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(71) Applicant *(for all designated States except US)*: **ERASMUS UNIVERSITY MEDICAL CENTER ROTTERDAM** [NL/NL]; Dr. Molewaterplein 50, NL-3015 GE Rotterdam (NL).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **UMAR, Arzu** [NL/NL]; c/o Erasmus University Medical Center Rotterdam, Dr. Molewaterplein 50, NL-3015 GE Rotterdam (NL). **FOEKENS, Johannes Albert** [NL/NL]; c/o Erasmus University Medical Center Rotterdam, Dr. Molewaterplein 50, NL-3015 GE Rotterdam (NL). **LUIDER, Theo, Marten** [NL/NL]; John Coltranestraat 37, NL-3069 XK Rotterdam (NL).

(74) Agent: **Hatzmann, M.J.**; Vereenigde, Johan de Wittlaan 7, NL-2517 JR Den Haag (NL).

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(54) Title: METHOD FOR ESTABLISHING AND PREDICTING RESISTANCE OR RESPONSIVENESS TO CHEMOTHERAPY USING PROTEIN PROFILE

(57) Abstract: The present invention is directed to a method to predict responsiveness or resistance to chemotherapy in breast cancer comprising providing an optionally processed sample from a breast cancer patient as a test sample, wherein the sample comprise peptides proteins and/or genes; determining the amount of individual peptides, proteins and/or genes in the sample comparing the amount of an individual peptide and/or protein or the presence of a gene present in the test sample with the amount of a corresponding peptide or protein in a reference sample, wherein the individual peptide or protein in the test sample is selected from the group consisting SEQ ID 1-73 or proteins identified in table 3 and 4 or genes encoding for the peptides or proteins.

Title: Method for establishing and predicting resistance or responsiveness to chemotherapy using protein profile

The invention

FIELD OF THE INVENTION

The invention relates to the field of medical diagnostics, more specifically to the field of cancer diagnostics, especially breast cancer.

BACKGROUND OF THE INVENTION

Chemotherapy is a well-established treatment of breast cancer for patients for whom targeted therapies are not, or no longer, available. Once metastasis occurs, patients have a very poor survival, due to intrinsic or acquired resistance to drugs used. Ultimately, drug resistance of metastatic tumor cells is the main cause of death among breast cancer patients. At the moment there are no clinical markers that sufficiently predict the type of response to chemotherapeutic drugs. It is therefore of pivotal importance to identify new markers that can better predict the type of response or can act as new drugable targets for future therapy.

WO2008/085024 describes a method with which markers can be identified for a large number of diseases, thereby providing diagnostic tools for medical and veterinary diagnosis. It uses a combination of different MALDI techniques for marker detection.

WO 2008/133493 discloses a method for predicting the responsiveness to anti-estrogen treatment in breast cancer patients by assaying for one or more of proteins, which have found to be differentially expressed in responders and non-responders.

In March 2010 at the European Breast Cancer Conference in Spain researches found that an abnormality on chromosome 17, called CEP17, is a "highly significant indicator" that the tumor will respond to chemotherapy drugs called anthracyclines.

However, there is until now no protein marker for chemotherapy resistance in breast cancer. Protein markers have advantages over genetic markers. Protein markers may be used for clinical specimens that do not contain genetic

information, such as serum. In addition, genetic markers may not always give the right information, as it is the expressed product, e.g. the protein that induces a certain effect. Often it is the level of expression of proteins that indicate whether a disease has a certain outcome or not, while at the DNA level there is no difference. In fact, it has been shown that RNA expression levels poorly correlate with protein levels [1, 2].

Therefore there is still a need for proteinaceous biomarkers that are able to identify patients who will not respond to given therapy, and to select patients for various tailored treatments. This will enable doctors to stratify patients for the most suitable, tailored, treatment option, and avoid giving them toxic drugs that pose a heavy burden on the patient without being beneficial.

Surprisingly we have found peptide and protein markers that reliably predict the response to anthracycline based combination chemotherapy in breast cancer patients.

SUMMARY OF THE INVENTION

The present invention is directed to a method to predict responsiveness or resistance to chemotherapy in breast cancer comprising

a) providing an optionally processed sample from a breast cancer patient as a test sample, wherein the sample comprise peptides and/or proteins;

b) determining the amount of individual peptides in the sample

c) comparing the amount of an individual peptide present in the test sample with the amount of a corresponding peptide in a reference sample, wherein the individual peptide in the test sample is selected from the group consisting SEQ ID 1-73. The method may also use proteins as identified in table 3 and 4, and genes encoding the peptides and proteins of the present invention.

DETAILED DESCRIPTION

Definitions

The term "breast cancer" as used herein refers to the erratic growth and proliferation of cells that originate in the breast tissue. A group of rapidly dividing cells may form a lump or mass of extra tissue. These masses are called tumors. Tumors can either be cancerous (malignant) or non-cancerous (benign). Malignant

tumors penetrate and destroy healthy body tissues. A group of cells within a tumor may also break away and spread to other parts of the body. Cells that spread from one region of the body into another are called metastases. In preferred embodiments of aspects of the invention the term breast cancer refers to a malignant tumor that has developed from cells in the breast. The teachings of the present invention could be extended to chemotherapy- and anthracycline resistance of other tumor types.

The term "predict" as used herein refers to identifying or forecasting whether a breast tumor will exhibit resistance or develop resistance against chemotherapy.

The term "resistance" as used herein with reference to resistance to treatment or therapy refers to the fact that the normal therapeutic efficacy of the drug is not attained and that, for instance, a tumor continues to grow. Preferably, a tumor is classified as being resistant if the patient from whom the tumor was derived is classified as being a non-responder according to, for instance, the criteria used or set forth by the European Organisation for Research and Treatment of Cancer (EORTC) or the Union International Contre le Cancer (UICC).

The term “reference sample” as used herein refers to a control sample that is a sample from a tumor which is resistant or that is a sample from a tumor that is not resistant to chemotherapy or that is a sample from a normal breast tissue, and which is used for comparison purpose with a test sample, that is, in order to classify the test sample – or more specifically the levels of certain proteins or peptides therein, as being indicative of a resistant or responsive tumor. In a preferred embodiment of a method according to the invention, the control sample is from a resistant tumor. The level of a protein or peptide of a control sample as described herein or an expression profile of proteins or peptides as described herein is compared to the level or profile in a suspected tumor sample. If there is no significant difference between the levels or profiles, then the suspected tumor sample is determined as having the same indication as that control sample. A control sample may be from a single individual or from multiple individuals.

The term “reference profile” as used herein refers to a collection of expression levels for a multitude of given proteins or peptides, which expression levels in combination and/or relative to each other provide specific information which can be used for the purpose of comparison with other profiles. The collection in aspects of this

invention is indicative for a resistant or responsive phenotype of the tumor and can be used as a reference for comparison with the test profile. Preferably, such reference profile is based on samples of one or more non-resistant breast tumors. Alternatively, the reference profile may be based on samples of one or more resistant breast tumors. The skilled person is well aware of methods for comparing data collections such as test and reference profiles of gene expression data, and such methods can suitably be used in aspects of the present invention.

The term "chemotherapy-resistant tumor" as used herein refers to a tumor, including the individual cells therein, that is or becomes refractory to treatment by a chemotherapy or anthracycline based therapy such as FEC (= fluorouracil, epirubicine, cyclophosphamide), or FAC (= fluorouracil, doxorubicine, cyclophosphamide). In specific embodiments, the chemotherapy-resistant tumor becomes resistant to chemo treatment after initiation of the treatment and may occur during the treatment. In further specific embodiments, the resistance to chemotherapy manifests at about 2-24 months while the patient is receiving chemotherapy. In de novo resistance, the patient does not respond to initial therapy. Acquired resistance is where the patient develops metastatic disease during therapy.

Acquired resistance to chemotherapy is well-known in the art. In particular, breast cancer patients while undergoing treatment with chemotherapy have recurrence of the disease. In specific embodiments, the disease metastasizes during therapy with anthracycline based chemotherapy, which results in resistant metastases.

The term "chemotherapy or anthracycline-sensitive tumor" as used herein refers to a tumor, including the individual cells therein, that is treatable with chemotherapy or anthracycline. In specific embodiments, the chemotherapy or anthracycline -sensitive tumor remains sensitive during the treatment. In further specific embodiments, the chemotherapy or anthracycline -sensitive tumor is still sensitive up to at least about seven to ten years. Preferably, a tumor is classified as being sensitive if the patient from whom the tumor was derived is classified as being a responder as can be classified using EORTC or UICC criteria. Preferably, the responder is classified using EORTC or UICC criteria.

The term “different” as used herein with reference to the comparison of expression profiles or expression levels refers to a degree of difference which is

statistically significantly increased or decreased compared to a reference or a control. The term "significantly" or "statistically significant" refers to statistical significance and generally means that values differ two standard deviations (SD). In preferred embodiments, the difference is classified as statistically significant if the expression level is at least a 20 percent increased or decreased compared to expression level of the same expression product in control individuals. Preferably, the increase or decrease is at least 20, 25, 30, 35, 40, 45, 50, 75, 100, 150, 200 or 250 percent. Most preferably, the increase or decrease is at least 100 percent (herein also referred to as "one fold").

The term "level" as used herein refers to the measurable absolute level or a measurable relative level compared to the level of another protein. If the expression level is determined of a protein from more than one individual (as a control), usually the median or mean expression level of these individuals is used for comparison.

Some of the most common chemotherapy combinations used for breast cancer are CMF (cyclophosphamide, methotrexate and fluorouracil), FEC (epirubicin, cyclophosphamide and fluorouracil), FAC (fluorouracil, doxorubicine, cyclophosphamide), FEC-T (epirubicin, cyclophosphamide, fluorouracil and taxotere), E-CMF (epirubicin, followed by CMF), AC (doxorubicin (adriamycin) and cyclophosphamide), MMM (methotrexate, mitozantrone and mitomycin), MM (methotrexate and mitozantrone).

Drug resistance of metastatic tumor cells is the main cause of death among breast cancer patients. By comparing the mass spectra of tissues from patients with either breast cancer resistant to chemotherapy or breast cancer responsive to chemotherapy, the inventors have found that there are significant differences in the expression of peptide and proteins. They found a list of peptides and proteins that were predominantly present in tissue from patients with breast cancer resistant to chemotherapy and found another list of peptides and proteins that were predominantly present in tissue from patients with breast cancer responsive to chemotherapy.

Peptides with SEQ ID NO 1-28 and proteins from table 3 are predominantly present in tissue from patients with breast cancer responsive to chemotherapy. The following proteins are predominantly present in tissue of breast cancer patients that are responsive to chemotherapy: 26S proteasome non-ATPase regulatory subunit 6,

40S ribosomal protein, 60S ribosomal protein, Aflatoxin B1 aldehyde reductase member 2, Alpha-enolase, Bifunctional aminoacyl-tRNA synthetase, Calumenin, Catechol O-methyltransferase , Clathrin heavy chain 1, Coatomer subunit gamma, Collagen alpha-3(VI) chain, D-3-phosphoglycerate dehydrogenase, Enolase-phosphatase E1, Eukaryotic translation initiation factor 5A-1, Extracellular superoxide dismutase [Cu-Zn], Ezrin, Fatty acid synthase, Filamin-A, Glyoxalase domain-containing protein 4, GTP cyclohydrolase 1 feedback regulatory protein, Hemoglobin subunit beta, Heterogeneous nuclear ribonucleoprotein U-like protein 1, Histone H2B type 1-J, Histone H2B type 1-K, Histone H2B type 2-F, Histone H4, Hydroxymethylglutaryl-CoA synthase, mitochondrial, Inner nuclear membrane protein Man1, Keratin, type I cytoskeletal 18, Keratin, type I cytoskeletal 19, Keratin, type II cytoskeletal 8, Lysyl-tRNA synthetase, Matrin-3, Moesin, Neuroblast differentiation-associated protein AHNAK, Peptidyl-prolyl cis-trans isomerase, mitochondrial, Platelet-activating factor acetylhydrolase IB subunit gamma, Inositol-3-phosphate synthase, Protein disulfide-isomerase A6, Receptor tyrosine-protein kinase erbB-2, Seryl-tRNA synthetase, cytoplasmic, SH3 domain-binding glutamic acid-rich-like protein 3, Stress-induced-phosphoprotein 1, Tetranectin, Thioredoxin, Tubulin beta chain, Tubulin beta-3 chain, UDP-glucose 6-dehydrogenase, UMP-CMP kinase, UPF0364 protein C6orf211, Valyl-tRNA synthetase, Zinc-alpha-2-glycoprotein, Inter-alpha-trypsin inhibitor heavy chain H4, Serine/threonine-protein phosphatase 2A catalytic subunit alpha isoform, NADH dehydrogenase [ubiquinone] iron-sulfur protein 5, and Complement factor I.

