high after the initiation of therapy; and
- the MCM4 gene expression is high after the initiation of therapy.

Preferably, when the expression level of the ASPM gene and/or the MCM4 gene is determined, the cancer is predicted to progress if:

- the ASPM gene expression is high two weeks after the initiation of therapy, and/or the ASPM gene expression is high three months after the initiation of therapy; and
- the MCM4 gene expression is high two weeks after the initiation of therapy, and/or the MCM4 gene expression is high three months after the initiation of therapy.

Preferably, when the expression level of the IL6ST gene and/or the NGFRAP1 gene and/or the ASPM gene and/or the MCM4 gene is determined, the cancer is predicted to progress if:

- IL6ST gene expression is low if when measured using a microarray (such as an Affymetrix GeneChip and/or an Illumina Beadarray) it has an approximate Log^2 mean expression of 7.7 or less; and
- the NGFRAP1 gene expression is low if when measured using a microarray (such as an Affymetrix GeneChip and/or an Illumina Beadarray) it has an approximate Log^2 mean expression of 8.9 or less; and
- the ASPM gene expression is high if when measured using a microarray (such as an Affymetrix GeneChip and/or an Illumina Beadarray) it has an approximate Log^2 mean expression of 5.4 or more; and
- the MCM4 gene expression is high if when measured using a microarray (such as an Affymetrix GeneChip and/or an Illumina Beadarray) it has an approximate Log^2 mean expression of 5.6 or more.

Preferably, in the fourth aspect of the invention it is determined that the cancer is predicted not to progress if:

- the IL6ST gene expression is high; and
- the LAX1 gene expression is high; and
- the HPRT1 gene expression is low.

Preferably, when the expression level of the IL6ST gene is determined the cancer is predicted not to progress if:

- the IL6ST gene expression is high if when measured using a microarray (such as an Affymetrix GeneChip and/or an Illumina Beadarray) it has an approximate Log^2

mean expression of 8.1 or more, and preferably between 8.1 and 8.3, for example a Log^2 mean expression of 8.1 or 8.3.

5 Preferably, in the fourth aspect of the invention it is determined that the cancer is predicted to progress if:

- the IL6ST gene expression is low; and
- the LAX1 gene expression is low; and
- the HPRT1 gene expression is high.

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Preferably, when the expression level of the IL6ST gene is determined, the cancer is predicted to progress if:

- IL6ST gene expression is low if when measured using a microarray (such as an Affymetrix GeneChip and/or an Illumina Beadarray) it has an approximate Log^2 mean expression of 7.7 or less.

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For the fourth aspect of the invention, by "high gene expression", "low gene expression" and " Log^2 mean expression" we include the definition of those terms as outlined for the first aspect of the invention.

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Preferably, in the fourth aspect of the invention there is a further determination for predicting the progression of cancer by measuring one or more parameter selected from the group comprising: the expression level of one or more proliferation-associated gene (such as a cyclin); the expression level of one or more mini-chromosome maintenance gene; the expression level of one or more mitotic spindle associated gene; the expression level of one or more glycolysis and oxidative phosphorylation gene; the expression level of one or more immune/inflammatory response gene; the expression level of one or more ECM stromal remodelling gene; tumour volume.

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Preferably, in the fourth aspect of the invention when a second sample is provided after the therapy the further determination occurs at least one day after the initiation of therapy and/or treatment; for example: at least two days, or at least three days, or at least four days, or at least five days, or at least six days, or at least seven days, or at least eight days, or at least nine days, or at least ten days, or at least 11 days, or at least 12 days, or at least 13 days, or at least 14 days, or at least 15 days, or at least 16 days, or at least 17 days, or at least 18 days, or at least 19 days, or at least 20 days, or at least 21 days, or at

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least 22 days, or at least 23 days, or at least 24 days, or at least 25 days, or at least 26 days, or at least 27 days, or at least 28 days, or at least five weeks, or at least six weeks, or at least seven weeks, or at least eight weeks, or at least nine weeks, or at least ten weeks, or at least 11 weeks, or at least three months, or at least four months, or at least five months, or at least six months, or at least seven months, or at least eight months, or at least nine months, or at least ten months, or at least 11 months, or at least 12 months after the initiation of therapy. Most preferably, in the fourth aspect of the invention when a second sample is provided after the therapy the further determination occurs 14 days (*i.e.* two weeks) after the initiation of therapy, or three months after the initiation of therapy.

In a fifth aspect, the invention provides a kit for performing a method as defined in the first aspect of the invention and/or the second aspect of the invention and/or the third aspect of the invention and/or the fourth aspect of the invention.

Preferably, the kit of the present invention comprises a reagent for detecting and/or measuring and/or quantifying the IL6ST gene.

Most preferably, the kit of the present invention comprises one or more reagent for detecting and/or measuring and/or quantifying the IL6ST gene selected from the group comprising: an IL6ST polynucleotide sequence; an anti-IL6ST antibody.

By "IL6ST polynucleotide sequence", we include one or more polynucleotide selected from the group comprising: a polynucleotide that can completely or partially anneal to the IL6ST gene or mRNA sequence; a polynucleotide that can be used to amplify the IL6ST gene or mRNA sequence (*e.g.* by PCR or RT-PCR); a polynucleotide comprising SEQ ID NO: 1; a polynucleotide comprising TTGCTTCTTCACTCCAGTCACT (SEQ ID NO: 5).

By "anti-IL6ST antibody", we include one or more antibody selected from the group comprising: a monoclonal anti-IL6ST antibody; a polyclonal anti-IL6ST antibody; the monoclonal antibody AM31176AF-N (available from Acris).

Preferably, the kit of the present invention further comprises a reagent for detecting and/or measuring and/or quantifying the NGFRAP1 gene.

Most preferably, the kit of the present invention comprises one or more reagent for detecting and/or measuring and/or quantifying the NGFRAP1 gene selected from the group comprising: an NGFRAP1 polynucleotide sequence; an anti-BEX3 antibody.

By "NGFRAP1 polynucleotide sequence", we include one or more polynucleotide selected from the group comprising: a polynucleotide that can completely or partially anneal to the NGFRAP1 gene or mRNA sequence; a polynucleotide that can be used to amplify the
5 NGFRAP1 gene or mRNA sequence (e.g. by PCR or RT-PCR); a polynucleotide comprising SEQ ID NO: 4; a polynucleotide comprising TGATGGTCATGGTGATTAGAGAG (SEQ ID NO: 6).

By "anti-BEX3 antibody", we include one or more antibody selected from the group
10 comprising: a monoclonal anti-BEX3 antibody; a polyclonal anti-BEX3 antibody.

Preferably, the kit of the present invention further comprises a reagent for detecting and/or measuring and/or quantifying the LAX1 gene.

15 Most preferably, the kit of the present invention comprises one or more reagent for detecting and/or measuring and/or quantifying the LAX1 gene selected from the group comprising: an LAX1 polynucleotide sequence; an anti-LAX1 antibody.

By "LAX1 polynucleotide sequence", we include one or more polynucleotide selected from
20 the group comprising: a polynucleotide that can completely or partially anneal to the LAX1 gene or mRNA sequence; a polynucleotide that can be used to amplify the LAX1 gene or mRNA sequence (e.g. by PCR or RT-PCR).

By "anti-LAX1 antibody", we include one or more antibody selected from the group
25 comprising: a monoclonal anti-LAX1 antibody; a polyclonal anti-LAX1 antibody; the monoclonal antibody EP8753 (available from ABCAM).

Preferably, the kit of the present invention further comprises a reagent for detecting and/or measuring and/or quantifying the HPRT1 gene.

30 Most preferably, the kit of the present invention comprises one or more reagent for detecting and/or measuring and/or quantifying the HPRT1 gene selected from the group comprising: a HPRT1 polynucleotide sequence; an anti-HGPRT antibody; the antibody ABIN453114 (available from Amersham - Ceballos-Picot et al. (2009) Hum Mol Genet
35 18(13):2317-27).

By "HPRT1 polynucleotide sequence", we include one or more polynucleotide selected from the group comprising: a polynucleotide that can completely or partially anneal to the HPRT1 gene or mRNA sequence; a polynucleotide that can be used to amplify the HPRT1 gene or mRNA sequence (*e.g.* by PCR or RT-PCR).

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By "anti-HGPRT antibody", we include one or more antibody selected from the group comprising: a monoclonal anti-HGPRT antibody; a polyclonal anti-HGPRT antibody.

Preferably, the kit of the present invention further comprises a reagent for detecting and/or measuring and/or quantifying the ASPM gene.

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Most preferably, the kit of the present invention comprises one or more reagent for detecting and/or measuring and/or quantifying the ASPM gene selected from the group comprising: an ASPM polynucleotide sequence; an anti-abnormal spindle-like microcephaly-associated protein antibody.

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By "ASPM polynucleotide sequence", we include one or more polynucleotide selected from the group comprising: a polynucleotide that can completely or partially anneal to the ASPM gene or mRNA sequence; a polynucleotide that can be used to amplify the ASPM gene or mRNA sequence (*e.g.* by PCR or RT-PCR); a polynucleotide comprising SEQ ID NO: 3; a polynucleotide comprising AGAAACAGACCAACAACAATAACTT (SEQ ID NO: 7).

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By "anti-abnormal spindle-like microcephaly-associated protein antibody", we include one or more antibody selected from the group comprising: a monoclonal anti-abnormal spindle-like microcephaly-associated protein antibody; a polyclonal anti-abnormal spindle-like microcephaly-associated protein antibody; the polyclonal antibody 09-066 (available from Millipore).

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Preferably, the kit of the present invention further comprises a reagent for detecting and/or measuring and/or quantifying the MCM4 gene.

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Most preferably, the kit of the present invention comprises one or more reagent for detecting and/or measuring and/or quantifying the MCM4 gene selected from the group comprising: an MCM4 polynucleotide sequence; an anti-MCM4 antibody.

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By "MCM4 polynucleotide sequence", we include one or more polynucleotide selected from the group comprising: a polynucleotide that can completely or partially anneal to the

MCM4 gene or mRNA sequence; a polynucleotide that can be used to amplify the MCM4 gene or mRNA sequence (e.g. by PCR or RT-PCR); a polynucleotide comprising SEQ ID NO: 2; a polynucleotide comprising CATGCCGCTGATGGTGATG (SEQ ID NO: 8).

- 5 By "anti-MCM4 antibody", we include one or more antibody selected from the group comprising: a monoclonal anti-MCM4 antibody; a polyclonal anti-MCM4 antibody; the monoclonal antibody sc-28317 (available from Santa Cruz); the monoclonal antibody D3H6N (available from Cell Signalling).
- 10 Preferably, the kit of the present invention further comprises a reagent for detecting and/or measuring and/or quantifying the MKI67 gene.

Most preferably, the kit of the present invention comprises one or more reagent for detecting and/or measuring and/or quantifying the MKI67 gene selected from the group
15 comprising: an MKI67 polynucleotide sequence; an anti-Ki67 antibody.

By "MKI67 polynucleotide sequence", we include one or more polynucleotide selected from the group comprising: a polynucleotide that can completely or partially anneal to the MKI67 gene or mRNA sequence; a polynucleotide that can be used to amplify the MKI67 gene or
20 mRNA sequence (e.g. by PCR or RT-PCR).

By "anti-Ki67 antibody", we include one or more antibody selected from the group comprising: a monoclonal anti-Ki67 antibody; a polyclonal anti-Ki67 antibody; the antibody FLEX Anti-Ki-67 Antigen (available from www.dako.com - Seshadri et al. Int J Cancer
25 1996;69:135-41).

Preferably, the kit of the present invention further comprises a reagent for detecting and/or measuring and/or quantifying a gene marker of cellular proliferation.

30 Most preferably, the kit of the present invention comprises one or more reagent for detecting and/or measuring and/or quantifying a gene marker of cellular proliferation selected from the group comprising: a gene marker of cellular proliferation polynucleotide sequence; an antibody to the protein product of a gene marker of cellular proliferation.

35 By "a gene marker of cellular proliferation polynucleotide sequence", we include one or more polynucleotide selected from the group comprising: a polynucleotide that can completely or partially anneal to a gene marker of cellular proliferation gene or mRNA

sequence; a polynucleotide that can be used to amplify the gene marker of cellular proliferation gene or mRNA sequence (e.g. by PCR or RT-PCR).

By "antibody to the protein product of a gene marker of cellular proliferation", we include
5 one or more antibody selected from the group comprising: a monoclonal antibody to the protein product of a gene marker of cellular proliferation; a polyclonal antibody to the protein product of a gene marker of cellular proliferation.

Preferably, the kit of the present invention comprises one or more reagent for detecting
10 and/or measuring and/or quantifying a housekeeping gene selected from the group comprising: a housekeeping gene polynucleotide sequence; an antibody to the protein product of a housekeeping gene.

The term housekeeping gene is well known in the art. A housekeeping gene is usually
15 constitutively expressed in all cells of an organism, and one or more housekeeping genes are often used as controls in experiments in which the relative level of a gene is calculated.

By "housekeeping gene", we include one or more gene selected from group comprising:
TBP (Kao et al. (1990) Science 29;248(4963):1646-50 PMID: 2194289); FKBP15
20 (Strausberg et al (2002) Proc Natl Acad Sci U S A. 99(26):16899-903 PMID: 12477932); PUM1 (Nagase et al. (1995) DNA Res. 1995;2(1):37-43 PMID: 7788527).

By "housekeeping gene polynucleotide sequence", we include one or more polynucleotide
selected from the group comprising: a polynucleotide comprising
25 GTTTGCCAAGGAAGAAAGTGAAC (SEQ ID NO: 9); a polynucleotide comprising GGGTCAGTCCAGTGCCAT (SEQ ID NO: 10); a polynucleotide comprising AGAGTAACCTGATGATGGAGAAGA (SEQ ID NO: 11); a polynucleotide comprising CTGCTAACTCCTCTGTCACCTT (SEQ ID NO: 12); a polynucleotide comprising GGACCATTTCGCCTTTAGGA (SEQ ID NO: 13); a polynucleotide comprising
30 TCAGAGAGTTGTTGCCGTAGAA (SEQ ID NO: 14).

Preferably, the kit of the fifth aspect of the invention is used *in vitro* or *ex vivo*.

