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Prognostic biomarkers for survival after treatment of a cancer disease with radiotherapy and/or chemotherapy.

FIELD OF THE INVENTION

The invention relates to the field of medical prognosis, more specifically to the field of cancer prognosis, particularly cervix cancer.

BACKGROUND OF THE INVENTION

The following discussion of the background of the invention is merely provided to aid the reader in understanding the invention and is not admitted to describe or constitute prior art to the present invention.

The acquisition of apoptosis resistance is a hallmark of cancer progression and is frequently observed e.g. in ovarian carcinoma. The standard treatment of advanced cancer is often chemotherapy or radiotherapy.

Radiation therapy (in American English), radiation oncology, or radiotherapy (in the UK, Canada and Australia), sometimes abbreviated to XRT or DXT, is the medical use of ionizing radiation, generally as part of cancer treatment to control or kill malignant cells. Radiation therapy may be curative in a number of types of cancer if they are localized to one area of the body. It may also be used as part of curative therapy, to prevent tumor recurrence after surgery to remove a primary malignant tumor (for example, early stages of breast cancer). Radiation therapy is synergistic with chemotherapy, and has been used before, during, and after chemotherapy in susceptible cancers. The response of a cancer to radiation is described by its radio-sensitivity. Highly radiosensitive cancer cells are rapidly killed by modest doses of radiation. These include leukemias, most lymphomas and germ cell tumors.

However, the majority of epithelial cancers are only moderately radiosensitive, and require a significantly higher dose of radiation (60-70Gy) to achieve a radical cure. Some types of cancer are notably radio-resistant, that is, much higher doses are required to produce a radical cure than may be safe in clinical practice. Renal cell cancer and melanoma are generally considered to be radio-resistant.

Chemotherapy is the treatment of cancer with an anti-neoplastic drug or with a combination of such drugs in a standardized treatment regimen. The most common chemotherapy agents act by killing cells that divide rapidly, one of the main properties of most cancer cells. This means that chemotherapy also harms cells that divide rapidly under normal circumstances: cells in the bone marrow, digestive tract, and hair follicles. This results in the most common side-effects of chemotherapy: myelosuppression (decreased production of blood cells, hence also immunosuppression), mucositis (inflammation of the lining of the digestive tract), and alopecia (hair loss).

In adults, cytotoxic chemotherapy became established in the 1970s as a curative treatment in advanced Hodgkin’s disease, non-Hodgkin’s lymphoma, teratoma of testis and as an adjuvant treatment for early breast cancer. The initial results suggested the potential use of cytotoxic
chemotherapy as a definitive treatment or as an adjuvant therapy in asymptomatic patients with the aim of improving survival. However, the early gains in a few tumour sites have not been seen in the more common cancers.

For most patients, the use of cytotoxic chemotherapy is for the palliation of symptoms and to improve quality of life, with prolongation of survival being a less important outcome. Some practitioners still remain optimistic that cytotoxic chemotherapy will significantly improve cancer survival. However, despite the use of new and expensive single and combination drugs to improve response rates and other agents to allow for dose escalation, there has been no change in some of the regimens used, and there has been little impact from the use of newer regimens. Examples are non-Hodgkin’s lymphoma and ovarian cancer, in which cyclophosphamide, adriamycin, vincristine and prednisolone (CHOP) and platinum, respectively, (introduced over 20 years ago) are still the ‘gold standard’ treatment. Similarly, in lung cancer, the median survival has increased by only 2 months during the same time period, and an overall survival benefit of less than 5% has been achieved in the adjuvant treatment of breast, colon, and head and neck cancers.

Additionally, despite initial response to therapy, it is often observed that different carcinomas acquire resistance to chemotherapeutic drugs or radiotherapy leading to tumor recurrence and frequent death of the patients. Often, it is then decided to switch to another chemotherapeutic drug or to higher dosages. However, often no improvement of the clinical situation is observed.

Consequently, there is currently a need to provide prognostic markers for survival after treatment of a cancer disease with radiotherapy and/or chemotherapy.

**BRIEF DESCRIPTION OF THE INVENTION**

A first aspect of the invention refers to a method of prognosticating or predicting the response to radio- and/or chemotherapy of a human subject suffering from a cancer disease, comprising but not restricted to ovarian, head and neck, larynx, colon, stomach, cervix, thyroid gland, lung, uterus, rectum, breast or kidney cancer, wherein said method comprises:

- using, as an indicator, expression levels of protein MAP17 or of mRNA encoding MAP17, in a biological sample originating from said human subject, wherein the expression levels of MAP17 are determined by an *in vitro* method and wherein the result is indicative of a positive response if the expression levels of protein MAP17, or of mRNA encoding MAP17, in said biological sample are over-expressed in comparison to a reference sample and/or a positive control.

In a preferred embodiment of the first aspect of the invention, over-expression is defined as a level of expression greater than 1/3 of the expression of protein MAP17 or mRNA encoding MAP17, in normal human kidney cells. In a still more preferred embodiment of the invention, over-expression is defined as a level of expression greater or equal to 1/2 of the expression of protein MAP17 or mRNA encoding MAP17, in normal human kidney cells.
A second aspect of the invention refers to a method of prognosticating or predicting the response to radio and/or chemotherapy of a human subject suffering from a cancer disease, comprising but not restricted to ovarian, head and neck, larynx, colon, stomach, cervix, thyroid gland, lung, uterus, rectum, breast or kidney cancer, wherein said method comprises:

- using, as an indicator, expression levels of SGLT1, or mRNA encoding SGLT1, in a biological sample originating from said human subject, wherein the expression levels of SGLT1 are determined by an in vitro method and wherein the result is indicative of a positive response if the expression levels of SGLT1, or mRNA encoding SGLT1, are increased in comparison to a reference sample and/or a positive control.

In a preferred embodiment of the second aspect of the invention, increased expression is defined as a level of expression greater than the expression produced in a sample of reference, preferably a normal epithelial human sample. In a more preferred embodiment of the invention, increased expression is defined as a level of expression greater than 1/10 of the expression of SGLT1 or mRNA encoding SGLT1, in normal human kidney cells, more preferably greater or equal to 1/3.

A third aspect of the present invention, refers to a method of prognosticating or predicting the response to radio and/or chemotherapy of a human subject suffering from a cancer disease, comprising but not restricted to ovarian, head and neck, larynx, colon, stomach, cervix, thyroid gland, lung, uterus, rectum, breast or kidney cancer, wherein said method comprises:

- using, as an indicator, expression levels of protein MAP17, or mRNA encoding MAP17, and protein SGLT1, or mRNA encoding SGLT1, in a biological sample originating from said human subject, wherein the expression levels of MAP17 and SGLT1 are determined by an in vitro method and wherein the result is indicative of a positive response if the expression levels of MAP17, or mRNA encoding MAP17, are over-expressed in comparison to a reference sample and/or a positive control and the expression levels of SGLT1, or mRNA encoding SGLT1, are increased in comparison to a reference sample and/or a positive control.