The enhanced level of these proteins are thus indicative of breast cancer that is responsive to chemotherapy, especially anthracycline based chemotherapy, in particular FEC and FAC. Preferred protein are selected from the group consisting of Aflatoxin B1 aldehyde reductase member 2 P, Alpha-enolase , Catechol O-methyltransferase , Coatomer subunit gamma , Matrin-3 P , Calumenin , Fatty acid synthase , Ezrin , Histone H4 4A P , Moesin , 26S proteasome non-ATPase regulatory subunit 6 P , Enolase-phosphatase E1 P , Receptor tyrosine-protein kinase erbB-2 P , Thioredoxin =1 , UDP-glucose 6-dehydrogenase , UMP-CMP kinase P , Heterogeneous nuclear ribonucleoprotein U-like protein 1 L1 P , Inner nuclear membrane protein Man1 P , Keratin, type II cytoskeletal 8 , Lysyl-tRNA synthetase , Zinc-alpha-2-glycoprotein P , GTP cyclohydrolase 1 feedback regulatory protein P ,

UPF0364 protein C6orf211 11 P , Clathrin heavy chain 1 , Extracellular superoxide dismutase [Cu-Zn] , Glyoxalase domain-containing protein 4 P , Aflatoxin B1 aldehyde reductase member 2 P , Alpha-enolase , Catechol O-methyltransferase , Coatomer subunit gamma.

Most preferred proteins are selected from the group consisting of Aflatoxin B1 aldehyde reductase member 2 P, Alpha-enolase , Catechol O-methyltransferase , Coatomer subunit gamma , Matrin-3 P , Calumenin , Fatty acid synthase , Ezrin , Histone H4 4A P , Moesin , 26S proteasome non-ATPase regulatory subunit 6 P , Enolase-phosphatase E1 P , Receptor tyrosine-protein kinase erbB-2 P , Thioredoxin =1 , UDP-glucose 6-dehydrogenase , UMP-CMP kinase P.

In contrast the peptides with SEQ ID 29-73 and proteins from table 4 are predominantly present in tissue from patients with breast cancer resistant to chemotherapy. The following proteins are predominantly present in tissue of breast cancer patients that are resistant to chemotherapy: 26S proteasome non-ATPase regulatory subunit 11, 60S ribosomal protein L21, 60S ribosomal protein L7, 6-phosphofructokinase, muscle type, Actin-related protein 2, Aldehyde dehydrogenase, mitochondrial, Alpha-1-antichymotrypsin Alpha-actinin-1,, Alpha-actinin-4, Amyloid beta A4 protein, Annexin A6, Annexin A6, Apoptosis-inducing factor 1, mitochondrial ATP-dependent DNA helicase 2 subunit 2, ATP-dependent RNA helicase A, Beta-glucuronidase, Calmodulin-like protein 5, Calnexin, Calumenin, Carbonic anhydrase 2, Clusterin, Collagen alpha-3(VI) chain, Complement C4-B, Cytosol aminopeptidase, Desmin, Dipeptidyl-peptidase 2, Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 2, Drebrin-like protein, Elongation factor 1-delta, Elongation factor 2,, Erythrocyte band 7 integral membrane protein, Extended-synaptotagmin-1, Fibronectin Filamin-A, Galectin-3, Glutamate--cysteine ligase regulatory subunit Glyceraldehyde-3-phosphate dehydrogenase, Heat shock 70 kDa protein 4, Heat shock protein HSP 90-alpha, Heat shock protein HSP 90-beta, Heterogeneous nuclear ribonucleoprotein U, Heterogeneous nuclear ribonucleoprotein U-like protein 1, Histone H2B type 1-K, Histone H2B type 2-F, Histone H4, Ig kappa chain C region, Importin-9, Inorganic pyrophosphatase, Proteasome subunit alpha type-6, Inositol-3-phosphate synthase, Inter-alpha-trypsin inhibitor heavy chain H2, Isocitrate dehydrogenase [NADP], mitochondrial, Keratin, type II cytoskeletal 1, Keratin, type II cytoskeletal 7, Keratin, type II cytoskeletal 8, KH domain-containing,

RNA-binding, signal transduction-associated protein 1, Leucine-rich repeat-containing protein 59, Mannose-6-phosphate receptor-binding protein 1, Moesin, Myosin-11, Myosin-9, Neuroblast differentiation-associated protein AHNAK, Nuclear autoantigenic sperm protein, Nucleolar phosphoprotein p130, Ornithine aminotransferase, mitochondrial, Pigment epithelium-derived factor, Plectin-1, Protein arginine N-methyltransferase 5, Protein disulfide-isomerase A3, Protein disulfide-isomerase A6, Protein-arginine deiminase type-2, Pyruvate dehydrogenase E1 component subunit alpha, somatic form, mitochondrial, Rab GDP dissociation inhibitor alpha, Rab GDP dissociation inhibitor alpha 1, Rab GDP dissociation inhibitor beta 2, Rab GDP dissociation inhibitor beta , Ras-related protein Rab-5B, RNA-binding protein Raly, Serum albumin, Serum albumin, Spectrin beta chain, brain 1, Stress-induced-phosphoprotein 1, Succinate dehydrogenase [ubiquinone] iron-sulfur subunit, mitochondrial, Talin-1, T-complex protein 1 subunit epsilon, Transketolase, Tubulin alpha-1A chain, Tubulin alpha-1B chain, Tubulin alpha-1C chain, Tubulin alpha-4A chain, Twinfilin-1, Ubiquitin-conjugating enzyme E2 N, Ubiquitin-conjugating enzyme E2 variant 1, UDP-glucose:glycoprotein glucosyltransferase 1, Voltage-dependent anion-selective channel protein 1, 40S ribosomal protein S19, Vimentin, Collagen alpha-1(VI) chain, Glyceraldehyde-3-phosphate dehydrogenase, Keratin, type I cytoskeletal 17, 40S ribosomal protein S5, and Beta-2-glycoprotein 1.

Preferred proteins are selected from the group consisting of Heterogeneous nuclear ribonucleoprotein U-like protein 1 P , Isocitrate dehydrogenase [NADP]mitochondrial 1 , Keratintype II cytoskeletal 1 1 , Pigment epithelium-derived factor P , UDP-glucose:glycoprotein glucosyltransferase 1 , Alpha-actinin-1 , Histone H4 P , Nuclear autoantigenic sperm protein 1 , T-complex protein 1 subunit epsilon 1 , Mannose-6-phosphate receptor-binding protein 1 P , Annexin A6 , Calumenin 1 , Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 2 1 , Extended-synaptotagmin-1 , Importin-9 1 , 6-phosphofructokinasemuscle type 1 , Beta-glucuronidase 1 , Fibronectin , Stress-induced-phosphoprotein 1 , Calmodulin-like protein 5 , Clusterin , ATP-dependent DNA helicase 2 subunit 2 , Desmin , Elongation factor 1-delta , Protein-arginine deiminase type-2 , Nucleolar phosphoprotein p130 , Calnexin 1 , Inorganic pyrophosphatase 1 , Collagen alpha-3(VI) chain , Heat shock 70 kDa protein 4 , Ig kappa chain C region 1 , Ornithine

aminotransferasemitochondrial , Rab GDP dissociation inhibitor alpha 1 , Rab GDP dissociation inhibitor beta 2 , Serum albumin , Alpha-actinin-4 , 60S ribosomal protein L7 1 , Histone H2B type 1-K K P , Histone H2B type 2-F F P , Ras-related protein Rab-5B , Transketolase , Alpha-1-antichymotrypsin P , 60S ribosomal protein L21 , Serum albumin , Drebrin-like protein 1 , Twinfilin-1 , Actin-related protein 2 , Glutamate--cysteine ligase regulatory subunit 1 , RNA-binding protein Raly 1 , Elongation factor 2 1 , Tubulin alpha-1A chain , Tubulin alpha-1B chain , Tubulin alpha-1C chain , Dipeptidyl-peptidase 2 1 , Protein disulfide-isomerase A3 , Amyloid beta A4 protein , and Voltage-dependent anion-selective channel protein 1.

More preferred proteins are selected from the group consisting of Heterogeneous nuclear ribonucleoprotein U-like protein 1 P , Isocitrate dehydrogenase [NADP]mitochondrial 1 , Keratintype II cytoskeletal 1 1 , Pigment epithelium-derived factor P , UDP-glucose:glycoprotein glucosyltransferase 1 , Alpha-actinin-1 , Histone H4 P , Nuclear autoantigenic sperm protein 1 , T-complex protein 1 subunit epsilon 1 , Mannose-6-phosphate receptor-binding protein 1 P , Annexin A6 , Calumenin 1 , Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 2 1 , Extended-synaptotagmin-1 , and Importin-9 1.

The enhanced level of these proteins are thus indicative of breast cancer that is resistant to chemotherapy, especially anthracycline based chemotherapy, in particular FEC and FAC.

In a first aspect the present invention is directed to a method to predict responsiveness or resistance to chemotherapy in breast cancer comprising

a) providing an optionally processed sample from a breast cancer patient as a test sample, wherein the sample comprise peptides and/or proteins;

b) determining the amount of an individual peptide in the test sample;

c) comparing the amount of the individual peptide present in the test sample with the amount of a corresponding peptide in a reference sample, wherein the individual peptide in the test sample is selected from the group consisting SEQ ID 1-73.

In a second aspect the present invention is directed to a method to predict responsiveness or resistance to chemotherapy in breast cancer comprising

a) providing an optionally processed sample from a breast cancer patient as a test sample, wherein the sample comprise peptides and/or proteins;

b) determining the amount of a individual protein in the test sample;

c) comparing the amount of an individual protein present in the test sample with the amount of a corresponding protein in a reference sample, wherein the individual protein in the test sample is selected from the group consisting of proteins as identified in table 3 and 4.

Also the genes coding for the peptides and proteins of the present invention may be used as markers for either responsiveness or resistance to chemotherapy. Therefore a third aspect of the present invention is related to a method to predict responsiveness or resistance to chemotherapy in breast cancer comprising

(a) providing an optionally processed sample from a breast cancer patient as a test sample, wherein the test sample comprises genetic information;

(b) determining the presence or the expression level of a gene in the test sample wherein the gene encodes for a peptide or protein selected from the group consisting of SEQ ID 1-73 and proteins as identified in table 3 and 4.

Preferably the reference sample is an optionally processed sample from a breast cancer patient being responsive to chemotherapy or from a breast cancer patient being resistant to chemotherapy. Suitably the reference sample is a pooled sample of more than one breast cancer patient, suitably more than 10, more suitably more than 50, and even more than 100 cancer patients. In a preferred embodiment the reference sample is a combined pooled sample from more than two breast cancer patients, both from a patient responsive and from a patient resistant to chemotherapy. By comparing the peptides and proteins profile of a tissue of a patient with breast cancer, still unknown whether the cancer is responsive to chemotherapy or resistant, to the list of peptides and proteins of the present invention, a doctor can see whether the patient will be responsive to chemotherapy or not.

