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# A method for the assessment of cancer in a biological sample obtained from a subject

## Field of the invention

The present invention relates to a method for the assessment and diagnosis of cancer. Specifically, the present invention relates to a method for the assessment and diagnosis of nasopharyngeal carcinoma (NPC) by determining the expression level of certain gene. The present invention also relates to polypeptides, antibodies and nucleic acids for the use in medicine and a kit for performing the method of the present invention.

## Background of the invention

Nasopharyngeal carcinoma (NPC) is a common cancer in southern China and South-East Asia, where more than 50 000 new cases are diagnosed each year. In Malaysia, NPC is the sixth most common cancer and the third most common in men. NPC is highly radiosensitive and chemosensitive; radiotherapy with concomitant chemotherapy has increased survival. Improved early detection of recurrence and application of appropriate surgical salvage procedures have further contributed to improved therapeutic results. However, survivors of NPC have impaired health-related quality of life. Patients who survive the disease can have several late complications, many of which result from the effects of radiation on the dose-limiting organs adjacent to the nasopharynx and neck nodes. The use of chemotherapy in more advanced cases adds to the side-effects, which include cytotoxicity associated with cisplatin. Therefore, alternative approaches for the treatment of NPC are required.

In NPC, unlike other head and neck cancers, clonal Epstein–Barr virus (EBV) genomes are detected in all cases. EBV latent protein expression is restricted to the EBNA1 nuclear antigen and to the latent membrane proteins (LMP1, LMP2A, LMP2B). However, the contribution of EBV to the development of

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NPC is not well understood. In an attempt to reveal how EBV contributes to the pathogenesis of this disease, we first compared cellular gene expression in EBV-positive NPC tumours with that in samples from cancer-free controls, using microarray analyses and we have identified a number of genes that may be associated with the pathogenesis of NPC.

Thus, there exists a need for a method for aiding assessment of a patient's risk of developing cancer, or likely severity or likelihood of progression of cancer, or aiding in selection of a cancer treatment regime for the patient, or aiding in assessment of a cancer treatment regime, particularly for NPC.

**Summary of the invention**

The first aspect of the present invention relates to a method for the assessment of nasopharyngeal carcinoma (NPC) in a biological sample obtained from a subject, comprising the steps of: (a) determining in said biological sample the expression level of a pre-determined gene; and (b) comparing the determined expression level of said gene with the level in a reference, wherein said pre-determined gene is Four-jointed Box 1 (FJX1) and wherein the difference of the expression levels may be indicative of the presence of nasopharyngeal cancerous cells.

Specifically, said reference is a normal or non-malignant cell or tissue.

In the present invention, expression level can be the RNA expression level of FJX 1 in said biological sample obtained from said subject.

In the preferred embodiment of the present invention, the method further comprising a step of determining RNA level of FJX1 in the sample.

The FJX1 level may be determined by using any suitable method that is well known in the art.

Preferably, the RNA level of FJX1 being determined in the present invention is FJX1 mRNA.

More preferably, the FJX1 mRNA level is determined by the use of at least two oligonucleotide primers comprising the sequences of:
SEQ ID NO. 1: 5'CCCGCAAAGGTGTCTAAAAACT3'; and
SEQ ID NO. 2: 5'TGCTGGCACAGTAAAGAATCCT3'
or their homologous in the complementary strands.

Alternatively, the FJX1 mRNA level may be determined by using microarray analysis method or quantitative real time PCR that are well known in the art.

It will be appreciated that the detection of the presence of an increased level of FJX1 mRNA in a cell compared to the level of a normal (non-malignant or non-cancerous) cell may aid in the assessment of a patient's risk of developing nasopharyngeal carcinoma (NPC). Increased expression level of FJX1 mRNA in a biological sample compared to the expression level found in a normal (non-malignant or non-cancerous) cell may be indicative of NPC. The detection of an increased level may also suggest that said subject will benefit from a particular form of treatment, such as treatment with a cancer vaccine as herein disclosed.

In the present invention, expression level can also be the protein expression level of FJX 1 in said biological sample obtained from said subject.

In another embodiment of the present invention, said method further comprising the detection and determination of FJX1 polypeptide or protein level in said biological sample.

The method of the present invention also includes the measurement and detection of FJX1 polypeptide in said biological sample, and their comparison with a reference sample. It will be appreciated that the detection of the presence of an increased level of FJX1 polypeptides in a biological sample

compared to the level of a normal (non-malignant or non-cancerous) cell may aid in the assessment of a patient's risk of developing nasopharyngeal carcinoma (NPC). Increased level of FJX1 polypeptides in a biological sample compared to the level found in a normal (non-malignant or non-cancerous) cell may be indicative of NPC.

The polypeptide or protein level of FJX1 may be determined by using any suitable protein quantitation method that is well known in the art. In particular, it is preferred if antibodies are used and that the amount of FJX1 is determined by using methods which include quantitative western blotting, enzyme-linked immunosorbent assays (ELISA) or quantitative immunohistochemistry.

As noted above, an increased level of FJX1 polypeptide in a sample compared with a known normal tissue reference sample is suggestive of a tumorigenic sample.

In another aspect of the present invention, it also provides a method of detecting cancer in a subject comprising administering to said subject an anti-FJX1 antibody and the use of said antibody in the manufacture of a medicament for treating nasopharyngeal carcinoma (NPC).

A further aspect of the present invention provides a method of treating nasopharyngeal carcinoma (NPC) in a patient comprising administering to said patient either an effective amount of FJX1 polypeptide, a molecule that modulates the activity of FJX1 gene, an antibody directed to FJX1, or molecules that are bound to FJX1.

Yet a further aspect of the present invention describes the uses of any one of the afore-mentioned FJX1 polypeptide, molecule that modulates the activity of FJX1 gene, antibody directed to FJX1 and molecules that are bound to FJX1 in the manufacture of a medicament for treating nasopharyngeal carcinoma (NPC).

Still, a further aspect of the present invention provides a kit for determining the expression level of FJX1 gene comprising at least an oligonucleotide primer pair comprising the sequences of SEQ ID NO. 1 and SEQ ID NO. 2 as herein dislcosed.

The afore-mentioned methods and uses will now be explained in more detail in the following disclosure and appended claims.

**Brief description of drawings**

In order to provide a better understanding, the present invention will now be described with reference to the accompanying drawings in which:

Figure 1(a) shows the mRNA sequence of FJX1;

Figure 1(b) shows the amino acid sequence of FJX1;

Figure 2 shows the quantitative PCR analysis of gene FJX1. Compared to two biopsies of normal nasopharynx, quantitative PCR showed that FJX1 mRNA was up-regulated in 14 NPC tumours.