In a preferred embodiment of the third aspect of the invention, over-expression is defined as a level of expression greater than 1/3 of the expression of protein MAP17 or mRNA encoding MAP17, in normal human kidney cells, more preferably more than ½.

In a preferred embodiment of the third aspect of the invention, increased expression is defined as a level of expression greater than the expression produced in a sample of reference. In a more preferred embodiment of the invention, increased expression is defined as a level of expression greater than 1/10 of the expression of SGLT1 or mRNA encoding SGLT1, in normal human kidney cells, more preferably greater or equal to 1/3.

In a preferred embodiment of any one of the preceding aspects of the invention, the human subject is suffering from a carcinoma. In a more preferred embodiment of the invention, the human subject suffers from cervix cancer. In a still more preferred embodiment of the present invention, the human subject is suffering from anyone of the following subtypes of cervix cancer: adenoma, adenosquamous carcinoma, clara cell carcinoma; squamous cell carcino-
ma; undifferentiated carcinoma; mucinous carcinoma, serous carcinoma, transitional carcinoma or endometroid.

In a further preferred embodiment of any one of the preceding aspects of the invention, the method is performed in vitro using a biological sample originating from the human subject, and wherein at the time point of taking the sample from the human subject, the human subject is not treated by chemotherapy and/or radiotherapy.

In a still further preferred embodiment of anyone of the preceding aspects of the invention, the method is performed in vitro using a biological sample originating from the human subject, and wherein at the time point of taking the sample from the human subject, the human subject is being treated or has been treated by chemotherapy and/or radiotherapy.

A fourth aspect of the invention refers to a method for allocating a human subject suffering from cancer in one of two groups, wherein group 1 comprises subjects identifiable by the method according to the first, second or third aspect of the invention; and wherein group 2 represents the remaining subjects.

A fifth aspect of the invention refers to a pharmaceutical composition comprising cisplatin and/or other compound acting through the activation of the oxidative stress pathway (comprising but not restricted to oxaliplatin, gencitabine, 5FU, taxol, taxotere, adriamycin, capecitabine, etoposide), for treating a human subject of group 1 as identifiable by the method of the fourth aspect of the invention.

In a particular aspect of the invention, the treatment of choice of a human subject suffering from cervix cancer of group 1, as identifiable by the method of the fourth aspect of the invention, is cisplatin and radiotherapy.

In a more particular aspect of the invention, a human subject not suffering from cervix cancer of group 1, as identifiable by the method of the fourth aspect of the invention, might be eligible to a change of treatment that includes but is not limited to the following types: radiotherapy, platinum coordination complexes, doxorubicin and other antracycins, camptothecin, procarbazine, cyclophosphamide, adriamycin or alkylating agents, photodynamic therapy and biologicals such as rituximab. The treatment of choice depends on many different factors such as the type of tumour and the stage of the cancer.

Thus a further aspect of the invention refers to a pharmaceutical composition comprising platinum coordination complexes, doxorubicin and other antracycins, camptothecin, procarbazine, cyclophosphamide, adriamycin or alkylating agents, photodynamic therapy and biologicals such as rituximab, for treating a human subject of group 1 as identifiable by the method of the fourth aspect of the invention.

A seventh aspect of the invention refers to a kit suitable for the determination of any of the preceding aspects of the invention, comprising at least one oligonucleotide(s) capable of hybridizing with the mRNAs of any of markers MAP17 and/or SGLT1.

A preferred embodiment refers to the kit of the seventh aspect of the invention which further comprises a positive control, preferably normal human kidney sample.
An eighth aspect of the invention refers to a kit suitable for detecting the level of expression of MAP17 and/or SGLT1 which comprises a media having at least a capture antibody capable of complexing with any of biomarker proteins MAP17 or SGLT1 or a fragment thereof and an assay for the detection of a complex of the biomarker and the capture antibody.

A preferred embodiment refers to the kit of the eighth aspect of the invention which comprises at least two capture antibodies each of which is capable of complexing with any of biomarker proteins MAP17 or SGLT1. In a more preferred aspect of the invention the kit comprises a positive control, preferably normal human kidney sample.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: Positive expression of MAP17 in cervix tumors. Representative images of MAP17 immunostaining are shown for different tumor types.

Figure 2: Expression of MAP17 in the cohort of tumors illustrated in Example 1. The graph correlates the percentage of cervical tumors with different MAP17 levels.

Figure 3: MAP17 expression distributed for tumor types in the cohort of tumors illustrated in Example 1. The distribution of the MAP17 expression levels among different cervical tumor types is shown. The maximum-levels shown in the graph refer to the maximum staining intensity observed in any part of the tumor (scale from 0 to 3). The normalized-levels (score) reflected in the graph refer to the maximum levels (0-3) scored by the percentage of cells (0-100). The normalized levels were obtained by multiplying the percentage of cells by the level of intensity observed.

Figure 4: Fig. 4a and 4b illustrate how the expression levels of MAP17 are a marker for survival after treatment with radio- and chemotherapy. Figure 4A) illustrates a Kaplan-Meier curve indicating that MAP17 could be a good prognostic marker for survival in cervical tumor patients treated with cisplatin and radiotherapy. Figure 4B) illustrates the accumulated impact of having high MAP17 levels in the survival of cervical tumor patients treated with cisplatin and radiotherapy.

Figure 5: Expression of SGLT1 in the cohort of tumors illustrated in Example 1. The graph correlates the percentage of cervical tumors with different SGLT1 levels. Additionally, a graph is shown representing the percentage of SGLT1-positive tumors by tumor subtype.

Figure 6: SGLT1 expression distributed for tumor types of the cohort of tumors illustrated in Example 1. The distribution of the SGLT1 expression levels among different cervical tumor types is shown. The maximum-levels reflected in the graph refer to the maximum staining intensity observed in any part of the tumor (scale from 0 to 3). The normalized-levels (score) reflected in the graph refer to the maximum levels (0-3) scored by the percentage of cells (0-100). The normalized levels were obtained by multiplying the percentage of cells by the level of intensity observed.
**Figure 7:** Fig 7a and 7b illustrate the correlation between the expression levels of proteins MAP17 and SGLT1 of the cohort of tumors illustrated in Example 1. A) A graph is shown depicting MAP17-SGLT1 correlations in all samples analyzed. Statistical analyses were performed using a 1-way ANOVA. B) Three representative examples of a positive correlation are shown (P1, P2 and P3).

**Figure 8:** 8A) A Kaplan-Meier curve is shown indicating that high levels of MAP17 and SGLT1 levels are good prognostic markers for survival in cervical tumor patients treated with cisplatin and radiotherapy. 8B) A Kaplan-Meier curve is shown indicating that SGLT1 levels could be a good prognostic marker for survival in cervical tumor patients treated with cisplatin and radiotherapy.