In a preferred embodiment the optionally processed sample is a body tissue sample or body fluid processed by subjecting the sample to laser capture microdissection to provide collections of microdissected cells, the collections preferably amounting to about 200- 3,000 cells. The body tissue sample or body fluid or collections of microdissected cells is suitably processed by subjection to protein digestion, preferably using trypsin. This treatment provides processed samples comprising peptide fragments from the proteins in said samples. Suitable body tissue

is selected from the group consisting of tissues from breast tumor, breast tumor stroma, and lymph node. Suitable body fluid is selected from the group consisting of blood, serum, cerebrospinal fluid (CSF), urine, saliva and nipple aspirate. Preferably the body fluid comprises about 0.05- 5 mg/ml of protein. In a preferred embodiment the test sample, consists of an amount of 1-10 µl containing 0.05-5 mg/ml protein is subjected to MALDI- FT-ICR mass spectrometry. Another suitable test sample may be circulating tumor cells that are collected from blood or other fluids. Body fluids and circulating tumor cells are more easily obtained from a patient than tissue sample that often need local anaesthetic to obtain. Preferably the peptides in the processed sample are in a mass range of 800 to 4,000 Da. In another preferred embodiment, the individual proteins are in a mass range of 4,000 to 75,000 Da.

The present inventors have found that especially individual peptide selected from the group consisting of SEQ ID NO 1-7, 25-54 and 73-79 give a good prediction to either responsiveness or resistant to chemotherapy. Even more, the peptide selected from the group consisting of SEQ ID NO 1-4, 25-34 and 73-79, give an even better prediction. In addition, especially the protein from the group consisting of proteins as identified in table 3 and 4 with a p value lower than 0.03, preferably lower than 0.01 are good predictors of responsiveness and resistance. Furthermore, the gene encoding for a peptide selected from the group consisting of SEQ ID NO 1-7, 25-54 and 73-79, more preferably selected from the group consisting of SEQ ID NO 1-4, 25-34, and 73-79, or the gene encoding for the a protein selected from the group comprising proteins as identified in table 3 and 4 with p value lower than 0.03, more preferably 0.01 are also good predictors.

The inventors have found that especially the peptides selected from the group consisting of SEQ ID NO 1-70, more preferably SEQ ID NO 1-7, 25-54 and 73-79 even more, SEQ ID NO 1-4, 25-34 and 73-79, are present in a higher amount in tissue from breast cancer patients that are responsive to chemotherapy. In a preferred method the amount of the individual peptide or protein present in the test sample is higher than the amount of a peptide or protein having a corresponding mass in the reference sample indicates a tumor responsive for chemotherapy. A preferred reference sample for determining responsiveness to chemotherapy is a reference sample that comprises one or more samples from one or more breast cancer patients being resistant to chemotherapy or a reference sample that comprises samples from

both breast cancer patients resistant to chemotherapy and from breast cancer patients that are responsive to chemotherapy. The advantage of the present invention is that only the individual peptide or protein selected from the group consisting of SEQ ID NO 1-28 preferably from the group consisting of SEQ ID NO 1-7 and 25-28, more preferably SEQ ID NO 1-4, 25-28, and proteins as indentified in table 3, preferably proteins from table 3 with a p value lower than 0.03, more preferably with a p value lower than 0.01, are measured. This saves time, and provides a more reliable method for determining whether a patient is responsve to chemotherapy or not.

The peptides and proteins selected from the group consisting of SEQ ID NO 29-79, preferably SEQ ID NO 29-54, and 73-79, more preferably SEQ ID NO 29-34, and 73-79, and proteins as identified in table 4, preferably with a p value lower than 0.03, more preferably 0.01, were found to be indicative for breast cancer resistant to chemotherapy. In order to determine whether a breast cancer patient may be resistant to chemotherapy the amount of the individual peptide or protein present in the test sample determined . If the amount of the peptide or protein is higher than the amount of a peptide or protein having a corresponding mass in the reference sample indicates a tumor resistant for chemotherapy. To determine chemotherapy resistance a preferred reference sample comprises one or more samples from one or more breast cancer patients being responsive to chemotherapy, or a reference sample that comprises samples from both breast cancer patients resistant to chemotherapy and from breast cancer patients that are responsive to chemotherapy is used. The advantage of the present invention is that only the individual peptide or protein from the test sample is selected from the group consisting of SEQ ID NO 29-73, preferably from the group consisting of SEQ ID NO 29-54 and 73-79, more preferably SEQ ID NO 29-34 and 73-79, and proteins as indentified in table 4, preferably proteins from table 4 with a p value lower than 0.03, more preferably lower than 0.01.

Suitably in practice, a tissue from a breast cancer patient unknown whether to respond or being resistant to chemotherapy will be compared to two reference samples, one comprising tissue from breast cancer patients responsive to chemotherapy, one comprising tissue from breast cancer patients resistant to chemotherapy. In a most preferred embodiment, the reference sample is a pooled combined sample from more than two patients, and from breast cancer patients

responsive to chemotherapy, and from breast cancer patients resistant to chemotherapy. In addition, suitably the peptides and/or proteins being indicative for responsiveness (i.e. SEQ ID NO 1-28, preferably 1-7, more preferably 1-4; proteins identified in table 3, preferably wit a p value lower than 0.03, more preferably lower than 0.01), together with the peptides and/or proteins being indicative for resistance (i.e. SEQ ID NO 29-79, preferably 29-54, and 73-79, more preferably 29-34, and 73-79; proteins identified in table 4, preferably with a p value lower than 0.03, more preferably lower than 0.01) are measured. This will give a differential peptide and/or protein profile that will indicate whether a patient may be responsive to chemotherapy (having more peptides and or proteins corresponding to SEQ ID NO 1-28 or proteins identified in table 3) or resistant to chemotherapy (having more peptides and/or protein corresponding to SEQ ID 29-79 or proteins identified in table 4).

For the present invention any method that is suitable to quantify the amount of proteins or peptides in a sample may be used. Preferred methods to determine the amount of peptide or protein is a method selected from the group consisting of mass spectrometry, peptide array, immuno-histochemical assay, ELISA, Protein array, Western Blot, and immunoaffinity chromatography.

In a preferred embodiment the amount of the individual peptide or protein in the test sample is at least 20% higher than the amount of the corresponding peptide or protein in the reference sample. Preferably the amount of the individual peptide or protein is at least 30%, more preferably at least 50%, even more preferably at least 60% or even at least 100% higher than the amount of the corresponding peptide or protein in the reference sample. Suitably the test sample is calibrated or normalised by e.g. an internal standard.

In an other preferred embodiment at least 5 of the individual peptides or proteins are present in a higher amount in the test sample than the corresponding peptides and proteins in the reference sample. Preferably at least 10, more preferably at least 20, or even at least 30 peptide or protein selected from the group consisting of SEQ ID NO 1-73, and proteins as indentified in table 3 and 4 are present in an higher amount in the test sample than the corresponding protein in the reference sample. Suitably, at least 2, more suitably at least 5, most suitably at least 10, or even at least 15 peptide or protein selected from the group consisting of SEQ ID NO 1-7, 25-54 and

73-79, andthe proteins from table 3 and 4 with a p value lower than 0.03 are present in an higher amount in the test sample than the corresponding peptide or protein in the reference sample. Most suitably, at least 2, more suitably at least 5, most suitably at least 10, peptide or protein selected from the group consisting of SEQ ID NO 1-4, 25-34 and 73-79, and the proteins from table 3 and 4 with a p value lower than 0.01 are present in an higher amount in the test sample than the corresponding peptide or protein in the reference sample.

The tissue from the breast cancer patient may also be genetically tested. In a preferred method the presence or level of a gene encoding for a peptide selected from the group consisting of SEQ ID NO 1-28, preferably from the group consisting of SEQ ID NO 1-7, 25-28, more preferably 1-4, 25-28 or wherein the presence of a gene encoding for a protein selected from the group consisting of proteins as indentified by table 3, preferably proteins from table 3 with a p value lower than 0.03, more preferably lower than 0.01, indicates a tumor responsive to chemotherapy.

Furthermore the presence or the expression level of a gene encoding for a peptide selected from the group consisting of SEQ ID 29-73, preferably from the group consisting of SEQ ID 29-54 and 73-79, more preferably 29-34 and 73-79 or wherein the presence of a gene encoding for a protein selected from the group consisting of proteins as indentified by table 4, preferably of proteins from table 4 with a p value lower than 0.03, more preferably lower than 0.01, indicates a tumor resistant to chemotherapy.

Any method to detect a gene or to determine the expression level of a gene is suitable for the method of the present invention. In a preferred embodiment the presence or expression level of a gene is identified with a method selected from the group consisting of DNA array, cDNA array, oligo dt array, exon array, SNP array, PCR, Q-PCR, RT-PCR. Preferably at least at least 5 of the genes encoding for a peptide or protein according to the present invention are present in the test sample. Preferably at least 10, more preferably at least 20, or even at least 30 genes encoding peptide or protein selected from the group consisting of SEQ ID 1-73, or from the group comprising proteins as indentified by table 3 and 4, are present. Suitably, at least 2, more suitably at least 5, most suitably at least 10, or even at least 15 genes encoding for a peptide or protein selected from the group consisting of SEQ ID NO 1-7, 25-54 and 73-79 and proteins from table 3 and 4 with a p value lower than 0.03, are

present in the test sample. Even more suitably Suitably, at least 2, more suitably at least 5, most suitably at least 10 genes encoding for a peptide or protein selected from the group consisting of SEQ ID NO 1-4, 25-34 and 73-79 and proteins from table 3 and 4 with a p value lower than 0.01, are present in the test sample.

The present invention is suitable to detect responsiveness or resistance to chemotherapy in breast cancer. More suitably the chemotherapy is anthracycline based preferably selected from the group consisting of FEC, FAC.

In yet another aspect of the invention antibodies and siRNA against peptides with SEQ ID NO 1-73 and proteins identified in table 3 and 4 may be used in a therapy against breast cancer. In a preferred embodiment antibodies and siRNA against peptides with SEQ ID NO 1-7, 25-54 and 73-79 and proteins of table 3 and 4 with a p value lower than 0.03, more preferably against peptides with SEQ ID NO 29-54 and 73-79 and proteins from table 4 with a p value lower than 0.03. In even more preferred embodiment antibodies and siRNA against peptides with SEQ ID NO 1-4, 25-34 and 73-79 and proteins of table 3 and 4 with a p value lower than 0.01, more preferably against peptides with SEQ ID NO 29-34 and 73-79 and proteins from table 4 with a p value lower than 0.01. As these peptides and proteins are differentially expressed in certain types of breast cancer, they may be suitable targets for breast cancer therapy.

Yet another aspect of the present invention is directed to the use of peptides with SEQ ID NO 1-73 or proteins as indentified in table 3 and 4 to identify compounds for use in a therapy against breast cancer. In a preferred embodiment peptides with SEQ ID NO 1-7, 25-54 and 73-79 and proteins of table 3 and 4 with a p value lower than 0.03 are used, more preferably peptides with SEQ ID NO 29-54 and 73-79 and proteins from table 4 with a p value lower than 0.03 are used. In an even more preferred embodiment peptides with SEQ ID NO 1-4, 25-34 and 73-79 and proteins of table 3 and 4 with a p value lower than 0.01 are used, more preferably peptides with SEQ ID NO 29-34 and 73-79 and proteins from table 4 with a p value lower than 0.01 are used.