Figure 3 shows an immunohistochemistry analysis of FJX1 protein where up-regulation of FJX1 protein expression in NPC is confirmed. FJX1 expression was detected in NPC tumour cells (white arrow), but not the surrounding non-malignant cell (black arrow).

Figure 4 shows the expression of FJX1 analysed using the Multiple Tissue cDNA Panel. FJX1 expression was barely detectable in most of the normal human organs analysed except for ovary, pancreas, placenta and small intestine. 1, Heart; 2, Brain; 3, Placenta; 4, Lung; 5, Liver; 6, Skeletal muscle; 7, Kidney; 8, Pancreas; 9, Spleen; 10, Thymus; 11, Prostate; 12, Testis; 13,

Ovary; 14, Small intestine; 15, Colon; 16, Peripheral blood leukocyte; +ve, Positive control for PCR reaction.

**Detailed description of the preferred embodiments**

The present invention describes a method for the assessment of nasopharyngeal carcinoma (NPC) via the determination of the expression level of gene Four-jointed Box 1 (FJX1).

In accordance with the first aspect of the present invention, it provides a method for the assessment of nasopharyngeal carcinoma (NPC) in a biological sample obtained from a subject, comprising the steps of: (a) determining in said biological sample the expression level of a pre-determined gene; and (b) comparing the determined expression level of said gene with the level in a reference, wherein said pre-determined gene is Four-jointed Box 1 (FJX1) and wherein the difference of the expression levels may be indicative of the presence of nasopharyngeal cancerous cells.

By "FJX1", it is meant to refer, as the context will make clear, to the gene or RNA product or protein product.

In accordance with the method of the present invention, the said expression level of a predetermined gene can be understand to be the RNA expression level of FJX1 in said biological sample obtained from said subject.

Preferably, the method further comprises determining the RNA level of FJX1 in the sample. The RNA level of FJX1 may be determined by the use of at least two oligonucleotide primers, for example, the primers selected from
SEQ ID NO. 1: 5'CCCGCAAAGGTGTCTAAAAACT3'; and
SEQ ID NO. 2: 5'TGCTGGCACAGTAAAGAATCCT3'
or their homologous in the complementary strands.

More preferably, the RNA level of FJX1 being determined in the present invention are FJX1 mRNA. The mRNA sequence is shown in the Figure 1. Increased FJX1 mRNA in a sample compared to that found in a normal (non-malignant or non-cancerous) tissue sample may be indicative of nasopharyngeal cancer.

The RNA level of FJX1 may be determined by using specific oligonucleotide primers and a nucleic acid amplification technique such as the polymerase chain reaction (PCR). Oligonucleotide primers can be synthesised using methods well known in the art, for example using solid-phase phosphoramidite chemistry. Preferably, the oligonucleotide primers are at least 20 nucleotides in length, more preferably at least 25 nucleotides in length and still more preferably at least 29 nucleotides in length.

Suitable conditions for PCR amplification include amplification in a suitable 1 x amplification buffer: 10 x amplification buffer is 500 mM KCl; 100 mM Tris. Cl (pH 8.3 at room temperature); 15 mM MgCl2; 0. 1 % gelatin. single-stranded DNA primers, suitable for use in a polymerase chain reaction, are particularly preferred.

It will be appreciated that FJX1 mRNA may be identified by reverse-transcriptase polymerase chain reaction (RT-PCR) using methods well known in the art.

Primers which are suitable for use in a polymerase chain reaction (PCR; Saiki et al (1988) Science 239,487-491) are preferred. Suitable PCR primers may have the following properties: It is well known that the sequence at the 5'end of the oligonucleotide need not match the target sequence to be amplified.

It is usual that the PCR primers do not contain any complementary structures with each other longer than 2 bases, especially at their 3'ends, as this feature may promote the formation of an artifactual product called "primer dimer". When the 3'ends of the two primers hybridize, they form a "primed template"

complex, and primer extension results in a short duplex product called "primer dimer".

Internal secondary structure should be avoided in primers. For symmetric PCR, a 40-60% G+C content is often recommended for both primers, with no long stretches of any one base. The classical melting temperature calculations used in conjunction with DNA probe hybridization studies often predict that a given primer should anneal at a specific temperature or that the 72°C extension temperature will dissociate the primer/template hybrid prematurely. In practice, the hybrids are more effective in the PCR process than generally predicted by simple Tm calculations.

Optimum annealing temperatures may be determined empirically and may be higher than predicted. Taq DNA polymerase does have activity in the 37-55°C region, so primer extension will occur during the annealing step and the hybrid will be stabilized. The concentrations of the primers are equal in conventional (symmetric) PCR and, typically, within 0.1- to 1- ,range.

Any of the nucleic acid amplification protocols can be used in the method of the invention including the polymerase chain reaction, QB replicase and ligase chain reaction. Also, NASBA (nucleic acid sequence based amplification), also called 3SR, can be used as described in Compton (1991) Nature 350,91-92 and AIDS (1993), Vol 7 (Suppl 2), S108 or SDA (strand displacement amplification) can be used as described in Walker et al (1992) Nucl. Acids Res. 20,1691-1696. The polymerase chain reaction is particularly preferred because of its simplicity.

When a pair of suitable nucleic acids of the invention is used in a PCR it is convenient to detect the product by gel electrophoresis and ethidium bromide staining. As an alternative to detecting the product of DNA amplification using agarose gel electrophoresis and ethidium bromide staining of the DNA, it is convenient to use a labelled oligonucleotide capable of hybridising to the amplified DNA as a probe. When the amplification is by a PCR the

oligonucleotide probe hybridises to the interprimer sequence as defined by the two primers. The oligonucleotide probe is preferably between 10 and 50 nucleotides long, more preferably between 15 and 30 nucleotides long. The probe may be labelled with a radionuclide such as $^{32}P$, $^{33}P$ and $^{35}S$ using standard techniques, or may be labelled with a fluorescent dye. When the oligonucleotide probe is fluorescently labelled, the amplified DNA product may be detected in solution (see for example Balaguer et al (1991) "Quantification of DNA sequences obtained by polymerase chain reaction using a bioluminescence adsorbent" Anal. Biochem. 195,105-110 and DiCesare et al (1993) "A high-sensitivity electrochemiluminescence-based detection system for automated PCR product quantitation "BioTechniques 15,152-157.

Amplification products can also be detected using a probe which may have a fluorophore-quencher pair or may be attached to a solid support or may have a biotin tag or they may be detected using a combination of a capture probe and a detector probe.

Fluorophore-quencher pairs are particularly suited to quantitative measurements of PCR reactions (eg RT-PCR). Fluorescence polarisation using a suitable probe may also be used to detect PCR products.

