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(54) Title: METHOD OF DIAGNOSING OR PROGNOSING EPITHELIAL OVARIAN CANCER

(57) Abstract: The present invention provides a binding moiety which selectively binds to Sox11 protein and/or mRNA for imaging, diagnosis or prognosis of epithelial ovarian cancer (EOC). Optionally, the moiety is an antibody or antigen-binding fragment thereof. Advantageously, moiety comprises a further, readily detectable moiety. The invention also provides methods of imaging EOC cells as well as methods of diagnosing or prognosing EOC in an individual. A further aspect of the present invention provides a method of identifying cells associated with EOC, the method comprising analysing the pattern of gene expression in a sample of cells to be tested and comparing it to the pattern of gene expression in a sample of known lymphomas cells. Preferably, the cells to be tested are identified as EOC cells if the expression of Sox11 is up-regulated compared to normal B-cells. Preferably EOC cells are identified as improved recurrence-free survival-associated if expression of Sox11 is up-regulated compared with non-cancerous epithelial ovarian cells. Preferably, EOC cells are identified as diminished recurrence-free survival-associated if expression of Sox11 is similar to, or down-regulated, compared with non-cancerous epithelial ovarian cells.

METHOD OF DIAGNOSING OR PROGNOSING EPITHELIAL OVARIAN CANCER

Field of Invention

The present invention relates to novel agents for the diagnosis, prognosis and imaging of epithelial ovarian cancer (EOC), and use of the same.

Introduction

Epithelial ovarian cancer (EOC) is the leading cause of death from gynaecological malignancy and the fifth most common cause of cancer related death in women. In 2008 it is estimated that 21,650 new ovarian cancer cases will be diagnosed in the United States and that 15,520 will succumb to the disease (Jemal A, Siegel R, Ward E, *et al.* Cancer statistics, 2008. CA: a cancer journal for clinicians 2008;58:71-96). The poor ratio of survival to incidence in EOC is related to the high percentage of cases that are diagnosed at an advance stage and the lack of effective therapies for advanced refractory disease. Despite improvements in surgical techniques and the advent of more targeted therapeutics such as bevacizimab, survival of patients with EOC stands at 45% at five years (Jemal A, Siegel R, Ward E, et al. Cancer statistics, 2008. CA: a cancer journal for clinicians 2008;58:71-96). Such poor prognostic statistics indicate that there is an urgent need to improve our understanding of the molecular mechanisms underlying EOC, so as to develop better prognostic and predictive assays and identify new therapeutic targets.

Epithelial ovarian cancer comprises three major histological subtypes; serous, mucinous and endometrioid. Serous EOC includes serous cystomas, serous benign cystadenomas, serous cystadenomas with proliferating activity of the epithelial cells and nuclear abnormalities but with no infiltrative destructive growth (low potential or borderline malignancy), and serous cystadenocarcinomas. Mucinous EOC includes mucinous cystomas, mucinous benign cystadenomas, mucinous cystadenomas with proliferating activity of the epithelial cells and nuclear abnormalities but with no infiltrative

destructive growth (low potential or borderline malignancy), and mucinous cystadenocarcinomas. Endometrioid EOC includes endometrioid tumours (similar to adenocarcinomas in the endometrium), endometrioid benign cysts, endometrioid tumours with proliferating activity of the epithelial cells and nuclear abnormalities but with no infiltrative destructive growth (low malignant potential or borderline malignancy), and endometrioid adenocarcinomas.

Two further, less-prevalent histological subtypes also exist, clear cell and undifferentiated.

In addition, EOC may be categorised by "stages", depending upon how far they have spread beyond the ovary. Thus, Stage I is defined as ovarian cancer that is confined to one or both ovaries. Stage II is defined as ovarian cancer that has spread to pelvic organs (*e.g.*, uterus, fallopian tubes), but has not spread to abdominal organs. Stage III is defined as ovarian cancer that has spread to abdominal organs or the lymphatic system (*e.g.*, pelvic or abdominal lymph nodes, on the liver, on the bowel). Finally, Stage IV is defined as ovarian cancer that has spread to distant sites (*e.g.*, lung, inside the liver, brain, lymph nodes in the neck).

EOCs may also be graded according to the appearance of the cancer cells. Low-grade (or Grade 1) means that the cancer cells look very like the normal cells of the ovary; they usually grow slowly and are less likely to spread. Moderate-grade (or Grade 2) means that the cells look more abnormal than low-grade cells. High-grade (or Grade 3) means that the cells look very abnormal. They are likely to grow more quickly and are more likely to spread.

EOC, like most other cancers, is thus a complex heterogeneous disease, influenced and controlled by multiple genetic and epigenetic alterations leading to an increasingly aggressive phenotype (Martin L and Schilder R. Novel approaches in advancing the treatment of epithelial ovarian cancer: the role of angiogenesis inhibition. Journal of clinical oncology 2007;25:2894-901; Naora H and Montell DJ. Ovarian cancer metastasis: integrating insights from disparate model organisms. Nature reviews 2005;5:355-66). It is now well recognised that the characteristics of an individual tumour and its life course results from multiple somatic mutations acquired over time (e.g. TP53, PTEN, RAS) and continual evolution of the host responses to environmental factors (e.g., estrogen or tobacco exposure) (West M, Ginsburg G, Huang A, and Nevins J.

Embracing the complexity of genomic data for personalized medicine. Genome Res 2006;16:559-66). In certain cases, these somatic mutations overlie inherent germline variations (e.g. BRCA1/2) (West M, Ginsburg G, Huang A, and Nevins J. Embracing the complexity of genomic data for personalized medicine. Genome Res 2006;16:559-66; Prat J, Ribe A, and Gallardo A. Hereditary ovarian cancer. Human pathology 2005;36:861-70). From a therapeutic standpoint EOC is best considered a collection of complex inter-related diseases represented by an immense natural heterogeneity in tumour phenotypes, disease outcomes, and response to treatment. A major challenge is consequently to identify and thoroughly validate diagnostic and prognostic biomarkers that can accurately describe the heterogeneity ascribed to EOC. In addition, accurate predictive biomarkers are required to guide current treatment protocols, as well as to guide the development and application of new targeted therapies.

Technologies, such as DNA microarrays, mass spectrometry-based proteomics and metabolomics have facilitated translational research over the last decade helping us to improve our understanding of the molecular and genetic basis of oncogenesis and by affording an opportunity to add new approaches to the practice of clinical oncology. The fundamental premise of "omic technologies" is that comprehensive examination of changes in the genome (DNA), transcriptome (mRNA), proteome (proteins), or metabolome (metabolites) can provide insight into the physiology and mechanism of disease, by the provision of superior diagnostic tests and therapeutic efficacy to that currently available (Brennan DJ, Kelly C, Rexhepaj E, et al. Contribution of DNA and Tissue Microarray Technology to the Identification and Validation of Biomarkers and Personalised Medicine in Breast Cancer. Cancer Genomics and Proteomics 2007;4:3-16). The application of DNA microarray technology to cancer biology, in particular, has led to an ever-growing comprehension of the complexity of the underlying pathophysiological pathways and interactions within a tumour (Duffy MJ, Kelly ZD, Culhane AC, O'Brien S, and Gallagher WM. DNA microarray-based gene expression profiling in cancer: aiding cancer diagnosis, assessing prognosis and predicting response to therapy. Current Pharmacogenomics 2005;3:289-304; Brennan DJ, O'Brien SL, Fagan A, et al. Application of DNA microarray technology in determining breast cancer prognosis and therapeutic response. Expert Opin Biol Ther 2005;5:1069-83). Transcriptomic screens have accelerated research into genotypic-phenotypic correlations, with a common aim of elucidating the functional taxonomy of genes in both normal tissues and disease states, such as cancer (Brennan D, O'Brien S, Fagan A, et

al. Application of DNA microarray technology in determining breast cancer prognosis and therapeutic response. Expert Opin Biol Ther 2005;5:1069-83).

However, there remains a need to improved agents and methods for the diagnosis and prognosis of EOC.

Summary of Invention

A first aspect of the invention provides a binding moiety which is capable of binding selectively to Sox11 protein, or to a nucleic acid molecule encoding the same, for use in diagnosing or prognosing epithelial ovarian cancer (EOC).

It will be appreciated that the invention also provides the use of a binding moiety which is capable of binding selectively to Sox11 protein, or to a nucleic acid molecule encoding the same, in the preparation of a diagnostic or prognostic agent for epithelial ovarian cancer (EOC).

By "Sox11 protein" we include the amino acid sequence of the human Sox11 protein as shown in Figure 4 herein, as well as naturally-occurring homologues thereof.

Thus, we also include the Sox11 proteins as identified by Database Accession Nos. BAA88122, AAH25789, AAB08518, AAH25789 and P35716.

The present inventors have surprisingly identified Sox11 as a novel diagnostic/prognostic antigen for EOC, using immunohistochemistry analysis. Not only is this is the first report showing Sox11 overexpression in EOC cells but also the differential expression of Sox11 in high risk versus low risk EOC cohorts. Thus, Sox11 provides a valuable marker for diagnosing EOC patients and facilitates accurate diagnosis and/or prognosis of this aggressive malignancy.

By "diagnosing" we include the act or process of identifying the existence and/or type of cancer from which an individual may be suffering. Thus, in one embodiment, diagnosis includes the differentiation of a particular cancer type, namely EOC, from one or more other cancers. In an alternative embodiment, binding moieties of the invention are for

use in classifying EOC patients into clinically relevant groups based on overall survival and/or cancer-specific survival.

By "prognosis" we include the act or process of predicting the probable course and outcome of a cancer, *e.g.* determining survival probability and/or recurrence-free survival (RFS) probability.

By "binding moiety" it is meant a molecule or entity which is capable of binding to Sox11 protein or mRNA encoding the same.

It will be appreciated by persons skilled in the art that the binding moieties of the invention may be used for the diagnosis or prognosis of EOC of any histological subtype (for example, serous, mucinous, endometrioid, clear cell, undifferentiated or unclassifiable).

In one embodiment, the binding moiety is for use in diagnosing or prognosing EOC belonging to a specific histological subtype. Thus, the EOC may belong to a histological subtype selected from the group consisting of serous, mucinous, endometrioid, clear cell and undifferentiated or unclassifiable.

Likewise, the binding moiety may be for use in diagnosing or prognosing EOC associated with cells of a specific grade (for example, high grade).

It will also be appreciated that the binding moieties of the invention may be used *in vivo* or *in vitro*.

In one embodiment, the binding moiety of the invention is for use in the detection of Sox11 expression as a sole biomarker for epithelial ovarian cancer (EOC). For example, Sox11 expression may be used as a sole biomarker for the differentiation of EOC from one or more other cancers.

Alternatively, the binding moiety of the invention may be for use in combination with one or more additional binding moieties for detecting one or more additional biomarkers for epithelial ovarian cancer (EOC). Thus, the binding moiety may be for use in combination with fewer than 20 additional binding moieties, for example fewer than 15, 10, 8, 6, 5, 4, 3, 2 or 1 additional binding moieties.

In one particular embodiment, the binding moiety of the invention is for detecting nuclear and/or cytoplasmic expression of Sox11.

By "binding selectively" we include binding moieties which bind more strongly to Sox11 than to another polypeptides or nucleic acids; preferably at least 10-fold more strongly, more preferably at least 50-fold more strongly and even more preferably, at least 100-fold more strongly. Preferably, the binding moieties bind only to Sox11 polypeptides or nucleic acids.

The term 'polypeptide' as used herein means a plurality of amino acids that are linked together via a peptide bond. The term 'peptide' may be used interchangeably with the term 'polypeptide' however a peptide may be composed of two or more polypeptides.

The term 'amino acid' as used herein includes the standard twenty genetically-encoded amino acids and their corresponding stereoisomers in the 'D' form (as compared to the natural 'L' form), omega-amino acids other naturally-occurring amino acids, unconventional amino acids (*e.g.* α, α -disubstituted amino acids, N-alkyl amino acids, *etc.*) and chemically derivatised amino acids.

When an amino acid is being specifically enumerated, such as 'alanine' or 'Ala' or 'A', the term refers to both L-alanine and D-alanine unless explicitly stated otherwise. Other unconventional amino acids may also be suitable components for polypeptides of the present invention, as long as the desired functional property is retained by the polypeptide. For the peptides shown, each encoded amino acid residue, where appropriate, is represented by a single letter designation, corresponding to the trivial name of the conventional amino acid.

Nucleic acid-based binding moieties of the invention are preferably DNA but may also be RNA or an artificial nucleic acid such as PNA.

The second aspect of the invention provides a binding moiety which is capable of binding selectively to Sox11 protein, or to a nucleic acid molecule encoding the same, for use in detecting epithelial ovarian cancer (EOC) cells in a sample.

In one embodiment, the binding moiety is for use in detecting EOC belonging to a specific histological subtype (*e.g.* serous, mucinous, endometrioid, clear cell and undifferentiated or unclassifiable).

Likewise, the binding moiety may be for use in detecting EOC associated with cells of a specific grade (*e.g.* high grade).