**Figure 9:** Expression of SGLT1 in the cohort of tumors illustrated in Example 1. Representative images are shown of SGLT1 immunostaining of different cervical tumor subtypes.

**Figure 10:** This figure shows that Hela tumor cells ectopically expressing MAP17 (B, C, D, F) are more sensitive to cisplatin treatment when cultured in vitro (in plastic dish) than parental Hela without MAP17 (P). Hela cancer cells expressing ectopic MAP17 cDNA were selected and analyzed for MAP17 protein expression by A) quantitative measurement of MAP17 mRNA expressed ectopically in Hela cells and B) immunodetection after cytopsin centrifugation onto slides. C) Map17 alters the transcription of genes involved in oxidative stress. A graph is shown depicting the distribution of gene transcriptional alterations induced by MAP17 in Hela cells. D) The IC50 values are shown for different chemotherapeutic drugs in Hela cells expressing MAP17 or with vector only.

**Figure 11:** T47D cancer cells expressing ectopic MAP17 cDNA were selected for MAP17 protein expression. The IC50 values are shown for bortezomib in T47D cells expressing MAP17 or with vector only. Table shows IC50 for bortezomib. Figure shows Graph of the log curve for the different concentrations performed in triplicate samples.

**Figure 12:** In a cohort of patients with lymphoma and treated with bortezomib (from Mulligan et al, BLOOD, 2007, 109( 8): 3177-88), the expression of MAP17 mRNA is higher in the responders (R). NR: Non responders. Non PARAMETRIC test MANN-WHITNEY, NR (N=84) vs. R(N=85) p(same): 0.01679; Monte Carlo p: 0.0168.

**DETAILED DESCRIPTION OF THE INVENTION**

The present invention provides new methods of using proteins markers MAP17 and/or SGLT1, or mRNA encoding these proteins, as indicators for survival after treatment of a human subject suffering from a cancer disease with radiotherapy and/or chemotherapy.

MAP17 is a small non-glycosylated membrane-associated protein of 17 kDa that locates to the plasma membrane and the Golgi apparatus. The protein sequence (Genebank accession number CR450304; NM_005764.3 GI: 41152089) shows a hydrophobic amino-terminus of 13 aminoacids encoding a PDZ binding domain and two transmembrane regions. MAP17 is highly expressed in renal proximal tubular cells and has been previously described to be associated with carcinomas.
We have studied whether MAP17 could be used as a prognostic or predictive marker for survival after treatment of a human subject suffering from a cancer disease with radiotherapy and/or chemotherapy. In this sense, we collected tumor tissue from a total of 264 female patients all of them suffering from cervix cancers that have been treated with radio- and chemo therapy.

From this cohort of 264 tumours, we analyzed the expression levels of MAP17 which were categorized into 3 grades: low expression defined as Hscore (Histological score assessment) = 0, a moderate expression defined as Hscore greater than 0 and below or equal to 100, and a high expression defined as Hscore greater than 100. Please refer herein below and to example 1 for an explanation on how the HScore has been determined.

From the results shown in Figure 4, it can be clearly concluded that high levels of expression (>100) of MAP17 significantly correlate with a better rate of survival after treatment with radio and chemo therapy. These results clearly demonstrate the usefulness of this marker, MAP17, as an indicator, for survival.

Additionally, in Maria V. Guijarro et al, “MAP17 over-expression is a common characteristic of carcinoma”, carcinogenesis, Vol. 28, no 8 pp. 1646-1652, 2007, we performed an in depth analysis of MAP17 over-expression in carcinomas by immunohistochemistry and mRNA expression and found that MAP17 protein is over-expressed in a large percentage of tumors and its expression significantly correlates with the tumor grade in ovarian and prostate carcinomas.

In this sense, the analysis of mRNA levels by Q-PCR or by hybridization comparing tumoral vs. non tumoral tissues of the same patients shows that a high percentage of tumor samples over-express protein MAP17. In tumors such as ovary, colon, stomach, cervix and thyroid gland, the percentage of over-expression in tumor samples is higher than 70%, while in lung, uterus and rectum it is around 50%. This data suggest that MAP17 expression is the most common marker of tumorigenesis in tumors, more specifically in carcinomas.

Thus, a first aspect of the invention refers to a method (from hereinafter first method of the invention) of prognosticating or predicting the response to radio- and/or chemotherapy of a human subject suffering from a cancer disease, comprising but not limited to ovarian, neck, colon, stomach, cervix, head and neck, larynx, thyroid gland, lung, uterus, rectum, breast or kidney cancer or carcinoma or a sarcoma, melanoma, myeloma leukaemia or lymphoma, wherein said method comprises:

- using, as an indicator, expression levels of protein MAP17, or of mRNA encoding MAP17, in a biological sample originating from said human subject, wherein the expression levels of MAP17 are determined by an in vitro method and wherein the result is indicative of a positive response if the expression levels of protein MAP17, or of mRNA encoding MAP17, in said biological sample are over-expressed in comparison to a reference sample and/or a positive control.
Secondly, Na-dependent glucose transporter 1 (SGLT1) (Genbank accession number NP_000334 for human isoform 1 or Genbank accession number NP_001243243 for isoform 2) is the main mediator of apical glucose uptake, whereas at the basolateral membrane the glucose transporter GLUT2 facilitates diffusive transport of intracellular glucose into the bloodstream. Previous studies demonstrated that activation of SGLT1 rescued enterocytes from cell apoptosis (Ching-Ying Huang1, Jong-Kai Hsiao Lab invest 2011), and inhibition of this membrane transport inhibit also MAP17-dependent Ros increase and proliferation (Guijarro et al b).

We have studied the presence of SGLT1 in the same cohort of tumors specified above, and determined its correlation with MAP17 and the relevance of this protein in cervix prognosis. The analysis of the correlation between the different levels of SGLT1 (Fig. 7) and MAP17 and the better or worse survival prognosis to treatment of cervix cancer with radio and chemotherapy resulted, similarly to the trend shown above for MAP17, in that moderate or high expression values of SGLT1 (Hscore > 0) correlate with a significantly better survival rate (Fig. 8b).

Consequently, a second aspect of the invention refers to a method of prognosticating or predicting the response to radio and/or chemotherapy of a human subject suffering from a cancer disease, preferably ovarian, neck, colon, stomach, cervix, head and neck, larynx, thyroid gland, lung, uterus, rectum, breast or kidney cancer or carcinoma or a sarcoma, melanoma, myeloma leukaemia or lymphoma, wherein said method comprises:

- using, as an indicator, expression levels of SGLT1, or of mRNA encoding SGLT1, in a biological sample originating from said human subject, wherein the expression levels of SGLT1 are determined by an in vitro method and wherein the result is indicative of a positive response if the expression levels of SGLT1, or of mRNA encoding SGLT1, are increased in comparison to a reference sample and/or a positive control

Thirdly, in the same cohort of tumours already illustrated above, we studied whether levels of MAP17 and SGLT1 influence the response to the standard combined treatment for cervix cancer with chemotherapy and radiotherapy. For this, we have compared whether patients with tumours expressing high levels of MAP17 and SGLT1 have a better survival rate than patients showing other different combinations.