Preferably, the kit of the fifth aspect of the invention comprises:

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- one or more reagent for detecting and/or measuring and/or quantifying the IL6ST gene and one or more reagent for detecting and/or measuring and/or quantifying the NGFRAP1 gene, or
- 5 • one or more reagent for detecting and/or measuring and/or quantifying the IL6ST gene and one or more reagent for detecting and/or measuring and/or quantifying the LAX1 gene, or
- one or more reagent for detecting and/or measuring and/or quantifying the IL6ST gene and one or more reagent for detecting and/or measuring and/or quantifying the HPRT1 gene, or
- 10 • one or more reagent for detecting and/or measuring and/or quantifying the IL6ST gene and one or more reagent for detecting and/or measuring and/or quantifying the ASPM gene, or
- one or more reagent for detecting and/or measuring and/or quantifying the IL6ST gene and one or more reagent for detecting and/or measuring and/or quantifying the MCM4 gene, or
- 15 • one or more reagent for detecting and/or measuring and/or quantifying the IL6ST gene and one or more reagent for detecting and/or measuring and/or quantifying the MKI67 gene, or
- one or more reagent for detecting and/or measuring and/or quantifying the IL6ST gene and one or more reagent for detecting and/or measuring and/or quantifying a gene marker of cellular proliferation, or
- 20 • one or more reagent for detecting and/or measuring and/or quantifying the IL6ST gene and one or more reagent for detecting and/or measuring and/or quantifying the NGFRAP1 gene and one or more reagent for detecting and/or measuring and/or quantifying the LAX1 gene, or
- 25 • one or more reagent for detecting and/or measuring and/or quantifying the IL6ST gene and one or more reagent for detecting and/or measuring and/or quantifying the NGFRAP1 gene and one or more reagent for detecting and/or measuring and/or quantifying the HPRT1 gene, or
- 30 • one or more reagent for detecting and/or measuring and/or quantifying the IL6ST gene and one or more reagent for detecting and/or measuring and/or quantifying the NGFRAP1 gene and one or more reagent for detecting and/or measuring and/or quantifying the ASPM gene, or
- 35 • one or more reagent for detecting and/or measuring and/or quantifying the IL6ST gene and one or more reagent for detecting and/or measuring and/or quantifying

the NGFRAP1 gene and one or more reagent for detecting and/or measuring and/or quantifying the MCM4 gene, or

- one or more reagent for detecting and/or measuring and/or quantifying the IL6ST gene and one or more reagent for detecting and/or measuring and/or quantifying the NGFRAP1 gene and one or more reagent for detecting and/or measuring and/or quantifying the MKI67 gene, or
- one or more reagent for detecting and/or measuring and/or quantifying the IL6ST gene and one or more reagent for detecting and/or measuring and/or quantifying the NGFRAP1 gene and one or more reagent for detecting and/or measuring and/or quantifying a gene marker of cellular proliferation, or
- one or more reagent for detecting and/or measuring and/or quantifying the IL6ST gene and one or more reagent for detecting and/or measuring and/or quantifying the LAX1 gene and one or more reagent for detecting and/or measuring and/or quantifying the HPRT1 gene, or
- one or more reagent for detecting and/or measuring and/or quantifying the IL6ST gene and one or more reagent for detecting and/or measuring and/or quantifying the LAX1 gene and one or more reagent for detecting and/or measuring and/or quantifying the ASPM gene, or
- one or more reagent for detecting and/or measuring and/or quantifying the IL6ST gene and one or more reagent for detecting and/or measuring and/or quantifying the LAX1 gene and one or more reagent for detecting and/or measuring and/or quantifying the MCM4 gene, or
- one or more reagent for detecting and/or measuring and/or quantifying the IL6ST gene and one or more reagent for detecting and/or measuring and/or quantifying the LAX1 gene and one or more reagent for detecting and/or measuring and/or quantifying the MKI67 gene, or
- one or more reagent for detecting and/or measuring and/or quantifying the IL6ST gene and one or more reagent for detecting and/or measuring and/or quantifying the LAX1 gene and one or more reagent for detecting and/or measuring and/or quantifying a gene marker of cellular proliferation, or
- determining the expression level of one or more reagent for detecting and/or measuring and/or quantifying the IL6ST gene and one or more reagent for detecting and/or measuring and/or quantifying the NGFRAP1 gene and one or more reagent for detecting and/or measuring and/or quantifying the LAX1 gene and one or more reagent for detecting and/or measuring and/or quantifying the HPRT1 gene, or

- [illegible]

detecting and/or measuring and/or quantifying the ASPM gene and one or more reagent for detecting and/or measuring and/or quantifying the MKI67 gene, or

- one or more reagent for detecting and/or measuring and/or quantifying the IL6ST gene and one or more reagent for detecting and/or measuring and/or quantifying the NGFRAP1 gene and one or more reagent for detecting and/or measuring and/or quantifying the LAX1 gene and one or more reagent for detecting and/or measuring and/or quantifying the HPRT1 gene and one or more reagent for detecting and/or measuring and/or quantifying the ASPM gene and one or more reagent for detecting and/or measuring and/or quantifying a gene marker of cellular proliferation, or
- one or more reagent for detecting and/or measuring and/or quantifying the IL6ST gene and one or more reagent for detecting and/or measuring and/or quantifying the NGFRAP1 gene and one or more reagent for detecting and/or measuring and/or quantifying the LAX1 gene and one or more reagent for detecting and/or measuring and/or quantifying the HPRT1 gene and one or more reagent for detecting and/or measuring and/or quantifying the ASPM gene and one or more reagent for detecting and/or measuring and/or quantifying the MCM4 gene and one or more reagent for detecting and/or measuring and/or quantifying the MKI67 gene, or
- one or more reagent for detecting and/or measuring and/or quantifying the IL6ST gene and one or more reagent for detecting and/or measuring and/or quantifying the NGFRAP1 gene and one or more reagent for detecting and/or measuring and/or quantifying the LAX1 gene and one or more reagent for detecting and/or measuring and/or quantifying the HPRT1 gene and one or more reagent for detecting and/or measuring and/or quantifying the ASPM gene and one or more reagent for detecting and/or measuring and/or quantifying the MCM4 gene and one or more reagent for detecting and/or measuring and/or quantifying a gene marker of cellular proliferation, or
- one or more reagent for detecting and/or measuring and/or quantifying the IL6ST gene and one or more reagent for detecting and/or measuring and/or quantifying the NGFRAP1 gene and one or more reagent for detecting and/or measuring and/or quantifying the LAX1 gene and one or more reagent for detecting and/or measuring and/or quantifying the HPRT1 gene and one or more reagent for detecting and/or measuring and/or quantifying the ASPM gene and one or more reagent for detecting and/or measuring and/or quantifying the MCM4 gene and one or more reagent for detecting and/or measuring and/or quantifying the MKI67 gene

and one or more reagent for detecting and/or measuring and/or quantifying a gene marker of cellular proliferation.

Preferably, the kit of the fifth aspect of the invention comprises one or more reagent
5 selected from the group comprising: one or more reagent for detecting and/or measuring
and/or quantifying the IL6ST gene; one or more reagent for detecting and/or measuring
and/or quantifying the NGFRAP1 gene; one or more reagent for detecting and/or
measuring and/or quantifying the LAX1 gene; one or more reagent for detecting and/or
measuring and/or quantifying the HPRT1 gene; one or more reagent for detecting and/or
10 measuring and/or quantifying the ASPM gene; one or more reagent for detecting and/or
measuring and/or quantifying the MCM4 gene; one or more reagent for detecting and/or
measuring and/or quantifying the MKI67 gene; one or more reagent for detecting and/or
measuring and/or quantifying a gene marker of cellular proliferation.

15 Preferably, the kit of the fifth aspect of the invention further comprises a set of instructions
for performing a method as defined in the first aspect of the invention and/or the second
aspect of the invention and/or the third aspect of the invention and/or the fourth aspect of
the invention.

20 In a sixth aspect, the invention provides the use of the IL6ST gene and/or the NGFRAP1
gene and/or the LAX1 gene and/or the HPRT1 gene and/or the ASPM gene and/or MCM4
gene and/or the MKI67 gene and/or a gene marker of cellular proliferation for predicting
the response to therapy of cancer in an individual; for example, as defined by the methods
of the invention.

25 For the sixth aspect of the invention, by "individual", "cancer", "the IL6ST gene", the
NGFRAP1 gene", "the LAX1 gene", "the ASPM gene", "the MCM4 gene", "the MKI67
gene", "a gene marker of cellular proliferation", "therapy", and "predicting the response to
therapy of cancer" we include the definition of those terms as outlined herein.

30 Preferably, the use of the sixth aspect of the invention is performed *in vitro* or *ex vivo*.

In a seventh aspect, the invention provides the use of the IL6ST gene and/or the
NGFRAP1 gene and/or the LAX1 gene and/or the HPRT1 gene and/or the ASPM gene
35 and/or MCM4 gene and/or the MKI67 gene and/or a gene marker of cellular proliferation
for selecting a treatment for an individual with cancer; for example, as defined by the
methods of the invention.

For the seventh aspect of the invention, by "individual", "cancer", "the IL6ST gene", the NGFRAP1 gene", "the LAX1 gene", "the ASPM gene", "the MCM4 gene", "the MKI67 gene", "selecting a treatment for an individual" and "selected treatment" we include the
5 definition of those terms as outlined herein.

Preferably, the use of the seventh aspect of the invention is performed *in vitro* or *ex vivo*.

In an eighth aspect, the invention provides the use of the IL6ST gene and/or the NGFRAP1
10 gene and/or the LAX1 gene and/or the HPRT1 gene and/or the ASPM gene and/or MCM4 gene and/or the MKI67 gene and/or a gene marker of cellular proliferation for predicting the prognosis of cancer in an individual; for example, as defined by the methods of the invention.

15 For the eighth aspect of the invention, by "individual", "cancer", "the IL6ST gene", the NGFRAP1 gene", "the LAX1 gene", "the ASPM gene", "the MCM4 gene", "the MKI67 gene", and "predicting the prognosis of cancer in an individual" we include the definition of those terms as outlined herein.

20 Preferably, the use of the eighth aspect of the invention is performed *in vitro* or *ex vivo*.

In a ninth aspect, the invention provides the use of the IL6ST gene and/or the NGFRAP1 gene and/or the LAX1 gene and/or the HPRT1 gene and/or the ASPM gene and/or MCM4 gene and/or the MKI67 gene and/or a gene marker of cellular proliferation for predicting
25 the progression of cancer in an individual; for example, as defined by the methods of the invention.

For the ninth aspect of the invention, by "individual", "cancer", "the IL6ST gene", the NGFRAP1 gene", "the LAX1 gene", "the ASPM gene", "the MCM4 gene", "the MKI67
30 gene", and "predicting the progression of cancer in an individual" we include the definition of those terms as outlined herein.

Preferably, the use of the ninth aspect of the invention is performed *in vitro* or *ex vivo*.

35 In a tenth aspect, the invention provides a method or a kit or a use substantially as described and/or claimed herein, with reference to the accompanying description and/or examples and/or drawings.

The listing or discussion in this specification of an apparently prior-published document should not necessarily be taken as an acknowledgement that the document is part of the state of the art or is common general knowledge.

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Preferred, non-limiting examples which embody certain aspects of the invention will now be described, with reference to the following figures:

Figure 1. Clinical and molecular response to letrozole in breast tumours. A, Relative
 10 changes in breast tumour size measured by 3D ultrasound in the Edinburgh training cohort. B, Multidimensional scaling plot with third dimension as time based upon the 500 most differentially expressed genes in quick stable responding tumours. Spheres are coloured based on clinical response pattern: 'quick stable response' (grey), 'slow response' (light grey) and 'non-response' (dark grey). C, Overlapping gene lists between
 15 the 73 matched pre- and post-letrazole treated samples among the quick stable responder (grey) and slow responder (light grey) groups of tumours using Rank Products analysis (Percentage False Present<0.01). D, Heatmaps summarising changes in gene expression over time in major functional groups in the three response groups. Samples are ordered left-right by patient ID for each time point. Dark grey and grey colours represent relative
 20 high and low log² gene expression values, respectively.

Figure 2. Breast cancer subtypes and proliferation genes do not accurately predict response prior to treatment, but lymphocytic infiltration pre-treatment and proliferation at 2 weeks is associated with clinical response. A, Proportions of
 25 tumours classed as luminal A (dark grey) and luminal B (light grey) molecular subtypes within the clinical response groups. B, Ordered proliferation gene set from low to high expression (left-right) in pre-treatment samples demonstrates no association with clinical response. However, after 2 weeks proliferation (sum of mean-centered expression values) is significantly higher in the non-response group (Wilcoxon test). Dark grey and grey
 30 colours in the heatmaps represent relative high and low log₂ gene expression values, respectively. QSR=Quick, stable responders, NR=non-responders. C, Lymphocytic infiltration as assessed by IHC analysis of CD45 is significantly higher (Chi-squared test) in the non-responding samples prior to treatment.

Figure 3. Generation and validation of a four gene signature to predict clinical response to letrozole. A, Decision tree diagram showing the hierarchical structure of the
 35 4 model and relationship between the constituent components. For each node, the right

and left branches indicate expression levels above and below the model defined splitter values, respectively. B, Pre-treatment 3 gene decision tree model. For each node, the right and left branches indicate expression levels above and below the model defined splitter values, respectively. C, Immunohistochemistry scores are significantly consistent with microarray gene expression measurements (Wilcoxon tests). D, and E, Kaplan Meier plots show significant association with recurrence-free survival (RFS) and breast cancer specific survival (BCS) for the 4 gene signature in the Edinburgh dataset.

Figure 4. Consort diagram showing the numbers of patients in the study.

Figure 5: Gene expression of IL6ST is associated with poor prognosis in 669 primary tumours from tamoxifen-treated patients. A, Ranked IL6ST expression is shown alongside some known markers of tamoxifen resistance. Heatmap colours are $\log^2$ mean-centered values, Light grey=high, grey=low. All possible cut-points for Kaplan Meier analysis are shown as a colour bar where white, p-val=1.0, black, p-val=0.002 (233 cut-points p<0.05). B, low (light grey) IL6ST is clearly associated with worse outcomes compared to high IL6ST (grey) using the optimum cut-point in a Kaplan Meier plot. C, Correlation with ER and proliferation marker AURKA. D, High grade tumours have lower expression levels of IL6ST.

Figure 6: A four gene signature predicts clinical response. Expression of the 4 genes in responders and non-responders over time. Low pre-treatment expression of the immune related gene IL6ST (GP130) and the apoptosis induction related gene NGFRAP1 (BEX3); along with high expression at 2 weeks treatment of two proliferation associated genes, ASPM and MCM4.

Figure 7. Measurement of Ki67 alone at 2 weeks is not significantly associated with response. Combining Ki67 at 2 weeks with IL6ST pre-treatment is more accurate, but less than the 4 gene signature. A, Immunochemistry assessment of Ki67 in 33 patients (26 responders and 7 non-responders), Wilcoxon test; B, comparison of prediction using Ki67 alone in combination with pre-treatment IL6ST or the 4 gene signature. Indications and assignment of poor response (low IL6ST and high Ki67) are highlighted in **bold** in the table and *italics* indicate wrongly predicted patients with each method; C, comparison of the log2 Hazard Ratios for two week Ki67 alone, pre-treatment IL6ST plus two week Ki67 combined and the 4 gene signature for recurrence-free and breast cancer specific survival.

EXAMPLE***Example 1: Experimental data*****5 Summary**

Purpose: Aromatase inhibitors (AIs) have an established role in breast cancer treatment. Response rates are only 50-70% in the neoadjuvant setting and lower in advanced disease. Accurate biomarkers are urgently needed to predict response in these settings and to determine which individuals will benefit from adjuvant AI therapy.

Participants and Methods: Pre- and on-treatment (after 2 weeks and 3 months) biopsies were obtained from 89 post-menopausal women with ER+ breast cancer receiving neoadjuvant letrozole for transcript profiling. Dynamic clinical response was assessed by three-dimensional ultrasound measurements.

Results: The molecular response to letrozole was characterised and a four gene classifier of clinical response was established (accuracy of 96%) based upon the level of two genes prior to treatment (one associated with immune signalling, IL6ST and the other with apoptosis, NGFRAP1) and two proliferation genes (ASPM, MCM4) at 2 weeks of therapy. The four gene signature was found to be 91% accurate in a blinded, completely independent validation dataset of patients treated with anastrozole. Matched 2 week on-treatment biopsies improved predictive power over pre-treatment biopsies alone. This signature also significantly predicted recurrence free survival ($p=0.048$ and $p=0.029$) and breast cancer specific survival ($p=0.025$ and $p=0.009$). It is demonstrated that the test can also be performed using quantitative PCR or immunohistochemistry.

Conclusion: A four gene predictive model of clinical response to AIs by 2 weeks has been generated and validated. Deregulated immune and apoptotic responses before treatment and a failure to reduce proliferation by 2 weeks are functional characteristics of breast tumours that do not respond to AIs.

Introduction

Third-generation aromatase inhibitors (AIs) such as letrozole and anastrozole have an established role in the treatment of estrogen-receptor alpha (ER) positive postmenopausal breast cancer ¹⁻⁵. Approximately 30-50% of patients do not respond to AIs ⁶⁻⁸, so there is an urgent clinical need to identify accurate biomarkers to predict which patients will respond to treatment. The majority of gene expression studies that have attempted to predict response have looked at associations between pre-treatment tumour samples and long term outcome ^{9,10}. A study comparing baseline molecular profiles of breast tumours from patients that failed to respond to endocrine therapy with a group of patients that remained disease-free for five years identified genes that were associated with a lack of response/resistance ¹¹. A potentially more powerful and informative approach is to compare sequential biopsies from the same patients, utilising the 'window of opportunity' afforded with neoadjuvant therapy ^{12,13}. Although matched-sample approaches have improved statistical power, these studies are challenging to perform and are highly dependent on analysing sufficient numbers of samples. Previous studies have compared only 5 'responders' with 8 'non-responders' treated with anastrozole alone ¹⁴ and three responding tumours from patients treated with exemestane alone with four non-responding tumours (three received exemestane plus tamoxifen and one exemestane only) ¹⁵. These studies are clearly underpowered, particularly as Miller *et al.* showed that there is greater molecular heterogeneity in the gene expression changes following letrozole treatment in non-responding compared with responding tumours ⁸. Looking specifically at changes in expression of estrogen-regulated and proliferation genes it was noted that some non-responding tumours had changes in expression that were very similar to changes in responding tumours ¹⁶. It has recently been demonstrated that the transcriptional response to letrozole in invasive ductal and lobular carcinomas is highly similar, despite clear histological and molecular differences between the subtypes before and on treatment ¹⁷.