One embodiment of the invention provides a binding moiety according to either the first or second aspect which is capable of binding selectively to Sox11 protein. Preferably, the Sox11 protein is a human protein.

Conveniently, the binding moiety is capable of binding selectively to a polypeptide comprising an amino acid sequence of SEQ ID NO:1 (see figure 4) and/or a natural variant thereof.

By "natural variant" we include Sox11 proteins which are found in nature, for example allelic variants.

Variants of polypeptides include polypeptides comprising a sequence with at least 60% identity to known amino acid sequences, preferably at least 70% or 80% or 85% or 90% identity to said sequences, and more preferably at least 95%, 96%, 97%, 98% or 99% identity to said amino acid sequence of SEQ ID NO:1.

Percent identity can be determined by, for example, the LALIGN program (Huang and Miller, *Adv. Appl. Math.* (1991) 12:337-357) at the Expasy facility site (http://www.ch.embnet.org/software/LALIGN_form.html) using as parameters the global alignment option, scoring matrix BLOSUM62, opening gap penalty –14, extending gap penalty –4. Alternatively, the percent sequence identity between two polypeptides may be determined using suitable computer programs, for example the GAP program of the University of Wisconsin Genetic Computing Group and it will be appreciated that percent identity is calculated in relation to polypeptides whose sequence has been aligned optimally.

Preferably the binding moiety comprises or consists of a polypeptide.

Polypeptide binding moieties can be identified by means of a screen. A suitable method or screen for identifying peptides or other molecules which selectively bind a target protein or polypeptide may comprise contacting the target protein or polypeptide with a test peptide or other molecule under conditions where binding can occur, and then determining if the test molecule or peptide has bound the target protein or peptide. Methods of detecting binding between two moieties are well known in the art of biochemistry. Preferably, the known technique of phage display is used to identify peptides or other ligand molecules suitable for use as binding moieties. An alternative method includes the yeast two hybrid system.

More preferably the binding moiety comprises or consists of an antibody, or an antigen-binding fragment or variant thereof.

By "antibody" we include substantially intact antibody molecules, as well as chimaeric antibodies, humanised antibodies, human antibodies (wherein at least one amino acid is mutated relative to the naturally occurring human antibodies), single chain antibodies, bispecific antibodies, antibody heavy chains, antibody light chains, homodimers and heterodimers of antibody heavy and/or light chains, and antigen binding fragments and derivatives of the same.

By "antigen-binding fragment" we mean a functional fragment of an antibody that is capable of binding to Sox11 protein.

Preferably, the antigen-binding fragment is selected from the group consisting of Fv fragments (*e.g.* single chain Fv and disulphide-bonded Fv), Fab-like fragments *(e.g.* Fab fragments, Fab' fragments and $F(ab)_2$ fragments), single variable domains (*e.g.* V_H and V_L domains) and domain antibodies (dAbs, including single and dual formats [*i.e.* dAb-linker-dAb]).

The advantages of using antibody fragments, rather than whole antibodies, are several-fold. The smaller size of the fragments may lead to improved pharmacological properties, such as better penetration of solid tissue. Moreover, antigen-binding fragments such as Fab, Fv, ScFv and dAb antibody fragments can be expressed in and secreted from *E. coli*, thus allowing the facile production of large amounts of the said fragments.

Also included within the scope of the invention are modified versions of antibodies and an antigen-binding fragments thereof, *e.g.* modified by the covalent attachment of polyethylene glycol or other suitable polymer.

Methods of generating antibodies and antibody fragments are well known in the art. For example, antibodies may be generated via any one of several methods which employ induction of *in vivo* production of antibody molecules, screening of immunoglobulin libraries (Orlandi. *et al*, 1989. *Proc. Natl. Acad. Sci. U.S.A.* **86**:3833-3837; Winter *et al.*, 1991, *Nature* **349**:293-299) or generation of monoclonal antibody molecules by cell lines in culture. These include, but are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the Epstein-Barr virus (EBV)-hybridoma technique (Kohler *et al.*, 1975. *Nature* **256**:4950497; Kozbor *et al.*, 1985. *J. Immunol. Methods* **81**:31-42; Cote *et al.*, 1983. *Proc. Natl. Acad. Sci. USA* **80**:2026-2030; Cole *et al.*, 1984. *Mol. Cell. Biol.* **62**:109-120).

Suitable monoclonal antibodies to selected antigens may be prepared by known techniques, for example those disclosed in *"Monoclonal Antibodies: A manual of techniques"*, H Zola (CRC Press, 1988) and in *"Monoclonal Hybridoma Antibodies: Techniques and Applications"*, J G R Hurrell (CRC Press, 1982).

Antibody fragments can be obtained using methods well known in the art (see, for example, Harlow & Lane, 1988, "*Antibodies: A Laboratory Manual*", Cold Spring Harbor Laboratory, New York). For example, antibody fragments according to the present invention can be prepared by proteolytic hydrolysis of the antibody or by expression in *E. coli* or mammalian cells (*e.g.* Chinese hamster ovary cell culture or other protein expression systems) of DNA encoding the fragment. Alternatively, antibody fragments can be obtained by pepsin or papain digestion of whole antibodies by conventional methods.

It will be appreciated by persons skilled in the art that for human therapy or diagnostics, humanised antibodies are preferably used. Humanised forms of non-human (*e.g.* murine) antibodies are genetically engineered chimaeric antibodies or antibody fragments having preferably minimal-portions derived from non-human antibodies. Humanised antibodies include antibodies in which complementary determining regions of a human antibody (recipient antibody) are replaced by residues from a complementary determining region of a non human species (donor antibody) such as mouse, rat of rabbit

having the desired functionality. In some instances, Fv framework residues of the human antibody are replaced by corresponding non-human residues. Humanised antibodies may also comprise residues which are found neither in the recipient antibody nor in the imported complementarity determining region or framework sequences. In general, the humanised antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the complementarity determining regions correspond to those of a non human antibody and all, or substantially all, of the framework regions correspond to those of a relevant human consensus sequence. Humanised antibodies optimally also include at least a portion of an antibody constant region, such as an Fc region, typically derived from a human antibody (see, for example, Jones *et al.*, 1986. *Nature* **321**:522-525; Riechmann *et al.*, 1988, *Nature* **332**:323-329; Presta, 1992, *Curr. Op. Struct. Biol.* **2**:593-596).

Methods for humanising non-human antibodies are well known in the art. Generally, the humanised antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues, often referred to as imported residues, are typically taken from an imported variable domain. Humanisation can be essentially performed as described (see, for example, Jones *et al.*, 1986, *Nature* **321**:522-525; Reichmann *et al.*, 1988. *Nature* **332**:323-327; Verhoeyen *et al.*, 1988, *Science* **239**:1534-1536l; US 4,816,567) by substituting human complementarity determining regions with corresponding rodent complementarity determining regions. Accordingly, such humanised antibodies are chimaeric antibodies, wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanised antibodies may be typically human antibodies in which some complementarity determining region residues and possibly some framework residues are substituted by residues from analogous sites in rodent antibodies.

Human antibodies can also be identified using various techniques known in the art, including phage display libraries (see, for example, Hoogenboom & Winter, 1991, *J. Mol. Biol.* **227**:381; Marks *et al.*, 1991, *J. Mol. Biol.* **222**:581; Cole *et al.*, 1985, In: *Monoclonal antibodies and Cancer Therapy*, Alan R. Liss, pp. 77; Boerner *et al.*, 1991. *J. Immunol.* **147**:86-95).

Once suitable antibodies are obtained, they may be tested for activity, for example by ELISA.

A further embodiment of the present invention provides a binding moiety capable of binding selectively to a nucleic acid molecule encoding Sox11 protein.

Preferably, the binding moiety is capable of binding selectively to a nucleic acid molecule encoding a polypeptide comprising an amino acid sequence of SEQ ID NO:1 and/or natural variants thereof.

More preferably the binding moiety comprises or consists of a nucleic acid molecule. Even more preferably the binding moiety comprises or consists of a DNA molecule. Advantageously the binding moiety comprises or consists of a fragment of the nucleotide sequence of SEQ ID NO:2 (see figure 5), or the complementyary sequence thereof, of a fragment or a variant of the same. Conveniently, the nucleic acid molecule is 5 to 100 nucleotides in length. More conveniently the nucleic acid molecule is 15 to 35 nucleotides in length.

Preferably the binding moiety comprises a detectable moiety.

By a "detectable moiety" we include the meaning that the moiety is one which, when located at the target site following administration of the compound of the invention into a patient, may be detected, typically non-invasively from outside the body and the site of the target located. Thus, the compounds of this embodiment of the invention are useful in imaging and diagnosis.

Typically, the detectable moiety is or comprises a radioactive atom which is useful in imaging. Suitable radioactive atoms include ^{99m}Tc and ^{123}I for scintigraphic studies. Other readily detectable moieties include, for example, spin labels for magnetic resonance imaging (MRI) such as ^{123}I again, ^{131}I, ^{111}In, ^{19}F, ^{13}C, ^{15}N, ^{17}O, gadolinium, manganese or iron. Clearly, the compound of the invention must have sufficient of the appropriate atomic isotopes in order for the molecule to be readily detectable.

The radio- or other labels may be incorporated in the compound of the invention in known ways. For example, if the binding moiety is a polypeptide it may be biosynthesised or may be synthesised by chemical amino acid synthesis using suitable amino acid precursors involving, for example, fluorine-19 in place of hydrogen. Labels such as ^{99m}Tc, ^{123}I, ^{186}Rh, ^{188}Rh and ^{111}In can, for example, be attached *via* cysteine

residues in the binding moiety. Yttrium-90 can be attached via a lysine residue. The IODOGEN method (Fraker *et al.*, 1978, *Biochem. Biophys. Res. Comm.* 80:49-57) can be used to incorporate ^{123}I. Reference ("Monoclonal Antibodies in Immunoscintigraphy", J-F Chatal, CRC Press, 1989) describes other methods in detail.

Thus, in a further embodiment of the invention the radioactive atom is selected from the group consisting of technetium-99m, iodine-123, iodine-125, iodine-131, indium-111, fluorine-19, carbon-13, nitrogen-15, oxygen-17, phosphorus-32, sulphur-35, deuterium, tritium, rhenium-186, rhenium-188 and yttrium-90.

A third aspect of the invention provides a method of diagnosing epithelial ovarian cancer (EOC) in an individual, the method comprising:

(a) providing a sample of epithelial ovarian cells from the individual; and

(b) determining the amount of Sox11 protein and/or mRNA in the sample of cells.

wherein the levels of Sox11 protein and/or mRNA are indicative of the individual having epithelial ovarian cancer (EOC).

In particular, high levels of Sox11 protein and/or mRNA are indicative of the individual having epithelial ovarian cancer (EOC).

In one embodiment, the method is for diagnosing EOC belonging to a specific histological subtype (*e.g.* serous, mucinous, endometrioid, clear cell and undifferentiated or unclassifiable).

Likewise, the method may be for diagnosing EOC associated with cells of a specific grade (*e.g.* high grade).

In one embodiment, the method further comprises conducting one or more additional diagnostic tests for EOC on the epithelial ovarian cells to confirm the diagnosis

A fourth aspect of the invention provides a method of prognosing epithelial ovarian cancer (EOC) in an individual, the method comprising:

(a) providing a sample of epithelial ovarian cancer cells from the individual; and

(b) determining the amount of Sox11 protein and/or mRNA in the sample of cells.

wherein the levels of Sox11 protein and/or mRNA are indicative of the chance of recurrence-free survival of the individual.

In one embodiment, the method is for prognosing EOC belonging to a specific histological subtype (*e.g.* serous, mucinous, endometrioid, clear cell and undifferentiated or unclassifiable).

Likewise, the method may be for prognosing EOC associated with cells of a specific grade (*e.g.* high grade).

By "chance of recurrence-free survival" we mean the probability of the individual surviving for a given period, such as one year, two years, three years, five years, ten years or more.

In one embodiment, high levels of Sox11 protein and/or mRNA is indicative of the individual having improved recurrence-free survival (RFS).

By "improved recurrence-free survival" we mean that the probability of recurrence-free survival is higher when compared to an average population of epithelial ovarian cancer (EOC) patients, for example the probability may be increased by at least 0.05, 0.1, 0.2, 0.3, 0.4 or 0.5 or more.

In an alternative embodiment, low levels of Sox11 protein and/or mRNA is indicative of the individual having diminished recurrence-free survival (RFS).

By "diminished recurrence-free survival" we mean that the probability of recurrence-free survival is lower when compared to an average population of epithelial ovarian cancer (EOC) patients, for example the probability may be lowered by at least 0.05, 0.1, 0.2, 0.3, 0.4 or 0.5 or more.

A fifth aspect of the invention provides a method of detecting an epithelial ovarian cancer (EOC) in an individual, the method comprising:

(a) providing a sample of cells from the individual; and

(b) determining the amount of Sox11 protein and/or mRNA in the sample of cells.

wherein the levels of Sox11 protein and/or mRNA are indicative of the individual having epithelial ovarian cancer (EOC) cells.