The results are illustrated in Figure 8a, wherein it is clearly illustrated how those patients having high levels of MAP17 (>100) and moderate or high levels of SGLT1 (> 0 or preferably > or = 100) have a significantly better survival rate (all of the patients were alive at the end of the present study) than those having low levels of MAP17 and/or SGLT1.

Therefore, a third aspect of the present invention, refers to a method of prognosticating or predicting the response to radio and/or chemotherapy of a human subject suffering from a cancer disease, comprising but not restricted to ovarian, head and neck, larynx, colon, stomach, cervix, thyroid gland, lung, uterus, rectum, breast or kidney cancer or carcinoma or a sarcoma, melanoma, myeloma leukaemia or lymphoma, wherein said method comprises:

- using, as an indicator, expression levels of protein MAP17, or of mRNA encoding MAP17, and protein SGLT1, or of mRNA encoding SGLT1, in a biological sample
originating from said human subject; wherein the expression levels of MAP17 and SGLT1 are determined by an in vitro method and wherein the result is indicative of a positive response if the expression levels of MAP17, or of mRNA encoding MAP17, are over-expressed in comparison to a reference sample and/or a positive control and the expression levels of SGLT1, or of mRNA encoding MAP17, are increased in comparison to a reference sample and/or a positive control.

The methods of the present invention may be applied with samples from individuals of either sex, i.e. men or women, and at any age. The profile determined by the present invention is predictive and prognostic.

In the context of the present invention “Response” refers to the clinical outcome of the subject. “Response” may be expressed as overall survival or progression-free survival. Survival of cancer patients is generally suitably expressed by Kaplan-Meier curves, named after Edward L. Kaplan and Paul Meier who first described it (Kaplan, Meier: Amer. Statist. Assn. 53:457–481). The Kaplan–Meier estimator is also known as the product limit estimator. It serves for estimating the survival function from life-time data. A plot of the Kaplan–Meier estimate of the survival function is a series of horizontal steps of declining magnitude which, when a large enough sample is taken, approaches the true survival function for that population. The value of the survival function between successive distinct sampled observations is assumed to be constant. With respect to the present invention, the Kaplan-Meier estimator may be used to measure the fraction of patients living for a certain amount of time after beginning of chemotherapy and/or radiotherapy. The clinical outcome predicted may be the (overall/progression-free) survival in months/years from the time point of taking the sample. It may be survival for a certain period from taking the sample, such as of six months or more, one year or more, two years or more, three years or more, four years or more, five years or more, six years or more. In each case, “survival” may refer to “overall survival” or “progression-free-survival”.

Thus, in one embodiment, the response is clinical outcome, which is “overall survival” (OS). “Overall survival” denotes the chances of a patient of staying alive for a group of individuals suffering from a cancer. The decisive question is whether the individual is dead or alive at a given time point.

In the context of the present invention the method of determining the response, i.e. the expression level of the mRNA of MAP17 or SGLT1, need not be particularly limited, and may be selected from a gene profiling method, such as a microarray, and/or a method comprising PCR, such as real time PCR; and/or Northern Blot or by using immunohistochemistry.

Real time quantitative PCR (RQ-PCR) is a sensitive and reproducible gene expression quantification technique which can particularly be used to profile mRNA expression in cells and tissues. Any method for evaluation of RT-PCR results may be used, and and the ΔΔCt-method may be preferred (Livak et al. Methods 2001, 25:402-408.) (Ct = Cycle threshold values). The ΔΔCt-method will involve a control sample and a treatment sample. For each sample, a target gene and an endogenous control (as described below) gene are included for PCR amplification from (typically serially diluted) aliquots. Typically several replicates are used for each diluted concentration to derive amplification efficiency. PCR amplification efficiency can be defined as percentage amplification (from 0 to 1). During the PPCR reac-
tion, a software typically measures for each sample the cycle number at which the fluorescence (indicator of PCR amplification) crosses an arbitrary line, the threshold. This crossing point is the Ct value. More dilute samples will cross at later Ct values. To quantify mRNA gene expression, the Ct for an RNA or DNA from the mRNA gene of interest is divided by Ct of nucleic acid from the endogenous control, such as non-tumoral tissue, to normalize for variation in the amount and quality of RNA between different samples. This normalization procedure is commonly called the ΔΔCt-method (Schefé et al., 2006, J. Mol. Med. 84: 901–10). ΔΔCt calculations express data in the context of test sample (here: mRNA) versus calibrator (endogenous control). If the ΔΔCt calculation is positive (for example +2.0), then: 2−ΔΔCt = 2−(2.0) = 0.25. The amount of target, normalized to an endogenous reference and relative to a calibrator, is given by: 2−ΔΔCt. Details of the ΔΔCt calculation method can be found in: Applied Biosystems user Bulletin No. 2 (P/N 4303859).

Without prejudice of the method used to determine the response (RQ-PCR, immunohistochemistry, an elisa-based method etc...), in the context of the present invention a “significantly increased expression” or “over-expression” can be defined in comparison to a normal sample/sample of reference and/or to a positive control.

In this sense, a “normal sample” or a “sample of reference” is defined as a sample that does not express proteins MAP17 and/or SGLT1 or mRNA encoding any of these proteins, i.e. a non tumoral sample originating from the same tissue of the biopsy of origin (in the case of lung cancer the control sample would be non tumoral lung tissue). Normal human Kidney expresses high levels of MAP17 and SGLT1 and thus it is excluded from the above stated definition.

In the context of the present invention a positive control sample are normal human kidney cells.

One non-limited manner of determining a significantly increased expression or over-expression of proteins MAP17 and/or SGLT1 or mRNA encoding any of these proteins, is to use a 0-300 scale wherein the expression levels in the normal sample are determined to be 0 and wherein the expression levels in the positive control are determined to be 300. In this context, a significantly increased expression or over-expression in a biological sample would be defined as a expression of protein MAP17 and/or SGLT1 or mRNA encoding any of these proteins, greater than 1/3 of the maximum score achieved in kidney (>100), preferably greater or equal to ½ (>150)

In a preferred embodiment of the invention, the expression of proteins MAP17 and/or SGLT1 or mRNA encoding any of these proteins, may be normalized in relation to a positive and/or negative endogenous control. The endogenous control is preferably a normal endogenous sample (i.e. in the same individual).

In the context of the present invention “increased expression” is defined in comparison to a normal sample/sample of reference and/or to a positive control as defined above.