METHODS

Experimental procedures

Reagents

PEN membrane (1 mm) covered glass slides and PALM caps were purchased from PALM laboratories (Carl Zeiss MicroImaging, GmbH, Munich, Germany), PCR clean LoBind 0.5ml tubes were from Eppendorf AG (Hamburg, Germany), trypsin gold mass spectrometry grade was obtained from Promega (Promega Benelux B.V., Leiden, Netherlands), RapiGest SF surfactant and LC glass vials were from Waters Corporation (Milford, MA, USA), MTP AnchorChip™ 600/384 T F target plate, 2,5-dihydroxybenzoic acid (DHB), and peptide calibration standard (containing angiotensin I and II, substance P, bombesin, rennin substrate, ACTH clip 1-17, ACTH clip 18-39, and somatostatin 28) were obtained from Bruker (Bruker Daltonik GmbH, Bremen, Germany), HPLC grade water and acetonitrile (ACN) were purchased from Fluka analytical (Sigma-Aldrich Corporation, St. Louis, MO, USA), and trifluoroacetic acid (TFA) was purchased from Thermo Fischer Scientific Inc. (Rockford, IL, USA).

Patients and tumor tissues

For the discovery phase of this study, we have used 50 snap frozen primary breast tumor tissues present in our N_2 bio bank, of which long-term clinical follow-up was available. Tissues were selected from patients that received anthracyclin-based chemotherapy; 13 FEC (5-fluorouracil + epirubicin + cyclofosfamide) / 37 FAC (5-fluorouracil + doxorubicin [Adriamycin®] + cyclofosfamide). Of these patients, 22 showed objective response (OR), 5 stable disease (SD) >6 months, 21 progressive disease (PD), and 5 SD <6 months upon treatment. Furthermore, 31 tumors were estrogen receptor (ER) negative, and 19 were ER positive, as assessed by ligand binding assay or enzyme-linked immuno sorbent assay (>=10 fmol/mg cytosolic protein). Clinical response was defined by standards of the International Union against Cancer criteria of tumor response [3].

This study was approved by the Medical Ethics Committee of the Erasmus MC Rotterdam, The Netherlands (MEC 02.953), and was performed in accordance to the Code of Conduct of the Federation of Medical Scientific Societies in the Netherlands.

Where ever possible we adhered to the Reporting Recommendations for Tumor Marker Prognostic Studies REMARK [4].

Laser Capture Microdissection (LCM)

LCM was performed on 10 µm tissue cryosections that were fixed in ice-cold 70% ethanol and stained with hematoxylin as previously described [5]. Laser microdissection and pressure catapulting (LMPC) was performed directly after staining. Tumor epithelial and stromal cells were separately collected, using a P.A.L.M. LMPC device, type P-MB (Carl Zeiss MicroImaging, GmbH, Munich, Germany). From each cryosection, an area of ~400,000 μm^2 that corresponds to ~4,000 cells (area x slide thickness / 1000 μm^3 cell volume) was collected in P.A.L.M. tube caps containing 10 µl of 0.1% RapiGest, and then spun down into 0.5 ml Eppendorf Protein LoBind tubes. Collected cells were stored at –80°C until further processing. Since we used small numbers of microdissected cells in this study, protein concentration was typically below the detection limit of any protein assay. Hence, protein concentration for samples undergoing MS analysis was estimated based on microdissected tissue area and extrapolations from protein assays performed on whole tissue lysates (i.e., ~4,000 cells corresponds to ~400 ng of total protein) as described previously [6].

Sample preparation

Microdissected cells were lysed by sonication directly in RapiGest solution in a cup horn sonifier bath, using an Ultrasonic Disruptor Sonifier II (Model W-250/W-450, Bransons Utrasonics, Danbury, CT, USA) for 1 min at 60% amplitude. Proteins were subsequently equilibrated for 2 min at 37°C, and denatured at 99°C for 5 min, and processed for overnight trypsin digestion at a 1:20 v/v ratio, as previously described [5] and according to the instructions of the RapiGest manufacturer. Peptides were lyophilized and stored in –80°C until further analysis. Prior to FTICR MS analysis, samples were reconstituted in 6 µl 50% ACN, 0.1% TFA. In order to fully cleave RapiGest in the samples, 0.6 µl 500mM HCl was added, shortly mixed, incubated at 37°C for 45 minutes, and centrifuged for 10 minutes at 10,600g to pellet any contaminating particulate material.

MALDI-FTICR MS

For MALDI-FTICR MS analysis, a matrix solution was prepared from 10 mg/ml DHB in 0.1% TFA in MilliQ water. The matrix solution was vortexed for 1 min prior to use. 0.5µl DHB matrix was spotted on an AnchorChip™ target plate, after which 0.5 µl prepared sample was added and mixed on the spot. Peptide calibration standard was spotted on distinct calibration positions together with DHB matrix. Samples were spotted in duplicate and left on the AnchorChip™ for drying prior to MALDI-FTICR analysis on the Apex IV Qe instrument with a 9.6 tesla magnet equipped with a 20 Hz nitrogen laser (Bruker Daltonik). For each measurement, 100 scans of 10 shots each at 60% laser power were accumulated, and spectra were acquired in the mass range of 800-4,000 m/z using XMass software v7.0.8 (Bruker). MALDI-FTICR data were acquired at 512K and further processed with a Gaussian filter and two zero fillings. For each sample, mass spectra were recorded from duplicate spots and accumulated in to one final spectrum. Prior to sample analysis, external calibration was performed on the peptide calibration standard using a quadratic equation. In addition, a post-acquisition internal calibration step was performed in DataAnalysis software, version 3.3, build 139 (Bruker) using auto-lytic trypsin MH+ peptide masses (842.50940, 1045.56370, 2211.10400 m/z), and ubiquitous keratin (1179.60100 m/z), actin (1198.70545, 1790.89186 m/z) and histone MH+ peptide masses (976.44824, 1515.74913, 2343.16486, 3183.61423 m/z), so that accuracy of <1ppm could be acquired. Spectra in which less than 5 calibration peaks were present, were excluded from further data analysis because of poor quality, which was the case for 1 OR tumor, 1 PD tumor, 4 OR stroma, and 3 PD stroma spectra.

Data analysis

All isotopic peaks with a signal-to-noise ratio >4 were annotated using DataAnalysis software package, version 3.3, build 139 (Bruker Daltonik). Peak lists were saved in general text format and imported into the home made script in the R-program, Peptrix v2.4.1. The Peptrix package was used to compare mass spectra for identification of differentially abundant peaks by label free quantitation using MS peak intensity. A matrix file was generated indicating presence, absence, and intensities of peaks in different samples. Peak masses present in less than 4 samples were omitted from the matrix. Within Peptrix, a univariate Wilcoxon-Mann-Whitney

rank sum test was performed for pair wise comparisons between tumor and stromal cells and between OR and PD patients. Peaks were considered to be differentially abundant if p-values were <0.05. Differentially abundant peak masses (annotated as doubly and triply charged peptide masses) were subjected to targeted MS/MS analysis for peptide identification purposes.

LC-MS/MS

Identification of differential peptide masses was performed by nLC-MS/MS analysis on an Orbitrap (Thermo Fischer Schientific, Germany) XL mass spectrometer, using an inclusion list of target peptide masses. Tryptic digests of whole tumor tissue lysates were subjected to nLC separation, using a 15 cm x 75 µm inner diameter C18 reversed phase column. Peptides were eluted from the column by the following binary gradient: from 100% solvent A (0.1% formic acid in water) to 75% solvent A/ 25% solvent B (80% acetonitrile and 0.08% formic acid in water) in 120 minutes, followed by 25-50% solvent B for a further 60 minutes, with a column flow rate of 300 nl/min. A data dependent acquisition was performed in the high resolution Orbitrap with a survey scan from 400-1800 Th. Based on this survey scan, up to 5 ions corresponding to the masses in the inclusion list were fragmented if present. If no target ions were present, the 5 most intensive ions were selected for collision-activated dissociation fragmentation, after which these masses were excluded for further MS/MS analysis for 3 minutes.

Protein identification and quantitation

Bioworks 3.2 software package (Thermo Fischer Scientific, Germany) was used for peak picking and MS/MS identification, and corresponding SEQUEST features, using HUPO criteria, were obtained from the UniProt and SwissProt databases. The number of allowed missed cleavages was set to 1, mass tolerance for precursor ions was 10 ppm, and for fragment ions 0.5 Da. The cut-off for matching identified peptides with theoretical mass was 2 ppm.

Precursor MS scan peptide mass intensities were used for peptide and protein quantitation.

Results

Primary breast cancer tissues from patients that received anthracycline-based chemotherapy in advanced stage of disease were subjected to global proteome analysis. For the identification of proteins that associate with chemotherapy resistance, we compared proteomes of tissues from patients responsive (OR + SD >6 months) to therapy with tissues from therapy-resistant (PD + SD <6 months) patients. Patient characteristics are summarized in Table 1. Tumor tissues were subjected to LCM, and, whenever possible, ~4000 tumor and surrounding stromal cells were collected from each tissue. Tumor cells were procured from 20 different OR, 10 SD, and 16 PD tissues, and stromal cells were collected from 18 OR, 10 SD, and 18 different PD tissues. Tryptic digests were prepared and analyzed in duplicate by MALDI-FTICR MS.

Comparative proteomics of breast cancer tissues

Mass spectra of sufficient quality were obtained for 19 OR tumor, 14 OR stroma, 15 PD tumor, 15 PD stroma, and all SD samples.... Using Peptrix software, OR and PD tumor, OR and PD stroma, and all tumor versus all stroma mass sprectra were compared based on peak intensity and peak count. All detected peaks in all mass spectra were populated into a matrix file, which was than used for further statistical analysis. In total, 3,948 peaks were detected in all tumor samples, taking all isotopic peaks into account. In stromal samples, a total of 4,715 peaks were detected. Wilcoxon-Mann-Whitney rank sum analysis revealed the presence of 135 differential peak masses ($p<0.05$) between OR and PD tumor cells, of which 28 peak masses had a p-value <0.01 (supplemental table S1). Furthermore, 231 differential peaks were observed between OR and PD stromal cells ($p<0.05$), of which 17 had a p-value <0.01. Comparison of tumor and stromal samples revealed a very large difference in sample composition

Identification of differentially abundant proteins

For the identification of peptide sequences corresponding to differentially abundant peak masses, a mass search was performed. Differential peak masses were matched to our list of identified proteins obtained through nLC-MS/MS analysis. From the 135 differential peak masses between OR and PD tumor cells, 70 were matched with

peptide sequences, representing 65 different proteins (see tables 1-4). 11 differential stromal peak masses were matched to peptides and their corresponding proteins.

Based on this putative peptide profile, chemotherapy responding breast cancer patients can be distinguished from chemotherapy resistant patients (fig 1). In a principal components analysis, it was shown that chemotherapy-resistant breast cancer patients (light dots) can be separated from chemotherapy responsive breast cancer patients (dark dots).

Figure 1. Principal Components Analysis of breast cancer patients. Groups can be separated from each other based on the differentiating peptide profile.