In one embodiment, the method is for detecting cells of an EOC belonging to a specific histological subtype (*e.g.* serous, mucinous, endometrioid, clear cell and undifferentiated or unclassifiable).

Likewise, the method may be for detecting cells of an EOC associated with cells of a specific grade (*e.g.* high grade).

In particular, high levels of Sox11 protein and/or mRNA are indicative of the individual having epithelial ovarian cancer (EOC).

By "high levels of Sox11 protein and/or mRNA" we mean the amount of Sox11 protein and/or mRNA is at least 10% higher than in non-cancerous (*e.g.* healthy)_epithelial ovarian cells, for example 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 120%, 150%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, 1000%, 1500%, 2000%, 3000%, 4000%, 5000%, 10000% higher, or more.

By "low levels of Sox11 protein and/or mRNA" we mean the amount of Sox11 protein and/or mRNA is not statistically different from that of non-cancerous epithelial ovarian cells.

It will be appreciated by skilled persons that the methods of the invention may be performed *in vivo* or *in vitro*.

In one embodiment, the method comprises the detection of Sox11 expression as a sole biomarker for the diagnosis or prognosis of epithelial ovarian cancer (EOC) (as discussed above).

Alternatively, the binding moiety of the invention may be used in combination with one or more additional binding moieties for detecting one or more additional biomarkers for the diagnosis or prognosis of EOC. Thus, the binding moiety may be used in combination with fewer than 20 additional binding moieties, for example fewer than 15, 10, 8, 6, 5, 4, 3, 2 or 1 additional binding moieties.

In one embodiment, the method comprises detecting nuclear and/or cytoplasmic expression of Sox11.

Preferably the sample of cells to be tested is in the form of a tissue sample.

Conveniently, the amount of Sox11 protein and/or mRNA in the sample is determined using a binding moiety according to the first or second aspect of the invention.

A further embodiment of the methods of the invention comprises comparing the amount of Sox11 protein and/or mRNA in the sample of cells to be tested with the amount of Sox11 protein and/or mRNA in a control sample.

Advantageously the control sample is a negative control sample comprising or consisting of non-cancerous epithelial ovarian cells.

Alternatively, or in addition, the control sample is a positive control sample comprising or consisting of epithelial ovarian cancer cells. The ovarian epithelial cells may be high recurrence-free survival (RFS)-associated EOC cells or low recurrence-free survival (RFS)-associated EOC cells.

Typically, step (b) of the methods of the invention is performed using a method selected from the group consisting of macroarray, microarray(including tissue microarray), nanoarray, reverse transcription PCR, real-time PCR or *in situ* PCR.

A further embodiment of the third or fourth aspects of the invention comprises determining the levels of additional EOC biomarker proteins and/or mRNA in the sample to cells to be tested, for example p53, estrogen receptor and/or progesterone receptor.

A sixth aspect of the invention provides a method of imaging epithelial ovarian cancer (EOC) cells in the body of an individual, the method comprising administering to the individual an effective amount of a binding moiety according to the first or second aspect of the invention.

In one embodiment, the method is for imaging cells of an EOC belonging to a specific histological subtype (*e.g.* serous, mucinous, endometrioid, clear cell and undifferentiated or unclassifiable).

Likewise, the method may be for imaging cells of an EOC associated with cells of a specific grade (*e.g.* high grade).

The term 'effective amount' as used herein, refers to that amount which provides a sufficiently detectable signal for a given administration regimen. This is a predetermined quantity of active material calculated to produce a desired signal strength in association with the required additive and diluent, *i.e.* a carrier or administration vehicle. As is appreciated by those skilled in the art, the amount of a compound may vary depending on its specific activity. Suitable dosage amounts may contain a predetermined quantity of active composition calculated to produce the desired signal strength in association with the required diluent. In the methods and use for manufacture of compositions of the invention, an effective amount of the active component is provided. An effective amount can be determined by the ordinary skilled medical or veterinary worker based on patient characteristics, such as age, weight, sex, condition, complications, other diseases, *etc.*, as is well known in the art.

Typically, the sixth aspect of the invention comprises the step of detecting the location of the binding moiety in the individual. Preferably Sox11 protein and/or mRNA encoding the same are used as a marker for epithelial ovarian cancer (EOC) cells.

A seventh aspect of the invention provides the use of a binding moiety as defined above in the preparation of a medicament for diagnosing or prognosing epithelial ovarian cancer (EOC).

The invention additionally provides as an eighth aspect the use of Sox11 protein and/or mRNA encoding the same as a biomarker for epithelial ovarian cancer (EOC) cells.

In one embodiment, the Sox11 protein and/or mRNA encoding the same is for use as a biomarker for EOC belonging to a specific histological subtype (*e.g.* serous, mucinous, endometrioid, clear cell and undifferentiated or unclassifiable).

Likewise, the Sox11 protein and/or mRNA encoding the same may be for use as a biomarker for EOC belonging to a specific grade (*e.g.* high grade).

It will be appreciated that Sox11 may be used as a sole biomarker for epithelial ovarian cancer (EOC).

Alternatively, Sox11 may be used in combination with one or more additional biomarkers. Preferably, however, Sox11 is used in combination with fewer than 20 additional biomarkers are used in the method, for example fewer than 15, 10, 8, 6, 5, 4, 3, 2 or 1 additional biomarkers.

A ninth aspect of the invention provides a method of screening for a molecule with efficacy in the diagnosis and/or prognosis of epithelial ovarian cancer (EOC), the method comprising the steps of:

(a) contacting a molecule to be tested with Sox11 protein and/or mRNA encoding the same (or with a fragment of said protein or mRNA); and

(b) detecting the presence of a complex containing the protein and/or mRNA (or fragment thereof) and the molecule to be tested.

In one embodiment, the method is for screening for a molecule with efficacy in the diagnosis and/or prognosis of EOC belonging to a specific histological subtype (*e.g.* serous, mucinous, endometrioid).

Likewise, the method may be for screening for a molecule with efficacy in the diagnosis and/or prognosis of EOC associated with cells of a specific grade (*e.g.* high grade).

Methods of detecting and/or measuring the concentration of protein and/or nucleic acid are well known to those skilled in the art, see for example Sambrook and Russell (*supra.*).

Preferred methods for detection and/or measurement of protein include Western blot, North-Western blot, immunosorbent assays (ELISA), antibody microarray, tissue microarray (TMA), immunoprecipitation, *in situ* hybridisation and other immunohistochemistry techniques, radioimmunoassay (RIA), immunoradiometric assays (IRMA) and immunoenzymatic assays (IEMA), including sandwich assays using monoclonal and/or polyclonal antibodies. Exemplary sandwich assays are described by David *et al.*, in US Patent Nos. 4,376,110 and 4,486,530, hereby incorporated by reference. Antibody staining of cells on slides may be used in methods well known in cytology laboratory diagnostic tests, as well known to those skilled in the art.

Typically, ELISA involves the use of enzymes which give a coloured reaction product, usually in solid phase assays. Enzymes such as horseradish peroxidase and phosphatase have been widely employed. A way of amplifying the phosphatase reaction is to use NADP as a substrate to generate NAD which now acts as a coenzyme for a second enzyme system. Pyrophosphatase from *Escherichia coli* provides a good conjugate because the enzyme is not present in tissues, is stable and gives a good reaction colour. Chemi-luminescent systems based on enzymes such as luciferase can also be used.

Conjugation with the vitamin biotin is frequently used since this can readily be detected by its reaction with enzyme-linked avidin or streptavidin to which it binds with great specificity and affinity.

Preferred methods for detection and/or measurement of nucleic acid (*e.g.* mRNA) include southern blot, northern blot, polymerase chain reaction (PCR), reverse transcriptase PCR (RT-PCR), quantitative real-time PCR (qRT-PCR), nanoarray, macroarray, autoradiography and *in situ* hybridisation.

In a typical embodiment the presence of epithelial ovarian cancer (EOC) cells is detected by detection of Sox11 protein and/or nucleic acid in cell nuclei and/or cytoplasm. Preferably, the nuclei/cytoplasm of lymphoma cells express Sox11 protein and/or nucleic

acid in a relatively high amount, as indicated, for example, by bright staining of the nuclei during *in situ* hybridisation analysis.

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

Figure 1. Sox11 protein expression in ovarian cancer.

Immunohistochemical staining of Sox 11 showing just cytoplasmic expression (A) and cytoplasmic and nuclear expression (B). Corresponding markup image of the deconvolution algorithm showing stroma in blue, cytoplasmic staining in yellow and orange and nuclear expression in red.

Figure 2. Sox11 protein expression and survival in ovarian cancer.

Sox11 expression classified into low, medium and high based on the histogram (A). Kaplan Meier estimate of RFS based on the three Sox11 groups (B). Kaplan Meier estimate of RFS based on comparison of high and medium levels of Sox11 to low levels of Sox11 (C).

Figure 3. Amino acid sequence of *Homo sapiens* Sox11 protein.

Figure 4. Nucleic acid sequence of *Homo sapiens SOX11* mRNA.

Figure 5. Overall survival (25 years) in endometroid ovarian cancer

The 25-year overall survival of patients with more or less than 10% Sox11 positive tumor cells are compared. When a patient is censored (removed from the study for other reason than death) this is indicated with a tick-mark on the line.

Figure 6. Overall survival (5 years) in endometroid ovarian cancer

The 5-year overall survival of patients with more or less than 10% Sox11 positive tumor cells are compared. When a patient is censored (removed from the study for other reason than death) this is indicated with a tick-mark on the line.

Figure 7. Cancer specific survival (5 years) in endometroid ovarian cancer

The 5-year cancer-specific survival of patients with more or less than 10% Sox11 positive tumor cells are compared. When a patient is censored (removed from the study for other reason than death) this is indicated with a tick-mark on the line.

Figure 8. Overall survival (25 years) for high grade EOC

The 25-year overall survival of high-grade patients with more or less than 10% Sox11 positive tumor cells are compared. When a patient is censored (removed from the study for other reason than death) this is indicated with a tick-mark on the line.

Figure 9. Cancer specific (25 years) survival for high grade EOC

The 25-year cancer-specific survival of high-grade patients with more or less than 10% Sox11 positive tumor cells are compared. When a patient is censored (removed from the study for other reason than death) this is indicated with a tick-mark on the line.

EXAMPLE A

Introduction

The transcription factor Sox11 is a member of the Sox gene family and has been mapped to chromosome 2p25.3 (Azuma T, Ao S, Saito Y, et al. Human SOX11, an upregulated gene during the neural differentiation, has a long 3' untranslated region. DNA research 1999;6:357-60). Sox proteins are identified as proteins that contain a DNA-binding high mobility group (HMG) domain with strong amino acid homology (usually >50%) to the HMG domain of the male sex determination gene, Sry (Wegner M. From head to toes: the multiple facets of Sox proteins. Nucleic acids research 1999;27:1409-20). More than 20 orthologous Sox genes have been identified in the human and mouse genomes, and family members are divided into eight subgroups according to the degree of homology within and outside the HMG-domain (Schepers GE, Teasdale RD, and Koopman P. Twenty pairs of sox: extent, homology, and nomenclature of the mouse and human sox transcription factor gene families. Developmental cell 2002;3:167-70). Sox proteins act as transcription factors by binding to the minor groove of DNA and inducing a sharp bend of DNA allowing them to play a key architectural role in the assembly of transcriptional enhancer complexes (Dy P, Penzo-Mendez A, Wang H, et al. The three SoxC proteins Sox4, Sox11 and Sox12 exhibit overlapping expression patterns and molecular properties. Nucleic acids research 2008;36:3101-17; van de Wetering M and Clevers H. Sequence-specific interaction of the HMG box proteins TCF-1 and SRY occurs within the minor groove of a Watson-Crick double helix. The EMBO journal 1992;11:3039-44). In addition to protein-DNA interactions, Sox proteins also interact with various other transcription factors to increase their efficiency and specificity of action (Dy P, Penzo-Mendez A, Wang H, et al. The three SoxC proteins Sox4, Sox11 and Sox12 exhibit overlapping expression patterns and molecular properties. Nucleic acids research 2008;36:3101-17).

Sox11 belongs to the C subgroup, along with Sox4 and Sox12 (Schepers GE, Teasdale RD, and Koopman P. Twenty pairs of sox: extent, homology, and nomenclature of the mouse and human sox transcription factor gene families. Developmental cell 2002;3:167-70), and all three proteins demonstrate a high degree of homology within both the C-terminal transactivation domain and the HMG domain (Dy P, Penzo-Mendez A, Wang H, et al. The three SoxC proteins Sox4, Sox11 and Sox12 exhibit overlapping expression patterns and molecular properties. Nucleic acids research 2008;36:3101-17;

Jay P, Goze C, Marsollier C, et al. The human SOX11 gene: cloning, chromosomal assignment and tissue expression. Genomics 1995;29:541-5). Sox11 and Sox 4 play major roles in cardiac, neuronal and other major embryonic processes, whilst less is known about Sox12 (Dy P, Penzo-Mendez A, Wang H, et al. The three SoxC proteins Sox4, Sox11 and Sox12 exhibit overlapping expression patterns and molecular properties. Nucleic acids research 2008;36:3101-17).