Other methods of detecting mRNA levels are included.

Methods for determining the relative amount of FJX1 mRNA include: in situ hybridisation (In Situ Hybridization Protocols. Methods in Molecular Biology Volume 33. Edited by K H A Choo. 1994, Humana Press Inc (Totowa, NJ, USA) pp 480p and In Situ Hybridization: A Practical Approach. Edited by D G Wilkinson. 1992, Oxford University Press, Oxford, pp 163), in situ amplification, northerns, nuclease protection, probe arrays, and amplification based systems; The mRNA may be amplified prior to or during detection and quantitation.'Real time' amplification methods wherein the product is measured for each amplification cycle may be particularly useful (eg Real time PCR Hid et al (1996) Genome Research 6,986-994, Gibson et al (1996)

Genome Research 6,995-1001; Real time NASBA Oehlenschlager et al (1996 Nov 12) PNAS (USA) 93 (23), 12811-6. Primers should be designed to preferentially amplify from an mRNA template rather than from the DNA, or be designed to create a product where the mRNA or DNA template origin can be distinguished by size or by probing. NASBA may be particularly useful as the process can be arranged such that only RNA is recognised as an initial substrate.

Detecting mRNA includes detecting mRNA in any context, or detecting that there are cells present which contain mRNA (for example, by in situ hybridisation, or in samples obtained from lysed cells). It is useful to detect the presence of mRNA or that certain cells are present (either generally or in a specific location) which can be detected by virtue of their expression of FJX1 mRNA. As noted, the presence versus absence of FJX1 mRNA may be a useful marker, or low levels versus high levels of FJX1 mRNA may be a useful marker, or specific quantified levels may be associated with a specific disease state. It will be appreciated that similar possibilities exist in relation to using the FJX1 polypeptide as a marker.

In accordance with the method of the present invention, the said expression level of a predetermined gene can also be understand to be the protein expression level of FJX1 in said biological sample obtained from said subject.

To further improve the assessment of nasopharyngeal carcinoma (NPC) the method of the present invention further comprises determining the protein level of FJX1 in the sample.

The methods of the invention also include the measurement and detection of the FJX1 polypeptide in test samples and their comparison in a reference sample. It will be appreciated that detecting the presence of an increased level of FJX1 polypeptides in a cell compared to the level present in a reference sample, .e.g a normal (non-malignant or non-cancerous) cell may aid in the assesssment of a patient's risk of developing nasopharyngeal carcinoma

(NPC). Increased FJX1 polypeptides in a sample compared to that found in a normal (non malignant or non-cancerous) tissue sample may be indicative of NPC.

The sample containing RNA and/or protein derived from the patient is conveniently a sample of the tissue in which nasopharyngeal carcinoma (NPC) is suspected or in which cancer may be or has been found. These methods may be used for any cancer, but they are particularly suitable in respect of NPC. The sample may also be blood, serum or lymph nodes which may be particularly useful in determining whether a cancer has spread. Alternatively, the sample may be tissue sample obtained surgically from a patient. Preferably, the tissue is epithelial tissues.

The methods of the invention involving detection of the FJX1 polypeptide are particularly useful in relation to historical samples such as those containing paraffin-embedded sections of tumour samples.

The amount of the FJX1 polypeptide may be determined in any suitable way.

It is preferred if the amount of the FJX1 polypeptide is determined by using a molecule which selectively binds to FJX1 polypeptide. Suitably, the molecule which selectively binds to FJX1 is an antibody. The antibody may also bind to a natural variant or fragment of FJX1 polypeptide.

By "variants" of the polypeptide we include insertions, deletions and substitutions, either conservative or non-conservative, where such changes do not substantially alter the activity of the said FJX1.

Variants and variations of the polynucleotide and polypeptide include natural variants, including allelic variants and naturally-occurring mutant forms.

By "fragment of FJX1", we include any fragment which retains activity or which is useful in some other way, for example, for use in raising antibodies or in a binding assay.

The antibodies for use in the methods of the invention may be monoclonal or polyclonal.

The protein level of FJX1 may be determined using any suitable protein quantitation method. In particular, it is preferred if antibodies are used and that the amount of FJX1 is determined using methods which include quantitative western blotting, enzyme-linked immunosorbent assays (ELISA) or quantitative immunohistochemistry.

As noted above, an increased level of FJX1 in a sample compared with a known normal tissue reference sample is suggestive of a tumorigenic sample.

In a preferred embodiment of the invention, antibodies will immunoprecipitate FJX1 protein from solution as well as react with FJX1 protein on western or immunoblots of polyacrylamide gels. In another preferred embodiment, antibodies will detect FJX1 protein in paraffin or frozen tissue sections, using immunocytochemical techniques.

Preferred embodiments relating to methods for detecting FJX1 include enzyme linked immunosorbent assays (ELISA), 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.

Methods for detection also include immuno-fluoresence. Automated and semi-automated image analysis systems may be of use. Several formats for quantitative immunoassays are known. Such systems may incorporate: more

than one antibody which binds the antigen; labelled or unlabelled antigen (in addition to any contained in the sample); and a variety of detection systems including radioisotope, colourimetric, fluorimetric, chemiluminescent, and enhanced chemiluminescent; enzyme catalysis may or may not be involved. Immunoassays may be homogenous systems, where no separation of bound and unbound reagents takes place, or heterogeneous systems involving a separation step.

Such assays are commonly referred to as for example enzyme-linked luminescent immunoassays (ELLIA), fluorescence enzyme immunoassay (FEIA), fluorescence immunoassay (FIA), enzyme immunoassay (EIA), luminescent immunoassay (LIA), latex photometrix immunoassay (LPIA).

Methods of cultivating the biological sample (e.g. sample cells) and isolating proteins are well known in the art. Cells can be harvested and lysed and the presence of the protein in the supernatant can be detected using antibodies. Such antibodies are useful in cancer diagnosis. Suitably, the antibodies of the invention are detectably labelled, for example they may be labelled in such a way that they may be directly or indirectly detected. Conveniently, the antibodies may be labelled with a radioactive moiety or a coloured moiety or a fluorescent moiety, or they may be linked to an enzyme. Typically, the enzyme is one which can convert a non-coloured (or non-fluorescent) substrate to a coloured (or fluorescent) product. The antibody may be labelled by biotin (or streptavidin) and then detected indirectly using streptavidin (or biotin) which has been labelled with a radioactive moiety or a coloured moiety or a fluorescent moiety, or the like or they may be linked to an enzyme of the type described above.