Table 1: Peptides identified that are OR:PD positive

SEQ ID NO	Mass (Da)	Peptide sequence	OR:PD	P-value	Tissue
1	1803.938924	AAVPSGASTGIYEALELR	+	0.003743	Tumor
1	1803.933324	AAVPSGASTGIYEALELR	+	0.025514	Tumor
2	1968.061424	IGPYQPNVPVGIDYVIPK	+	0.003802	Tumor
3	1309.695424	TVTAMDVVYALK	+	0.008458	Tumor
4	1478.755424	YGYTHLSAGELLR	+	0.009611	Tumor
5	1351.669424	TEMENEFVLIK	+	0.016072	Tumor
6	2904.330324	FVDTDIWNQYLEYQQSLLNESDGK	+	0.016072	Tumor
7	1336.673924	AVVVHAGEDDLGR	+	0.017989	Tumor
8	1614.829824	AILVDLEPGTMDSVR	+	0.031053	Tumor
9	1697.852824	FTPGTFTNQIQAAFR	+	0.031312	Tumor
10	1279.710824	RLNKHEENLK	+	0.031312	Tumor
11	1443.803324	KLNVTEQEKIDK	+	0.031312	Tumor
12	1697.852824	THVADFAPEVAWVTR	+	0.031312	Tumor
13	1612.846224	SLDEISQPAQELKR	+	0.031312	Tumor
14	1297.733624	VHLVGIDIFTGK	+	0.031312	Tumor
15	2471.299324	LHLSGIDANPNALFPPVEFPAPR	+	0.031312	Tumor
16	1908.909624	EGPYSISVLYGDEEVPR	+	0.031312	Tumor
17	1743.791024	AMGIMNSFVNDIFER	+	0.031312	Tumor
18	1738.758024	CVSPEEFTEIMNQR	+	0.031312	Tumor
19	1359.727024	VGPVAATYPMLNK	+	0.031312	Tumor
20	1697.852824	IDVTAPDVSIEEPEGK	+	0.031312	Tumor
21	989.555524	VLDLDLFR	+	0.031312	Tumor
22	2306.139324	IQYQLVDISQDNALRDEMR	+	0.031312	Tumor
23	1040.598824	IVLQIDNAR	+	0.036303	Tumor
24	1451.698424	DQLPYICQFGIV	+	0.045333	Tumor
25	1563.795024	EKAEAQAQYSAAVAK	+	0.006495	Stroma
26	1703.789324	ELDQWIEQLNECK	+	0.008981	Stroma
27	1162.619724	MPFLDIQKR	+	0.009882	Stroma
28	1191.626424	VFSLQWGEVK	+	0.009882	Stroma

OR:PD positive means that these peptides were found to be more present in tumor or stroma tissue from patients that were responsive to chemotherapy.

Table 2: Peptides identified that are OR:PD negative

SEQ ID NO	Mass (Da)	Peptide sequence	OR:PD	P-value	Tissue
29	1382.685624	TAAVANSMNYLTK	-	0.000284	Tumor
30	1991.891624	MVSDINNAWGCLEQVEK	-	0.000499	Tumor
31	1609.888424	IAILTCPFEPPKPK	-	0.002715	Tumor
32	1570.772824	LEPQIASASEYAHR	-	0.003743	Tumor
33	1531.741624	TNEQMHQLVAAYK	-	0.003802	Tumor
34	1939.075824	EALVDTLTGILSPVQEVR	-	0.006279	Tumor
35	2167.063224	SLDPSRPVTFVSNSNYAADK	-	0.010876	Tumor
36	2263.117124	AFSAVDTDGNGTINAQELGAALK	-	0.011881	Tumor
37	2313.182824	VTTVASHTSDSDVPSGVTEVVVK	-	0.013752	Tumor
38	2904.371124	VEQIAAIAQELNELDYYDSHNVNTR	-	0.018889	Tumor
39	1862.923724	KIPNPDFFEDLEPFR	-	0.018889	Tumor
40	1085.584224	QVEVLTNQR	-	0.018889	Tumor
41	1862.923724	GQYISPFHDIPIYADK	-	0.018889	Tumor
42	1686.973124	VVPSDLYPLVLGFLR	-	0.018889	Tumor
43	2140.092724	VAIAALEVLEEENLAENADK	-	0.018889	Tumor
44	2140.092724	SPYLYPLYGLGELPQGFAR	-	0.018889	Tumor
45	1639.908824	VYNVTQHAVGIVVNK	-	0.02324	Tumor
46	1420.662424	DEELSCTVVELK	-	0.02324	Tumor
47	1718.863824	FQDVGPQAPVGSVYQK	-	0.02324	Tumor
48	1770.894824	TLNEWSSQINPDLVR	-	0.02324	Tumor
49	1264.634024	KESYSVYVYK	-	0.02324	Tumor
50	2249.183724	VLAGQTLDINMAGEPKPDRPK	-	0.02324	Tumor
51	1367.778524	LDNLVAILDINR	-	0.02324	Tumor
52	2006.882224	TIGGGDDSFNTFFSETGAGK	-	0.025514	Tumor
53	1190.594824	LAPEYEAAATR	-	0.026061	Tumor
54	1373.652224	WTEYGLTFTEK	-	0.029336	Tumor
55	1713.907424	SSGPTSLFAVTVAPPGAR	-	0.030101	Tumor
56	1482.712424	GSFSEQGINEFLR	-	0.030101	Tumor
57	1714.910124	AVFVDLEPTVIDEIR	-	0.030101	Tumor
58	2245.094324	DSLDPSFTHAMQLLTAEIEK	-	0.031053	Tumor
60	2312.181824	DKLDGNELDLSLSDLNEVPVK	-	0.031053	Tumor
61	2312.181824	EQELQQTLQQEQSVLDQLR	-	0.031053	Tumor
62	1959.980124	FCEVIPGLNDTAENLLR	-	0.031312	Tumor
63	2448.288524	LEGPDVSLKGPGVDLPSVNLSMPK	-	0.034763	Tumor
64	1044.557424	ALTSELANAR	-	0.036303	Tumor
65	1530.737424	TIPIDGDFFSYTR	-	0.043153	Tumor
66	2139.085924	YDPSLKPLSVSYDQATSLR	-	0.045333	Tumor
67	1648.846224	VAVNDAHLLQYNHR	-	0.045333	Tumor
68	1410.769224	GALQNIIPASTGAAK	-	0.045333	Tumor
69	1359.729624	LLDSSTVTHLFK	-	0.045333	Tumor

70	1671.819324	DLVPDLSNFYAQYK	-	0.045333	Tumor
71	2195.075424	YFHVVIAGPQDSPFEGGTFK	-	0.045333	Tumor
72	2150.077424	FDLGQDVIDFTGHALALYR	-	0.049085	Tumor
73	1312.743224	LKVPEWVDTVK	-	0.002111	Stroma
74	1569.888624	ISLPLPNFSSLNLR	-	0.003995	Stroma
75	1579.838424	VFSVAITPDHLEPR	-	0.005947	Stroma
76	1612.894824	LVINGNPITIFQER	-	0.006502	Stroma
77	1515.746524	LLEGEDAHLTQYK	-	0.008981	Stroma
78	1630.825024	TIAECLADELINAAK	-	0.009882	Stroma
79	1912.997224	TCPKPDDLPFSTVVPLK	-	0.009882	Stroma

OR:PD negative means that these peptides were found to be more present in tumor or stroma tissue from patients that were resistant to chemotherapy.

Identification of proteins

Table 3: proteins identified that are OR:PD positive

Mass (Da)	OR PD	P-value	Accession	Protein description	Tissue
1478.755424	+	0.009611013	Q15008	26S proteasome non-ATPase regulatory subunit 6 OS=Homo sapiens GN=PSMD6 PE=1 SV=1	Tumor
1697.852824	+	0.03131214	P08865	40S ribosomal protein SA OS=Homo sapiens GN=RPSA PE=1 SV=4	Tumor
1279.710824	+	0.03131214	P42766	60S ribosomal protein L35 OS=Homo sapiens GN=RPL35 PE=1 SV=2	Tumor
1803.933324	+	0.0255138	O43488	Aflatoxin B1 aldehyde reductase member 2 OS=Homo sapiens GN=AKR7A2 PE=1 SV=3	Tumor
1803.938924	+	0.003742871	O43488	Aflatoxin B1 aldehyde reductase member 2 OS=Homo sapiens GN=AKR7A2 PE=1 SV=3	Tumor
1443.803324	+	0.03131214	P06733	Alpha-enolase OS=Homo sapiens GN=ENO1 PE=1 SV=2	Tumor
1803.933324	+	0.0255138	P06733	Alpha-enolase OS=Homo sapiens GN=ENO1 PE=1 SV=2	Tumor
1803.938924	+	0.003742871	P06733	Alpha-enolase OS=Homo sapiens GN=ENO1 PE=1 SV=2	Tumor
1697.852824	+	0.03131214	P07814	Bifunctional aminoacyl-tRNA synthetase OS=Homo sapiens GN=EPRS PE=1 SV=3	Tumor
1649.819324	+	0.007955847	O43852	Calumenin OS=Homo sapiens GN=CALU PE=1 SV=2	Tumor

1803.933324	+	0.0255138	P21964	Catechol O-methyltransferase OS=Homo sapiens GN=COMT PE=1 SV=2	Tumor
1803.938924	+	0.003742871	P21964	Catechol O-methyltransferase OS=Homo sapiens GN=COMT PE=1 SV=2	Tumor
1336.673924	+	0.01798863	Q00610	Clathrin heavy chain 1 OS=Homo sapiens GN=CLTC PE=1 SV=5	Tumor
1803.933324	+	0.0255138	Q9Y678	Coatomer subunit gamma OS=Homo sapiens GN=COPG PE=1 SV=1	Tumor
1803.938924	+	0.003742871	Q9Y678	Coatomer subunit gamma OS=Homo sapiens GN=COPG PE=1 SV=1	Tumor
1612.846224	+	0.03131214	P12111	Collagen alpha-3(VI) chain OS=Homo sapiens GN=COL6A3 PE=1 SV=3	Tumor
1297.733624	+	0.03131214	O43175	D-3-phosphoglycerate dehydrogenase OS=Homo sapiens GN=PHGDH PE=1 SV=4	Tumor
1478.755424	+	0.009611013	Q9UHY7	Enolase-phosphatase E1 OS=Homo sapiens GN=ENOPH1 PE=1 SV=1	Tumor
1297.733624	+	0.03131214	P63241	Eukaryotic translation initiation factor 5A-1 OS=Homo sapiens GN=EIF5A PE=1 SV=2	Tumor
1336.673924	+	0.01798863	P08294	Extracellular superoxide dismutase [Cu-Zn] OS=Homo sapiens GN=SOD3 PE=1 SV=2	Tumor
1309.695424	+	0.00845795	P15311	Ezrin OS=Homo sapiens GN=EZR PE=1 SV=4	Tumor
1649.819324	+	0.007955847	P49327	Fatty acid synthase OS=Homo sapiens GN=FASN PE=1 SV=2	Tumor
2471.299324	+	0.03131214	P49327	Fatty acid synthase OS=Homo sapiens GN=FASN PE=1 SV=2	Tumor
1908.909624	+	0.03131214	P21333	Filamin-A OS=Homo sapiens GN=FLNA PE=1 SV=4	Tumor
1336.673924	+	0.01798863	Q9HC38	Glyoxalase domain-containing protein 4 OS=Homo sapiens GN=GLOD4 PE=1 SV=1	Tumor
2685.231224	+	0.01607209	P30047	GTP cyclohydrolase 1 feedback regulatory protein OS=Homo sapiens GN=GCHFR PE=1 SV=3	Tumor
2073.933724	+	0.036303	P68871	Hemoglobin subunit beta OS=Homo sapiens GN=HBB PE=1 SV=2	Tumor
1351.669424	+	0.01607209	Q9BUJ2	Heterogeneous nuclear ribonucleoprotein U-like protein 1 OS=Homo sapiens GN=HNRNPUL1 PE=1 SV=2	Tumor