We have recently demonstrated the nuclear Sox11 is specifically up-regulated in mantle cell lymphoma (MCL) and distinguishes MCL from other B-cell lymphomas (Ek S, Dictor M, Jerkeman M, Jirstrom K, and Borrebaeck CA. Nuclear expression of the non B cell lineage Sox11 transcription factor identifies mantle cell lymphoma. Blood 2008;111:800-5). Sox4 is a prominent transcription factor in lymphocytes of both the B and T-cell lineage (Wegner M. From head to toes: the multiple facets of Sox proteins. Nucleic acids research 1999;27:1409-20; van de Wetering M, Oosterwegel M, van Norren K, and Clevers H. Sox-4, an Sry-like HMG box protein, is a transcriptional activator in lymphocytes. The EMBO journal 1993;12:3847-54) and is crucial for B lymphopoiesis (Smith E and Sigvardsson M. The roles of transcription factors in B lymphocyte commitment, development, and transformation. Journal of leukocyte biology 2004;75:973-81), whilst Sox11 has no known lymphopoietic function and is not expressed in B cells (Ek S, Dictor M, Jerkeman M, Jirstrom K, and Borrebaeck CA. Nuclear expression of the non B-cell lineage Sox11 transcription factor identifies mantle cell lymphoma. Blood 2008;111:800-5). Both Sox4 and Sox11 are expressed in medulloblastoma (Lee CJ, Appleby VJ, Orme AT, Chan WI, and Scotting PJ. Differential expression of SOX4 and SOX11 in medulloblastoma. Journal of neuro-oncology 2002;57:201-14) and Sox11 is also overexpressed in malignant glioma (Weigle B, Ebner R, Temme A, et al. Highly specific overexpression of the transcription factor SOX11 in human malignant gliomas. Oncology reports 2005;13:139-44). Additionally Sox4 is expressed in bladder cancer with increased levels of expression associated with improved patient outcome (Aaboe M, Birkenkamp-Demtroder K, Wiuf C, et al. SOX4 expression in bladder carcinoma: clinical aspects and in vitro functional characterization. Cancer research 2006;66:3434-42).

This study outlines the expression of Sox11 mRNA across a large number of normal tissues and tumours, and reveals Sox11 mRNA to be overexpressed in a large number of malignant tissues. In addition, we specifically examined Sox11 protein expression in

EOC and demonstrated that increased levels of Sox11 protein, as determined by image analysis, were associated with an improve recurrence free survival (RFS).

Materials and Methods

Transcriptional profiling

SOX11 gene mRNA expression levels across a large number of human tissues were retrieved from the In Silico Transcriptomics (IST) database, containing data from a meta-analysis of 14,095 samples analyzed using the Affymetrix gene expression microarrays.

Patients and tumour samples

The TMA, used in this study, was constructed from a consecutive cohort of 76 patients diagnosed with primary invasive epithelial ovarian cancer at the National Maternity Hospital, Dublin, with a median follow-up of of 4.3 years. The patient cohort is summarised in table 1. The standard surgical approach was a total abdominal hysterectomy, bilateral salpingo-oophorectomy and omentectomy with cytological evaluation of peritonea fluid or washings. Residual disease was resected to less than 2 cm where possible. Stage and volume of residual disease (no residual disease, residual disease greater or less than 2 cm) was recorded in all cases. Adjuvant chemotherapy consisted of cisplatin or carboplatin prior to 1992 and combined with paclitaxel from 1992 to 2002. No patient received neo-adjuvant chemotherapy. Benign or borderline ovarian cancers, non-epithelial ovarian cancer and cases with histological features typical of secondary ovarian cancer were excluded from the study. Diagnostic specimens were all formalin fixed and paraffin embedded in the Department of Pathology at the National Maternity Hospital, Dublin, Ireland. All tissue blocks were stored in this department prior to construction of the TMA. Full ethical approval was obtained from the ethics committee of the National Maternity Hospital, Dublin.

Tissue microarrays and immunohistochemistry

Seventy six paraffin-embedded tumour specimens were used for tissue microarray (TMA) construction. Areas representative of invasive cancer were marked on haematoxylin and eosin-stained slides and the TMA was constructed, using a manual tissue arrayer (MTA-1, Beecher Inc, WI). The array consisted of four cores per patient.

Two 1.0 mm cores were extracted from each donor block and assembled in a recipient block. Recipient blocks were limited to approximately 100 cores each. In general, cores were taken from the peripheral part of the tumour in cases where the tumour had well-defined borders. In more diffusely growing tumours, areas with the highest tumour cell density were primarily targeted. Necrotic tissue was avoided.

TMA sections (4 µm) were dried, deparaffinized, rehydrated and put through descending concentrations of ethanol. Heat mediated antigen retrieval was performed in a BORGdecloaker (Biocare, Concord, CA, USA) at pH 9.0 and sections were then stained with the primary rabbit anti-human Sox11 antibody (1:100) at room temperature for 25 minutes. This specific antibody was raised, as previously described (Ek S, Dictor M, Jerkeman M, Jirstrom K, and Borrebaeck CA. Nuclear expression of the non B-cell lineage Sox11 transcription factor identifies mantle cell lymphoma. Blood 2008; 111:800-5) and targeted the following protein sequence:

FMVWSKIERRKIMEQSPDMHNAEISKRLGKRWKMLKDSEKIPFIREAERLRLKHMADYP
DYKYRPRKKPKMDPSAKPSASQSPEKSAAGGGGGSAGGGAGGAKTSKGSSKK

[SEQ ID NO:3]

Signal was detected, using the Dako REAL Detection system, containing the secondary biotinylated goat anti-rabbit/mouse antibody, the strepavidin/horseradish peroxidase complex and 3,3´- diaminobenzidine, according to the manufacturer's protocol. Slides were counterstained with Mayers hematoxylin (Sigma-Aldrich, St Louis, MO).

Image Acquisition, Management and Automated analysis

The Aperio ScanScope XT Slide Scanner (Aperio Technologies, Vista, CA) system was used to capture whole slide digital images with a 20X objective. Slides were de-arrayed to visualise individual cores, using TMA Lab (Aperio). A color deconvolution algorithm (Aperio) was used to develop a quantitative scoring model for Sox11 expression.

Statistical analysis

Spearman's Rho correlation was used estimate the relationship between cores from individual tumours, Differences in distribution of clinical data and tumour characteristics between samples with a high and low Sox11 expression (described below) were

evaluated using the χ^2 test. Kaplan-Meier analysis and the log rank test were used to illustrate differences between RFS. Cox regression proportional hazards models were used to estimate the relationship to RFS and Sox11, stage and grade. All calculations were performed, using SPSS version 11.0 (SPSS Inc, Chicago, IL). P value < 0.05 was considered statistically significant.

Results

Sox11 mRNA expression in normal and tumour tissues

A metanalysis of Sox11 mRNA expression levels was performed in 14 095 samples analyzed using the Affymetrix gene expression microarrays.

Increased levels of Sox11 mRNA expression were evident in epithelial ovarian carcinoma samples (data not shown).

Sox11 protein expression in ovarian cancer

Having identified Sox11 as a gene that was overexpressed in epithelial ovarian carcinoma cells, Sox11 protein expression was examined using IHC in EOC as illustrated in Figure 1. Sox11 expression was seen exclusively in tumour epithelium and IHC signal was evident in both the nucleus and the cytoplasm. Nuclear expression of Sox11 was present only when accompanied by cytoplasmic signal, whereas a proportion (49%) of tumours did demonstrate cytoplasmic expression in the absence of nuclear signal (Fig. 1).

Quantitative determination of Sox11 expression as determined by image analysis

Quantitative determination of Sox11 expression was then ascertained, using an image analysis approach, in particular via the use of a commercial colour deconvolution algorithm (Aperio). A pseudo-colour "mark-up" image was generated as an algorithm result, thus allowing confirmation that the algorithm was accurately identifying epithelial and stromal pixels (Fig. 1). A full description of the algorithm was recently published (Brennan DJ, Rexhepaj E, O'Brien SL, et al. Altered Cytoplasmic-to-Nuclear Ratio of Survivin Is a Prognostic Indicator in Breast Cancer. Clinical cancer research 2008;14:2681-9).

The algorithm was used to calculate a total intensity (TI) for Sox11 for the each core. There was a strong correlation between quadruplicate cores from individual tumours for TI (Spearman's Rho = 0.858, $p < 0.001$), indicating that Sox11 has a homogenous pattern of expression in ovarian cancer and is suitable for TMA based analysis. As tumours were arrayed in quadruplicate, the median value for each tumour was used for further analysis. The algorithm accurately distinguished between nuclear and cytoplasmic staining in all cores, as confirmed by a histopathologist.

A histogram of Sox11 image analysis data for the entire cohort is shown in Figure 2a. Using this histogram, the tumours were placed into three categories - high, medium and low level of Sox11 expression, as determined by image analysis. Based on image analysis categorization, 20% (n = 17) of tumours were classified as having high levels 43% (n = 35) medium levels and 29% (n = 24) low/negative levels of Sox11 expression, as determined by image analysis. Tumours in the high expression group all showed expression of nuclear and cytoplasmic Sox11, whilst those in the medium group generally exhibited only cytoplasmic Sox11. No association was found between Sox11 expression and age, grade or stage of disease.

<u>Associations between Sox11 expression as determined by automated image analysis and survival</u>

Kaplan Meier analysis of RFS based on the expression of Sox11 revealed a stepwise decrease in RFS between the high, medium and low groups ($p = 0.033$) (Fig. 2b). Further subset analysis revealed a markedly reduced RFS in patients with low levels of Sox11 expression, as determined by image analysis compared to high and medium Sox11 expressers ($p = 0.02$) (Fig. 2c). We proceeded to perform a multivariate Cox regression analysis of RFS, which revealed that Sox11 expression was an independent predictor of RFS when compared to stage and grade (HR = 0.56, 95% CI = 0.319 – 0.997, $p = 0.049$).

Discussion

In this study we combined a meta-analysis of transcriptomic data, TMAs and automated image analysis to identify and validate Sox11 as a prognostic biomarker in epithelial ovarian carcinoma (EOC).This study is the first to describe the relationship between Sox11 expression and prognosis in EOC. Having previously identified Sox11 as a new diagnostic marker in MCL (Ek S, Dictor M, Jerkeman M, Jirstrom K, and Borrebaeck CA. Nuclear expression of the non B-cell lineage Sox11 transcription factor identifies mantle cell lymphoma. Blood 2008;111:800-5), we proceeded to use the IST database containing data from a meta-analysis of 14 095 samples analyzed, using the Affymetrix gene expression microarrays to profile the expression of Sox11 mRNA in EOC cells.

We then proceeded to use TMAs and a quantitative automated analysis of IHC to evaluate Sox11 protein expression in EOC in relation to recurrence free survival. This revealed that epithelial-specific Sox11 expression in both the nuclear and cytoplasmic compartments. Increased levels of Sox11, particularly nuclear Sox11, was associated with an increased RFS and Cox regression multivariate analysis revealed Sox11 was an independent predictor of RFS when controlling for grade and stage (see Table 2).

Sox11 plays an important role in embryogenesis and tissue remodeling, and consequently is present during gastrulation and early post-gastrulation development throughout the embryo (Hargrave M, Wright E, Kun J, et al. Expression of the Sox11 gene in mouse embryos suggests roles in neuronal maturation and epithelio-mesenchymal induction. Developmental dynamics 1997;210:79-86; Sock E, Rettig SD, Enderich J, et al. Gene targeting reveals a widespread role for the high-mobility-group transcription factor Sox11 in tissue remodeling. Molecular and cellular biology 2004;24:6635-44). Later during development, Sox11 is prominently expressed in the developing nervous system and at many sites throughout the embryo where epithelial-mesenchymal interactions occur (Hargrave M, Wright E, Kun J, et al. Expression of the Sox11 gene in mouse embryos suggests roles in neuronal maturation and epithelio-mesenchymal induction. Developmental dynamics 1997;210:79-86). At sites of such epithelial-mesenchymal interactions, Sox11 can be found in the mesenchymal or epithelial compartment, and it has been postulated to be involved in inductive remodelling (Hargrave M, et al., 1997*supra*.). Sox11 expression in most tissues is transient and as a consequence, little Sox11 expression has been found in terminally differentiated adult tissues, in contrast to its widespread expression during

embryogenesis (Sock E, Rettig SD, Enderich J, et al. Gene targeting reveals a widespread role for the high-mobility-group transcription factor Sox11 in tissue remodeling. Molecular and cellular biology 2004;24:6635-44). Our findings complement these data, whereby Sox11 expression was absent in normal tissue.