Anti-"FJX1" antibodies or fragments or derivatives thereof such as humanised antibodies or ScFv fragments or dAbs or other fragments which retain antigen-binding specificity may be useful for imaging, such as imaging of tumours in the patient using, for example, radioimmunoscintigraphy. Conveniently, the

antibodies or fragments or derivatives thereof are labelled with a moiety which allows detection.

Suitably, the label is a radioactive moiety and, preferably, it contains 99mTc, or other suitable isotopes of technetium, or suitable isotopes of yttrium, indium, iodine or the like, all of which are well known in the art.

Preferably, the antibody is a monoclonal antibody or fragment thereof.

Anti-FJX1 antibodies or fragments or derivatives thereof may be used therapeutically. For example, unconjugated antibodies or fragments or derivatives thereof may be used to induce an anti-idiotype response.

Alternatively, antibodies or fragments or derivatives thereof may be conjugated to a moiety which is directly or indirectly cytotoxic. Directly cytotoxic agents include, for example, radioisotopes and toxins such as ricin; indirectly cytotoxic agents include, for example, enzymes which can convert a relatively non-toxic prodrug into a cytotoxic drug.

It is particularly preferred if peptides are made, based on the amino acid sequence of FJX1 (as shown in Figure 1(b)), which allow for specific antibodies to be made.

Preferably, the reference is normal cell or tissue, more preferably normal or non-malignant cell or tissue. The reference, to which the determined expression levels the genes is compared, may be normal (healthy) tissue, such as normal epithelial cells, or any other reference tissue. The reference tissue can be non-cancerous tissue composed of normal tissue levels. A person skilled in the art will be able to determine, based on the appearance and histology of the tissue sample, whether the sample tissue is normal (healthy) or cancerous. The reference tissue can either originate from the subject the biological sample is collected from or from any other adequate

source, for example, a subject not suffering from NPC. Alternatively, cell lines, e.g human NPC cell lines, may be used to exogenously express the FJX1.

The term "biological sample" may be any biological material taken either directly from the human being or animal or after culturing. Biological sample may be e.g. body fluid, blood, serum, lymph nodes, biopsies, colonies, liquid cultures etc.

Preferably, the biological sample is selected from the group consisting of oral mucosal tissue, nasopharyngeal tissue, nasopharyngeal swab and mouth washing.

In accordance with another aspect of the invention, there is provided a method of detecting nasopharyngeal carcinoma (NPC) in a patient, the method comprising administering to the patient an anti-FJX1 antibody or a fragment or derivative thereof labelled with a detectable label, allowing the labelled antibody to locate to the cancer, and imaging the cancer.

A further aspect of the invention provides the use of a nucleic acid which selectively hybridises to FJX1 mRNA in the manufacture of a reagent for diagnosing nasopharyngeal carcinoma (NPC). Yet a further aspect of the invention provides the use of a nucleic acid which selectively hybridises to FJX1 mRNA in a method of diagnosing nasopharyngeal carcinoma (NPC).

A further aspect of the invention provides the use of a molecule which selectively binds to FJX1 polypeptide or a natural fragment or variant thereof in the manufacture of a reagent for diagnosing or imaging cancer.

In another aspect of the invention, there is provided a method of treating nasopharyngeal carcinoma (NPC), the method comprising administering to the patient an effective amount of FJX1 polypeptide or a variant or fusion or fragment thereof, or an effective amount of a nucleic acid encoding a FJX1 polypeptide or a variant or fragment or fusion thereof, wherein the amount of

said polypeptide or amount of said nucleic acid is effective to provoke an anti-cancer cell immune response in said patient.

The peptide or peptide-encoding nucleic acid constitutes a tumour or cancer vaccine. It may be administered directly into the patient, into the affected organ or systemically, or applied ex vivo to cells derived from the patient or a human cell line which are subsequently administered to the patient, or used in vitro to select a subpopulation from immune cells derived from the patient, which are then re-administered to the patient. If the nucleic acid is administered to cells in vitro, it may be useful for the cells to be transfected so as to co-express immune-stimulating cytokines, such as interleukin-2. The FJX1 polypeptide or peptide fragment may be substantially pure, or combined with an immune-stimulating adjuvant such as Detox, or used in combination with immune-stimulatory cytokines, or be administered with a suitable delivery system, for example liposomes.

The FJX1 polypeptide or peptide fragment may also be conjugated to a suitable cancer such as keyhole limpet haemocyanin (KLH) or mannan (see WO 95/18145 and Longenecker et al (1993) Ann. NYAcad. Sci. 690, 276-291). The peptide may also be tagged, or be a fusion protein. The nucleic acid may be substantially pure, or contained in a suitable vector or delivery system. Suitable vectors and delivery systems include viral, such as systems based on adenovirus, vaccinia virus, retroviruses, herpes virus, adeno-associated virus or hybrids containing elements of more than one virus. Non-viral delivery systems include cationic lipids and cationic polymers as are well known in the art of DNA delivery. Physical delivery, such as via a "gene-gun" may also be used. The peptide or peptide encoded by the nucleic acid may be a fusion protein, for example with ß2-microglobulin.

The peptide fragment for use in a cancer vaccine may be any suitable length fragment of the FJX1 polypeptide. In particular, it may be a suitable 9-mer peptide or a suitable 7-mer or 8-mer peptide. Longer peptides may also be suitable, but 9-mer peptides are preferred. Multiple epitopes, derived from the

FJX1 polypeptide, may also be used. The term peptide includes a peptidomimetic. It also includes glycopeptides.

Suitably, any nucleic acid or peptide administered to the patient is sterile and pyrogen free. Naked DNA may be given intramuscularly or intradermally or subcutaneously. The peptides may be given intramuscularly, intradermally or subcutaneously.

It is particularly useful if the cancer vaccine is administered in a manner which produces a cellular immune response, resulting in cytoxic tumour cell killing by NK cells or cytotoxic T cells (CTLs). Strategies of administration which activate T helper cells are particularly useful. It may also be useful to stimulate a humoral response. It may be useful to co-administer certain cytokines to promote such a response, for example interleukin-2, interleukin-12, interleukin-6, or interleukin-10. In addition, it may be useful to combine vaccination with strategies which increase MHC presentation on the surface of tumour cells, for example by co- administration of interferon-gamma or retinoic as is described in Nouri et al (1992) Eur. J. Cancer 28A, 1110-1115 and Seliger et al (1997) Scand. J. Immunol. 46,625-632. It may also be desirable to make modifications to the antigen (FJX1 polypeptide or part thereof) to enhance its presentation to the immune system.