1743.791024	+	0.03131214	P06899	Histone H2B type 1-J OS=Homo sapiens GN=HIST1H2BJ PE=1 SV=3	Tumor
1743.791024	+	0.03131214	O60814	Histone H2B type 1-K OS=Homo sapiens GN=HIST1H2BK PE=1 SV=3	Tumor
1743.791024	+	0.03131214	Q5QNW6	Histone H2B type 2-F OS=Homo sapiens GN=HIST2H2BF PE=1 SV=3	Tumor
1309.695424	+	0.00845795	P62805	Histone H4 OS=Homo sapiens GN=HIST1H4A PE=1 SV=2	Tumor
1738.758024	+	0.03131214	P54868	Hydroxymethylglutaryl-CoA synthase, mitochondrial OS=Homo sapiens GN=HMGCS2 PE=1 SV=1	Tumor
1351.669424	+	0.01607209	Q9Y2U8	Inner nuclear membrane protein Man1 OS=Homo sapiens GN=LEMD3 PE=1 SV=2	Tumor
1040.598824	+	0.036303	P05783#	Keratin, type I cytoskeletal 18 OS=Homo sapiens GN=KRT18 PE=1 SV=2	Tumor
1040.598824	+	0.036303	P08727#	Keratin, type I cytoskeletal 19 OS=Homo sapiens GN=KRT19 PE=1 SV=3	Tumor
1351.669424	+	0.01607209	P05787	Keratin, type II cytoskeletal 8 OS=Homo sapiens GN=KRT8 PE=1 SV=7	Tumor
1351.669424	+	0.01607209	Q15046	Lysyl-tRNA synthetase OS=Homo sapiens GN=KARS PE=1 SV=3	Tumor
1968.061424	+	0.003801999	P43243	Matrin-3 OS=Homo sapiens GN=MATR3 PE=1 SV=2	Tumor
1309.695424	+	0.00845795	P26038	Moesin OS=Homo sapiens GN=MSN PE=1 SV=3	Tumor
1697.852824	+	0.03131214	Q09666	Neuroblast differentiation-associated protein AHNAK OS=Homo sapiens GN=AHNAK PE=1 SV=2	Tumor
2832.356924	+	0.04795378	P30405	Peptidyl-prolyl cis-trans isomerase, mitochondrial OS=Homo sapiens GN=PPIF PE=1 SV=1	Tumor
1040.598824	+	0.036303	Q15102	Platelet-activating factor acetylhydrolase IB subunit gamma OS=Homo sapiens GN=PAFAH1B3 PE=1 SV=1	Tumor
1359.727024	+	0.03131214	P60900	Inositol-3-phosphate synthase OS=Homo sapiens GN=INO1 PE=1 SV=1	Tumor
1614.829824	+	0.03105303	Q15084	Protein disulfide-isomerase A6 OS=Homo sapiens GN=PDIA6 PE=1 SV=1	Tumor

1478.755424	+	0.009611013	P04626	Receptor tyrosine-protein kinase erbB-2 OS=Homo sapiens GN=ERBB2 PE=1 SV=1	Tumor
989.555524	+	0.03131214	P49591	Seryl-tRNA synthetase, cytoplasmic OS=Homo sapiens GN=SARS PE=1 SV=3	Tumor
2306.139324	+	0.03131214	Q9H299	SH3 domain-binding glutamic acid-rich-like protein 3 OS=Homo sapiens GN=SH3BGRL3 PE=1 SV=1	Tumor
860.431924	+	0.03131214	P31948	Stress-induced-phosphoprotein 1 OS=Homo sapiens GN=STIP1 PE=1 SV=1	Tumor
1451.698424	+	0.04533272	P05452	Tetranectin OS=Homo sapiens GN=CLEC3B PE=1 SV=2	Tumor
1478.755424	+	0.009611013	P10599	Thioredoxin OS=Homo sapiens GN=TXN PE=1 SV=3	Tumor
1614.829824	+	0.03105303	P07437	Tubulin beta chain OS=Homo sapiens GN=TUBB PE=1 SV=2	Tumor
1614.829824	+	0.03105303	Q13885	Tubulin beta-2A chain OS=Homo sapiens GN=TUBB2A PE=1 SV=1	Tumor
1614.829824	+	0.03105303	Q13509	Tubulin beta-3 chain OS=Homo sapiens GN=TUBB3 PE=1 SV=2	Tumor
1478.755424	+	0.009611013	O60701	UDP-glucose 6-dehydrogenase OS=Homo sapiens GN=UGDH PE=1 SV=1	Tumor
1478.755424	+	0.009611013	P30085	UMP-CMP kinase OS=Homo sapiens GN=CMPK1 PE=1 SV=3	Tumor
2904.330324	+	0.01607209	Q9H993	UPF0364 protein C6orf211 OS=Homo sapiens GN=C6orf211 PE=1 SV=1	Tumor
1697.852824	+	0.03131214	P26640	Valyl-tRNA synthetase OS=Homo sapiens GN=VARS PE=1 SV=4	Tumor
1351.669424	+	0.01607209	P25311	Zinc-alpha-2-glycoprotein OS=Homo sapiens GN=AZGP1 PE=1 SV=1	Tumor
1563.795024	+	0.006495	Q14624	Inter-alpha-trypsin inhibitor heavy chain H4 OS=Homo sapiens GN=ITIH4 PE=1 SV=3	Stroma
1703.789324	+	0.008981	P67775	Serine/threonine-protein phosphatase 2A catalytic subunit alpha isoform OS=Homo sapiens GN=PPP2CA PE=1 SV=1	Stroma
1162.619724	+	0.009882	Q00341	NADH dehydrogenase [ubiquinone] iron-sulfur protein 5 OS=Homo sapiens GN=NDUFS5 PE=1 SV=3	Stroma

1191.626424	+	0.009882	P05156	Complement factor I OS=Homo sapiens GN=CFI PE=1 SV=1	Stroma

Accession number is the number in the swiss prot database (ref). OS indicates the organism from which the protein is orginated. GN is the gene identity code.

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Table 4: Peptides identified that are OR:PD negative

Mass (Da)	OR PD	P-value	Accession	Protein description	Tissue
1713.907424	-	0.03010091	O00231	26S proteasome non-ATPase regulatory subunit 11 OS=Homo sapiens GN=PSMD11 PE=1 SV=3	Tumor
1639.908824	-	0.02324039	P46778	60S ribosomal protein L21 OS=Homo sapiens GN=RPL21 PE=1 SV=2	Tumor
1264.634024	-	0.02324039	P18124	60S ribosomal protein L7 OS=Homo sapiens GN=RPL7 PE=1 SV=1	Tumor
2167.063224	-	0.01087639	P08237	6-phosphofructokinase, muscle type OS=Homo sapiens GN=PFKM PE=1 SV=2	Tumor
1770.894824	-	0.02324039	P61160	Actin-related protein 2 OS=Homo sapiens GN=ACTR2 PE=1 SV=1	Tumor
1530.737424	-	0.0431534	P05091	Aldehyde dehydrogenase, mitochondrial OS=Homo sapiens GN=ALDH2 PE=1 SV=2	Tumor
1420.662424	-	0.02324039	P01011	Alpha-1-antichymotrypsin OS=Homo sapiens GN=SERPINA3 PE=1 SV=2	Tumor
1991.891624	-	0.000499394	P12814	Alpha-actinin-1 OS=Homo sapiens GN=ACTN1 PE=1 SV=2	Tumor
2904.371124	-	0.01888901	O43707	Alpha-actinin-4 OS=Homo sapiens GN=ACTN4 PE=1 SV=2	Tumor
1373.652224	-	0.02933639	P05067	Amyloid beta A4 protein OS=Homo sapiens GN=APP PE=1 SV=3	Tumor
1531.741624	-	0.003801999	P08133	Annexin A6 OS=Homo sapiens GN=ANXA6 PE=1 SV=3	Tumor
1716.853824	-	0.04533272	P08133	Annexin A6 OS=Homo sapiens GN=ANXA6 PE=1 SV=3	Tumor
1443.803324	-	0.03131214	O95831	Apoptosis-inducing factor 1, mitochondrial OS=Homo sapiens GN=AIFM1 PE=1 SV=1	Tumor
1085.584224	-	0.01888901	P13010	ATP-dependent DNA helicase 2 subunit 2 OS=Homo sapiens GN=XRCC5 PE=1 SV=3	Tumor
1713.907424	-	0.03010091	Q08211	ATP-dependent RNA helicase A OS=Homo sapiens GN=DHX9 PE=1 SV=4	Tumor
2167.063224	-	0.01087639	P08236	Beta-glucuronidase OS=Homo sapiens GN=GUSB PE=1 SV=2	Tumor
2263.117124	-	0.01188119	Q9NZT1	Calmodulin-like protein 5 OS=Homo sapiens GN=CALML5 PE=1 SV=1	Tumor
1862.923724	-	0.01888901	P27824	Calnexin OS=Homo sapiens GN=CANX PE=1 SV=2	Tumor
1531.741624	-	0.003801999	O43852	Calumenin OS=Homo sapiens GN=CALU PE=1 SV=2	Tumor
2139.085924	-	0.04533272	P00918	Carbonic anhydrase 2 OS=Homo	Tumor

				sapiens GN=CA2 PE=1 SV=2	
2313.182824	-	0.01375215	P10909	Clusterin OS=Homo sapiens GN=CLU PE=1 SV=1	Tumor
2140.092724	-	0.01888901	P12111	Collagen alpha-3(VI) chain OS=Homo sapiens GN=COL6A3 PE=1 SV=3	Tumor
2139.085924	-	0.04533272	P0C0L5	Complement C4-B OS=Homo sapiens GN=C4B PE=1 SV=1	Tumor
1671.819324	-	0.04533272	P28838	Cytosol aminopeptidase OS=Homo sapiens GN=LAP3 PE=1 SV=3	Tumor
1085.584224	-	0.01888901	P17661	Desmin OS=Homo sapiens GN=DES PE=1 SV=3	Tumor
1190.594824	-	0.02606067	Q9UHL4	Dipeptidyl-peptidase 2 OS=Homo sapiens GN=DPP7 PE=1 SV=2	Tumor
1531.741624	-	0.003801999	P04844	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 2 OS=Homo sapiens GN=RPN2 PE=1 SV=3	Tumor
1718.863824	-	0.02324039	Q9UJU6	Drebrin-like protein OS=Homo sapiens GN=DBNL PE=1 SV=1	Tumor
1085.584224	-	0.01888901	P29692	Elongation factor 1-delta OS=Homo sapiens GN=EEF1D PE=1 SV=5	Tumor
1713.907424	-	0.03010091	P13639	Elongation factor 2 OS=Homo sapiens GN=EEF2 PE=1 SV=4	Tumor
2006.882224	-	0.0255138	P13639	Elongation factor 2 OS=Homo sapiens GN=EEF2 PE=1 SV=4	Tumor
1714.910124	-	0.03010091	P27105	Erythrocyte band 7 integral membrane protein OS=Homo sapiens GN=STOM PE=1 SV=3	Tumor
1531.741624	-	0.003801999	Q9BSJ8	Extended-synaptotagmin-1 OS=Homo sapiens GN=FAM62A PE=1 SV=1	Tumor
1713.907424	-	0.03010091	Q9BSJ8	Extended-synaptotagmin-1 OS=Homo sapiens GN=FAM62A PE=1 SV=1	Tumor
2167.063224	-	0.01087639	P02751	Fibronectin OS=Homo sapiens GN=FN1 PE=1 SV=3	Tumor
2448.288524	-	0.03476276	P21333	Filamin-A OS=Homo sapiens GN=FLNA PE=1 SV=4	Tumor
1648.846224	-	0.04533272	P17931	Galectin-3 OS=Homo sapiens GN=LGALS3 PE=1 SV=4	Tumor
1770.894824	-	0.02324039	P48507	Glutamate--cysteine ligase regulatory subunit OS=Homo sapiens GN=GCLM PE=1 SV=1	Tumor
1410.769224	-	0.04533272	P04406	Glyceraldehyde-3-phosphate dehydrogenase OS=Homo sapiens GN=GAPDH PE=1 SV=3	Tumor
2140.092724	-	0.01888901	P34932	Heat shock 70 kDa protein 4 OS=Homo sapiens GN=HSPA4 PE=1 SV=3	Tumor