One non-limited manner of determining an increased expression of proteins MAP17 and/or SGLT1 or mRNA encoding these proteins, is to use a 0-300 scale wherein the expression levels in the normal sample are determined to be 0 and wherein the expression levels in the
positive control are determined to be 300. In this context, an increased expression in a biological sample would be defined as an expression of protein MAP17 and/or SGLT1 or mRNA encoding any of these proteins, greater than the expression produced in a sample of reference.

5 In a more preferred embodiment of the invention, increased expression is defined as a level of expression greater than 1/10 of the expression of SGLT1 or MAP17, or mRNA encoding any of these proteins, in normal human kidney cells, more preferably greater or equal to 1/3.

In a particularly preferred aspect of the present invention the response is determine by using immunohistochemistry. In a still more preferred aspect of the invention, the used of this technique entails the determination of the Histological score value.

In the context of the present invention the Histological score (Hscore) is determined per biological sample according to (i) staining intensity and (ii) the percentage of positive staining tumor cells by using the following formula:

\[ \text{Intensity reader} \times \text{Percentage reader} \]

Staining intensities are classified as follows:

<table>
<thead>
<tr>
<th>Intensity</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>No staining</td>
</tr>
<tr>
<td>0.5</td>
<td>Very weak staining</td>
</tr>
<tr>
<td>1</td>
<td>Positive staining, clear but weak</td>
</tr>
<tr>
<td>1.5</td>
<td>Positive staining</td>
</tr>
<tr>
<td>2</td>
<td>Positive, strong staining</td>
</tr>
<tr>
<td>3</td>
<td>Positive very strong staining</td>
</tr>
</tbody>
</table>

The staining intensity is scored in the following 4 grades:

<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>no staining or very weak staining (intensity = 0);</td>
</tr>
<tr>
<td>2</td>
<td>positive staining or clear but weak staining (intensity= 100, 1 or +);</td>
</tr>
<tr>
<td>3</td>
<td>positive strong staining (intensity= 200, 2 or ++); and</td>
</tr>
<tr>
<td>4</td>
<td>very strong staining (intensity= 300, 3 or +++).</td>
</tr>
</tbody>
</table>

35 Scale could be assessed by staining normal healthy human epithelia (must be 0 since there is no expression) and normal human kidney cells, (must be 300 since normal human kidney has high expression levels of SGLT1 and MAP17). Scale could be done as stated above from 0 to 300 or optionally from 0 to 3 or from 0 to ++, or being acquired automatically by software that measures intensity such as Attovision.

40 The assessment of the staining intensity and the percentage of positive staining tumor cells can be determined by any means known to the skilled person including but not limited to a panel of at least two independent pathologists with no knowledge about clinical data scoring all immunohistochemical stainings. In case, the panel of pathologist were to disagree in the scores it is convenient to expand the panel of independent pathologist to at least 5.

Once the staining intensity and the percentage of positive staining tumor cells have been determined, the value of the Hscore shall be obtained by applying the above formula.
The resulting value of the Hscore determines the level of expression. In the case of using a scale from 0 to 300 the results would be as follows:

1. low expression is defined as Hscore = 0;
2. a moderate or “increased expression” is defined as Hscore > 0 and < or = 100; and
3. a high expression or over-expression (significantly high expression) is defined as Hscore > 100.

In the context of the present invention illustrative non-limiting examples of a biological sample include different types of samples from tissues, as well as from biological fluids, such as blood, serum, plasma, cerebrospinal fluid, peritoneal fluid, faeces. Preferably, said samples are from tissues and most preferably, said samples of tissues originate from tumour tissue of the individual the response of which is to be predicted, and may originate from biopsies.

Additionally, it is known that MAP17 is expressed in a great variety of human carcinomas. Hence, in a still more preferred embodiment of the invention, the cancer disease as defined in the first and third method of the invention is a carcinoma, preferably a carcinoma selected from the list consisting of ovarian, neck, colon, stomach, cervix, thyroid gland, lung, uterus, rectum, breast or kidney carcinoma.

In a further preferred embodiment of the invention, the cancer disease as defined in any of the methods of the invention is cervix cancer, preferably anyone of the following subtypes: adenosquamous carcinoma, clara cell carcinoma; squamous cell carcinoma; undifferentiated carcinoma; mucinous carcinoma, serous carcinoma, transitional carcinoma or endometroid.

Cervical cancer is malignant neoplasm of the cervix uteri or cervical area. One of the most common symptoms is abnormal vaginal bleeding, but in some cases there may be no obvious symptoms until the cancer is in its advanced stages. Treatment consists of surgery (including local excision) in early stages and chemotherapy and radiotherapy in advanced stages of the disease.

Prognosis depends on the stage of the cancer i.e. thirty-five percent of patients with invasive cervical cancer have persistent or recurrent disease after treatment. In this sense, it is important to find good prognosis markers for survival after treatment for this specific disease and thereby the usefulness of the biomarkers of the present invention in the prognosis of this disease.

A fourth aspect of the present invention refers to anyone of the methods of the invention, wherein the method is a predictive method which is performed in vitro using a biological sample originating from the human subject, and wherein at the time point of taking the sample from the human subject, the human subject is not treated by chemotherapy and/or radiotherapy.

A fifth aspect of the present invention refers to anyone of the methods of the invention, wherein the method is a prognostic method which is performed in vitro using a biological sample originating from the human subject, and wherein at the time point of taking the sam-
ple from the human subject, the human subject is being or has been treated by chemotherapy and/or radiotherapy.

Additionally, the present inventors have identified a novel subgroup of patients that will profit from chemotherapy and/or radiotherapy. Hence, a sixth aspect of the invention refers to a method for allocating a human subject suffering from cancer in one of two groups, wherein group 1 comprises subjects identifiable by the method according to the first, second or third aspect of the invention; and wherein group 2 represents the remaining subjects.

A seventh aspect of the invention refers to a pharmaceutical composition comprising cisplatin and/or hycaam for treating a human subject of group 1 as identifiable by the method of the third aspect of the invention.

In a particular aspect of the invention, the treatment of choice of a human subject suffering from cervix cancer of group 1, as identifiable by the method of the fourth aspect of the invention, is cisplatin and radiotherapy.

In a more particular aspect of the invention, a human subject not suffering from cervix cancer of group 1, as identifiable by the method of the fourth aspect of the invention, might be eligible to a change of treatment that includes but is not limited to the following types: radiotherapy, platinum coordination complexes, doxorubicin and other antracyccins, bortezomib, camptothecin, procarbazine, cyclophosphamide, adriamycin or alkylating agents, photodynamic therapy and biologicals such as rituximab. The change of treatment depends on many different factors such as the type of tumour and the stage of the cancer, which will be apparent to the skill person.

A further aspect of the invention refers to a pharmaceutical composition comprising platinum coordination complexes, doxorubicin and other antracyccins, bortezomib, camptothecin, procarbazine, cyclophosphamide, adriamycin or alkylating agents, photodynamic therapy and biologicals such as rituximab, for treating a human subject of group 1 as identifiable by the method of the fourth aspect of the invention.