It may also be useful to target the vaccine to specific cell populations, for example antigen presenting cells, either by the site of injection, use of targeting vectors and delivery systems, or selective purification of such a cell population from the patient and ex vivo administration of the peptide or nucleic acid (for example dendritic cells may be sorted as described in Zhou et al (1995) Blood 86, 3295-3301; Roth et al (1996) Scand. J. Immunology 43,646-651). For example, targeting vectors may comprise a tissue-or tumour-specific promoter which directs expression of the antigen at a suitable place.

Patients to whom the therapy is to be given, may have their tumours typed for overexpression or abnormal expression of FJX1.

A further aspect of the invention provides the use of an effective amount of FJX1 polypeptide or a variant or fusion or fragment thereof, or an effective amount of a nucleic acid encoding a FJX1 polypeptide or a variant or fragment or fusion thereof, in the manufacture of a medicament for treating nasopharyngeal carcinoma (NPC).

In any aspect of the invention, there is provided a nasopharyngeal carcinoma (NPC) vaccine comprising a FJX1 polypeptide or variant or fragment thereof, or a nucleic acid encoding FJX1 polypeptide or fragment or variant thereof. It is known that inoculation with a nucleic acid vaccine, such as a DNA vaccine, encoding a polypeptide leads to a T cell response. In particular, MHC class I and class II-mediated interactions can be elicited.

Peptide products derived by cytosolic degradation of fragments of tumour-specific proteins, expressed de novo, are believed to gain access to the presentational pathways, mimicking the presentation of, for example, viral proteins, in infected cells. Presentation as neo-antigens or surrogate antigens in this novel context is believed to be a means of breaking immunological tolerance, and may lead to the generation of a tumour-specific immune response.

Conveniently, the nucleic acid vaccine may comprise any suitable nucleic acid delivery means. The nucleic acid, preferably DNA, may be naked (i.e. with substantially no other components to be administered) or it may be delivered in a liposome or as part of a viral vector delivery system.

It is believed that uptake of the nucleic acid and expression of the encoded polypeptide by dendritic cells may be the mechanism of priming of the immune response.

It is preferred if the vaccine, such as DNA vaccine, is administered into the muscle. It is also preferred if the vaccine is administered onto the skin.

It is preferred if the nucleic acid vaccine is administered with an adjuvant such as BCG or alum. Other suitable adjuvants include Aquila's QS21 stimulon (Aquila Biotech, Worcester, MA, USA) which is derived from saponin, mycobacterial extracts and synthetic bacterial cell wall mimics, and proprietory adjuvants such as Ribi's Detox. Quil A, another saponin- derived adjuvant, may also be used (Superfos, Denmark).

Other adjuvants such as Freund's may also be useful. It may also be useful to give the FJX1 antigen conjugated to keyhole limpet haemocyanin, preferably also with an adjuvant.

Polynucleotide-mediated immunisation therapy of cancer is described in Conry et al (1996) Seminars in Oncology 23,135-147; Condon et al (1996) Nature Medicine 2,1122-1127; Gong et al (1997) Nature Medicine 3,558-561; Zhai et al (1996) J. Immunol. 156,700-710; Graham et al (1996) Int J. Cancer 65, 664-670; and Burchell et al (1996) pp 309-313 In: Breast Cancer, Advances in biology and therapeutics, Calvo et al (eds), John Libbey Eurotext, all of which are incorporated herein by reference.

The FJX1 polypeptide is an appropriate target for a cell-mediated response to cancer or tumour cells which express the FJX1 polypeptide.

Therapeutic response to a cancer vaccine may usefully be monitored. Suitably, FJX1 specific antibody and CTL responses are monitored using methods well known in the art to assess the efficacy of the therapeutic response. Lymphoblastic transformation assays, lymphokine release assays, CTL response assays and serologic assays may be used. Detection of antigen-specific T lymphocytes by fluorescent-activated cell sorting (FACS) may also be used and is described in Altman et al (1996) Science 274, 94-96 and in WO 96/26962.

Preferably, the nucleic acid of the invention may be detectably labelled. For example, they may be labelled in such a way that they may be directly or indirectly detected. Conveniently, the nucleic acid is labelled with a radioactive moiety or a coloured moiety or a fluorescent moiety or some other suitable detectable moiety such as digoxygenin and luminescent or chemiluminescent moieties. The polynucleotides may be linked to an enzyme, or they may be linked to biotin (or streptavidin) and detected in a similar way as described for antibodies of the invention. Also preferably the nucleic acid of the invention may be bound to a solid support (including arrays, beads, magnetic beads, sample containers and the like).

The nucleic acid of the invention may also incorporate a "tag" nucleotide sequence which tag sequence can subsequently be recognised by a further nucleic acid probe. Suitable labels or tags may also be used for the selective capture of the hybridised (or non-hybridised) polynucleotide using methods well known in the art.

In another aspect of the invention, there is provided a method of treating cancer in a patient, the method comprising administering to the patient a molecule that modulates the activity of the FJX1 gene or their products.

By “modulates the activity of the FJX1 gene or their products”, it is meant to refer to the activation, inhibition, delay, repression or interference of one or more of; the activity of FJX1; the RNA splicing or posttranslational processing to FJX1; the phosphorylation of FJX1; the level of expression of FJX1 including both mRNA expression and protein expression; or the sub-cellular localisation of FJX1. Preferably, the term “modulates” refers to inhibition of the level of expression of FJX1.

By “treating cancer” or “combating cancer”, it is meant to include treating, preventing or ameliorating the symptoms of cancer and also includes preventing the recurrence of cancer.

Preferably, the molecule that modulates the activity of the FJX1 gene or their products is a FJX1 antisense agent, siRNA or antibody.

By "antisense agent", it is meant to include agents which bind to FJX1 mRNA and, preferably, inhibit its translation; also included are agents which bind to the FJX1 gene and inhibit its transcription. Antisense agents can be designed by reference to the FJX1 sequences. Preferably, the antisense agent is an oligonucleotide.

Oligonucleotides are subject to being degraded or inactivated by cellular endogenous nucleases. To counter this problem, it is possible to use modified oligonucleotides, eg having altered internucleotide linkages, in which the naturally occurring phosphodiester linkages have been replaced with another linkage. For example, Agrawal et al (1988) Proc. Natl. Acad. Sci. USA 85, 7079-7083 showed increased inhibition in tissue culture of HIV-1 using oligonucleotide phosphoramidates and phosphorothioates. Sarin et al (1988) Proc. Natl. Acad. Sci. USA 85, 7448-7451 demonstrated increased inhibition of HIV-1 using oligonucleotide methylphosphonates. Agrawal et al (1989) Proc. Natl. Acad. Sci. USA 86, 7790-7794 showed inhibition of HIV-1 replication in both early-infected and chronically infected cell cultures, using nucleotide sequence-specific oligonucleotide phosphorothioates. Leither et al (1990) Proc. Natl. Acad. Sci. USA 87, 3430-3434 report inhibition in tissue culture of influenza virus replication by oligonucleotide phosphorothioates.