1671.819324	-	0.04533272	P07900	Heat shock protein HSP 90-alpha OS=Homo sapiens GN=HSP90AA1 PE=1 SV=5	Tumor
1671.819324	-	0.04533272	P08238	Heat shock protein HSP 90-beta OS=Homo sapiens GN=HSP90AB1 PE=1 SV=4	Tumor
1713.907424	-	0.03010091	Q00839	Heterogeneous nuclear ribonucleoprotein U OS=Homo sapiens GN=HNRNPU PE=1 SV=5	Tumor
1382.685624	-	0.000284132	Q9BUJ2	Heterogeneous nuclear ribonucleoprotein U-like protein 1 OS=Homo sapiens GN=HNRNPUL1 PE=1 SV=2	Tumor
1264.634024	-	0.02324039	O60814	Histone H2B type 1-K OS=Homo sapiens GN=HIST1H2BK PE=1 SV=3	Tumor
1264.634024	-	0.02324039	Q5QNW6#	Histone H2B type 2-F OS=Homo sapiens GN=HIST2H2BF PE=1 SV=3	Tumor
1609.888424	-	0.002714834	P62805	Histone H4 OS=Homo sapiens GN=HIST1H4A PE=1 SV=2	Tumor
2140.092724	-	0.01888901	P01834	Ig kappa chain C region OS=Homo sapiens GN=IGKC PE=1 SV=1	Tumor
1939.075824	-	0.006278819	Q96P70	Importin-9 OS=Homo sapiens GN=IPO9 PE=1 SV=3	Tumor
1862.923724	-	0.01888901	Q15181	Inorganic pyrophosphatase OS=Homo sapiens GN=PPA1 PE=1 SV=2	Tumor
1359.729624	-	0.04533272	Q9NPH2	Proteasome subunit alpha type-6 OS=Homo sapiens GN=PSMA6 PE=1 SV=1	Tumor
1959.980124	-	0.03131214	Q9NPH2	Inositol-3-phosphate synthase OS=Homo sapiens GN=INO1 PE=1 SV=1	Tumor
1648.846224	-	0.04533272	P19823	Inter-alpha-trypsin inhibitor heavy chain H2 OS=Homo sapiens GN=ITIH2 PE=1 SV=1	Tumor
1382.685624	-	0.000284132	P48735	Isocitrate dehydrogenase [NADP], mitochondrial OS=Homo sapiens GN=IDH2 PE=1 SV=2	Tumor
1382.685624	-	0.000284132	P04264	Keratin, type II cytoskeletal 1 OS=Homo sapiens GN=KRT1 PE=1 SV=5	Tumor
1044.557424	-	0.036303	P08729	Keratin, type II cytoskeletal 7 OS=Homo sapiens GN=KRT7 PE=1 SV=3	Tumor
1044.557424	-	0.036303	P05787	Keratin, type II cytoskeletal 8 OS=Homo sapiens GN=KRT8 PE=1 SV=7	Tumor
2245.094324	-	0.03105303	Q07666	KH domain-containing, RNA-binding, signal transduction-	Tumor

				associated protein 1 OS=Homo sapiens GN=KHDRBS1 PE=1 SV=1	
2312.181824	-	0.03105303	Q96AG4	Leucine-rich repeat-containing protein 59 OS=Homo sapiens GN=LRRC59 PE=1 SV=1	Tumor
1570.772824	-	0.003742871	O60664	Mannose-6-phosphate receptor-binding protein 1 OS=Homo sapiens GN=M6PRBP1 PE=1 SV=2	Tumor
1044.557424	-	0.036303	P26038	Moesin OS=Homo sapiens GN=MSN PE=1 SV=3	Tumor
1044.557424	-	0.036303	P35749	Myosin-11 OS=Homo sapiens GN=MYH11 PE=1 SV=3	Tumor
1044.557424	-	0.036303	P35579	Myosin-9 OS=Homo sapiens GN=MYH9 PE=1 SV=4	Tumor
2448.288524	-	0.03476276	Q09666!	Neuroblast differentiation-associated protein AHNAK OS=Homo sapiens GN=AHNAK PE=1 SV=2	Tumor
1609.888424	-	0.002714834	P49321	Nuclear autoantigenic sperm protein OS=Homo sapiens GN=NASP PE=1 SV=2	Tumor
1686.973124	-	0.01888901	Q14978	Nucleolar phosphoprotein p130 OS=Homo sapiens GN=NOLC1 PE=1 SV=2	Tumor
2140.092724	-	0.01888901	P04181	Ornithine aminotransferase, mitochondrial OS=Homo sapiens GN=OAT PE=1 SV=1	Tumor
1382.685624	-	0.000284132	P36955	Pigment epithelium-derived factor OS=Homo sapiens GN=SERPINF1 PE=1 SV=3	Tumor
2312.181824	-	0.03105303	Q15149	Plectin-1 OS=Homo sapiens GN=PLEC1 PE=1 SV=2	Tumor
1044.557424	-	0.036303	O14744	Protein arginine N-methyltransferase 5 OS=Homo sapiens GN=PRMT5 PE=1 SV=4	Tumor
1190.594824	-	0.02606067	P30101	Protein disulfide-isomerase A3 OS=Homo sapiens GN=PDIA3 PE=1 SV=4	Tumor
1482.712424	-	0.03010091	Q15084	Protein disulfide-isomerase A6 OS=Homo sapiens GN=PDIA6 PE=1 SV=1	Tumor
1085.584224	-	0.01888901	Q9Y2J8	Protein-arginine deiminase type-2 OS=Homo sapiens GN=PADI2 PE=1 SV=2	Tumor
1410.769224	-	0.04533272	P08559	Pyruvate dehydrogenase E1 component subunit alpha, somatic form, mitochondrial OS=Homo sapiens GN=PDHA1 PE=1 SV=3	Tumor
2150.077424	-	0.04908471	P31150	Rab GDP dissociation inhibitor alpha OS=Homo sapiens GN=GDI1 PE=1 SV=2	Tumor

2140.092724	-	0.01888901	P31150#	Rab GDP dissociation inhibitor alpha OS=Homo sapiens GN=GDI1 PE=1 SV=2	Tumor
2150.077424	-	0.04908471	P50395	Rab GDP dissociation inhibitor beta OS=Homo sapiens GN=GDI2 PE=2 SV=2	Tumor
2140.092724	-	0.01888901	P50395#	Rab GDP dissociation inhibitor beta OS=Homo sapiens GN=GDI2 PE=2 SV=2	Tumor
1367.778524	-	0.02324039	P61020	Ras-related protein Rab-5B OS=Homo sapiens GN=RAB5B PE=1 SV=1	Tumor
2249.183724	-	0.02324039	Q9UKM9	RNA-binding protein Raly OS=Homo sapiens GN=RALY PE=1 SV=1	Tumor
1639.908824	-	0.02324039	P02768	Serum albumin OS=Homo sapiens GN=ALB PE=1 SV=2	Tumor
2140.092724	-	0.01888901	P02768	Serum albumin OS=Homo sapiens GN=ALB PE=1 SV=2	Tumor
2150.077424	-	0.04908471	Q01082	Spectrin beta chain, brain 1 OS=Homo sapiens GN=SPTBN1 PE=1 SV=2	Tumor
1780.794624	-	0.01188119	P31948	Stress-induced-phosphoprotein 1 OS=Homo sapiens GN=STIP1 PE=1 SV=1	Tumor
1671.819324	-	0.04533272	P21912	Succinate dehydrogenase [ubiquinone] iron-sulfur subunit, mitochondrial OS=Homo sapiens GN=SDHB PE=1 SV=3	Tumor
2139.085924	-	0.04533272	Q9Y490	Talin-1 OS=Homo sapiens GN=TLN1 PE=1 SV=3	Tumor
1609.888424	-	0.002714834	P48643	T-complex protein 1 subunit epsilon OS=Homo sapiens GN=CCT5 PE=1 SV=1	Tumor
1367.778524	-	0.02324039	P29401	Transketolase OS=Homo sapiens GN=TKT PE=1 SV=3	Tumor
2006.882224	-	0.0255138	Q71U36#	Tubulin alpha-1A chain OS=Homo sapiens GN=TUBA1A PE=1 SV=1	Tumor
2006.882224	-	0.0255138	P68363#	Tubulin alpha-1B chain OS=Homo sapiens GN=TUBA1B PE=1 SV=1	Tumor
2006.882224	-	0.0255138	Q9BQE3#	Tubulin alpha-1C chain OS=Homo sapiens GN=TUBA1C PE=1 SV=1	Tumor
1714.910124	-	0.03010091	P68366	Tubulin alpha-4A chain OS=Homo sapiens GN=TUBA4A PE=1 SV=1	Tumor
1718.863824	-	0.02324039	Q12792	Twinfilin-1 OS=Homo sapiens GN=TWF1 PE=1 SV=2	Tumor
2195.075424	-	0.04533272	P61088	Ubiquitin-conjugating enzyme E2 N OS=Homo sapiens GN=UBE2N PE=1 SV=1	Tumor

1671.819324	-	0.04533272	Q13404	Ubiquitin-conjugating enzyme E2 variant 1 OS=Homo sapiens GN=UBE2V1 PE=1 SV=1	Tumor
1382.685624	-	0.000284132	Q9NYU2	UDP-glucose:glycoprotein glucosyltransferase 1 OS=Homo sapiens GN=UGCGL1 PE=1 SV=2	Tumor
1373.652224	-	0.02933639	P21796	Voltage-dependent anion-selective channel protein 1 OS=Homo sapiens GN=VDAC1 PE=1 SV=2	Tumor
1312.743224	-	0.002111	Q8TD06	40S ribosomal protein S19 OS=Homo sapiens GN=RPS19 PE=1 SV=2	Stroma
1569.888624	-	0.003995	P08670	Vimentin OS=Homo sapiens GN=VIM PE=1 SV=4	Stroma
1579.838424	-	0.005947	P12109	Collagen alpha-1(VI) chain OS=Homo sapiens GN=COL6A1 PE=1 SV=3	Stroma
1612.894824	-	0.006502	P04406	Glyceraldehyde-3-phosphate dehydrogenase OS=Homo sapiens GN=GAPDH PE=1 SV=3	Stroma
1515.746524	-	0.008981	Q04695	Keratin, type I cytoskeletal 17 OS=Homo sapiens GN=KRT17 PE=1 SV=2	Stroma
1630.825024	-	0.009882	P07437	40S ribosomal protein S5 OS=Homo sapiens GN=RPS5 PE=1 SV=4	Stroma
1912.997224	-	0.009882	P02749	Beta-2-glycoprotein 1 OS=Homo sapiens GN=APOH PE=1 SV=3	Stroma

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Claims

1. Method to predict responsiveness or resistance to chemotherapy in breast cancer comprising

a) providing an optionally processed sample from a breast cancer patient as a test sample, wherein the sample comprise peptides and/or proteins;

b) determining the amount of individual peptides in the sample

c) comparing the amount of an individual peptide present in the test sample with the amount of a corresponding peptide in a reference sample, wherein the individual peptide in the test sample is selected from the group consisting SEQ ID 1-73

2. Method to predict responsiveness or resistance to chemotherapy in breast cancer comprising

a) providing an optionally processed sample from a breast cancer patient as a test sample, wherein the sample comprise peptides and/or proteins;

b) determining the amount of individual proteins in the sample;

c) comparing the amount of an individual protein present in the test sample with the amount of a corresponding protein in a reference sample, wherein the individual protein in the test sample is selected from the group consisting of proteins as identified in table 3 and 4.

3. Method to predict responsiveness or resistance to chemotherapy in breast cancer comprising

(a) providing an optionally processed sample from a breast cancer patient as a test sample, wherein the test sample comprises genetic information;

b) determining the presence or the expression level of a gene in the test sample wherein the gene encodes for a peptide or protein selected from the group consisting of SEQ ID 1-73 and proteins as identified in table 3 and 4.

4. Method according to any one of the preceding claims wherein the reference sample is an optionally processed sample from a breast cancer patient being responsive to chemotherapy or from a breast cancer patient being resistant to chemotherapy.

5. Method according to claim any one of the preceding claims wherein the optionally processed sample is a body tissue sample processed by subjecting the sample to laser capture microdissection to provide collections of microdissected cells, the collections preferably amounting to about 200- 3,000 cells.

6. Method according to any one of the preceding claims, wherein the optionally processed sample is a body tissue sample, body fluid sample, or collections of microdissected cells processed by subjection to protein digestion, preferably using trypsin, to provide processed samples comprising peptide fragments from the proteins in said samples.