The role played by Sox11 in tumourogenesis remains to be fully elucidated. As mentioned previously, a marked upregulation of Sox11 mRNA was evident in EOC cells. The exact functional role of Sox11 in adult tissues is not fully understood, although the Sox proteins appear to play a dual role (i) DNA binding and (ii) transcriptional partner selection, which may permit selective recruitment of individual Sox proteins to specific genes (Ek S, Dictor M, Jerkeman M, Jirstrom K, and Borrebaeck CA. Nuclear expression of the non B-cell lineage Sox11 transcription factor identifies mantle cell lymphoma. Blood 2008;111:800-5). Whilst a number of studies have described Sox11 expression in gliomas (Weigle B, Ebner R, Temme A, et al. Highly specific overexpression of the transcription factor SOX11 in human malignant gliomas. Oncology reports 2005;13:139-44), neuroblastomas (Lee CJ, Appleby VJ, Orme AT, Chan WI, and Scotting PJ. Differential expression of SOX4 and SOX11 in medulloblastoma. Journal of neuro-oncology 2002;57:201-14) and MCL (Ek S, Dictor M, Jerkeman M, Jirstrom K, and Borrebaeck CA. Nuclear expression of the non B-cell lineage Sox11 transcription factor identifies mantle cell lymphoma. Blood 2008;111:800-5), its functional role in these tumours is not completely understood.

In summary, this is the first description of the differential expression of Sox11 in EOC. Our findings demonstrate that Sox11 is an independent predictor of improved RFS in EOC.

Table 1. Patient and tumour characteristics

Age	
Median (Range)	52 (31-77)
Histology	
Serous	50
Mucinous	4
Endometroid	17
Clear cell	1
Other	4
Grade	
Well Differentiated	12
Moderately Differentiated	29
Poorly Differentiated	35
Stage	
1	0
2	21
3	54
4	1

Table 2. Multivariate* Cox regression analysis of RFS

	HR	95.0% CI	P value
Sox11 *(high/ medium v's low)*	0.56	0.319 - 0.997	0.049
Stage *(continuous)*	1.92	0.971 - 3.800	0.061
Grade *(Well and moderately diff v's poorly Diff)*	1.08	0.740 - 1.562	0.702

*Adjusted for all other variables in the table

Abbreviations: HR = Hazard ratio, 95% CI = 95% Confidence intervals

EXAMPLE B

Introduction

Epithelial ovarian cancer (EOC) comprises three major histological subtypes (serous, mucinous and endometrioid) and can also be subgrouped based on stage and grade. Endometrioid tumors make up about 2 to 4 percent of all ovarian tumors and most of them (about 80 percent) are malignant, representing 10 to 20 percent of all ovarian carcinomas.

Material & Methods

Sections of high grade EOC and endometrioid EOC were stained for Sox11 and analyzed as previously described (Brennan *et al.*, 2009, *European Journal of Cancer*, **45**(8):1510-1517).

Results & Discussion

As shown in Figures 5, 6 and 7, overall and cancer specific survival can be predicted using Sox11 for endometrioid EOCs. Also, as shown in Figures 8 and 9, overall and cancer specific survival can be predicted using Sox11 for high grade EOCs.

When calculating cancer-specific survival probability only data for cancer-related deaths are used, in contrast to when calculating overall survival.

These data indicate that Sox11 can be used in both high grade EOC and endometrioid ovarian cancer to stratify patients into clinically relevant groups based on overall and cancer specific survival.

In conclusion, Sox11 is not only a useful biomarker for EOC as a group, but can be used for subgroups of patients with different clinical and/or histological features.

CLAIMS

1. A binding moiety which is capable of binding selectively to Sox11 protein, or to a nucleic acid molecule encoding the same, for use in diagnosing and/or prognosing epithelial ovarian cancer (EOC).

2. A binding moiety which is capable of binding selectively to Sox11 protein, or to a nucleic acid molecule encoding the same, for detecting epithelial ovarian cancer (EOC) cells.

3. A binding moiety according to Claim 1 for the diagnosis of epithelial ovarian cancer (EOC).

4. A binding moiety according to Claim 1 for the prognosis of epithelial ovarian cancer (EOC).

5. A binding moiety according to any one of the preceding claims wherein the EOC belongs to a histological subtype selected from the group consisting of serous, mucinous, endometrioid, clear cell and undifferentiated or unclassifiable.

6. A binding moiety according to Claim 5 wherein the EOC is serous EOC.

7. A binding moiety according to Claim 5 wherein the EOC is mucinous EOC.

8. A binding moiety according to Claim 5 wherein the EOC is endometrioid EOC.

9. A binding moiety according to Claim 5 wherein the EOC is clear cell EOC.

10. A binding moiety according to Claim 5 wherein the EOC is undifferentiated or unclassifiable EOC.

11. A binding moiety according to any one of the preceding claims for use *in vivo*.

12. A binding moiety according to any one of the preceding claims for use *in vitro*.

13. A binding moiety according to any one of the preceding claims for use in the detection of Sox11 expression as a sole biomarker for diagnosing or prognosing epithelial ovarian cancer (EOC).

14. A binding moiety according to any one of the preceding claims for use in combination with one or more additional binding moieties for detecting one or more additional biomarkers for diagnosing or prognosing epithelial ovarian cancer (EOC).

15. A binding moiety according to Claim 14 for use in combination with fewer than 20 additional binding moieties, for example fewer than 15, 10, 8, 6, 5, 4, 3, 2 or 1 additional binding moieties.

16. A binding moiety according to any one of the preceding claims for detecting nuclear and/or cytoplasmic expression of Sox11.

17. A binding moiety according to any one of the preceding claims wherein the binding moiety is capable of binding selectively to Sox11 protein.

18. A binding moiety according to Claim 17 wherein the binding moiety is capable of binding selectively to a polypeptide comprising an amino acid sequence of SEQ ID NO: 1 and/or a natural variant thereof.

19. A binding moiety according to any one of the preceding claims wherein the binding moiety comprises or consists of a polypeptide.

20. A binding moiety according to Claim 19 wherein the binding moiety comprises or consists of an antibody, or an antigen-binding fragment or variant thereof.

21. A binding moiety according to Claim 20 wherein the antibody is a monoclonal antibody.

22. A binding moiety according to Claim 20 or 21 wherein the antibody or antigen-binding fragment or variant thereof is selected from the group consisting of Fv fragments, Fab-like fragments, single variable domains and domain antibodies.

23. A binding moiety according to any one of Claims 20 to 22 wherein the antibody or an antigen-binding fragment or variant thereof is humanised.

24. A binding moiety according to any one of Claims 1 to 16 wherein the binding moiety is capable of binding selectively to a nucleic acid molecule encoding Sox11 protein.

25. A binding moiety according to Claim 24 wherein the binding moiety is capable of binding selectively to a nucleic acid molecule encoding a polypeptide comprising an amino acid sequence of SEQ ID NO: 1 and/or natural variants thereof.

26. A binding moiety according to Claim 24 or 25 wherein the binding moiety comprises or consists of a nucleic acid molecule.

27. A binding moiety according to Claim 24 wherein the binding moiety comprises or consists of a DNA molecule.

28. A binding moiety according to Claim 24 to 27 wherein the binding moiety comprises or consists of a fragment of the nucleotide sequence of SEQ ID NO:2, or the complementary sequence thereof, or a variant of the same.

29. A binding moiety according to any one of Claims 26 to 28 wherein the nucleic acid molecule is 5 to 100 nucleotides in length.

30. A binding moiety according to Claim 29 wherein the nucleic acid molecule is 15 to 35 nucleotides in length

31. A binding moiety according to any one of the preceding claims wherein the binding moiety comprises a detectable moiety.

32. A binding moiety according to Claim 31 wherein the detectable moiety comprises or consists of a radioactive atom.

33. A binding moiety according to Claim 32 wherein the radioactive atom is selected from the group consisting of technetium-99m, iodine-123, iodine-125, iodine-131, indium-

111, fluorine-19, carbon-13, nitrogen-15, oxygen-17, phosphorus-32, sulphur-35, deuterium, tritium, rhenium-186, rhenium-188 and yttrium-90.

34. A method of diagnosing epithelial ovarian cancer (EOC) in an individual, the method comprising:

(a) providing a sample of epithelial ovarian cells from the individual; and

(b) determining the amount of Sox11 protein and/or mRNA in the sample of cells.

wherein the levels of Sox11 protein and/or mRNA are indicative of the individual having epithelial ovarian cancer (EOC).

35. A method according to Claim 34 wherein high levels of Sox11 protein and/or mRNA are indicative of the individual having epithelial ovarian cancer (EOC).

36. A method according to Claim 34 or 35 wherein the EOC belongs to a histological subtype selected from the group consisting of serous, mucinous, endometrioid, clear cell and undifferentiated or unclassifiable.

37. A method according to Claim 36 wherein the EOC is serous EOC.

38. A method according to Claim 36 wherein the EOC is mucinous EOC.

39. A method according to Claim 36 wherein the EOC is endometrioid EOC.

40. A method according to Claim 36 wherein the EOC is clear cell EOC.

41. A method according to Claim 36 wherein the EOC is undifferentiated or unclassifiable EOC.

42. A method of prognosing epithelial ovarian cancer (EOC) in an individual, the method comprising:

(a) providing a sample of epithelial ovarian cancer cells from the individual; and

(b) determining the amount of Sox11 protein and/or mRNA in the sample of cells.

wherein the levels of Sox11 protein and/or mRNA are indicative the individual having improved recurrence-free survival (RFS).

43. A method according to Claim 42 wherein the EOC belongs to a histological subtype selected from the group consisting of serous, mucinous, endometrioid, clear cell and undifferentiated or unclassifiable.

44. A method according to Claim 43 wherein the EOC is serous EOC.

45. A method according to Claim 43 wherein the EOC is mucinous EOC.

46. A method according to Claim 43 wherein the EOC is endometrioid EOC.

47. A method according to Claim 43 wherein the EOC is clear cell EOC.

48. A method according to Claim 43 wherein the EOC is undifferentiated or unclassifiable EOC.

49. A method according to any one of Claims 42 to 48 wherein high levels of Sox11 protein and/or mRNA is indicative of the individual having improved recurrence-free survival (RFS).

50. A method according to any one of Claims 42 to 48 wherein low levels of Sox11 protein and/or mRNA is indicative of the individual having diminished recurrence-free survival (RFS).

51. A method of detecting epithelial ovarian cancer (EOC) cells in an individual, the method comprising:

(a) providing a sample of epithelial ovarian cells from the individual; and

(b) determining the amount of Sox11 protein and/or mRNA in the sample of cells.

wherein the levels of Sox11 protein and/or mRNA are indicative of the individual having epithelial ovarian cancer (EOC) cells.

52. A method according to Claim 51 wherein high levels of Sox11 protein and/or mRNA are indicative of the cells being epithelial ovarian cancer (EOC) cells.

53. A method according to Claim 51 to 52 wherein the EOC belongs to a histological subtype selected from the group consisting of serous, mucinous, endometrioid, clear cell and undifferentiated or unclassifiable.

54. A method according to Claim 53 wherein the EOC is serous EOC.

55. A method according to Claim 53 wherein the EOC is mucinous EOC.

56. A method according to Claim 53 wherein the EOC is endometrioid EOC.

57. A method according to Claim 53 wherein the EOC is clear cell EOC.

58. A method according to Claim 53 wherein the EOC is undifferentiated or unclassifiable EOC.

59. A method according to any one of Claims 34 to 58 wherein the method is performed *in vivo*.

60. A method according to any one of Claims 34 to 58 wherein the method is performed *in vitro*.

61. A method according to any one of Clams 34 to 60 wherein Sox11 is used as a sole biomarker.

62. A method according to any one of Clams 34 to 61 wherein Sox11 is used in combination with one or more additional biomarkers for diagnosing or prognosing EOC.

63. A method according to Claim 62 wherein fewer than 20 additional biomarkers are used in the method, for example fewer than 15, 10, 8, 6, 5, 4, 3, 2 or 1 additional biomarkers.

64. A method according to any one of Claims 34 to 63 wherein the method comprises detecting nuclear and/or cytoplasmic expression of Sox11.

65. A method according to any one of 34 32 to 64 wherein the sample of cells to be tested is in the form of a tissue sample.

66. A method according to any one of Claims 34 to 65 wherein determining the amount of Sox11 protein and/or mRNA in the sample is performed using a binding moiety according to any one of Claims 1 to 31.

67. A method according to any one of Claims 34 to 66 further comprising comparing the amount of Sox11 protein and/or mRNA in the sample of cells to be tested with the amount of Sox11 protein and/or mRNA in a control sample.

68. A method according to Claim 67 wherein the control sample is a negative control sample comprising or consisting of non-cancerous epithelial ovarian cells.

69. A method according to Claim 67 wherein the control sample is a positive control sample comprising or consisting of epithelial ovarian cancer (EOC) cells.

70. A method according to Claim 67 wherein the epithelial ovarian cancer (EOC) cells are high recurrence-free survival (RFS)-associated EOC cells.