The present invention also provides a kit suitable to put into practice the method of the invention, comprising at least one oligonucleotide(s) capable of hybridizing with the mRNAs of either of MAP17 and/or SGLT1.

The kit is based on the prognostic power of the method of the present invention. In the particular case of the kit, the reference value indicative for non-response (and/or a reference value indicative for response) may be provided with the kit. With the help of the kit, the expression of each target mRNA can be calculated, i.e. relative to, such as the endogenous control samples exemplified above. The endogenous control can thus also be comprised within the kit.

It is preferred that said oligonucleotide(s) hybridizes with two mismatches or less, and preferably with no mismatch, with respect to the mRNA to be determined. As far as hybridization of the oligonucleotide(s) is concerned, it is preferred that said oligonucleotide(s) is capable to do so under stringent conditions. Stringency is a term used in hybridization experiments. Stringency reflects the degree of complementarity between the oligonucleotide and the nucle-
ic acid (which is in this case the mRNA to be detected); the higher the stringency, the higher percent homology between the probe and filter bound nucleic acid. It is well known to the skilled person that the temperature and salt concentrations have a direct effect upon the results that are obtained. It is recognized that the hybridization results are related to the number of degrees below the Tm (melting temperature) of DNA at which the experiment is performed. Often, stringent conditions are defined as a wash with 0.1X SSC (saline-sodium citrate (SSC) buffer at 65°C. (SSC is typically provided as 20X stock solution, which consists of 3 M sodium chloride and 300 mM trisodium citrate (adjusted to pH 7.0 with HCl)).

In particular embodiments, the kit is selected from (a) a kit suitable for PCR, (b) a kit suitable for Northern Blot and (c) a kit suitable for microarray analysis. Any two or more of these embodiments may also be combined, so that the kit may comprise, for example both (a) and (c).

As regards (a) a kit suitable for PCR, this PCR is typically real-time quantitative PCR (RQ-PCR), a sensitive and reproducible gene expression quantification technique.

A Northern Blot involves the use of electrophoresis to separate RNA samples by size and subsequent detection with an oligonucleotide(s) (hybridization probe) complementary to (part of) the target sequence of the RNA of interest.

It is also possible that the oligonucleotide(s) are immobilized in spots on a (preferably solid) surface. In one embodiment thereof, the kit comprises a microarray. An RNA microarray is an array on a solid substrate (usually a glass slide or silicon thin-film cell) that assays large amounts of different RNAs which are detectable via specific probes immobilized on spots on the solid substrate. Each spot contains a specific nucleic acid sequence, typically a DNA sequence, known as probes (or reporters). While the number of spots is not as such limited, there is a preferred embodiment in which the microarray is customized to the methods of the invention. In one embodiment, such a customized microarray comprises fifty spots or less, such as thirty spots or less, including twenty spots or less.

A further embodiment of the invention refers to a kit suitable for detecting the level of expression of MAP17 and/or SGLT1 which comprises a media having affixed thereto a capture antibody capable of complexing with any of biomarker proteins MAP17 or SGLT1 or a fragment thereof and an assay for the detection of a complex of the biomarker and the capture antibody.

As with the previous kit, this kit is based on the prognostic power of the method of the present invention. In the particular case of the kit, the reference value indicative for non-response (and/or a reference value indicative for response) may be provided with the kit. With the help of the kit, the expression of each target mRNA can be calculated, i.e. relative to, such as the endogenous control samples exemplified above. The endogenous control can thus also be comprised within the kit. Thus, in a more preferred aspect of the invention the kit comprises a positive control, preferably normal human kidney sample.

The kit may be used and the use is not particularly limited, although use in the method of the invention in any of its embodiments is preferred.
The following examples serve to illustrate the present invention; these examples are in no way intended to limit the scope of the invention.

5 EXAMPLES

- Example 1. Expression of protein MAP17 as prognostic marker for persons suffering from cervix tumour after treatment with radio and chemotherapy.

A total of 264 patients suffering from cervix tumour were chosen to analyse whether the levels of MAP17 influences the response to the standard combined treatment with chemotherapy and radiotherapy.

The characteristics of the patients included in the present study are detailed in Table I herein below.

<table>
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<tr>
<th>Table I</th>
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<tr>
<td><strong>Sex</strong></td>
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<td>3</td>
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<tr>
<td>Nd</td>
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<td>IB</td>
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<td>IIA</td>
</tr>
<tr>
<td>IIIB</td>
</tr>
<tr>
<td>III</td>
</tr>
<tr>
<td><strong>Treatment</strong></td>
</tr>
<tr>
<td>Combined therapy: Radio and chemotherapy</td>
</tr>
</tbody>
</table>

Tumour tissue was collected from all 264 patients, quickly frozen and stored at -80°C immediately after surgery. All tumours were histologically examined to confirm the diagnosis of cervix tumour.

Three-micrometer slices were sectioned from the tumour block and applied to special immunochemistry coated slides (DAKO, Glostrup, Denmark). The slides were baked overnight in a 56°C oven, deparaffinised in xylene for 20 min, rehydrated through a graded ethanol series, and washed with phosphate-buffered saline. A heat-induced epitope retrieval step was performed in a solution of sodium citrate buffer, pH 6.5. The slide was then heated for 2 min in a conventional pressure cooker and after heating was incubated with proteinase K for 10 min and rinsed in cool running water for 5 min. Endogeneous peroxide activity was quenched with 1.5% hydrogen peroxidise (DAKO) in methanol for 10 min and incubation with the primary antibodies αMAP17 (1:250) was performed (40 min).
Antibody αMAP17 was generated from bacterial purified GST-MAP17 protein as illustrated in Guijarro et al, “MAP17 over-expression is a common characteristic of carcinomas”, Carcinogenesis Vol. 28, no.8 pp. 1646-1652, 2007.

After incubation, immunodetection was carried out with EnVision (DAKO) visualization system using diaminobenzidine chromogen as substrate, according to manufacturer’s instructions. Immunostaining was performed in a TechMate 500 automatic immunostaining device (DAKO).

Histological score (Hscore) assessment: Two independent pathologists with no knowledge about clinical data scored all immunohistochemical stainings of MAP17 according to staining intensity and the percentage of positive staining tumor cells. Staining intensities were classified as follows:

<table>
<thead>
<tr>
<th>Value</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No staining</td>
</tr>
<tr>
<td>0.5</td>
<td>Very weak staining</td>
</tr>
<tr>
<td>1</td>
<td>Positive staining, clear but weak</td>
</tr>
<tr>
<td>1.5</td>
<td>Positive staining</td>
</tr>
<tr>
<td>2</td>
<td>Positive, strong staining</td>
</tr>
<tr>
<td>3</td>
<td>Positive very strong staining</td>
</tr>
</tbody>
</table>

The staining intensity was scored in 4 grades for MAP17: no staining or very weak staining (intensity = 0); positive staining or clear but weak staining (intensity= 100); positive strong staining (intensity= 200) and very strong staining (intensity=300).