7. Method according to any one of the preceding claims, wherein the body tissue is selected from the group consisting of tissues from breast tumor, breast tumor stroma, and lymph node.

8. Method according to any one of the preceding claims, wherein the body fluid is selected from the group consisting of blood, serum, cerebrospinal fluid (CSF), urine, saliva and nipple aspirate.

9. Method according to any one of the preceding claims, wherein the sample is a body fluid sample, preferably a body fluid comprising about 0.05- 5 mg/ml of protein, and wherein in step (b) an amount of 1-10 µl of optionally processed body fluid is subjected to MALDI- FT-ICR mass spectrometry.

10. Method according to any of the preceding claims wherein the sample is a sample comprising circulating tumor cells that are collected from blood or other fluids.

11. Method according to any one of the preceding claims, wherein in step (b) the individual peptides are in a mass range of 800 to 4,000 Da, and the individual proteins are in a mass range of 4,000 to 75,000 Da.

12. Method according to any one of the preceding claims, wherein the individual peptide from the test sample is selected from the group consisting of SEQ ID NO 1-7, 25-54 and 73-79, preferably 1-4, 25-34 and 73-79 or wherein the individual protein from the test sample is selected from the group consisting of proteins as identified in table 3 and 4 with a p value lower than 0.03, preferably lower than 0.01.

13. Method according to any one of the preceding claims wherein the gene encodes for a peptide selected from the group consisting of SEQ ID NO 1-7, 25-54 and 73-79, preferably 1-4, 25-34, and 73-79 or wherein the gene encodes for the a protein

selected from the group comprising proteins as identified in table 3 and 4 with p value lower than 0.03, preferably lower than 0.01.

14. Method according to any one of the preceding claims, wherein the amount of the individual peptide or protein present in the test sample is higher than the amount of a peptide or protein having a corresponding mass in the reference sample indicates a tumor responsive for chemotherapy, wherein the individual peptide or protein from the test sample is selected from the group consisting of SEQ ID NO 1-28 preferably from the group consisting of SEQ ID NO 1-7 and 25-28, more preferably from the group consisting of SEQ ID NO 1-4, and 25-28 , and proteins as indentified in table 3, preferably proteins from table 3 with a p value lower than 0.03, more preferably lower than 0.01.

15. Method according to any one of the preceding claims, wherein the amount of the individual peptide or protein present in the test sample is higher than the amount of a peptide or protein having a corresponding mass in the reference sample indicates a tumor resistant for chemotherapy, wherein the individual peptide or protein from the test sample is selected from the group consisting of SEQ ID NO 29-73, preferably from the group consisting of SEQ ID NO 29-54 and 73-79, more preferably from the group consisting of SEQ ID NO 29-34 and 73-79, and proteins as indentified in table 4, preferably proteins from table 4 with a p value lower than 0.03, more preferably lower than 0.01.

16. Method according to any of the preceding claims wherein the amount of peptide or protein is determined by a method selected from the group consisting of mass spectrometry, peptide array, immuno-histochemical assay, ELISA, Protein array, Western Blot, and immunoaffinity chromatography.

17. Method according to any one of the preceding claims wherein the amount of the individual peptide or protein in the test sample is at least 20% higher than the amount of the corresponding peptide or protein in the reference sample, and wherein the individual peptide or protein from the test sample is selected from the group consisting of SEQ ID NO 1-73, preferably from the group consisting of SEQ ID NO 1-7, 25-54 and 73-79, more preferably from the group consisting of SEQ ID NO 1-4, 25-34, and 73-79, and proteins as indentified in table 3 and 4, preferably proteins from table 3 and 4 with a p value lower than 0.03, more preferably lower than 0.01.

18. Method according to any one of the preceding claims wherein at least 5 of the individual peptides or proteins are present in a higher amount in the test sample than the corresponding peptides and proteins in the reference sample, and wherein the individual peptide or protein from the test sample is selected from the group consisting of SEQ ID NO 1-73, preferably from the group consisting of SEQ ID NO 1-7, 25-54 and 73-79, and proteins as indentified in table 3 and 4, preferably the proteins from table 3 and 4 with a p value lower than 0.03.

19. Method according to any of the preceding claims wherein the presence or level of a gene encoding for a peptide selected from the group consisting of SEQ ID NO 1-28, preferably from the group consisting of SEQ ID NO 1-7, 25-28, more preferably from the group consisting of SEQ ID NO 1-4 and 25-28 or wherein the presence of a gene encoding for a protein selected from the group consisting of proteins as indentified by table 3, preferably proteins from table 3 with a p value lower than 0.03, more preferably lower than 0.01, indicates a tumor responsive to chemotherapy.

20. Method according to any of the preceding claims wherein the presence of a gene encoding for a peptide selected from the group consisting of SEQ ID 29-73, preferably from the group consisting of SEQ ID 29-54 and 73-79, more preferably from the group consisting of SEQ ID NO 29-34 and 73-79 or wherein the presence of a gene encoding for a protein selected from the group consisting of proteins as indentified by table 4, preferably of proteins from table 4 with a p value lower than 0.03, more preferably lower than 0.01, indicates a tumor resistant to chemotherapy.

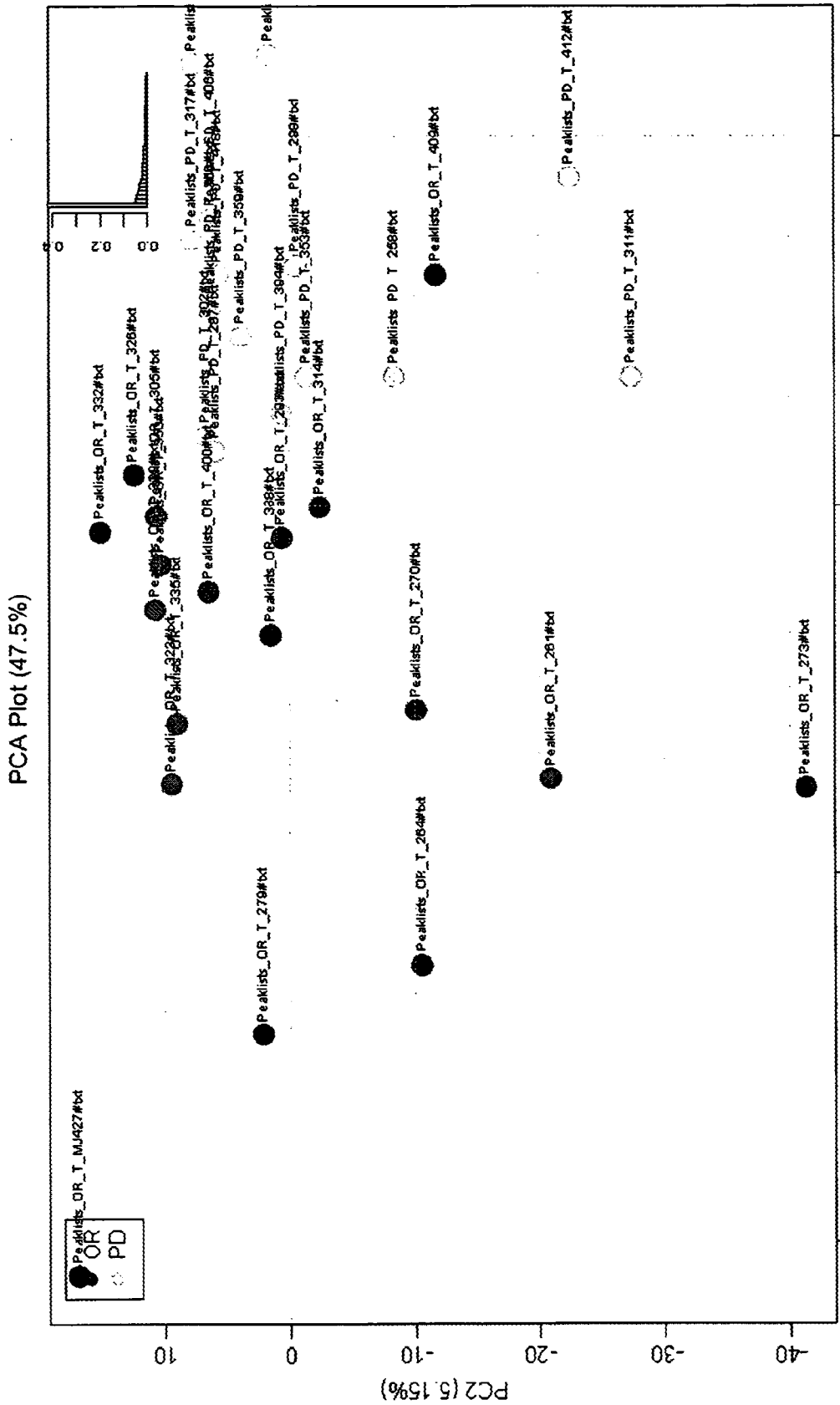
21. Method according to any one of the preceding claims wherein the presence or expression level of a gene is identified with a method selected from the group consisting of DNA array, cDNA array, oligo dt array, exon array, SNP array, PCR, Q-PCR, RT-PCR.

22. Method according to any one of the preceding claims wherein at least 10 of the genes encoding for a peptide selected from the group consisting of SEQ ID 1-73, preferably from the group consisting of SEQ ID NO 1-7, 25-54 and 73-79 or wherein at least 10 of the genes encoding for a protein selected from the group comprising proteins as indentified by table 3 and 4, preferably proteins from table 3 and 4 with a p value lower than 0.03, are present in the test sample.

23. Method according to any one of preceding claims wherein the chemotherapy is anthracycline based preferably selected from the group consisting of FEC, FAC.

24. Antibodies and siRNA against peptides with SEQ ID NO 1-73 and proteins identified in table 3 and 4 for use in a therapy against breast cancer.

25. Use of peptides with SEQ ID NO 1-73 or proteins as indentified in table 3 and 4 to identify compounds for use in a therapy against breast cancer.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PC1 (42.4%)
PC2 (5.15%)
OR
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Peaklists_OR_T_332#txt
Peaklists_OR_T_326#txt
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Peaklists_OR_T_264#txt
Peaklists_OR_T_279#txt
Peaklists_OR_T_273#txt
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Fig. 1

INTERNATIONAL SEARCH REPORT

International application No
PCT/NL2010/050199

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N33/574 G01N33/68
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)
G01N

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

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, COMPENDEX, 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	UMAR A ET AL: "Identification of a Putative Protein Profile Associated with Tamoxifen Therapy Resistance in Breast Cancer" MOL CELL PROTEOMICS, vol. 8, no. 6, 1 June 2009 (2009-06-01), pages 1278-1294, XP002587882 [retrieved on 2009-03-27]	1-23
A	the whole document -& UMAR A ET AL: "Supplemental table S4" MOL CELL PROTEOMICS vol. 8, no. 6, 1 June 2009 (2009-06-01), XP002599946 Retrieved from the Internet: URL:http://www.mcponline.org/content/suppl/2009/04/01/M800493-MCP200.DC1/S4_Peptides_for_targeted_MSMS.xls [retrieved on 2010-09-09]	24,25

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[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 September 2010	29/09/2010

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Weber, Peter

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INTERNATIONAL SEARCH REPORT

International application No
PCT/NL2010/050199

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	UMAR A ET AL: "High-throughput proteomics of breast carcinoma cells: a focus on FTICR-MS" EXP REV PROTEOMICS, vol. 5, no. 3, 1 June 2008 (2008-06-01), pages 445-455, XP009135020	1-23
A	the whole document, in particular figure 1	24,25
X	CEN S ET AL: "Retrovirus-specific packaging of aminoacyl-tRNA synthetases with cognate primer tRNAs." J VIROL, vol. 76, no. 24, December 2002 (2002-12), pages 13111-13115, XP002599947 the whole document, in particular abstract	24