The current Example uses the largest dataset yet generated of matched breast cancer samples collected before and during endocrine therapy to assess the effects of letrozole. The aim was to generate and validate a signature to accurately predict clinical response and long-term outcome to treatment with aromatase inhibitors.

Materials and Methods

Patients

A total of 89 patients from a consecutive series of 255 postmenopausal women presenting to Edinburgh Western General Hospital with large primary histologically confirmed estrogen receptor (ER) rich (Allred Scores 6-8) invasive breast cancer recruited between 2003 and 2011 fulfilled the requirements to be included in this study (Table 1).

Table 1. Comparison of the patient characteristics of the Edinburgh and Royal Marsden Datasets. Logistic regression analysis performed to compare each variable between the two datasets and cohorts.

	Edinburgh (training) Cohort				Royal Marsden (test) Cohort		Cohort p-val	Cohort p-val
	Affymetrix	Illumina	Total	Platform p-val	a	b	a	b
No. of patients	55	34	89		44	44		
Tumour size				0.13			0.003	>0.001
T1	1(2%)	5(16%)	6		0	0		
T2	40(73%)	14(44%)	54		25	25		
T3	4(7%)	5(16%)	9		17	17		
T4	10(18%)	8(24%)	18		0	0		
Nodes				0.22			0.75	0.30
N0	42(76%)	21(66%)	63		38	28		
N1	13(24%)	11(34%)	24		16	16		
Metastasis				0.35			0.12	0.12
M0	51(93%)	28(88%)	79		44	44		
M1	3(5%)	2(6%)	5		0	0		
MX	1(2%)	2(6%)	3		0	0		
Tumour grade				0.27			0.19	0.58
1	5(9%)	6(18%)	11		10	8		
2	39(71%)	23(68%)	62		23	29		
3	11(20%)	5(15%)	16		8	6		
ER (IHC Allred Score)				0.23				
6	1(2%)	1(3%)	2					
7	10(18%)	5(15%)	15					
8	44(80%)	28(82%)	72					
H-score								
Mean					223.4	223.4		
Range					112-292	112-292		
Molecular subtype							>0.001	>0.001
Luminal A	43	28	71	0.32	17	17		
Luminal B	7	6	13		3	3		
Normal-like			0		15	15		
ERBB2			0		8	8		
Basal			0		1	1		
NA	5	0	5					

Patients were excluded on strict predefined criteria: (i) tumour was multifocal, mucinous or tubular, (ii) low cellularity or less than 40% malignancy, (iii) insufficient quantity and quality RNA, (iv) follow-up records were incomplete or sample unavailable, (v) drug was changed

from letrozole to another agent during the 3 month treatment window. All patients gave informed consent and the study was approved by the local ethics committee (LREC; 2001/8/80 and 2001/8/81). Patients were treated with letrozole (Femara, 2.5mg) daily for at least 3 months (see consort diagram Figure 4).

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Tumour Samples and Response Assessment

Tumour biopsies were taken with a 14-guage needle: before, and approximately 2 weeks and 3 months following commencement of continuous letrozole treatment as described previously¹⁸. Samples were snap-frozen in liquid nitrogen and frozen sections taken for staining with haematoxylin and eosin (H&E) to assess cellularity and percentage of invasive cancer by a pathologist. Clinical response was determined using dynamic changes in tumour volumes assessed by repeated measurements taken over the 3 month treatment period (Figure 1A) by a single observer (JMD) and verified by mammographic measurements. Strictly defined clinical response criteria were used as described in the results section. The earliest subsequent event (local or distant recurrence or a second primary) was used for recurrence-free survival analysis.

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RNA Processing, Microarray Hybridisation and Data analysis

RNA was extracted, labelled and hybridised to Human HT-12v4 Illumina BeadChips according to the standard protocol for NuGEN amplified samples as previously described^{17,19}. Raw and normalised gene expression files representing 75 samples from 25 patients not previously published are available from NCBI GEO²⁰ under the accession GSE59515. The analysis also includes Illumina data from 10 patients and Affymetrix data from 54 patients from previous studies^{8,17}, available from NCBI GEO under GSE55374 and GSE20181. There were no significant differences in clinical or pathological features of patients or tumours from the two datasets (Table 1). Illumina and Affymetrix data were independently normalised (quantile using *lumi*²¹ and RMA implemented in *affy* packages) and mapped to Ensembl gene identifiers using reMOAT²² and custom Chip Definition File (CDF)²³ respectively, before detection filtering and batch correction with cross-platform normalisation (XPN)²⁴, as described and evaluated previously¹⁹. Differential gene expression analysis was performed using Rank Products²⁵. Affymetrix data representing 669 tamoxifen-treated tumours from four published microarray datasets (GSE6532, GSE9195, GSE17705, GSE12093) were summarized with Ensembl alternative CDF²³, normalized with RMA and integrated using ComBat²⁶ to remove dataset-specific bias. Pathway enrichment analysis was performed using DAVID bioinformatics resources²⁷. The most informative features differentiating between responders and non-responders were identified using Random Forest²⁸ and predictive signatures were assessed using

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Support Vector Machines (SVM), centroid classification and logistic regression (*glm* package). The Cluster²⁹ and TreeView programs were used to generated heatmaps.

Royal Marsden Validation Dataset

5 Illumina gene expression data was generated from primary breast tumour tissue biopsies before, and after 2 weeks of continuous anastrozole treatment in the anastrozole-only arm of a neoadjuvant clinical trial^{30,31}. RNA was extracted, amplified and labelled as previously described³², before hybridising to HumanWG-6 v2 Expression BeadChips (Illumina) according to the standard protocol. The analysis was performed blind in that the Royal
10 Marsden group were not informed of the identity of the genes and the Edinburgh group were not aware of the response status, until the analysis was complete.

Quantitative polymerase chain reaction (qPCR)

RNA was reverse transcribed (and qPCR was performed using SYBR-green RNA-to-Ct
15 one-stop Taqman chemistry with off-the-shelf validated assays for each of the 4 genes as follows: IL6ST (CATCCACCCGATCTTCATTAC) [SEQ ID NO: 1] (Hs00174360_m1), MCM4 (CTTTGACCGTTACCCTGACTC) [SEQ ID NO: 2] (Hs00907398_m1), ASPM (TCTGAATCGCCTACTTTGGAATC) [SEQ ID NO: 3] (Hs00411505_m1) and NGFRAP1 (TGGGATGGGTGGAGATGGA) [SEQ ID NO: 4] (Hs00918411_s1) (Life Technologies).
20 Serial dilutions of universal cDNA were used as a calibrator for the standard curve. The 4 genes of interest were normalised to the geometric mean of the three housekeeping genes (TBP, RPL37A and PUM1) prior to further analysis.

Immunohistochemistry

25 FFPE sections were stained with a mouse monoclonal antibody to CD45 (Cell Signalling Technology Inc.) diluted x100, antigen retrieval used 0.1M sodium citrate/0.1M citric acid pH6 and was detected using the EnVisionTM kit (Dako, Agilent Technologies) using standard protocol. Sections were counterstained using haematoxylin and scored by two independent scorers. Monoclonal antibodies to IL6ST (Acris AM31176AF-N, Unmasking:
30 TRIS/EDTA, dilution: 1/10, 1hr), MCM4 (Santa Cruz sc-28317, Unmasking: Tris/EDTA, dilution: 1/25, 1hr) and MCM4 (Cell Signalling D3H6N, Unmasking: Na Citrate, Dilution: 1/25, 1hr), along with a polyclonal antibody to ASPM (Millipore 09-066, Unmasking: Na Citrate, Dilution: 1/200, 1hr) were used.

35

Results

Clinical and molecular response

Over 90% of tumours had changes in volume on treatment that conformed to three clearly defined categories of response (Figure 1A): (i) 'quick stable response': reduction of at least 50% by day 45 and at least 70% by 3 months (n=30), (ii) 'slow response': reduction between 0 and 50% by day 45 and at least 70% by 3 months (n=24), (iii) 'non-responder': increase in tumour volume or a partial reduction that never exceeded 50% (n=19). Despite evident differences in tumour size measurements, 'quick stable' and 'slow' responding tumours had very similar gene expression changes upon treatment as noted by significantly overlapping gene lists (Figure 1B). Unsupervised analysis demonstrated that non-responders could be more clearly distinguished from responders at 3 months than by 2 weeks (Figure 1C). However, proliferation-associated genes including cyclins (CCNA2, CCNB1 and CCND1), mini chromosome maintenance genes (MCM2, MCM4 and MCM6) and mitotic spindle associated genes (ASPM and AURKA) were found to be significantly down-regulated in responding tumours by 2 weeks. These genes were also down-regulated in some patients classed as non-responders, but to a much lesser extent. Glycolysis and oxidative phosphorylation genes were significantly and consistently down-regulated by 3 months in responding tumours, but not in the non-responders. Genes involved with the immune/inflammatory response and ECM/stromal remodelling were significantly co-expressed and up-regulated by 3 months in the majority of responding tumours, but remained relatively unchanged in most non-responders (Figure 1D).

Proliferation and immune response

Whilst there is some dispute as to the exact definition, Luminal B cancers tend to have a more aggressive phenotype with lower progesterone receptor expression, high proliferation and high grade, and generally poorer responses to hormone therapy, compared to Luminal A tumours³⁴. Although the majority of luminal B tumours (8/13) did not respond to treatment, numerically more of the non-responders were classified as luminal A tumours (11/60, Figure 2A), the predictive value of luminal B status was only 30% (95% CI: 14%-50%) indicating that molecular subtype before treatment is not an accurate marker of response. Similarly, expression of a proliferation gene set in pre-treated samples was not significantly associated with clinical response ($p=0.62$, Wilcoxon, Figure 2B). However, proliferation genes were more highly expression in non-responders after 2 weeks of treatment than in non-responders ($p=0.002$, Wilcoxon, Figure 2B). The observation that a local immune/inflammatory response occurs in some tumours and is significantly associated with a positive response to therapy, led us to assess leukocyte

infiltration using immunohistochemistry of tumour sections for the leukocyte common antigen (CD45). Leukocyte infiltration was significantly greater amongst non-responding pre-treatment samples compared to the responsive groups (Chi-square $p=0.028$, Figure 2C), consistent with a previous study³¹.

5

Generation and validation of a 4-gene signature to predict clinical response

As the molecular changes in the 'quick stable' and 'slow' responders were highly consistent (Figure 1C), these groups were combined to identify accurate response prediction biomarkers. Random Forest analysis identified the 200 most informative variables
10 differentiating between responsive and non-responsive tumours; these were 82 genes between pre-treatment samples, 82 genes between two week samples and 36 genes that were changed between the two week and pre-treatment samples. Classification and regression tree (CART) modelling was used to generate the optimal model comprising just 4 genes (Figure 3A). Response was predicted by high expression of the immune-related
15 gene IL6ST and the apoptosis induction related gene NGFRAP1 before treatment and low expression of two proliferation-associated genes, ASPM and MCM4 after 2 weeks treatment (Figure 3B). Overall the model had 96% accuracy, 89% sensitivity and 98% specificity, with a positive predictive value (PPV) of 89% and a negative predictive value (NPV) of 96%. The area under the receiver operating characteristic AUC (ROC) curve
20 was 0.96 units², only failing to predict response accurately in 3 of 73 patients (Table 2).

Table 2. Comparison of a pre- and on-treatment with a pre-treatment only gene expression signatures in the discovery and validation cohorts. PPV=Positive predictive value, NPV=Negative predictive value, CI=Confidence interval

	Edinburgh Cohort	Royal Marsden Cohort
Aromatase Inhibitor	Letrozole	Anastrozole
4 gene model (Pre and 2 week biopsy)		
Accuracy	70/73 (96%)	40/44 (91%)
Responders (PPV)	53/54 (98%)	28/29 (97%)
Non-responders (NPV)	17/19 (89%)	12/15 (80%)
Sensitivity (95% CI)	96% (87-99%)	90% (74-98%)
Specificity (95% CI)	94% (72-99%)	92% (64-99%)
3 gene model (Pre-treatment biopsy only)		
Accuracy	68/73 (93%)	37/44 (84%)
Responders (PPV)	49/54 (91%)	22/29 (76%)
Non-responders (NPV)	19/19 (100%)	12/15 (80%)
Sensitivity (95% CI)	100% (93-100%)	88% (68-97%)
Specificity (95% CI)	79% (58-93%)	63% (38-83%)
qPCR 4 gene model (Pre and 2 week biopsy)		
Accuracy	37/39 (95%)	
Responders (PPV)	25/25 (100%)	
Non-responders (NPV)	12/14 (86%)	
Sensitivity (95% CI)	93% (76-99%)	
Specificity (95% CI)	100% (73-100%)	
HistoScore (3 protein model) (Pre IL6ST, 2 week MCM4 and ASPM)		
Accuracy	42/46 (91%)	
Responders (PPV)	33/36 (92%)	
Non-responders (NPV)	9/10 (90%)	
Sensitivity (95% CI)	97% (84-100%)	
Specificity (95% CI)	75% (43-94%)	

- 5 For independent validation, double-blind testing of the four gene signature was performed on a recently published dataset from a similar patient cohort treated with anastrozole³¹ at the Royal Marsden Hospital. The signature was found to correctly predict the response status in 40 out of 44 patients (Accuracy=91%) with 90% sensitivity, 92% specificity (Table 2).

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The use of a three gene model

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The pre-treatment level of IL6ST had an accuracy of 85% alone, and the optimal pre-treatment CART model also utilised IL6ST as the primary splitter, but also included the immune cell signalling associated gene LAX1 and HPRT1, which plays an essential role in purine metabolism via the purine salvage pathway [397]. This three gene pre-treatment model (Figure 3B) correctly predicted response status of 68/73 patients (Accuracy=93%, Sensitivity= 100%, Specificity=79%, Table 2) in the Edinburgh training dataset. However

the performance in the anastrozole-treated test set (37/44, Accuracy=84%, Sensitivity=88%, Specificity=63%, Table 2) was not as good as the four gene pre and 2 week sample model.

5 The use of the gene modelling in a clinical test

To establish whether the findings based upon microarray data could be translated into a clinical test, we performed validation by qPCR and found that gene expression values from microarray and qPCR were significantly correlated (Pearson correlations, $R=0.87$, $R=0.89$, $R=0.86$, $R=0.90$, $p<0.0001$) for all four genes and that a subset of tumours from our training
 10 set ($n=39$) could be classified with 96% accuracy using cut-points extrapolated from the microarray data. All but two (12/14) non-responders were correctly identified and 25/25 responders were identified correctly (Table 2). As current clinical practice is still focused on measuring the protein levels of ER, PR, HER2 and Ki67 by immunohistochemistry, we also wanted to establish whether it would be possible to use IHC measurements of the 4
 15 genes to predict response to endocrine treatment. As with the qPCR results, the IHC levels of IL6ST, ASPM and MCM (2 different antibodies) were significantly correlated ($p<0.0005$) with the microarray data (Figure 3C). Unfortunately we were unable to validate an antibody for NGFRAP. Despite this, using the subjective low/high cut-points for of three proteins, IL6ST before, and 2 week levels of MCM4 and ASPM we were able to predict
 20 response to letrozole in a subset of our training set ($n=46$) with 91% accuracy (Table 2). All but one (9/10) of the non-responders were identified correctly and 33 out of 36 responders were identified correctly, demonstrating that this test could realistically be translated to the clinic.