71. A method according to Claim 67 wherein the epithelial ovarian cancer (EOC) cells are low recurrence free survival (RFS)-associated EOC cells.

72. A method according to any one of Claims 34 to 71 wherein step (b) is performed using a method selected from the group consisting of macroarray screening, microarray screening, nanoarray screening, reverse transcription PCR, real-time PCR or *in situ* PCR.

73. A method of imaging epithelial ovarian cancer (EOC) cells in the body of an individual, the method comprising administering to the individual an effective amount of a binding moiety as defined in any one of Claims 1 to 33.

74. A method according to Claim 73 further comprising the step of detecting the location of the binding moiety in the individual.

75. Use of a binding moiety as defined in any one of Claims 1 to 33 in the preparation of a medicament for diagnosing epithelial ovarian cancer (EOC).

76. Use of a binding moiety as defined in any one of Claims 1 to 33 in the preparation of a medicament for prognosing epithelial ovarian cancer (EOC).

77. Use of Sox11 protein and/or mRNA encoding the same as a biomarker for diagnosing epithelial ovarian cancer (EOC) cells.

78. Use of Sox11 protein and/or mRNA encoding the same as a biomarker for prognosing epithelial ovarian cancer (EOC) cells.

79. The use according to any one of Claims 75 to 78 wherein the EOC belongs to a histological subtype selected from the group consisting of serous, mucinous, endometrioid, clear cell and undifferentiated or unclassifiable.

80. The use according to Claim 79 wherein the EOC is serous EOC.

81. The use according to Claim 79 wherein the EOC us mucinous EOC.

82. The use according to Claim 79 wherein the EOC is endometrioid EOC.

83. The use according to Claim 79 wherein the EOC is clear cell EOC.

84. The use according to Claim 79 wherein the EOC is undifferentiated or unclassifiable EOC.

85. The use according to any one of Claims 77 to 84 wherein Sox11 is used as a sole biomarker.

86. The use according to any one of Claims 77 to 84 wherein Sox11 is used in combination with one or more additional biomarkers.

87. The use according to Claim 86 wherein fewer than 20 additional biomarkers are used in the method, for example fewer than 15, 10, 8, 6, 5, 4, 3, 2 or 1 additional biomarkers.

88. A method of screening for a molecule with efficacy in the diagnosis and/or prognosis of epithelial ovarian cancer (EOC), the method comprising the steps of:

(a) contacting a molecule to be tested with Sox11 protein and/or mRNA encoding the same (or with a fragment of said protein or mRNA); and

(b) detecting the presence of a complex containing the protein and/or mRNA (or fragment thereof) and the molecule to be tested.

89. A method according to Claim 88 wherein the EOC belongs to a histological group selected from the group consisting of serous, mucinous, endometrioid, clear cell and undifferentiated or unclassifiable.

90. The method according to Claim 89 wherein the EOC is serous EOC.

91. The method according to Claim 89 wherein the EOC us mucinous EOC.

92. The method according to Claim 89 wherein the EOC is endometrioid EOC.

93. A method according to Claim 89 wherein the EOC is clear cell EOC.

94. A method according to Claim 89 wherein the EOC is undifferentiated or unclassifiable EOC.

95. A binding moiety for diagnosing or prognosing epithelial ovarian cancer (EOC) substantially as herein described with reference to the description.

96. A binding moiety for detecting epithelial ovarian cancer (EOC) cells in a sample substantially as herein described with reference to the description.

97. A method of diagnosing or prognosing epithelial ovarian cancer (EOC) in an individual substantially as herein described with reference to the description.

98. A method of imaging epithelial ovarian cancer (EOC) cells in the body of an individual substantially as herein described with reference to the description.

99. Use of a binding moiety in the preparation of a medicament for diagnosing or prognosing epithelial ovarian cancer (EOC) substantially as herein described with reference to the description.

100. Use of Sox11 protein and/or mRNA encoding the same as a marker for epithelial ovarian cancer (EOC) cells substantially as herein described with reference to the description.

101. A method of screening for a molecule with efficacy in the diagnosis, prognosis and/or treatment of epithelial ovarian cancer (EOC) substantially as herein described with reference to the description.

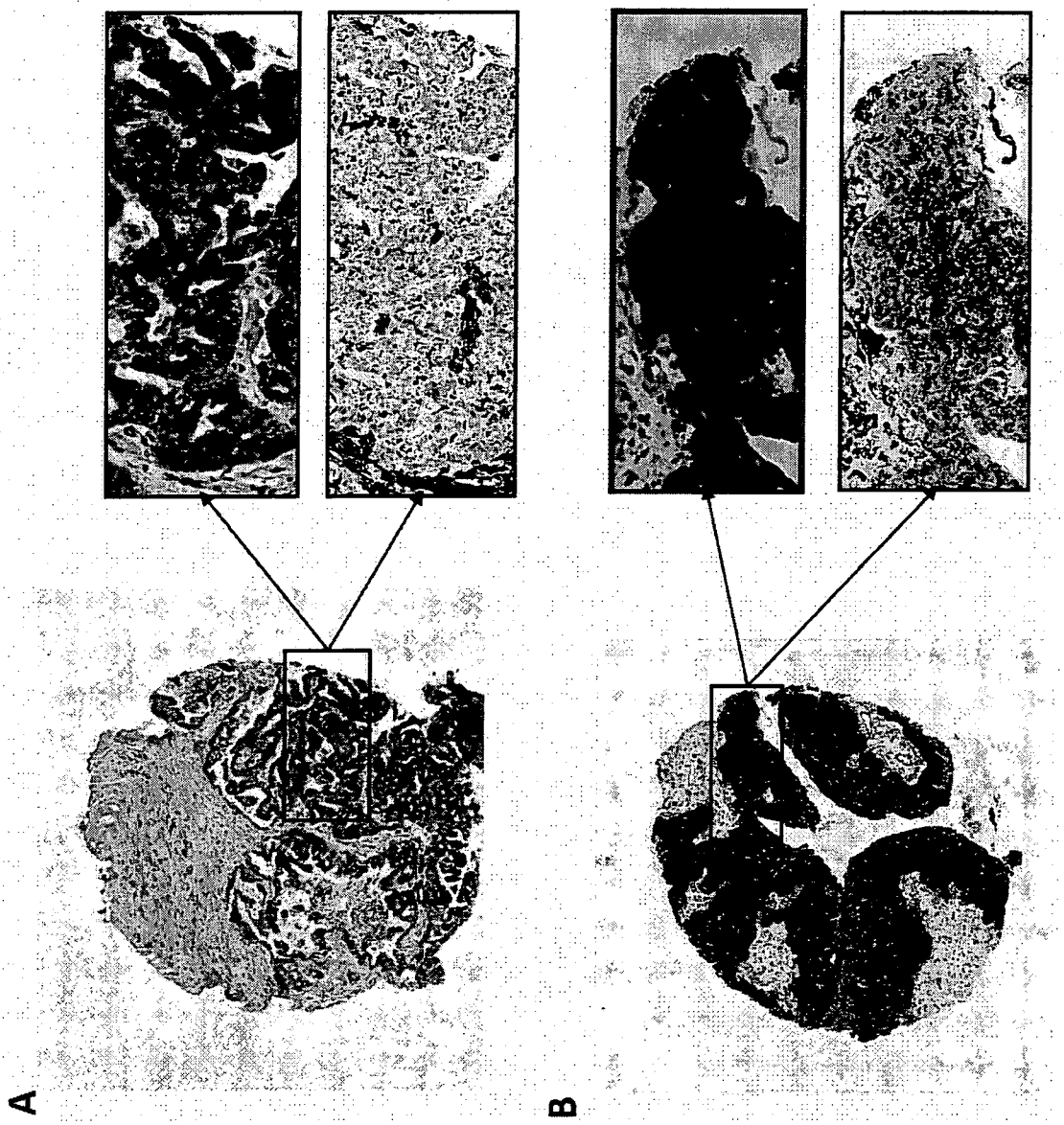

FIGURE 1

FIGURE 2(A)

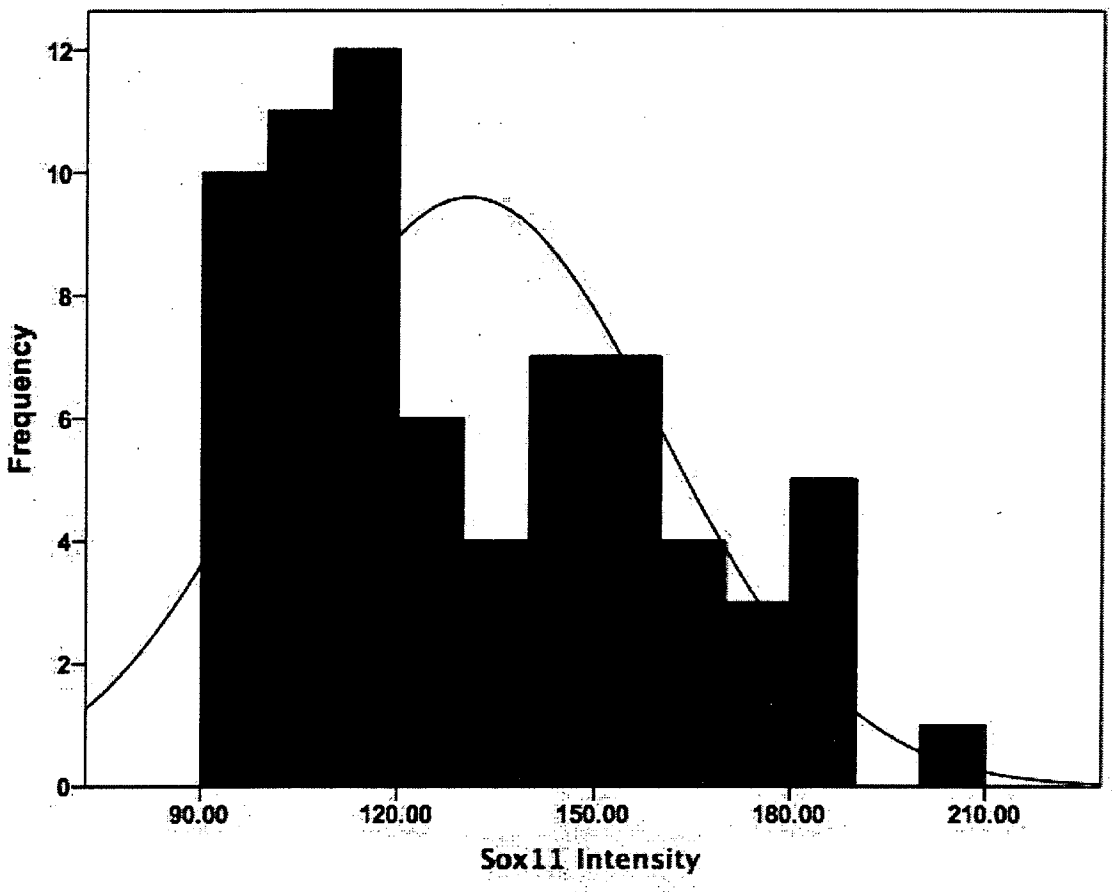
Frequency
0
2
4
6
8
10
12
90.00
120.00
150.00
180.00
210.00
Sox11 Intensity

FIGURE 2(B)

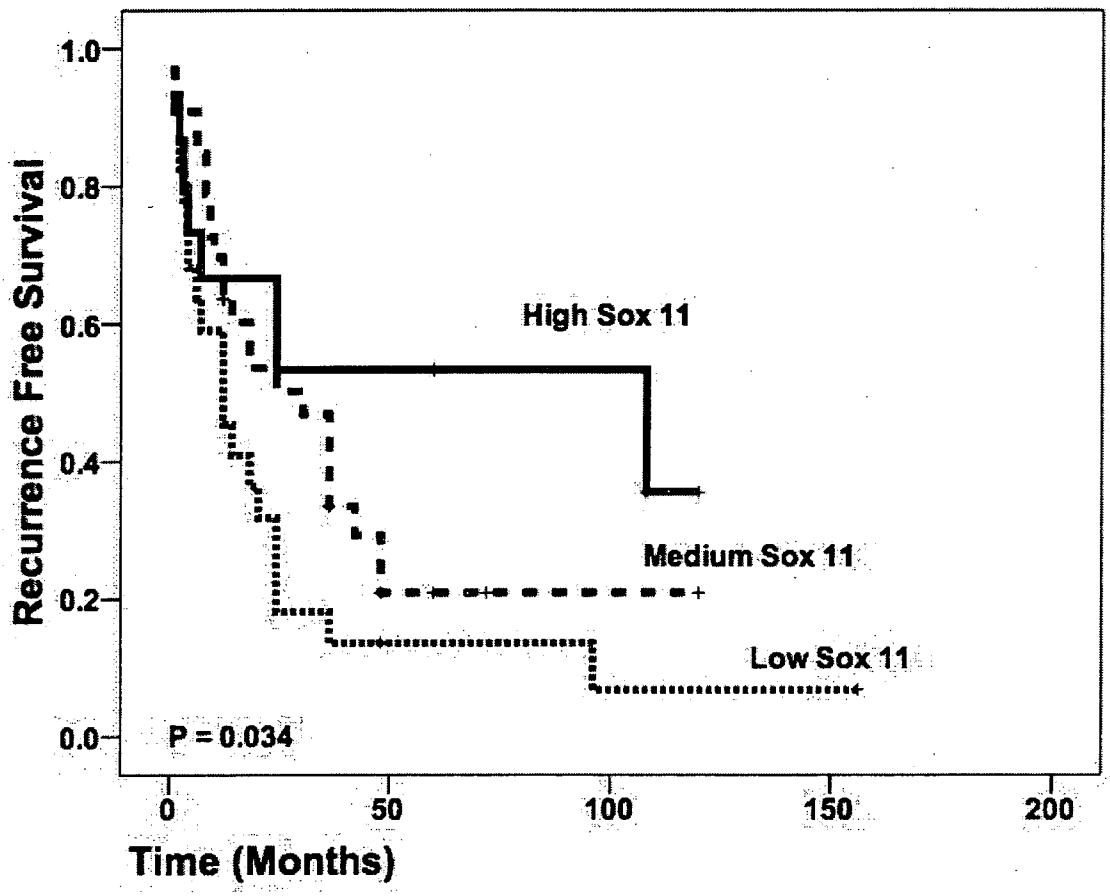
Recurrence Free Survival
1.0
0.8
0.6
0.4
0.2
0.0
High Sox 11
Medium Sox 11
Low Sox 11
P = 0.034
0
50
100
150
200
Time (Months)