The tumor cells staining positive for each immunohistochemical staining was scored according to their percentage over the total number of cells.

Intensity and percentage of positive staining tumor cells was scored after counting at least 10 high power fields, final magnification 10x40. Mean Hscores were calculated as follows:

\[
\frac{[(\text{Intensity reader 1 } \times \text{Percentage reader 1 }) + (\text{Intensity reader 2 } \times \text{Percentage reader 2})]}{2}
\]

Expression of MAP17 was categorized into 3 grades: low expression defined as Hscore = 0, a moderate expression defined as Hscore < or = 100, and a high expression defined as Hscore > 100.

A representative staining of positive tumours for MAP17 is shown in Figure 1 for the following cervix tumour subtypes: clara cell carcinoma, squamous cell carcinoma, undifferentiated carcinoma and mucinous carcinoma.

The results of the MAP17 expression in the cohort of tumours is illustrated in figure 2, wherein 18% of the samples showed no expression of MAP17, 61.1% showed low expression of MAP17 and 20.9% highly expressed MAP17. These results distributed by cervix tumours subtypes are illustrated in Figure 3.
Lastly, we analysed to what end the different levels of MAP17 correlated with better or worse survival prognosis to treatment of cervix cancer with radio and chemotherapy. The results are illustrated in Figure 4.

These results clearly support the fact that high levels of expression of MAP17 significantly correlate with a better rate of survival.

- Example 2. Expression of protein SGLT1 as a prognostic marker for persons suffering from cervix tumour after treatment with radio and chemotherapy.

We have studied the presence of SGLT1 in the same cohort of tumours already illustrated in Example 1 above.

Immunohistochemical staining: The formalin fixed, paraffin-embedded pathology specimens of 264 cervix cancer tissue samples were examined. Sections of 5-um-thick cut from paraffin block were put on glass slides. The slides were dried in the incubator at 60°C, deparaffinised in xylene, and then rehydrated in a downgraded series of ethanol. After flushing in water, antigen retrieval with citrate buffer was performed under high temperature and high pressure conditions. The sections cooled down for 20 min, flushed in PBS twice for 5 min, and then incubated in serum for 10 min. The primary antibody (SGLT1 from Abcam USA; EGFR from Cell Signal, USA), 1/50 diluted in 1% PBS, was incubated for 45 min after tipping serum, and then the anti-rabbit secondary antibody (from Invitrogen, USA) was incubated for 30 min after flushed in PBS twice for 5 min. Diaminobezidine (DAB, from Invitrogen, USA) was used for 10 min to visualize immunolabeling after flushed in PBS twice for 5 min. After washing, the sections were counterstained with hematoxylin (from Invitrogen, USA).

Two independent pathologists with no knowledge about clinical data scored all immunohistochemical stainings of SGLT1 according to staining intensity and the percentage of positive staining tumor cells as already explained in example 1 above.

The expression of SGLT1 in the cohort of tumours is illustrated in Figure 5, wherein it can be seen that 58.3% of the tumours do not express SGLT1, 39.5% express low or moderate levels of SGLT1 and 2.20% express high levels of SGLT1. The distribution of the levels of expression of SGLT1 among tumour types is illustrated in Figure 6.

The analysis of the correlation between the different levels of SGLT1 and the better or worse survival prognosis to treatment of cervix cancer with radio and chemotherapy resulted, similarly to the trend shown in Example 1 above for MAP17, in that moderate or high expression values of SGLT1 correlate with a better survival rate (see figures 7 and 8b).

- Example 3. Expression of protein SGLT1 and MAP17 as prognostic markers for persons suffering from cervix tumour after treatment with radio and chemotherapy.

In the same cohort of tumours already illustrated in Example 1 above, we studied whether levels of MAP17 and SGLT1 influence the response to the standard combined treatment for
cervix cancer with chemotherapy and radiotherapy. For this, we have compared whether patients with tumours expressing high levels of MAP17 and SGLT1 have a better survival rate than patients showing other different combinations.

The results are illustrated in Figure 8b, wherein it is clearly illustrated how those patients having high levels of MAP17 (>100) and moderate or high levels of SGLT1 (> or = 100) have a significantly better survival rate (all of the patients were alive at the end of the present study) than those having low levels of MAP17 and/or SGLT1.

- Example 4. Susceptibility to cisplatin of T47D tumor cells overexpressing MAP17.

We have overexpressed MAP17 in T47D tumor cells (Fig. 10 B, C, D, F) and found that these are more sensitive to cisplatin treatment when cultured in vitro (in plastic dish) than parental T47D without MAP17 (P). Similar results are illustrated in Figures 11 and 12 for bortezomib.

This result clearly illustrates that the present invention can certainly predict the response of a human subject suffering from a cancer disease to radio and/or chemotherapy.

The above stated results demonstrate that each marker (SGLT1 and MAP17) can be used to predict or prognosticate the response of a human subject to radio and/or chemotherapy.

One skilled in the art readily appreciates that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those inherent therein. The examples provided herein are representative of preferred embodiments, are exemplary, and are not intended as limitations on the scope of the invention.

It will be readily apparent to a person skilled in the art that varying substitutions and modifications may be made to the invention disclosed herein without departing from the scope and spirit of the invention.

All publications mentioned in the specification are indicative of the levels of those ordinary skills in the art to which the invention pertains. All publications are herein incorporated by reference to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference.

The invention illustratively described herein suitably may be practiced in the absence of any element or elements, limitation or limitations which is not specifically disclosed herein. Thus, for example, in each instance herein any of the terms “comprising”, “consisting essentially of” and “consisting of” may be replaced with either of the other two terms. The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention that in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the concepts herein dis-
closed may be resorted to by those skilled in the art, and that such modifications and variations to be within the scope of this invention as defined by the appended claims.
CLAIMS

1. A method of predicting or prognosticating the response of a human subject to radio and/or chemotherapy, wherein the subject is suffering from a cancer disease, and wherein the method comprises using, as an indicator, expression levels of MAP17 from a biological sample of the subject; wherein the expression levels of MAP17 are determined by an *in vitro* method and wherein the result is indicative of a positive response if the expression levels of MAP17 are over-expressed in comparison to a reference sample and/or a positive control.

2. The method of claim 1 wherein protein MAP17 or mRNA that encodes MAP17 is used as an indicator.

3. The method of claim 1 or claim 2, wherein the expression levels of MAP17 are determined by
   a. a gene profiling method, such as a microarray, and/or
   b. a method comprising PCR, such as real time PCR; and/or
   c. northern Blot and/or
   d. an immunohistochemistry method; and/or
   e. an elisa-based method

4. The method of any one of the preceding claims, wherein over-expressed is defined as a level of expression greater than 1/3 of the maximum score achieved in normal human kidney cells.