25 Long term outcome

Although the focus of this study was on short-term clinical response, we now have follow-up data of up to eleven years for the patients treated with Letrozole in Edinburgh. Kaplan Meier analysis demonstrated a significant association with recurrence-free survival ($p=0.048$ and $p=0.029$) and a significant association with overall survival ($p=0.025$ and
 30 $p=0.009$) with the predictions of clinical response using the 4 gene signature (Figure 4D and 4E). This is consistent with the P024 neoadjuvant endocrine therapy trial which found clinical response predicts prognosis of endocrine-treated patients ³⁵, however our approach predicts response after 2 weeks rather than after 4 months of treatment. In addition we confirmed that pre-treatment IL6ST alone is a good marker of benefit to
 35 adjuvant tamoxifen therapy (Figure 5), and similar results were found for the other pre-treatment markers, LAX1 and HPRT1, suggesting that these genes may also be useful for helping to predict response to non-AI endocrine agents.

Discussion

To realise the goal of personalised medicine we urgently need biomarkers that identify accurately which cancer patients will benefit from specific therapies ³⁶. In this Example, the largest dataset yet generated of matched breast cancer samples taken before and during therapy has been utilised to assess the molecular effects of letrozole on tumours, before generating and validating a four gene signature that predicts clinical response to aromatase inhibitors. We have demonstrated that assessment of proliferation after 2 weeks of treatment improves the accuracy of prediction, beyond that of a pre-treatment only biopsy. The four predictive genes have established and meaningful roles in cancer biology, giving confidence as to their value in treatment decision-making. IL6ST was the most informative pre-treatment marker of clinical response and is included in among the 8 genes of the Endopredict test ³⁷, which has been developed to predict distant recurrence in ER+/HER2- breast cancers treated with adjuvant endocrine therapy. This test is currently only available in several specially qualified molecular pathology labs in Germany, Austria and Switzerland. Only a few relatively small patient-matched molecular endocrine response studies have previously been published ^{14,15} and most of these have lacked statistical power. For example the previously described gene expression signature of 25 pre-treatment genes derived from 7 patients ¹⁵ was found to be only 64% accurate in the current dataset.

The four gene signature includes two proliferation markers after 2 weeks of treatment, which is consistent with previous studies suggesting that a short-term change in Ki67 is a good biomarker of recurrence-free survival ³⁸, as well as an endpoint for assessing efficacy. A single marker of proliferation may be less reliable, although more rigorous standardisation of ki67 measurement ³⁹ could be combined with pre-treatment assessment of IL6ST and/or NGFRAP1 by qPCR or IHC for accurate prediction of short and long-term response to endocrine treatment.

The level of Ki67 measured by IHC at two weeks post treatment was not significantly different ($p=0.13$, Wilcoxon test) between a subset of 26 responders and 7 non-responders in this study (Figure 7A). However combining the Ki67 status at 2 weeks with the pre-treatment IL6ST level was more accurate at predicting clinical response (up to 85% depending upon how applied) than Ki67 level alone (67%), but still less than the 4 gene signature (100%, Figure 7B). Only the 4-gene signature was significantly associated with recurrence-free and breast cancer specific survival (Figure 7C).

Given that these four biomarkers can be measured by qPCR and IHC, this predictive signature has huge clinical potential. ER and HER2 status are available within a few days of performing a core biopsy and most patients have an interval between core biopsy and surgery, so patients with an ER rich breast cancer could be given a 10-14 day course of endocrine therapy before surgery. The current study has shown that changes in proliferation in response to treatment with letrozole are evident within this period. Therefore, one could envisage patients routinely having approximately 2 weeks of endocrine therapy prior to surgery and measuring the level of the 4 genes/proteins to provide evidence in individual patients of the likely benefit of adjuvant endocrine therapy. Alternative or additional therapies could then be given to those with cancers that are predicted to be endocrine resistant. This would be a significant shift towards truly 'individualised therapy', measuring and utilising "response" to treatment as well as baseline tumour biology.

This investigation represents the largest dynamic molecular endocrine response study to-date and has resulted in identification of a 4-gene clinical response predictor that has been validated in an independent dataset and can be assessed by qPCR and IHC. It requires further prospective validation incorporating other known prognostic factors, but demonstrates that delivering personalised endocrine therapy may require not only on baseline criteria, but also incorporate response to therapy to predict long term benefits of treatment.

Table 3. Implementing the prediction signature using immunohistochemistry
 Indications and assignment of poor response (0/1+ IL6ST, 1/2+ ASPM or MCM4) are highlighted in bold. Italics indicate the 4/46 patients wrongly predicted

Patients		IHC Histoscores			Prediction
Clinical Response	ID	IL6ST (Pre)	MCM4 (2w)	ASPM (2w)	
Non-responder	84	1+	0	1+	Non-responder
Non-responder	27	0	1+	1+	Non-responder
Non-responder	331	1+	1+	1+	Non-responder
Non-responder	367	1+	1+	1+	Non-responder
Non-responder	8	1+	1+	1+	Non-responder
Non-responder	109	0	2+	1+	Non-responder
Non-responder	124	1+	2+	2+	Non-responder
Non-responder	364	2+	2+	2+	Non-responder
Non-responder	179	2+	2+	1+	Non-responder
Non-responder	81	1+	0	0	<i>Responder</i>
Responder	87	1+	0	1+	<i>Non-responder</i>
Responder	328	1+	0	2+	<i>Non-responder</i>
Responder	226	2+	2+	2+	<i>Non-responder</i>
Responder	77	1+	0	0	Responder
Responder	115	1+	0	0	Responder
Responder	116	1+	0	0	Responder
Responder	309	1+	0	0	Responder
Responder	25	1+	0	0	Responder
Responder	35	1+	0	0	Responder
Responder	67	1+	0	0	Responder
Responder	89	1+	0	0	Responder
Responder	110	1+	0	0	Responder
Responder	299	1+	0	0	Responder
Responder	61	1+	0	0	Responder
Responder	100	1+	0	0	Responder
Responder	101	1+	0	0	Responder
Responder	291	2+	0	0	Responder
Responder	200	2+	0	2+	Responder
Responder	69	2+	0	0	Responder
Responder	209	2+	0	0	Responder
Responder	329	2+	0	0	Responder
Responder	341	2+	0	0	Responder
Responder	343	2+	0	0	Responder
Responder	350	2+	0	2+	Responder
Responder	48	3+	0	0	Responder
Responder	71	3+	0	0	Responder
Responder	357	3+	0	0	Responder
Responder	10	3+	0	1+	Responder
Responder	215	3+	0	0	Responder
Responder	172	3+	0	1+	Responder
Responder	59	1+	1+	0	Responder
Responder	120	1+	1+	2+	Responder
Responder	311	2+	1+	0	Responder
Responder	373R	2+	1+	0	Responder
Responder	94	1+	2+	0	Responder
Responder	36	1+	2+	0	Responder

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CLAIMS

1. A method for predicting the response to therapy of cancer in an individual, comprising the steps of:
 - (i) providing a first sample comprising one or more cancer cell from the individual before the therapy,
 - (ii) determining in the first sample the expression level of the IL6ST gene; and
 - (iii) predicting the response to the therapy of cancer in the individual on the basis of the determination in step (ii).
2. The method according to Claim 1, wherein step (ii) further comprises determining in the first sample the expression level of the NGFRAP1 gene.
3. The method according to Claims 1 or 2, wherein step (ii) further comprises determining in the first sample the expression level of the LAX1 gene and/or HPRT1 gene.
4. The method according to any of Claims 1-3, wherein:
 - step (i) further comprises providing a second sample comprising one or more cancer cell from the individual after the therapy; and
 - step (ii) further comprises determining in the second sample the expression level of the ASPM gene and/or the MCM4 gene and/or the MKI67 gene and/or a gene marker of cellular proliferation.
5. The method according to Claim 4, wherein:
 - step (i) further comprises providing a third sample comprising one or more cancer cell from the individual after the therapy, and after the provision of the second sample; and
 - step (ii) further comprises determining in the third sample the expression level of the ASPM gene and/or the MCM4 gene and/or the MKI67 gene and/or a gene marker of cellular proliferation.
6. The method according to any preceding claim, wherein the therapy is a neoadjuvant therapy.

7. The method according to any of Claims 1-5, wherein the therapy is one or more therapy selected from the group comprising: an aromatase inhibitor; an Estrogen Receptor modulator; an Estrogen Receptor down-regulator.
- 5 8. The method according to Claim 6, wherein the neoadjuvant therapy is one or more neoadjuvant therapy selected from the group comprising: an aromatase inhibitor; an Estrogen Receptor modulator; an Estrogen Receptor down-regulator.
- 10 9. The method according to Claim 7 or 8, wherein the aromatase inhibitor is one or more aromatase inhibitor selected from the group comprising: anastrozole; letrozole; exemestane; vorozole; formestane; fadrozole.
- 15 10. The method according to Claim 7 or 8, wherein the Estrogen Receptor modulator and/or the Estrogen Receptor down-regulator is one or more selected from the group comprising: tamoxifen; fulvestrant; raloxifene; toremifene; lasofoxifene.
- 20 11. The method according any preceding claim, wherein the cancer is one or more cancer selected from the group comprising: breast cancer; endometrial cancer; ovarian cancer; an estrogen-dependent cancer.
- 25 12. The method according to Claim 11, wherein the estrogen-dependent cancer is one of more estrogen-dependent cancer selected from the group comprising: estrogen-dependent breast cancer; estrogen-dependent endometrial cancer; estrogen-dependent ovarian cancer.
- 30 13. The method according Claims 11 or 12, wherein the breast cancer is one or more breast cancer selected from the group comprising: an Estrogen Receptor-positive breast cancer; a luminal A breast cancer; a luminal B breast cancer; an invasive breast cancer; a lobular breast cancer; a ductal breast cancer; an Estrogen Receptor-positive lobular breast cancer; an Estrogen Receptor-negative lobular breast cancer; an Estrogen Receptor-positive ductal breast cancer; Estrogen Receptor-negative ductal breast cancer.
- 35 14. The method according to any of Claims 11-13, wherein the breast cancer is one or more breast cancer selected from the group comprising: an invasive Estrogen

Receptor-positive breast cancer; an invasive luminal A breast cancer; an invasive luminal B breast cancer; a non-invasive Estrogen Receptor-positive breast cancer.

15. The method according to any preceding claim, wherein the individual is a female.
16. The method according to any preceding claim, wherein the individual is human or non-human, for example, a non-human mammal (*i.e.* any mammal other than a human), such as one or more non-human mammal selected from the group comprising: a horse; a cow; a goat; a sheep; a pig; a dog; a cat; a rodent; a rabbit; a mouse; a rat.
17. The method according to Claim 15 or 16, wherein the female individual is one or more female individual selected from the group comprising: a post-menopausal female; a pre-menopausal female; a perimenopausal female.
18. The method according to any preceding claim, wherein the gene expression level is determined as a high gene expression or a low gene expression.
19. The method according to Claim 18, wherein the high gene expression or low gene expression is calculated relative to data in a training set and/or to data in a reference set.
20. The method according to Claim 19, wherein the data in a training set and/or to data in a reference set is provided from a group of subjects.
21. The method according to any preceding claim, wherein the therapy is administered to the individual after the provision of the first sample.
22. The method according to Claim 21, wherein the therapy is administered at least once per day to the individual, for example: once per day, or once per two days, or once per three days, or once per four days, or once per five days, or once per six days, or once per seven days, or once per eight days, or once per nine days, or once per ten days, or once per eleven days, or once per twelve days, or once per thirteen days, or once per two weeks, or once per three weeks, or once per four weeks, or once per five weeks, or once per six weeks, or once per seven weeks, or once per eight weeks to the individual.

23. The method according to any of Claims 4-22, wherein the second sample is provided from the individual at least one day after the initiation of therapy, for example: at least two days, or at least three days, or at least four days, or at least five days, or at least six days, or at least seven days, or at least eight days, or at least nine days, or at least ten days, or at least 11 days, or at least 12 days, or at least 13 days, or at least 14 days, or at least 15 days, or at least 16 days, or at least 17 days, or at least 18 days, or at least 19 days, or at least 20 days, or at least 21 days, or at least 22 days, or at least 23 days, or at least 24 days, or at least 25 days, or at least 26 days, or at least 27 days, or at least 28 days, or at least five weeks, or at least six weeks, or at least seven weeks, or at least eight weeks, or at least nine weeks, or at least ten weeks, or at least 11 weeks, or at least three months, or at least four months, or at least five months, or at least six months, or at least seven months, or at least eight months, or at least nine months, or at least ten months, or at least 11 months, or at least 12 months after the initiation of therapy.
24. The method according to any preceding claim, wherein the predicted response is one or more predicted response selected from the group comprising: a response; a non-response.
25. The method according to Claim 24, wherein a response or a non-response is selected using a decision tree algorithm.
26. The method according to Claim 24 or 25 wherein, when the expression level of the IL6ST gene and/or the NGFRAP1 gene and/or the ASPM gene and/or the MCM4 gene and/or the LAX1 gene and/or the HPRT1 gene is determined, a response is determined if:
- the IL6ST gene expression is high; and/or
 - the NGFRAP1 gene expression is high; and/or
 - the LAX1 gene expression is high; and/or
 - the HPRT1 gene expression is low; and/or
 - the ASPM gene expression is low after the initiation of therapy; and/or
 - the MCM4 gene expression is low after the initiation of therapy.
27. The method according to Claim 26, wherein

- the ASPM gene expression is low two weeks after the initiation of therapy; and/or
 - the ASPM gene expression is low three months after the initiation of therapy; and/or
 - the MCM4 gene expression is low two weeks after the initiation of therapy; and/or
 - 5 • the MCM4 gene expression is low three months after the initiation of therapy.
28. The method according to Claim 24 or 25 wherein, when the expression level of the IL6ST gene and/or the NGFRAP1 gene and/or the ASPM gene and/or the MCM4
- 10 gene and/or the LAX1 gene and/or the HPRT1 gene is determined, a non-response is determined if:
- the IL6ST gene expression is low; and/or
 - the NGFRAP1 gene expression is low; and/or
 - 15 • the LAX1 gene expression is low; and/or
 - the HPRT1 gene expression; and/or
 - the ASPM gene expression is high after the initiation of therapy; and/or
 - the MCM4 gene expression is high after the initiation of therapy.
- 20 29. The method according to Claim 28, wherein
- the ASPM gene expression is high two weeks after the initiation of therapy; and/or
 - the ASPM gene expression is high three months after the initiation of therapy; and/or
 - 25 • the MCM4 gene expression is high two weeks after the initiation of therapy; and/or
 - the MCM4 gene expression is high three months after the initiation of therapy.
30. The method according to any of Claims 24-29, wherein the response or non-response is further characterised by measuring one or more selected from the
- 30 group comprising: the expression level of one or more proliferation-associated gene (such as a cyclin); the expression level of one or more mini-chromosome maintenance gene; the expression level of one or more mitotic spindle associated gene; the expression level of one or more glycolysis and oxidative phosphorylation gene; the expression level of one or more immune/inflammatory response gene;
- 35 the expression level of one or more ECM stromal remodelling gene; tumour volume.

31. The method according to Claim 30, wherein the proliferation-associated gene is one or more gene selected from the group comprising: CCNA2; CCNB1; CCND1.
- 5 32. The method according to Claim 30, wherein the mini chromosome maintenance gene is one or more gene selected from the group comprising: MCM2; MCM4; MCM6.
- 10 33. The method according to Claim 30, wherein the mitotic spindle associated gene is one or more gene selected from the group comprising: ASPM; AURKA.
34. The method according to any preceding claim, wherein the expression level is determined by measuring the presence and/or amount of one or more product of the gene, for example: protein or mRNA.
- 15 35. The method according to Claim 34, wherein protein is measured using one or more method selected from the group comprising: Raman spectroscopy; Acoustic Membrane MicroParticle technology; immunohistochemistry; an antibody-based detection method, for example, RPPA or AQUA.
- 20 36. The method according to Claim 34, wherein mRNA is measured using one or more method selected from the group comprising: a PCR-based approach (such as RT-PCR); microarray technology; NanoString.
- 25 37. The method according to any preceding claim wherein the sample is one or more sample selected from the group comprising: a tumour biopsy; surgical tumour resection; blood; serum; plasma; lymphatic fluid; urine; nipple aspirate fluid.
- 30 38. A method for selecting a treatment for an individual with cancer, comprising the steps of:
- a) providing a first sample comprising one or more cancer cell from the individual;
 - b) determining in the first sample the expression level of the IL6ST gene; and
 - 35 c) selecting a treatment for the individual on the basis of the determination in step (b).