FIGURE 2(C)

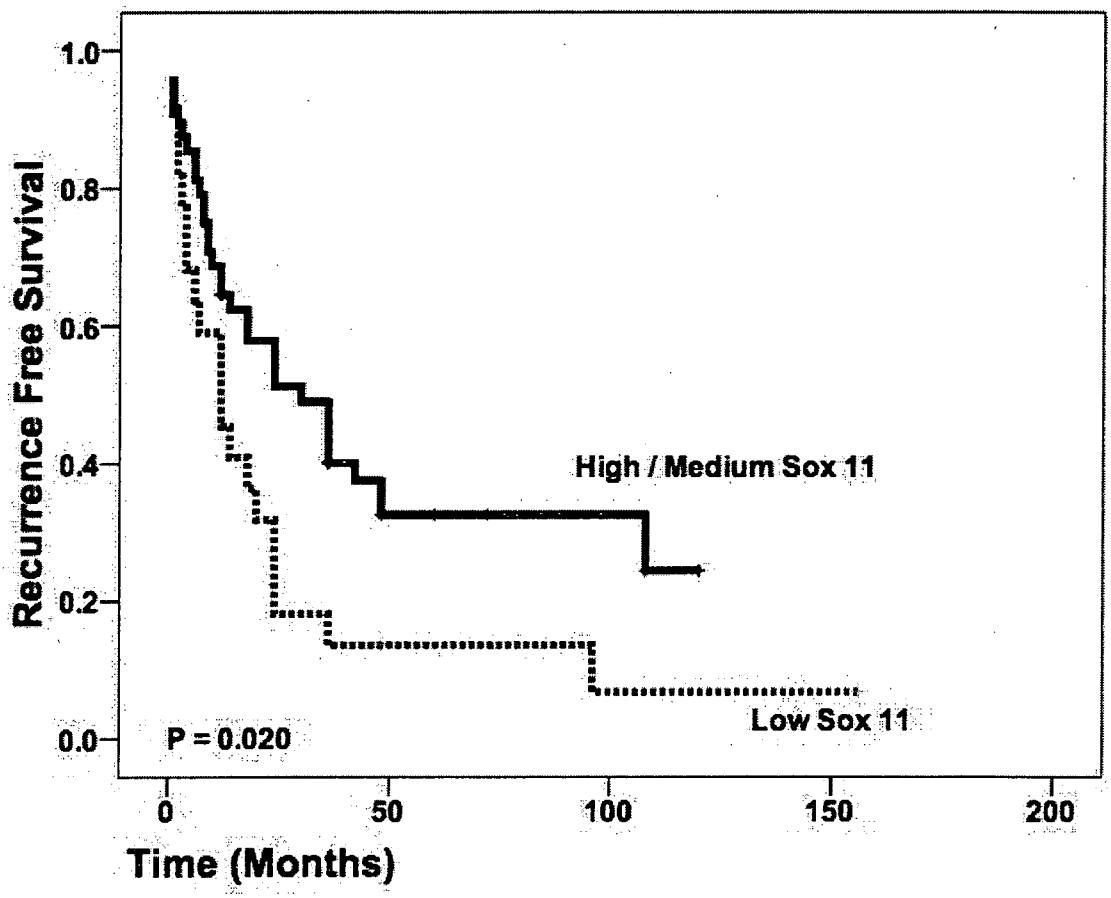
Recurrence Free Survival
1.0
0.8
0.6
0.4
0.2
0.0
High / Medium Sox 11
Low Sox 11
P = 0.020
0
50
100
150
200
Time (Months)

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FIGURE 3

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FIGURE 4

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FIGURE 4 (CONT.)

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FIGURE 4 (CONT.)

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FIGURE 4 (CONT.)

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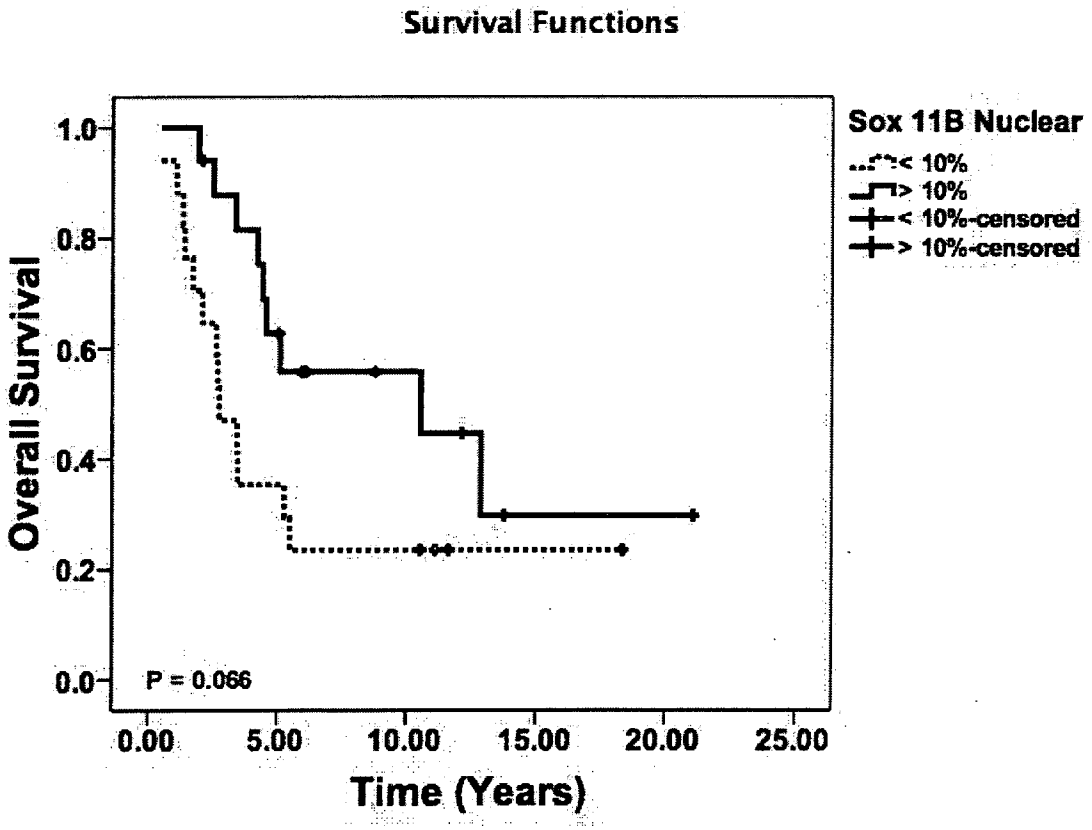
FIGURE 4 (CONT.)

FIGURE 5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0.8
0.6
0.4
0.2
0.0
P = 0.066
0.00
5.00
10.00
15.00
20.00
25.00
Time (Years)
Sox 11B Nuclear
< 10%
> 10%
< 10%-censored
> 10%-censored

FIGURE 6

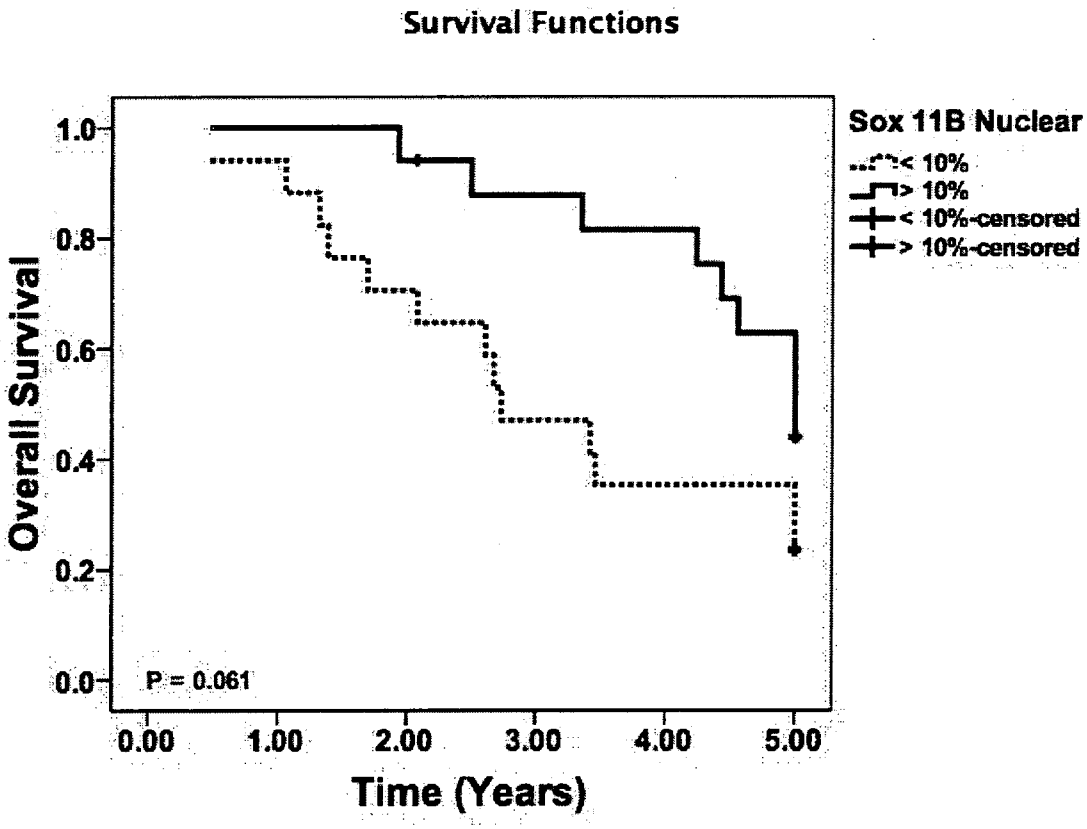

Survival Functions
Overall Survival
1.0
0.8
0.6
0.4
0.2
0.0
P = 0.061
0.00
1.00
2.00
3.00
4.00
5.00
Time (Years)
Sox 11B Nuclear
< 10%
> 10%
< 10%-censored
> 10%-censored