Oligonucleotides having artificial linkages have been shown to be resistant to degradation in vivo. For example, Shaw et al (1991) in Nucleic Acids Res. 19,747-750, report that otherwise unmodified oligonucleotides become more resistant to nucleases in vivo when they are blocked at the 3'end by certain capping structures and that uncapped oligonucleotide phosphorothioates are not degraded in vivo.

A detailed description of the H-phosphonate approach to synthesizing oligonucleoside phosphorothioates is provided in Agrawal and Tang (1990)

Tetrahedron Letters 31, 7541-7544, the teachings of which are hereby incorporated herein by reference. Syntheses of oligonucleoside methylphosphonates, phosphorodithioates, phosphoramidates, phosphate esters, bridged phosphoramidates and bridge phosphorothioates are known in the art. See, for example, Agrawal and Goodchild (1987) Tetrahedron Letters 28,3539; Nielsen et al (1988) Tetrahedron Letters 29,2911; Jager et al (1988) Biochemistry 27,7237; Uznanski et al (1987) Tetrahedron Letters 28,3401; Bannwarth (1988) Helv. Chim. Acta. 71,1517; Crosstick and Vyle (1989) Tetrahedron Letters 30,4693; Agrawal et al (1990) Proc. Natl. Acad. Sci. USA 87,1401-1405, the teachings of which are incorporated herein by reference. Other methods for synthesis or production also are possible. In a preferred embodiment the oligonucleotide is a deoxyribonucleic acid (DNA), although ribonucleic acid (RNA) sequences may also be synthesized and applied.

The oligonucleotides useful in the invention preferably are designed to resist degradation by endogenous nucleolytic enzymes. In vivo degradation of oligonucleotides produces oligonucleotide breakdown products of reduced length. Such breakdown products are more likely to engage in non-specific hybridization and are less likely to be effective, relative to their full-length counterparts. Thus, it is desirable to use oligonucleotides that are resistant to degradation in the body and which are able to reach the targeted cells. The present oligonucleotides can be rendered more resistant to degradation in vivo by substituting one or more internal artificial internucleotide linkages for the native phosphodiester linkages, for example, by replacing phosphate with sulphur in the linkage. Examples of linkages that may be used include phosphorothioates, methylphosphonates, sulphone, sulphate, ketyl, phosphorodithioates, various phosphoramidates, phosphate esters, bridged phosphorothioates and bridged phosphoramidates. Such examples are illustrative, rather than limiting, since other intemucleotide linkages are known in the art. See, for example, Cohen, (1990) Trends in Biotechnology. The synthesis of oligonucleotides having one or more of these linkages substituted for the phosphodiester internucleotide linkages is well known in the art,

including synthetic pathways for producing oligonucleotides having mixed internucleotide linkages.

Oligonucleotides can be made resistant to extension by endogenous enzymes by "capping" or incorporating similar groups on the 5'or 3 terminal nucleotides. A reagent for capping is commercially available as Amino- Link II from Applied BioSystems Inc, Foster City, CA. Methods for capping are described, for example, by Shaw et al (1991) Nucleic Acids Res. 19,747-750 and Agrawal et al (1991) Proc. Natl. Acad. Sci. USA 88 (17), 7595-7599, the teachings of which are hereby incorporated herein by reference.

A further method of making oligonucleotides resistant to nuclease attack is for them to be "self-stabilised" as described by Tang et al (1993) Nucl. Acids Res. 21,2729-2735 incorporated herein by reference. Self-stabilized oligonucleotides have hairpin loop structures at their 3'ends, and show increased resistance to degradation by snake venom phosphodiesterase, DNA polymerase I and fetal bovine serum. The self-stabilized region of the oligonucleotide does not interfere in hybridization with complementary nucleic acids, and pharmacokinetic and stability studies in mice have shown increased in vivo persistence of self-stabilized oligonucleotides with respect to their linear counterparts.

In accordance with the invention, the inherent binding specificity of antisense oligonucleotides characteristic of base pairing is enhanced by limiting the availability of the antisense compound to its intend locus in vivo, permitting lower dosages to be used and minimizing systemic effects.

Thus, oligonucleotides are applied locally to achieve the desired effect. The concentration of the oligonucleotides at the desired locus is much higher than if the oligonucleotides were administered systemically, and the therapeutic effect can be achieved using a significantly lower total amount.

The local high concentration of oligonucleotides enhances penetration of the targeted cells and effectively blocks translation of the target nucleic acid sequences.

The oligonucleotides can be delivered to the locus by any means appropriate for localised administration of a drug. For example, a solution of the oligonucleotides can be injected directly to the site or can be delivered by infusion using an infusion pump. The oligonucleotides also can be incorporated into an implantable device which when placed at the desired site, permits the oligonucleotides to be released into the surrounding locus.

The oligonucleotides are most preferably administered via a hydrogel material. The hydrogel is noninflammatory and biodegradable. Many such materials now are known, including those made from natural and synthetic polymers. In a preferred embodiment, the method exploits a hydrogel which is liquid below body temperature but gels to form a shape-retaining semisolid hydrogel at or near body temperature. Preferred hydrogel are polymers of ethylene oxide-propylene oxide repeating units. The properties of the polymer are dependent on the molecular weight of the polymer and the relative percentage of polyethylene oxide and polypropylene oxide in the polymer. Preferred hydrogels contain from about 10 to about 80% by weight ethylene oxide and from about 20 to about 90% by weight propylene oxide. A particularly preferred hydrogel contains about 70% polyethylene oxide and 30% polypropylene oxide. Hydrogels which can be used are available, for example, from BASF Corp., Parsippany, NJ, under the tradename PluronicR.

In this embodiment, the hydrogel is cooled to a liquid state and the oligonucleotides are admixed into the liquid to a concentration of about 1 mg oligonucleotide per gram of hydrogel. The resulting mixture then is applied onto the surface to be treated, for example by spraying or painting during surgery or using a catheter or endoscopic procedures. As the polymer warms, it solidifies to form a gel, and the oligonucleotides diffuse out of the gel into the

surrounding cells over a period of time defined by the exact composition of the gel.

The oligonucleotides can be administered by means of other implants that are commercially available or described in the scientific literature, including liposomes, microcapsules and implantable devices. For example, implants made of biodegradable materials such as polyanhydrides, polyorthoesters, polylactic acid and polyglycolic acid and copolymers thereof, collagen, and protein polymers, or non-biodegradable materials such as ethylenevinyl acetate (EVAc), polyvinyl acetate, ethylene vinyl alcohol, and derivatives thereof can be used to locally deliver the oligonucleotides. The oligonucleotides can be incorporated into the material as it is polymerized or solidified, using melt or solvent evaporation techniques, or mechanically mixed with the material. In one embodiment, the oligonucleotides are mixed into or applied onto coatings for implantable devices such as dextran coated silica beads, stents, or catheters.