39. The method according to any of Claim 38, further comprising the step of administering the selected treatment to the individual.
40. The method according to Claim 38 or 39, wherein the selected treatment is one or more selected treatment selected from the group comprising: a continuation of the earlier treatment; surgery; radiotherapy; chemotherapy; an mTOR inhibitor; an anti-HER2 therapy.
41. A method for predicting the prognosis of cancer in an individual, comprising the steps of:
- 1) providing a first sample comprising one or more cancer cell from the individual;
 - 2) determining in the first sample the expression level of the IL6ST gene; and
 - 3) predicting the prognosis of cancer on the basis of the determination in step (2).
42. A method for predicting the progression of cancer in an individual, comprising the steps of:
- A) providing a first sample comprising one or more cancer cell from the individual;
 - B) determining in the first sample the expression level of the IL6ST gene; and
 - C) predicting the progression of cancer on the basis of the determination in step (B).
43. The method according to Claim 41 or 42, wherein the step of predicting the prognosis or progression of cancer in the individual is selected from the group comprising: percentage response of the individual to cancer treatment; overall survival of the individual; disease-specific survival of the individual; progression-free survival of the individual.
44. A kit for performing a method as defined in any of Claims 1 to 43, comprising one or more reagent selected from the group comprising: one or more reagent for detecting and/or measuring and/or quantifying the IL6ST gene; one or more reagent for detecting and/or measuring and/or quantifying the NGFRAP1 gene; one or more reagent for detecting and/or measuring and/or quantifying the LAX1

gene; one or more reagent for detecting and/or measuring and/or quantifying the HPRT1 gene; one or more reagent for detecting and/or measuring and/or quantifying the ASPM gene; one or more reagent for detecting and/or measuring and/or quantifying the MCM4 gene; one or more reagent for detecting and/or measuring and/or quantifying the MKI67 gene; one or more reagent for detecting and/or measuring and/or quantifying a gene marker of cellular proliferation.

45. A method or a kit or a use substantially as described and/or claimed herein, with reference to the accompanying description and/or examples and/or drawings.

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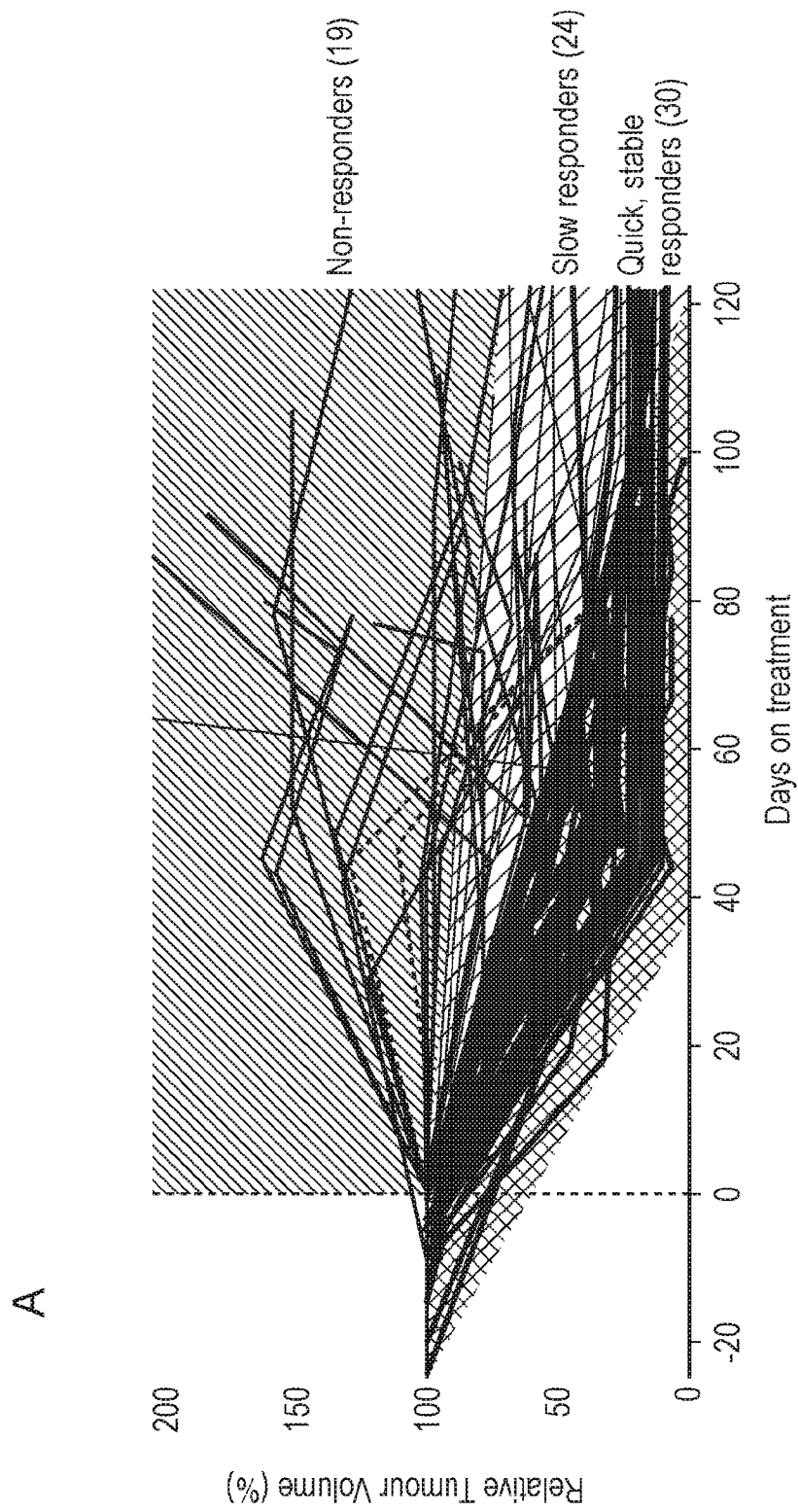


FIG. 1a

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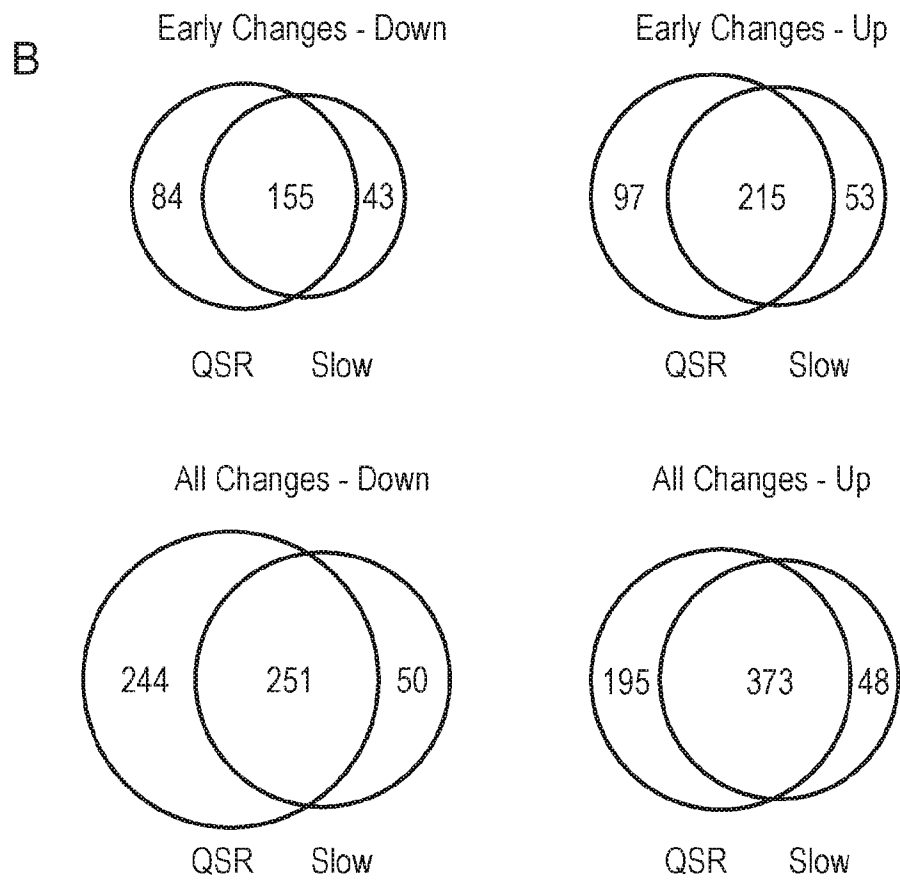


FIG. 1b

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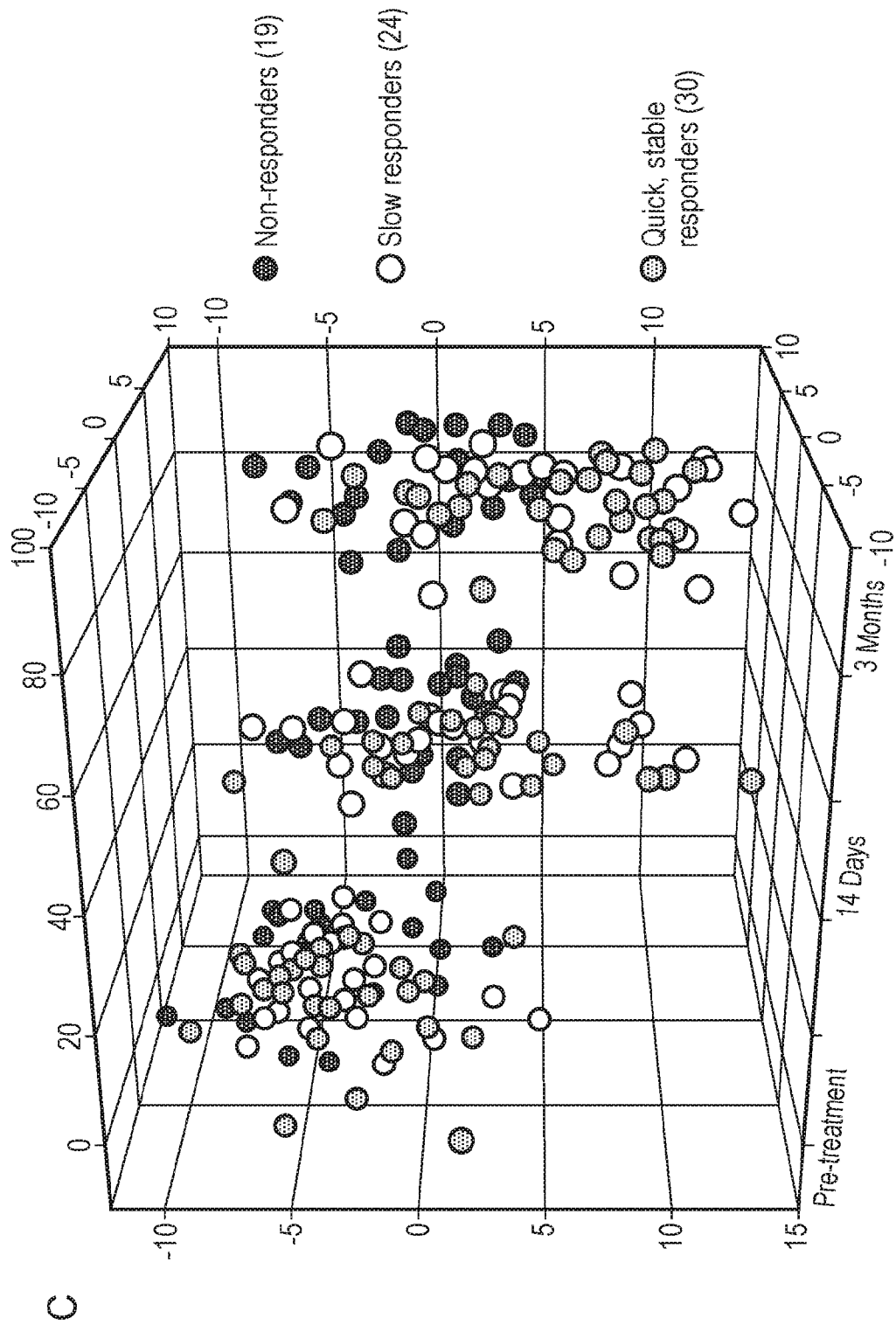