5. The method of any one of claims 1-3, wherein over-expressed is defined as a level of expression greater or equal to ½ of the maximum score achieved in normal human kidney cells.

6. A method of prognosticating the response of a human subject to radio and/or chemotherapy, wherein the subject is suffering from a cancer disease, and wherein the method comprises using, as an indicator, expression levels of both MAP17 and SGLT1 from a biological sample of a subject; wherein the expression levels of MAP17 and SGLT1 are determined by an *in vitro* method and wherein the result is indicative of a positive response if the expression levels of MAP17 are over-expressed in comparison to a reference sample and/or a positive control and the expression levels of SGLT1 are increased in comparison to a reference sample and/or a positive control.

7. The method of claim 6 wherein protein SGLT1 or mRNA that encodes SGLT1 and protein MAP17 or mRNA that encodes MAP17 are used as indicators.

8. The method of claim 6 or claim 7, wherein the expression levels of MAP17 and SGLT1 are determined by
   a. a gene profiling method, such as a microarray, and/or
   b. a method comprising PCR, such as real time PCR; and/or
c. Northern Blot. 1, and/or
d. an immunohistochemistry method; and
e. an elisa-based method

9. The method of any one of claims 6-8, wherein increased expression is defined as a level of expression greater than 1/3 of the expression of SGLT1 or mRNA encoding SGLT1, in normal human kidney cells, and wherein over-expressed is defined as in anyone of claims 4 or 5.

10. The method of any one of the preceding claims, wherein the response refers to the overall survival rate.

11. The method of any one of the preceding claims, wherein the cancer disease is a carcinoma or adenocarcinoma, sarcoma, melanoma, myeloma leukaemia or lymphoma.

12. The method of any one of the preceding claims, wherein the cancer or carcinoma is selected from the list consisting of ovarian, head and neck, larynx, colon, stomach, cervix, thyroid gland, lung, uterus, rectum, breast or kidney cancer or carcinoma or a sarcoma, melanoma, myeloma leukaemia or lymphoma.

13. The method claim 12, wherein the cancer disease is cervix cancer.

14. The method of claim 13, wherein the cervix cancer is selected from anyone of the following subtypes: adenoma, adenosquamous carcinoma, clara cell carcinoma; squamous cell carcinoma; undifferentiated carcinoma; mucinous carcinoma, serous carcinoma, transitional carcinoma or endometroid.

15. The method of any one of the precedent claims, wherein the biological sample is fresh tissue, paraffin embed tissue or RNA extracted from a tissue from a patient with cancer.

16. The method of any one of the precedent claims, wherein the method is a predictive method which is performed in vitro using a biological sample originating from the human subject, and wherein at the time point of taking the sample from the human subject, the human subject is not treated by chemotherapy and/or radiotherapy.

17. The method of any one of the preceding claims, wherein the method is a prognostic method which is performed in vitro using a biological sample originating from the human subject, and wherein at the time point of taking the sample from the human subject, the human subject is being treated or has been treated by chemotherapy and/or radiotherapy.

18. The method of any one of the preceding claims, wherein the chemotherapy comprises administration of cisplatin and/or other compound acting through the activation of the oxidative stress pathway.

19. The method of claim 18 which further comprises the administration of radiotherapy.
20. A method for allocating a human subject suffering from cancer in one of two groups, wherein group 1 comprises subjects identifiable by the method according to claims 1, 6 or any of the claims dependent thereon; and wherein group 2 represents the remaining subjects.

21. A pharmaceutical composition comprising cisplatin and/or other compound acting through the activation of the oxidative stress pathway, for treating a human subject of group 1 as identifiable by the method of claim 20.

22. A pharmaceutical composition comprising platinum coordination complexes, doxorubicin, anthracyclins, camptothecin, bortezomib, procarbazine, cyclophosphamide, adriamycin or alkylating agents, photodynamic therapy and rituximab, for treating a human subject of group 1 as identifiable by the method of claim 20.

23. A kit suitable for the determination of any of the preceding claims, comprising at least one oligonucleotide(s) capable of hybridizing with the mRNAs of any of markers MAP17 and/or SGLT1.

24. A kit suitable for detecting the level of expression of MAP17 and/or SGLT1 which comprises a media having affixed thereto a capture antibody capable of complexing with any of biomarker proteins MAP17 or SGLT1 or a fragment thereof and an assay for the detection of a complex of the biomarker and the capture antibody.

25. The kit of anyone of claims 23 or 24 which further comprises a positive control sample.

26. The kit of claim 25, wherein the positive control is a normal human kidney sample.
Expression of MAP17 in cervix tumors

Fig. 1
Fig. 2

- Null (0): n=43
- Low (0.5-1): n=146
- High (1.5-3): n=50

Fig. 3

- Transitional Ca (n=7)
- Serous Ca (n=8)
- Mucinous Ca (n=32)
- Undifferentiated Ca (n=9)
- Escamos Ca (n=97)
- Endometroid Ca (n=12)
- Clear Cell Carcinoma (n=10)
- Adenoescamous Ca (n=7)
- Adenoma (n=3)
Fig. 4A

Survival Curve

Cum Survival

Days to M17

MAP17

High MAP17

Low MAP17

MAP17

Fig. 4B

Hazard Curve

Cum Hazard

Days to M17

MAP17

Low MAP17

High MAP17

MAP17

MAP17 > 100

MAP17 ≤ 100

MAP17 > 100

MAP17 ≤ 100
Expression of SGLT1 in cervix tumors

Fig. 5
Distribution of the levels of expression of SGLT1 among tumor types

Fig.6
Correlation between MAP17 and SGLT1 expression
1 wayanova,
Fig. 9
Fig. 10

A

MAP7 mRNA (Log 10 fold)

Vector MAP7

B

control MAP7

C

Log10(group 1 2^ΔCt)

D

<table>
<thead>
<tr>
<th>Compound</th>
<th>Hela IC50 (μM)</th>
<th>Hela MAP7 IC50 (μM)</th>
</tr>
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<tbody>
<tr>
<td>cisplatin</td>
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<td>0.744</td>
</tr>
<tr>
<td>oxaliplatin</td>
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<td>0.160</td>
</tr>
<tr>
<td>gemcitabine</td>
<td>0.049</td>
<td>0.026</td>
</tr>
<tr>
<td>Bortezomib</td>
<td>[nM]</td>
<td></td>
</tr>
<tr>
<td>------------</td>
<td>------</td>
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<tr>
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Fig. 11
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<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
</table>

**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
- "E" earlier application or patent but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "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
- "S" document member of the same patent family

**Date of the actual completion of the international search**
26 April 2013

**Date of mailing of the international search report**
10/05/2013

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Tel: (+31-70) 340-2000, Fax: (+31-70) 340-3016

Reuter, Uwe
<table>
<thead>
<tr>
<th>Category</th>
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