The dose of oligonucleotides is dependent on the size of the oligonucleotides and the purpose for which is it administered. In general, the range is calculated based on the surface area of tissue to be treated. The effective dose of oligonucleotide is somewhat dependent on the length and chemical composition of the oligonucleotide but is generally in the range of about 30 to 3000 ug per square centimetre of tissue surface area.

The oligonucleotides may be administered to the patient systemically for both therapeutic and prophylactic purposes. The oligonucleotides may be administered by any effective method, for example, parenterally (eg intravenously, subcutaneously, intramuscularly) or by oral, nasal or other means which permit the oligonucleotides to access and circulate in the patient's bloodstream. Oligonucleotides administered systemically preferably are given in addition to locally administered oligonucleotides, but also have utility in the absence of local administration. A dosage in the range of from

about 0.1 to about 10 grams per administration to an adult human generally will be effective for this purpose.

It will be appreciated from the foregoing that the invention contemplates the use of a polynucleotide, or antibody, to detect a cell expressing FJX1.

A further aspect of the invention provides the use of a molecule that modulates the activity of the FJX1 gene or their products in the manufacture of a medicament for combating cancer.

In accordance with another aspect of the invention, there is provided a method of combating cancer in a patient, the method comprising administering to the patient an antibody directed to FJX1.

It will be appreciated that, with the advancements in antibody technology, it may not be necessary to immunise an animal in order to produce an antibody. Synthetic systems, such as phage display libraries, may be used. The use of such systems is included in the methods of the invention.

Monoclonal antibodies which will bind to FJX1 antigens can be prepared. The antigen-binding portion may be a part of an antibody (for example a Fab fragment) or a synthetic antibody fragment (for example a single chain Fv fragment [ScFv]). 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).

Chimaeric antibodies are discussed by Neuberger et al (1988,8th International Biotechnology Symposium Part 2,792-799).

Suitably prepared non-human antibodies can be "humanised" in known ways, for example by inserting the CDR regions of mouse antibodies into the framework of human antibodies.

The variable heavy ($V_H$) and variable light ($V_L$) domains of the antibody are involved in antigen recognition, a fact first recognised by early protease digestion experiments. Further confirmation was found by "humanisation" of rodent antibodies. Variable domains of rodent origin may be fused to constant domains of human origin such that the resultant antibody retains the antigenic specificity of the rodent parented antibody (Morrison et al (1984) Proc. Natl. Acad. Sci. USA 81,6851-6855).

That antigenic specificity is conferred by variable domains and is independent of the constant domains is known from experiments involving the bacterial expression of antibody fragments, all containing one or more variable domains. These molecules include Fab-like molecules (Better et al (1988) Science 240,1041); Fv molecules (Skerra et al (1988) Science 240, 1038); single-chain Fv (ScFv) molecules where the $V_H$ and $V_L$ partner domains are linked via a flexible oligopeptide (Bird et al (1988) Science 242,423; Huston et al (1988) Proc. Natl. Acad. Sci. USA 85,5879) and single domain antibodies (dAbs) comprising isolated V domains (Ward et al (1989) Nature 341,544). A general review of the techniques involved in the synthesis of antibody fragments which retain their specific binding sites is to be found in Winter & Milstein (1991) Nature 349,293-299.

By "ScFv molecules" we mean molecules wherein the $V_H$ and $V_L$ partner domains are linked via a flexible oligopeptide.

Fab, Fv, ScFv and dAb antibody fragments can all be made and expressed in and secreted from, for example, E. coli, thus allowing the facile production of large amounts of the said fragments.

Whole antibodies, and F (ab') 2 fragments are "bivalent". By "bivalent" we mean that the said antibodies and F (ab') 2 fragments have two antigen combining sites. In contrast, Fab, Fv, ScFv and dAb fragments are monovalent, having only one antigen combining sites.

The antibody may be labelled with a directly or indirectly cytotoxic agent. Various of these agents have previously been attached to antibodies and other target site-delivery agents, and may readily be made by the person skilled in the art. In particular, the cytotoxic agent is preferably directly or indirectly toxic to cells in neovasculature or cells which are in close proximity to and associated with neovasculature.

By "directly cytotoxic" we include the meaning that the agent is one which on its own is cytotoxic. By "indirectly cytotoxic" we include the meaning that the agent is one which, although is not itself cytotoxic, can induce cytotoxicity, for example by its action on a further molecule or by further action on it.

In one embodiment the cytotoxic agent is a cytotoxic chemotherapeutic agent. Cytotoxic chemotherapeutic agents are well known in the art.

Cytotoxic chemotherapeutic agents, such as anticancer agents, include: alkylating agents including nitrogen mustards such as mechlorethamine ($HN_2$), cyclophosphamide, ifosfamide, melphalan (L-sarcolysin) and chlorambucil; ethylenimines and methylmelamines such as hexamethylmelamine, thiotepa; alkyl sulphonates such as busulfan; nitrosoureas such as carmustine (BCNU), lomustine (CCNU), semustine (methyl-CCNU) and streptozocin (streptozotocin); and triazenes such as decarbazine (DTIC; dimethyltriazenoimidazole-carboxamide); Antimetabolites including folic acid analogues such as methotrexate (amethopterin); pyrimidine analogues such as fluorouracil (5-fluorouracil; 5-FU), floxuridine (fluorodeoxyuridine; FUdR) and cytarabine (cytosine arabinoside); and purine analogues and related inhibitors such as mercaptopurine (6-mercaptopurine; 6-MP), thioguanine (6-thioguanine; TG) and pentostatin (2'-deoxycoformycin). Natural Products including vinca alkaloids

such as vinblastine (VLB) and vincristine; epipodophyllotoxins such as etoposide and teniposide; antibiotics such as dactinomycin (actinomycin D), daunorubicin (daunomycin; rubidomycin), doxorubicin, bleomycin, plicamycin (mithramycin) and mitomycin (mitomycin C); enzymes such as L-asparaginase; and biological response modifiers such as interferon alphenomes. Miscellaneous agents including platinum coordination complexes such as cisplatin (*cis*-DDP) and carboplatin; anthracenedione such as mitoxantrone and anthracycline; substituted urea such as hydroxyurea; methyl hydrazine derivative such as procarbazine (N-methylhydrazine, MIH); and adrenocortical suppressant such as mitotane (*o,pN*-DDD) and aminoglutethimide; taxol and analogues/derivatives; and hormone agonists/antagonists such as flutamide and tamoxifen.