FIG. 1c

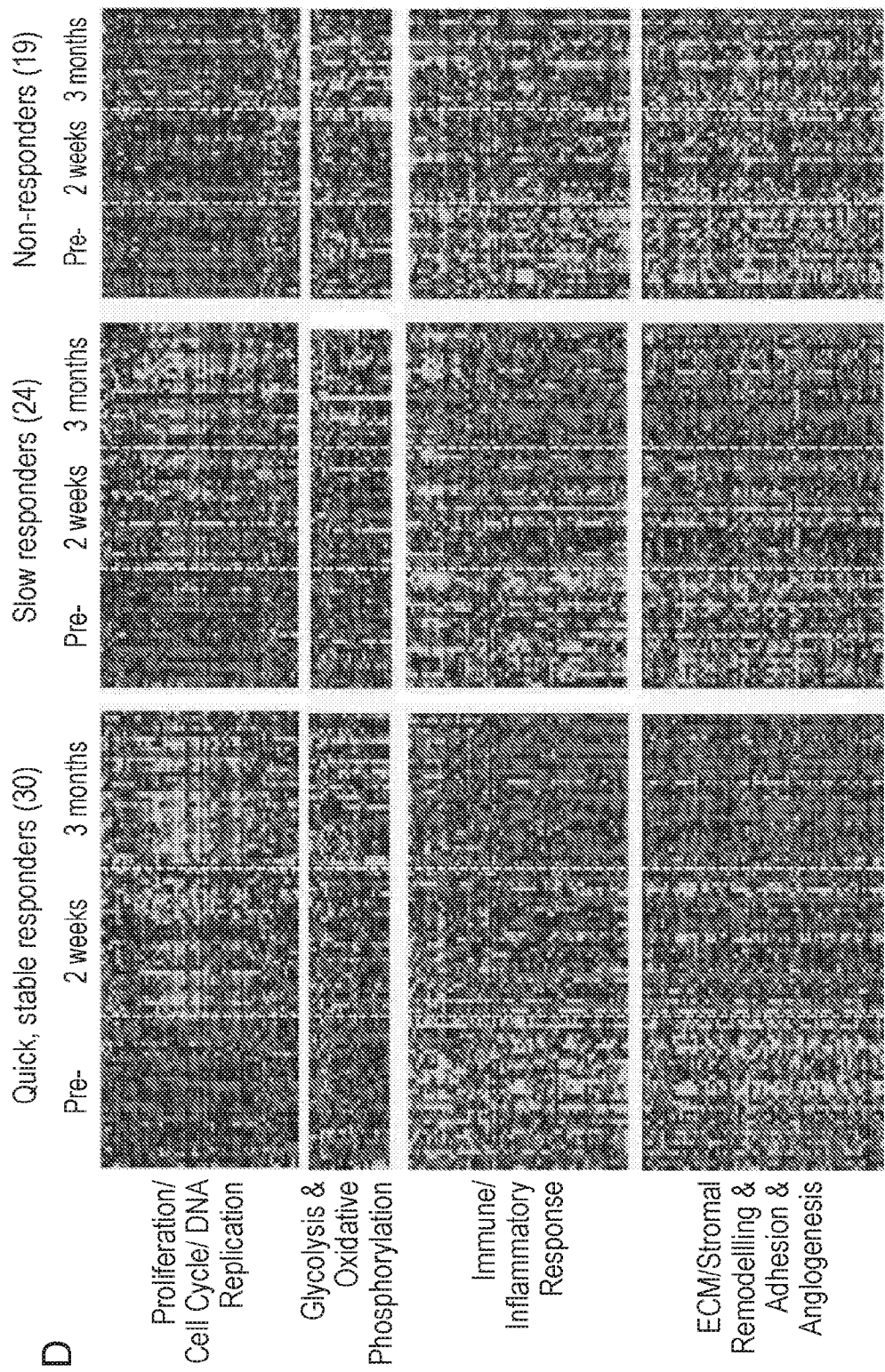


FIG. 1d

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A

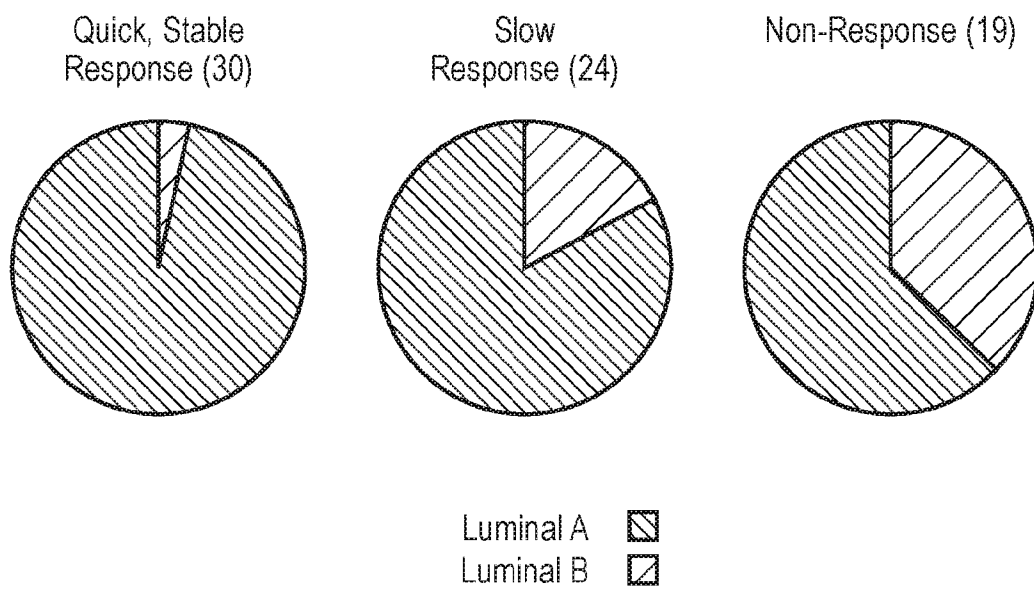


FIG. 2a

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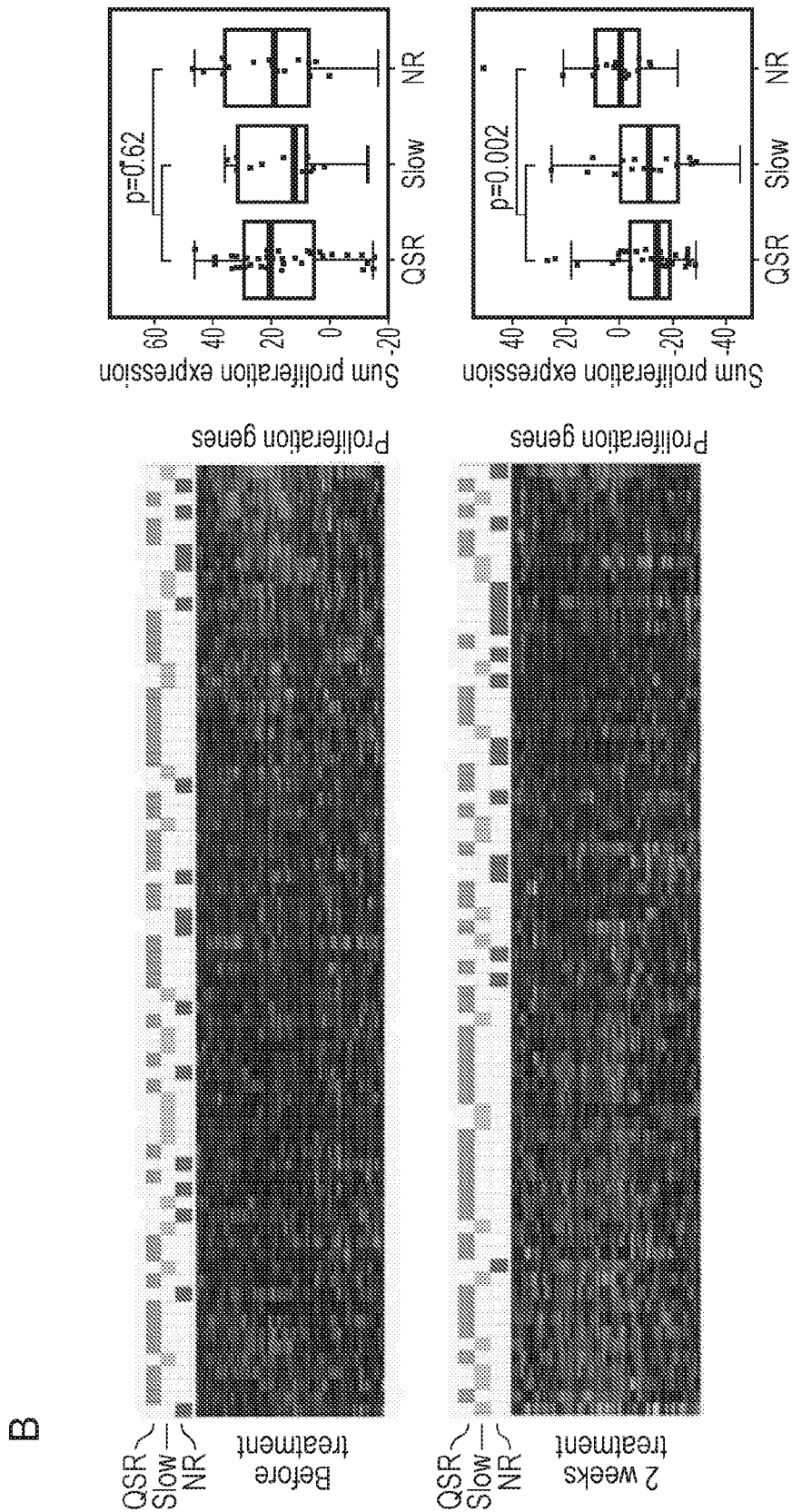


FIG. 2b

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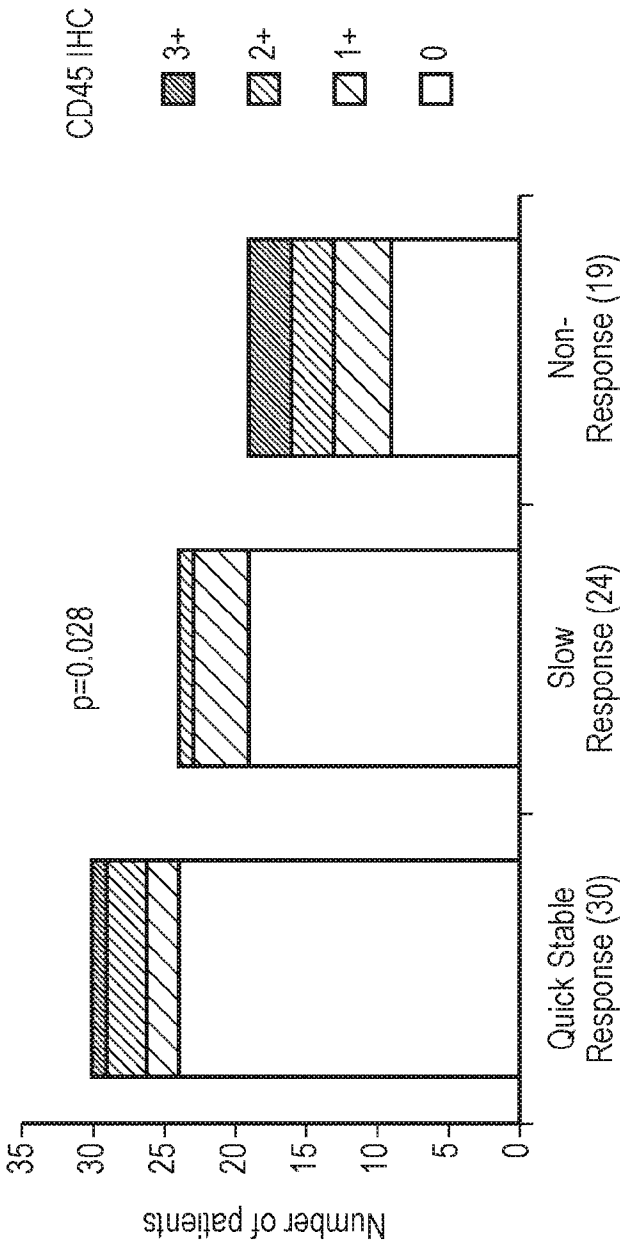


FIG. 2c

C

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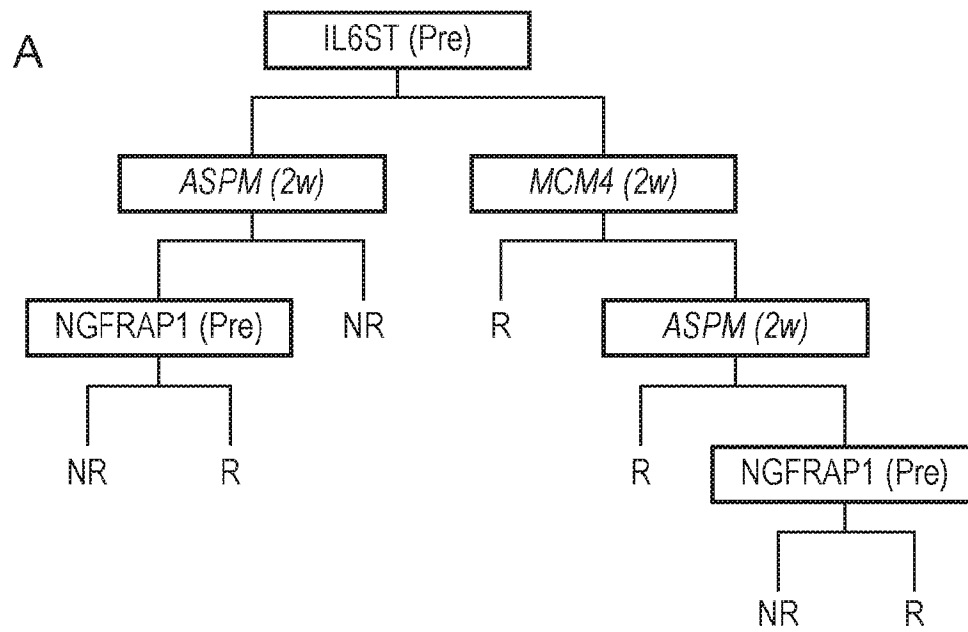


FIG. 3a

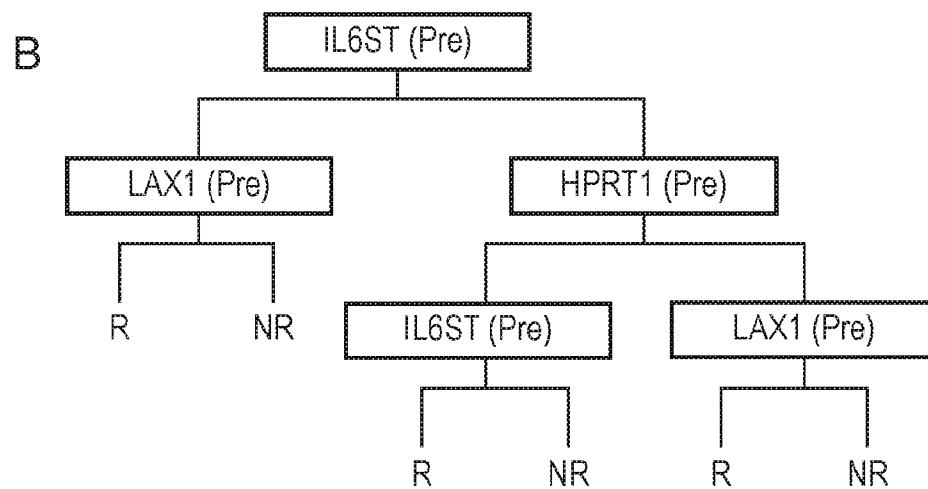


FIG. 3b

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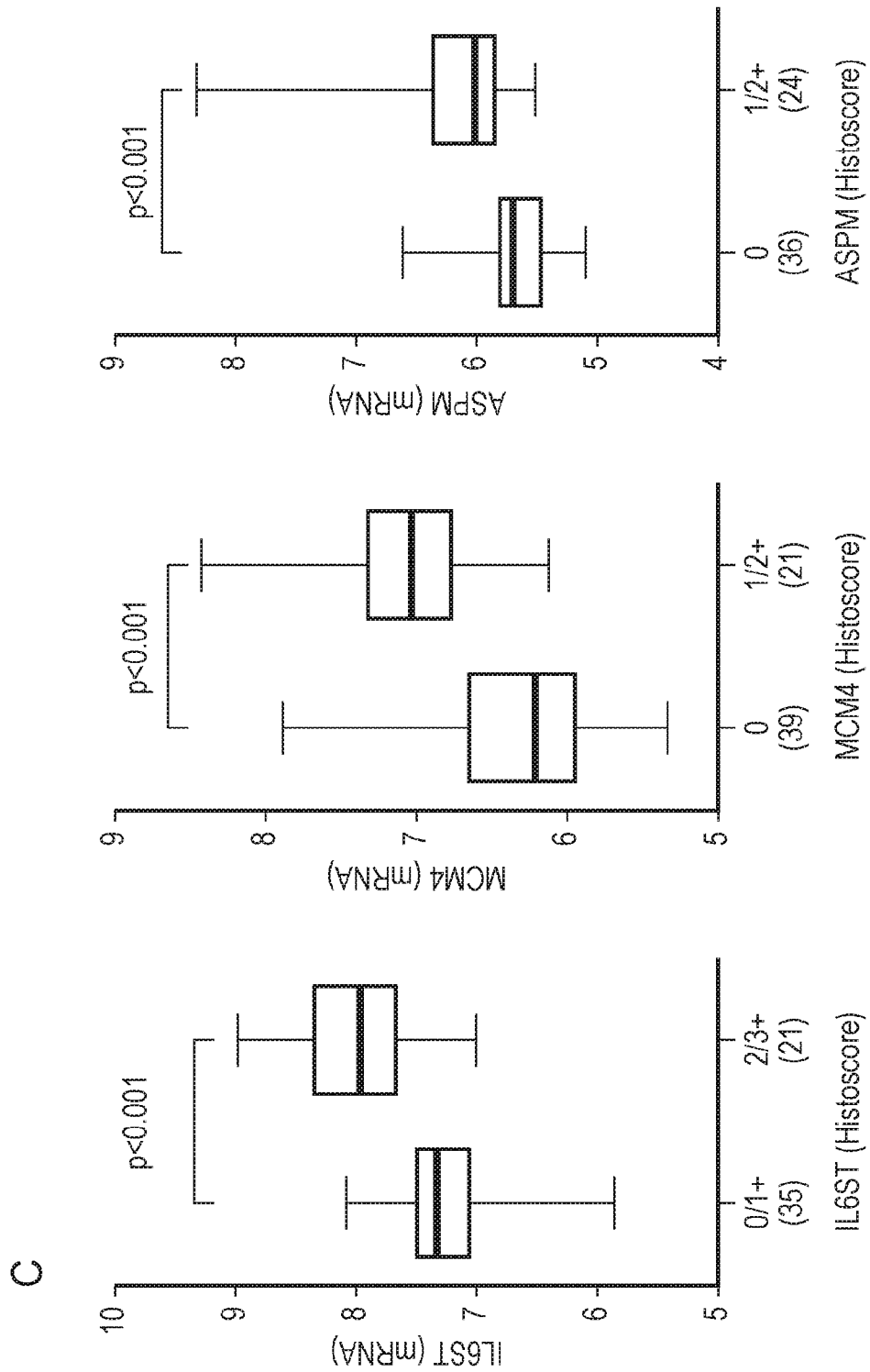