FIGURE 7

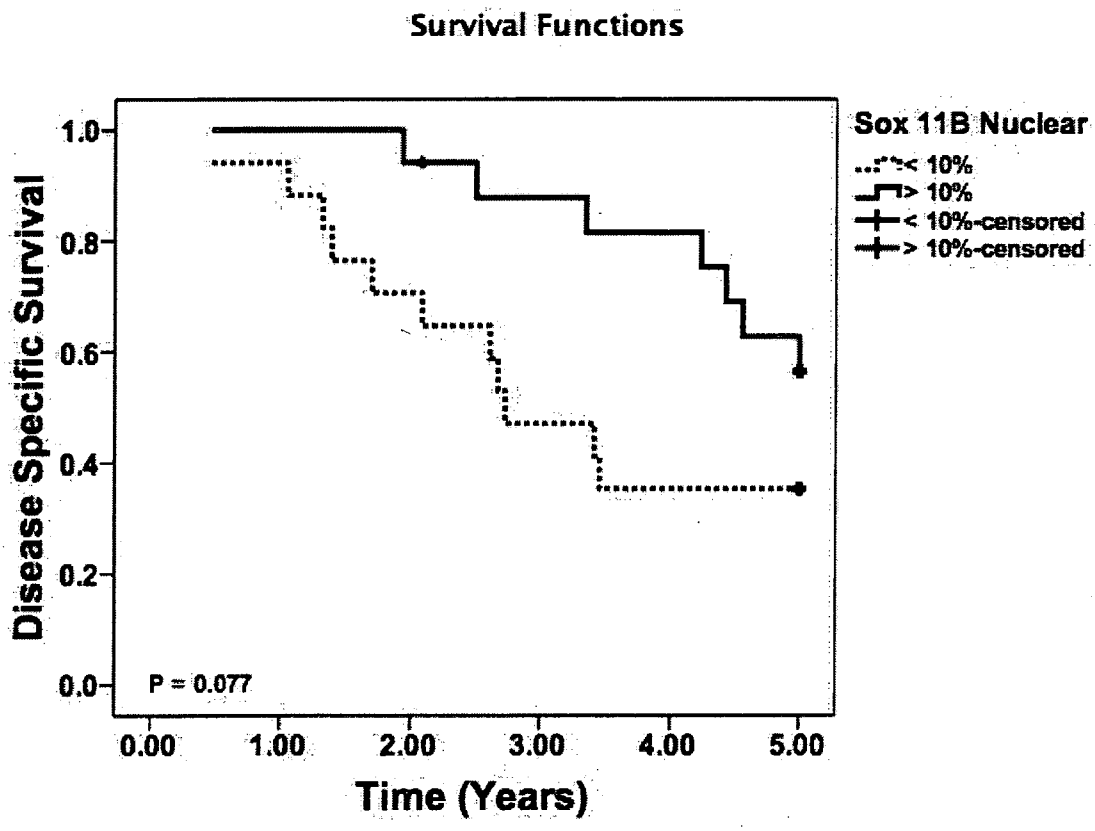

Survival Functions
Disease Specific Survival
1.0
0.8
0.6
0.4
0.2
0.0
P = 0.077
0.00
1.00
2.00
3.00
4.00
5.00
Time (Years)
Sox 11B Nuclear
< 10%
> 10%
< 10%-censored
> 10%-censored

FIGURE 8

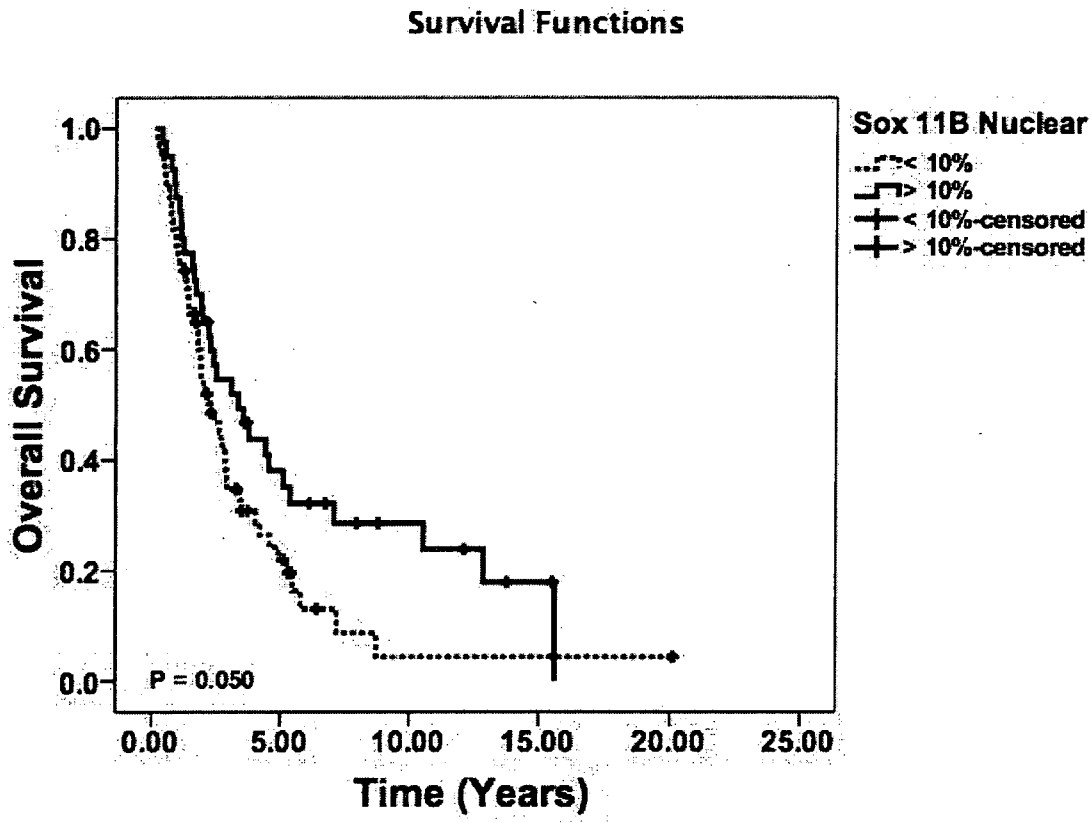
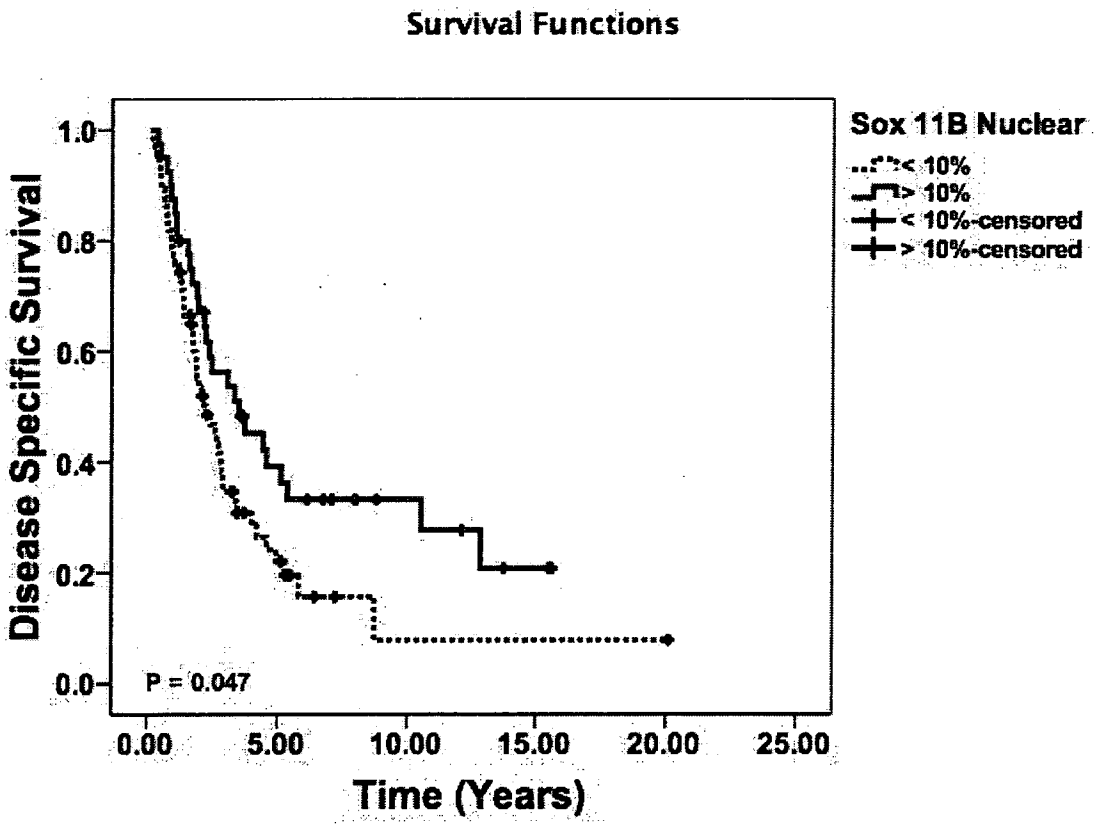

Survival Functions
Overall Survival
1.0
0.8
0.6
0.4
0.2
0.0
P = 0.050
0.00
5.00
10.00
15.00
20.00
25.00
Time (Years)
Sox 11B Nuclear
< 10%
> 10%
< 10%-censored
> 10%-censored

FIGURE 9


Survival Functions
Disease Specific Survival
1.0
0.8
0.6
0.4
0.2
0.0
P = 0.047
0.00
5.00
10.00
15.00
20.00
25.00
Time (Years)
Sox 11B Nuclear
< 10%
> 10%
< 10%-censored
> 10%-censored

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2009/002098

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

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12Q G01N

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2005/002417 A2 (AVALON PHARMACEUTICALS [US]; WEIGLE BERND [DE]; EBNER REINHARD [US]) 13 January 2005 (2005-01-13) the whole document	1-33
X	EK S ET AL: "Nuclear expression of the non B-cell lineage Sox11 transcription factor identifies mantle cell lymphoma" BLOOD CONF- 50TH ANNUAL MEETING OF THE AMERICAN- SOCIETY-OF-HEMATOLOGY; SAN FRANCISCO, CA, USA; DECEMBER 06 -09, 2008, AMERICAN SOCIETY OF HEMATOLOGY, vol. 111, no. 2, 12 October 2007 (2007-10-12), pages 800-805, XP007905438 ISSN: 0006-4971 page 801, left-hand column, last paragraph - right-hand column, last paragraph	1-33

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

[X] 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 document 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.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 January 2010	22/01/2010

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	Ulbrecht, Matthias

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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LEE CHING-JUNG ET AL: "Differential expression of SOX4 and SOX11 in medulloblastoma" JOURNAL OF NEURO-ONCOLOGY, KLUWER, BOSTON, US, vol. 57, no. 3, 1 May 2002 (2002-05-01), pages 201-214, XP002492273 ISSN: 0167-594X page 203, left-hand column, last paragraph - right-hand column, paragraph 1	1-33
X	WEIGLE BERND ET AL: "Highly specific overexpression of the transcription factor SOX11 in human malignant gliomas" ONCOLOGY REPORTS, NATIONAL HELLENIC RESEARCH FOUNDATION, ATHENS, GR, vol. 13, no. 1, 1 January 2005 (2005-01-01), pages 139-144, XP009104552 ISSN: 1021-335X page 140, left-hand column, paragraph 3 - right-hand column, paragraph 3	1-33
X	PANAGIOTIS A KONSTANTINOPOULOS ET AL: "Gene-expression profiling in epithelial ovarian cancer" NATURE CLINICAL PRACTICE ONCOLOGY, NATURE PUBLISHING GROUP, vol. 5, no. 10, 22 July 2008 (2008-07-22), pages 577-587, XP007910974 ISSN: 1743-4254 page 582, right-hand column, last paragraph - page 583, left-hand column, last paragraph	34-94
X	WO 2005/005661 A2 (NOVARTIS AG [CH]; NOVARTIS PHARMA GMBH [AT]; LAVEDAN CHRISTIAN NICOLAS) 20 January 2005 (2005-01-20) the whole document	34-41, 51-75, 77,79-94
X	EP 1 806 413 A1 (OLIGENE GMBH [DE]) 11 July 2007 (2007-07-11) the whole document	42-50, 59-72, 76,78-94

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3

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SPENTZOS D ET AL: "Gene expression signature with independent prognostic significance in epithelial ovarian cancer" JOURNAL OF CLINICAL ONCOLOGY, AMERICAN SOCIETY OF CLINICAL ONCOLOGY, US, vol. 22, no. 23, 1 December 2004 (2004-12-01), pages 4700-4710, XP002382645 ISSN: 0732-183X the whole document	42-50, 59-72, 76,78-94
X	TAMMELA J ET AL: "Gene expression and prognostic significance in ovarian cancer" MINERVA GINECOLOGICA, TORINO, IT, vol. 56, no. 6, 1 December 2004 (2004-12-01), pages 495-502, XP009126778 ISSN: 0026-4784 the whole document	42-50, 59-72, 76,78-94
X,P	BRENNAN DONAL J ET AL: "The transcription factor Sox11 is a prognostic factor for improved recurrence-free survival in epithelial ovarian cancer." EUROPEAN JOURNAL OF CANCER (OXFORD, ENGLAND : 1990) MAY 2009, vol. 45, no. 8, May 2009 (2009-05), pages 1510-1517, XP026056403 ISSN: 1879-0852 the whole document	1-94

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box II.2

Claims Nos.: 95-101

Claims 95-101 contain the wording "substantially as herein described with reference to the description". The limiting effect of this wording is not determinable and hence the technical features defining the binding moiety of claims 95 and 96, and the methods and uses according to claims 97-101 remain elusive (Art. 6 PCT). Consequently, a meaningful search of these claims could not be carried out.

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guideline C-VI, 8.2), should the problems which led to the Article 17(2) declaration be overcome.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB2009/002098

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 95-101
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/GB2009/002098

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2005002417 A2	13-01-2005	NONE	
WO 2005005661 A2	20-01-2005	AU 2004256182 A1	20-01-2005
		BR PI0412110 A	21-11-2006
		CA 2531091 A1	20-01-2005
		CN 1845999 A	11-10-2006
		EP 1644522 A2	12-04-2006
		JP 2007526749 T	20-09-2007
		MX PA05014220 A	09-03-2006
EP 1806413 A1	11-07-2007	NONE	