FIG. 3b

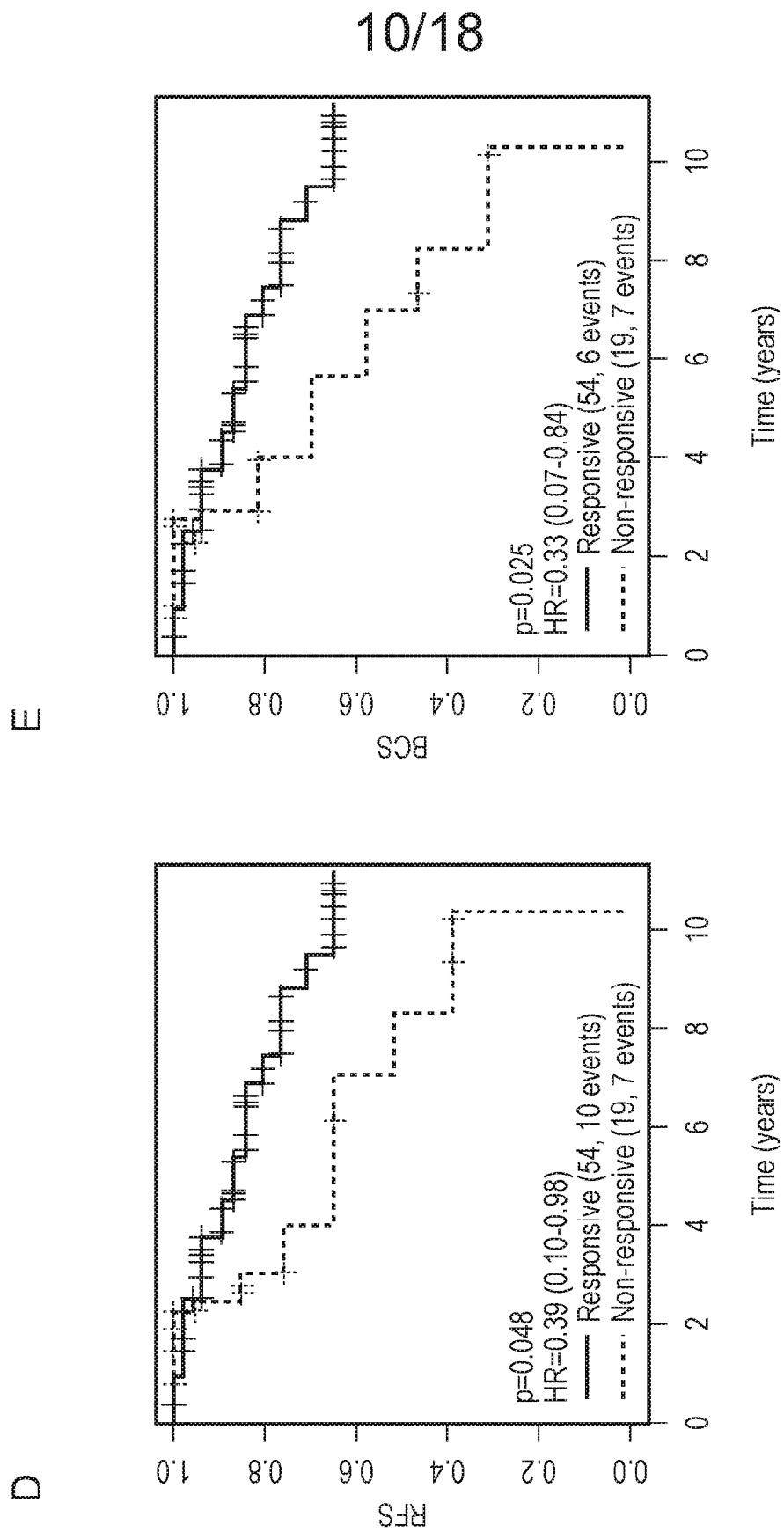


FIG. 3b (Continued)

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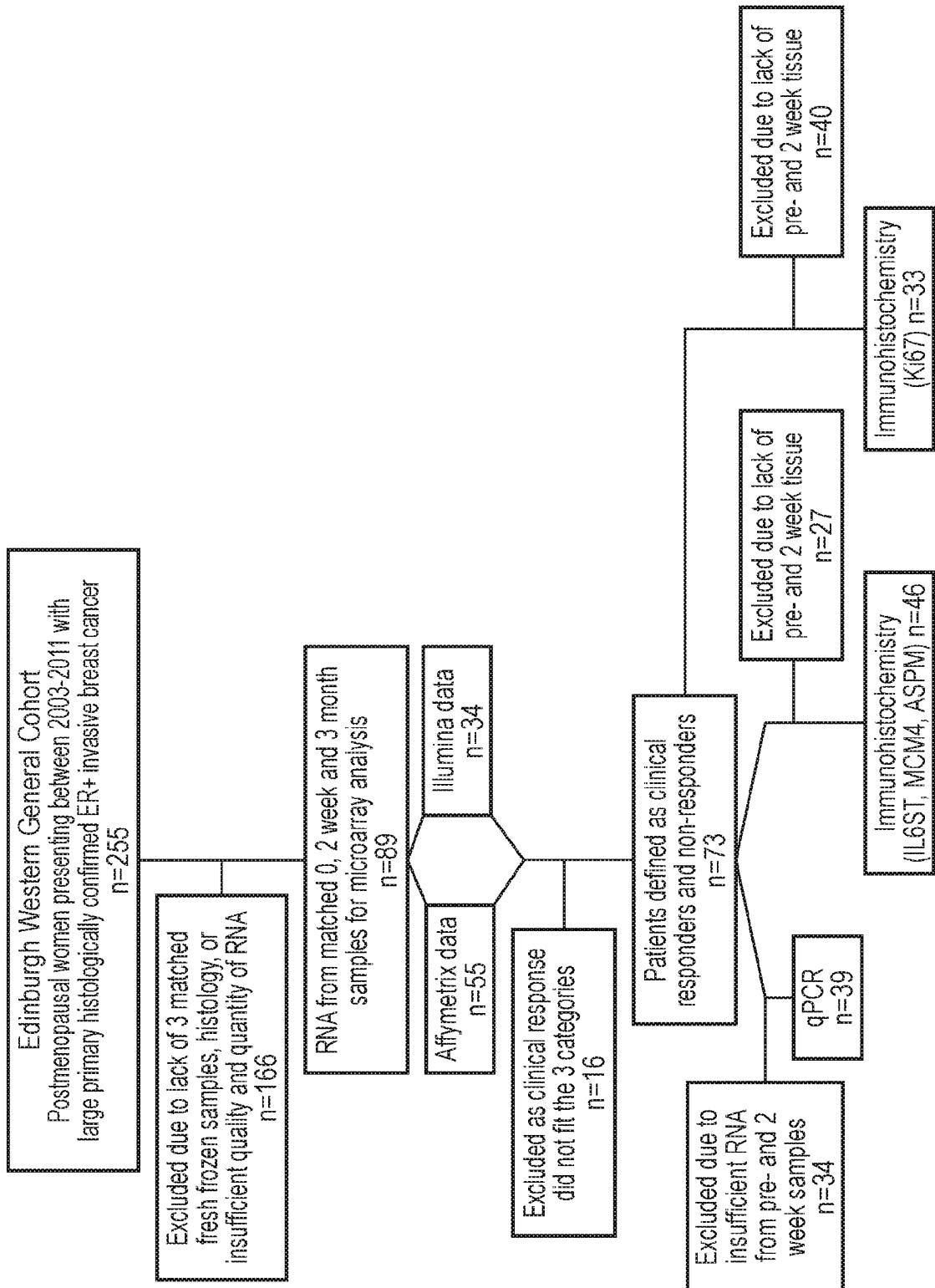


FIG. 4a

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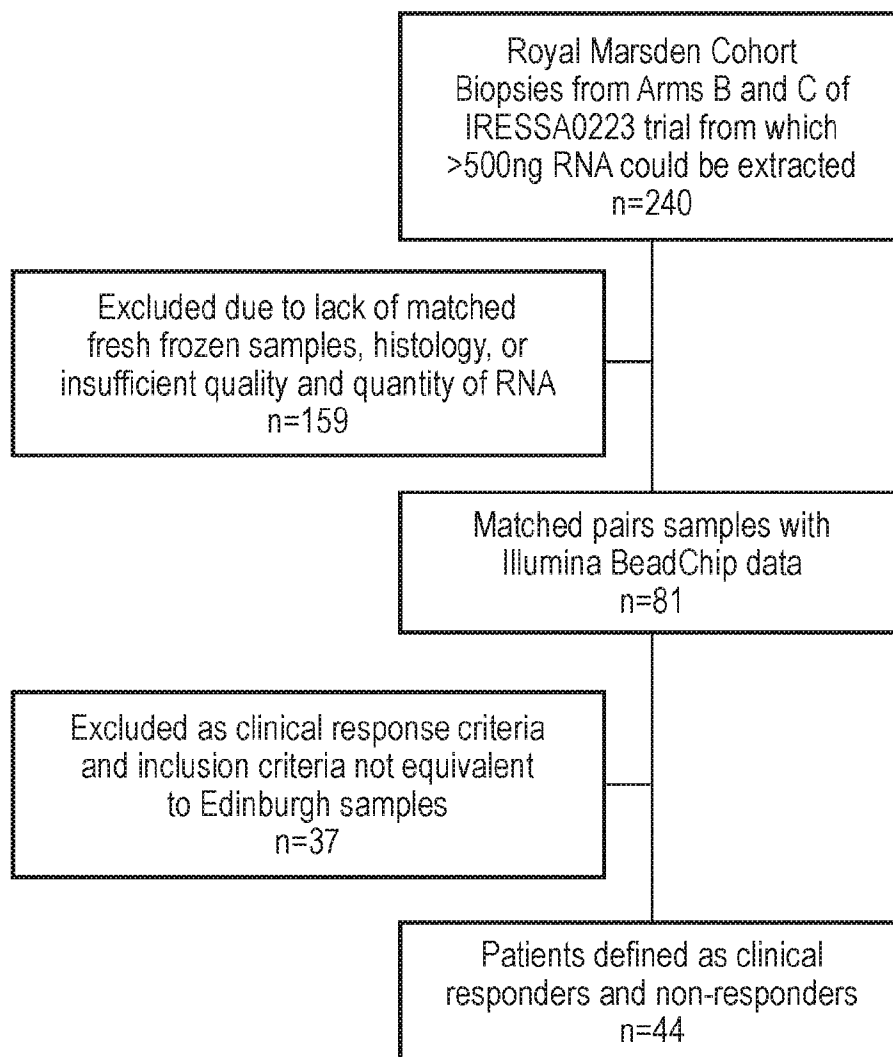


FIG. 4b

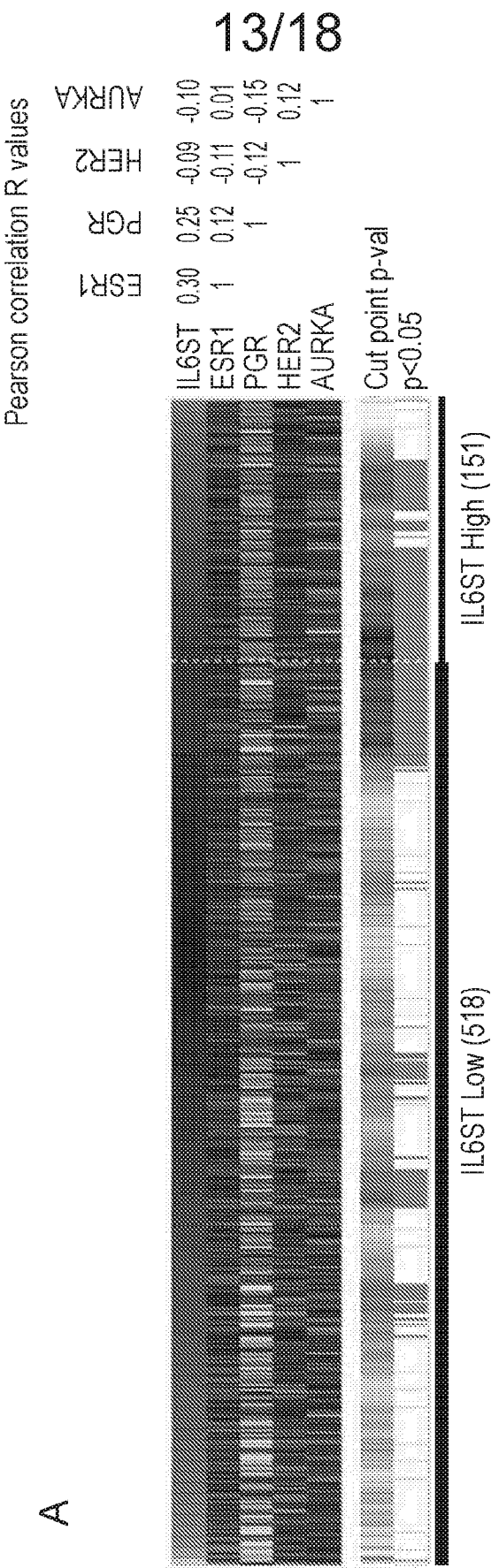


FIG. 5a

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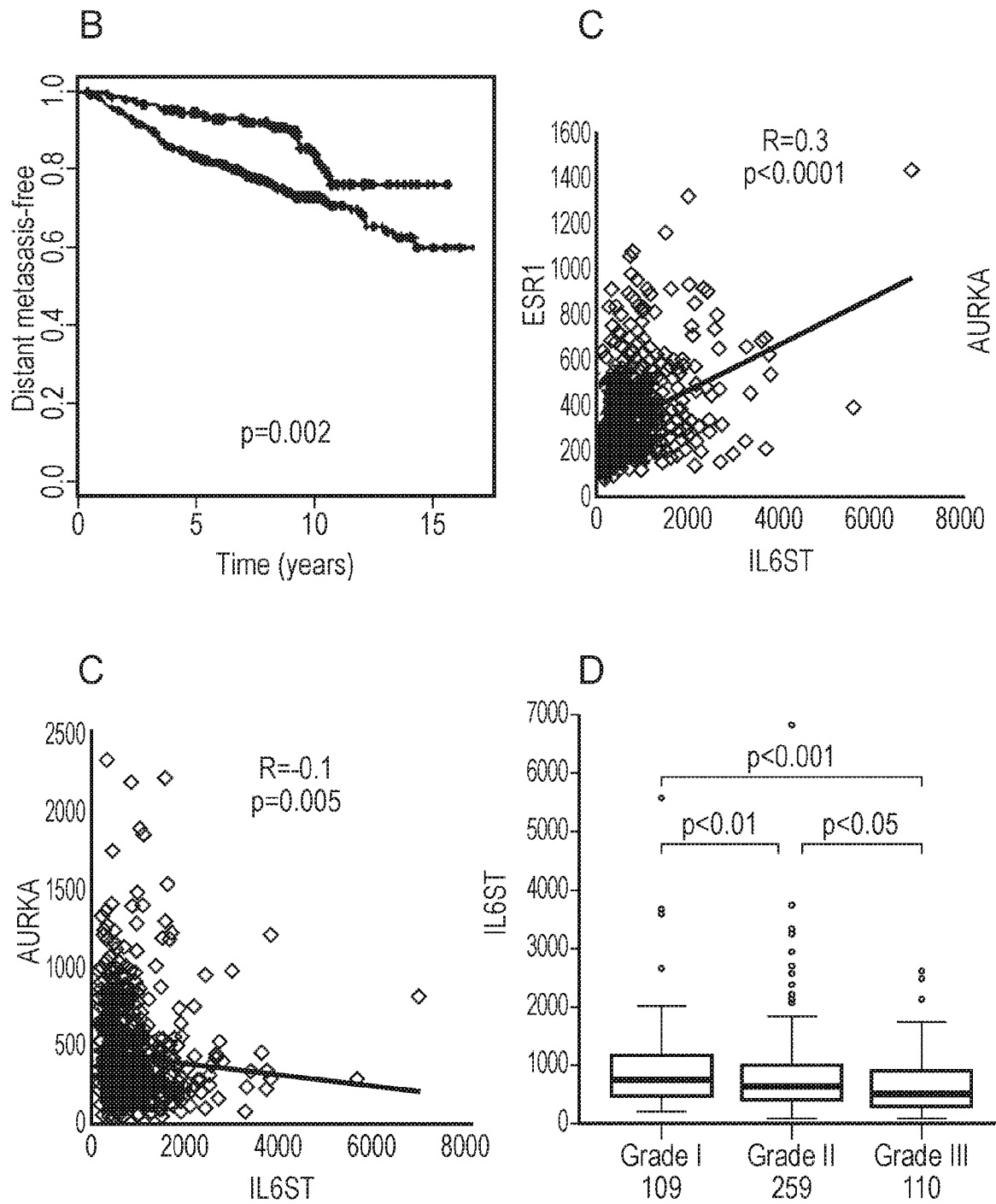


FIG. 5b

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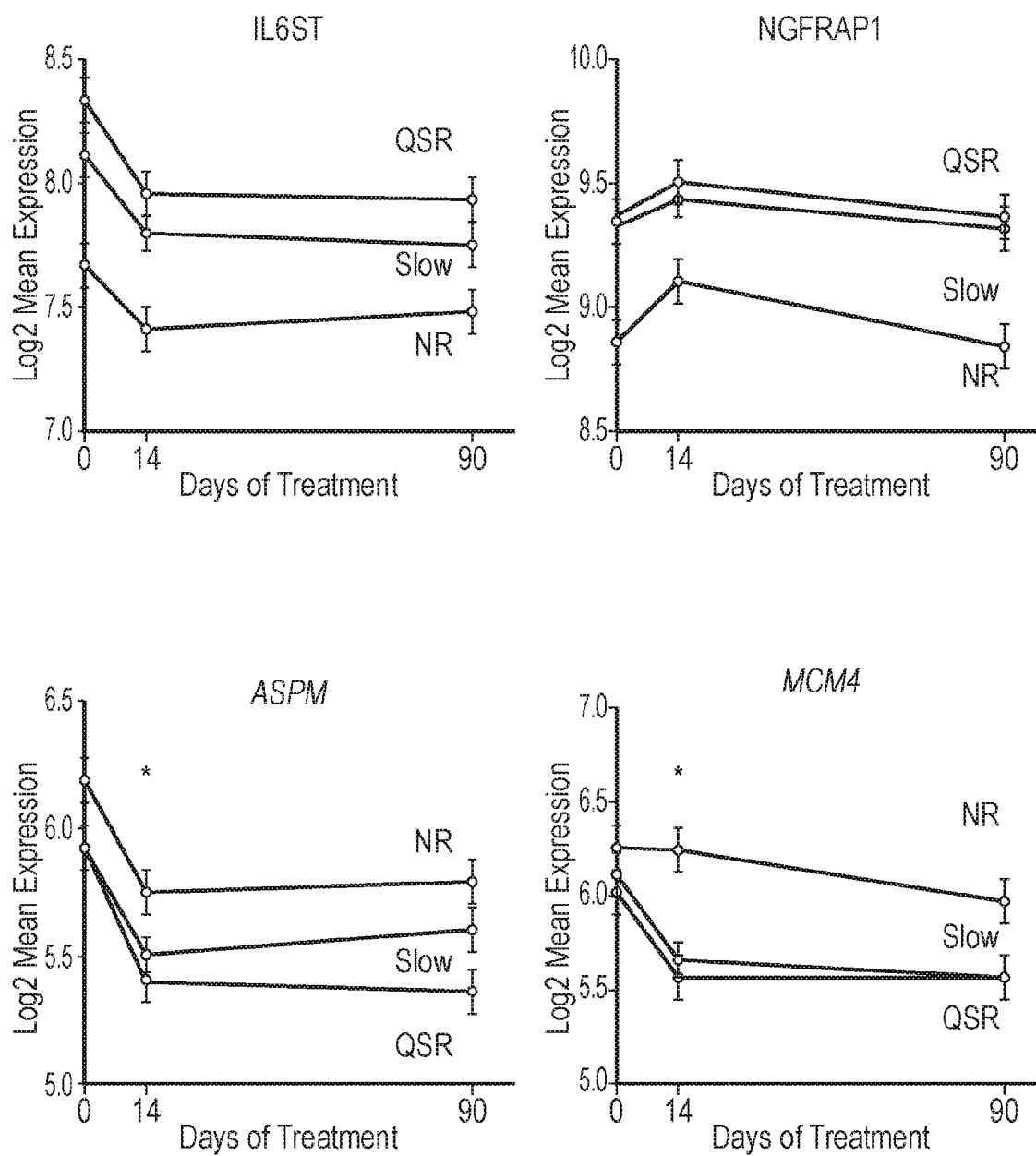


FIG. 6

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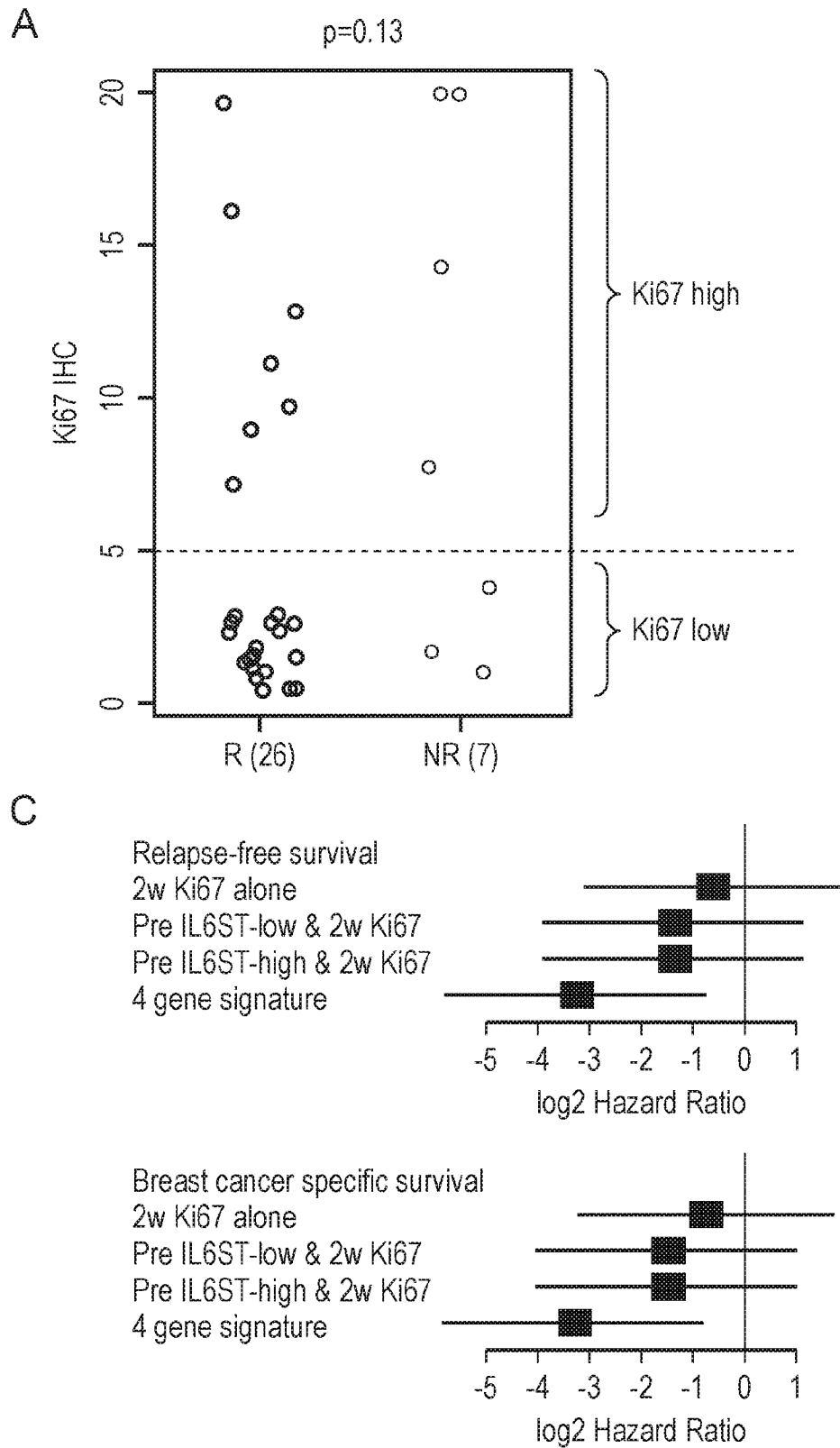


FIG. 7

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B

IL6ST-low and Ki67 IL6ST-high and Ki67

IL6ST (pre) IL6ST (pre)

R R NR R NR NR

Ki67 (2w) Ki67 (2w) Ki67 (2w)

Clinical Response	ID	IHC Histocores		Prediction (2w Ki67 alone)	Prediction (pre IL6ST-low and 2w Ki67)	Prediction (pre IL6ST-high and 2w Ki67)	Prediction (4gene signature)
		IL6ST (Pre)	Ki67 (2w)				
Non-responder	76	IL6ST high	Ki67 low	Responder	Responder	Responder	Non-responder
Non-responder	95	IL6ST low	Ki67 low	Responder	Responder	Non-responder	Non-responder
Non-responder	8	IL6ST low	Ki67 low	Responder	Responder	Non-responder	Non-responder
Non-responder	27	IL6ST low	Ki67 high	Non-responder	Non-responder	Non-responder	Non-responder
Non-responder	121	IL6ST low	Ki67 high	Non-responder	Non-responder	Non-responder	Non-responder
Non-responder	84	IL6ST low	Ki67 high	Non-responder	Non-responder	Non-responder	Non-responder
Non-responder	109	IL6ST low	Ki67 high	Non-responder	Non-responder	Non-responder	Non-responder
Responder	56	IL6ST low	Ki67 low	Responder	Responder	Non-responder	Responder
Responder	35	IL6ST low	Ki67 low	Responder	Responder	Non-responder	Responder
Responder	25	IL6ST low	Ki67 high	Non-responder	Non-responder	Non-responder	Responder
Responder	94	IL6ST low	Ki67 high	Non-responder	Non-responder	Non-responder	Responder
Responder	34	IL6ST high	Ki67 high	Non-responder	Responder	Non-responder	Responder
Responder	125	IL6ST high	Ki67 high	Non-responder	Responder	Non-responder	Responder
Responder	90	IL6ST high	Ki67 high	Non-responder	Responder	Non-responder	Responder
Responder	2	IL6ST high	Ki67 high	Non-responder	Responder	Non-responder	Responder

FIG. 7 (Continued)

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Responder	120	IL6ST high	Ki67 high	Non-responder	Responder	Non-responder	Responder
Responder	87	IL6ST high	Ki67 high	Non-responder	Responder	Non-responder	Responder
Responder	119	IL6ST high	KJ67 low	Responder	Responder	Responder	Responder
Responder	67	IL6ST high	KJ67 low	Responder	Responder	Responder	Responder
Responder	102	IL6ST high	KJ67 low	Responder	Responder	Responder	Responder
Responder	89	IL6ST high	KJ67 low	Responder	Responder	Responder	Responder
Responder	111	IL6ST high	KJ67 low	Responder	Responder	Responder	Responder
Responder	71	IL6ST high	KJ67 low	Responder	Responder	Responder	Responder
Responder	115	IL6ST high	KJ67 low	Responder	Responder	Responder	Responder
Responder	48	IL6ST high	KJ67 low	Responder	Responder	Responder	Responder
Responder	110	IL6ST high	KJ67 low	Responder	Responder	Responder	Responder
Responder	59	IL6ST high	KJ67 low	Responder	Responder	Responder	Responder
Responder	116	IL6ST high	KJ67 low	Responder	Responder	Responder	Responder
Responder	10	IL6ST high	KJ67 low	Responder	Responder	Responder	Responder
Responder	77	IL6ST high	KJ67 low	Responder	Responder	Responder	Responder
Responder	69	IL6ST high	KJ67 low	Responder	Responder	Responder	Responder
Responder	130	IL6ST high	KJ67 low	Responder	Responder	Responder	Responder
Responder	78	IL6ST high	KJ67 low	Responder	Responder	Responder	Responder
Accuracy (n=33)				22/33 (67%)	28/33 (85%)	22/33 (67%)	33/33 (100%)
Responders (PPV)				4/7 (57%)	4/7 (57%)	6/7 (80%)	7/7 (100%)
Non-responders (NPV)				18/26 (69%)	24/26 (92%)	16/26 (62%)	26/26 (100%)
Sensitivity (95% CI)				33% (21-55%)	67% (24-94%)	38% (16-64%)	100% (56-100%)
Specificity (95% CI)				80% (63-96%)	89% (70-97%)	94% (69-100%)	100% (84-100%)
Recurrence-free survival				p=0.64	p=0.259	p=0.25	p=0.002
Breast cancer specific survival				p=0.55	p=0.204	p=0.208	p=0.001

FIG. 7 (Continued)

INTERNATIONAL SEARCH REPORT

International application No

PCT/GB2015/053767

A. CLASSIFICATION OF SUBJECT MATTER

INV. C12Q1/68

ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C12Q

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

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

EPO-Internal, BIOSIS, Sequence Search, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2011/063274 A2 (GENOMIC HEALTH INC [US]; BAKER JOFFRE B [US]; CRONIN MAUREEN T [US]; C) 26 May 2011 (2011-05-26)	1,6-26, 28,30, 34-43,45
Y	claims 1, 4, 6, 8; tables 1, 5 ----- -/--	2-5,27, 29,31-33



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

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Date of the actual completion of the international search

21 March 2016

Date of mailing of the international search report

31/03/2016

Name and mailing address of the ISA/

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Authorized officer

Santagati, Fabio

INTERNATIONAL SEARCH REPORT

International application No

PCT/GB2015/053767

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ALDONA KARCZEWSKA ET AL: "Expression of interleukin-6, interleukin-6 receptor, and glycoprotein 130 correlates with good prognoses for patients with breast carcinoma", CANCER., vol. 88, no. 9, 1 May 2000 (2000-05-01), pages 2061-2071, XP055237631, US ISSN: 0008-543X, DOI: 10.1002/(SICI)1097-0142(20000501)88:9<2061::AID-CNCR12>3.0.CO;2-0 page 2070, left-hand column; figure 1; table 4	1,11-13, 15,18, 24,26, 30,34, 36-43,45
Y	WO 2011/130495 A1 (NUVERA BIOSCIENCES INC [US]; UNIV TEXAS [US]; HATZIS CHRISTOS [US]; SY) 20 October 2011 (2011-10-20) claim 1; tables 2-4	2-5,27, 29,31-33
Y	WO 2011/120984 A1 (SIVIDON DIAGNOSTICS GMBH [DE]; DARTMANN MAREIKE [DE]; FEDER INKE SABIN) 6 October 2011 (2011-10-06) claim 1	2-5,27, 29,31-33
X	WANG Y ET AL: "Autocrine production of interleukin-6 confers cisplatin and paclitaxel resistance in ovarian cancer cells", CANCER LETTERS, NEW YORK, NY, US, vol. 295, no. 1, 1 September 2010 (2010-09-01), pages 110-123, XP027075306, ISSN: 0304-3835, DOI: 10.1016/J.CANLET.2010.02.019 [retrieved on 2010-03-16] figures 1C-E	1,6,11, 15-18, 24,34, 36-40
X	"Data Sheet: Gene Expression Array-Based Gene Expression Analysis", illumina, 1 January 2011 (2011-01-01), XP055259707, Retrieved from the Internet: URL:http://support.illumina.com/content/dam/illumina-marketing/documents/products/datasheets/datasheet_gene_exp_analysis.pdf [retrieved on 2016-03-18] page 2; table 1	44
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International application No

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
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X,P	TURNBULL ARRAN K ET AL: "Accurate Prediction and Validation of Response to Endocrine Therapy in Breast Cancer", JOURNAL OF CLINICAL ONCOLOGY, LIPPINCOTT WILLIAMS & WILKINS, USA, vol. 33, no. 20, 10 July 2015 (2015-07-10), pages 2270-2278, XP008179469, ISSN: 1527-7755, DOI: 10.1200/JCO.2014.57.8963 [retrieved on 2015-06-01] the whole document -----	1-45
A	WEI HU: "Identifying predictive markers of chemosensitivity of breast cancer with random forests", JOURNAL OF BIOMEDICAL SCIENCE AND ENGINEERING, vol. 03, no. 01, 1 January 2010 (2010-01-01), pages 59-64, XP055259396, ISSN: 1937-6871, DOI: 10.4236/jbise.2010.31009 table 3 -----	1-45

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Information on patent family members

International application No

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