An example of a cytotoxic agent attached to antibodies is carbodiimide conjugation (Bauminger & Wilchek (1980) *Methods Enzymol.* 70, 151-159; incorporated herein by reference) may be used to conjugate a variety of agents, including doxorubicin, to antibodies or peptides.

Carbodiimides comprise a group of compounds that have the general formula R-N=C=N-RN, where R and RN can be aliphatic or aromatic, and are used for synthesis of peptide bonds. The preparative procedure is simple, relatively fast, and is carried out under mild conditions. Carbodiimide compounds attack carboxylic groups to change them into reactive sites for free amino groups.

The water soluble carbodiimide, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) is particularly useful for conjugating a functional agent to a binding agent and may be used to conjugate doxorubicin to tumour homing peptides. The conjugation of doxorubicin and a binding moiety requires the presence of an amino group, which is provided by doxorubicin, and a carboxyl group, which is provided by the binding moiety such as an antibody or peptide.

In addition to using carbodiimides for the direct formation of peptide bonds, EDC also can be used to prepare active esters such as N-hydroxysuccinimide (NHS) ester. The NHS ester, which binds only to amino groups, then can be used to induce the formation of an amide bond with the single amino group of the doxorubicin. The use of EDC and NHS in combination is commonly used for conjugation in order to increase yield of conjugate formation (Bauminger & Wilchek, *supra*, 1980).

Other methods for conjugating a functional agent to a binding agent also can be used. For example, sodium periodate oxidation followed by reductive alkylation of appropriate reactants can be used, as can glutaraldehyde crosslinking. However, it is recognised that, regardless of which method of producing a conjugate of the invention is selected, a determination must be made that the binding moiety maintains its targeting ability and that the functional moiety maintains its relevant function.

In a further embodiment of the invention, the cytotoxic moiety is a cytotoxic peptide or polypeptide moiety by which we include any moiety which leads to cell death. Cytotoxic peptide and polypeptide moieties are well known in the art and include, for example, ricin, abrin, *Pseudomonas* exotoxin, tissue factor and the like. Methods for linking them to antibodies are also known in the art. The use of ricin as a cytotoxic agent is described in Burrows & Thorpe (1993) *Proc. Natl. Acad. Sci. USA* 90, 8996-9000, incorporated herein by reference, and the use of tissue factor, which leads to localised blood clotting and infarction of a tumour, has been described by Ran *et al* (1998) *Cancer Res.* 58, 4646-4653 and Huang *et al* (1997) *Science* 275, 547-550. Tsai *et al* (1995) *Dis. Colon Rectum* 38, 1067-1074 describes the abrin A chain conjugated to a monoclonal antibody and is incorporated herein by reference. Other ribosome inactivating proteins are described as cytotoxic agents in WO 96/06641. Pseudomonas exotoxin may also be used as the cytotoxic polypeptide agent (see, for example, Aiello *et al* (1995) *Proc. Natl. Acad. Sci. USA* 92, 10457-10461; incorporated herein by reference).

Certain cytokines, such as TNFI and IL-2, may also be useful as cytotoxic agents.

Certain radioactive atoms may also be cytotoxic if delivered in sufficient doses. Thus, the cytotoxic agent may comprise a radioactive atom which, in use, delivers a sufficient quantity of radioactivity to the target site so as to be cytotoxic. Suitable radioactive atoms include phosphorus-32, iodine-125, iodine-131, indium-111, rhenium-186, rhenium-188 or yttrium-90, or any other isotope which emits enough energy to destroy neighbouring cells, organelles or nucleic acid. Preferably, the isotopes and density of radioactive atoms in the compound of the invention are such that a dose of more than 4000 cGy (preferably at least 6000, 8000 or 10000 cGy) is delivered to the target site and, preferably, to the cells at the target site and their organelles, particularly the nucleus.

The radioactive atom may be attached to the binding agent in known ways. For example EDTA or another chelating agent may be attached to the binding moiety and used to attach $^{111}$In or $^{90}$Y. Tyrosine residues may be labelled with $^{125}$I or $^{131}$I.

The cytotoxic agent may be a suitable indirectly cytotoxic polypeptide. In a particularly preferred embodiment, the indirectly cytotoxic polpeptide is a polypeptide which has enzymatic activity and can convert a relatively non-toxic prodrug into a cytotoxic drug. When the targeting moiety is an antibody this type of system is often referred to as ADEPT (Antibody-Directed Enzyme Prodrug Therapy). The system requires that the targeting moiety locates the enzymatic portion to the desired site in the body of the patient (ie the site expressing I5-integrin, such as new vascular tissue associated with a tumour) and after allowing time for the enzyme to localise at the site, administering a prodrug which is a substrate for the enzyme, the end product of the catalysis being a cytotoxic compound. The object of the approach is to maximise the concentration of drug at the desired site and to minimise the concentration of

drug in normal tissues (see Senter, P.D. *et al* (1988) Anti-tumour effects of antibody-alkaline phosphatase conjugates in combination with etoposide phosphate *Proc. Natl. Acad. Sci. USA* 85, 4842-4846; Bagshawe (1987) *Br. J. Cancer* 56, 531-2; and Bagshawe, K.D. *et al* (1988). A cytotoxic agent can be generated selectively at cancer sites *Br. J. Cancer.* 58, 700-703.)

The cytotoxic agent may be a radiosensitiser. Radiosensitisers include fluoropyrimidines, thymidine analogues, hydroxyurea, gemcitabine, fludarabine, nicotinamide, halogenated pyrimidines, 3-aminobenzamide, 3-aminobenzodiamide, etanixadole, pimonidazole and misonidazole (see, for example, McGinn *et al* (1996) *J. Natl. Cancer Inst.* 88, 1193-11203; Shewach & Lawrence (1996) *Invest. New Drugs* 14, 257-263; Horsman (1995) *Acta Oncol.* 34, 571-587; Shenoy & Singh (1992) *Clin. Invest.* 10, 533-551; Mitchell *et al* (1989) *Int. J. Radiat. Biol.* 56, 827-836; Iliakis & Kurtzman (1989) *Int. J. Radiat. Oncol. Biol. Phys.* 16, 1235-1241; Brown (1989) *Int. J. Radiat. Oncol. Biol. Phys.* 16, 987-993; Brown (1985) *Cancer* 55, 2222-2228).

Also, delivery of genes into cells can radiosensitise them, for example delivery of the p53 gene or cyclin D (Lang *et al* (1998) *J. Neurosurg.* 89, 125-132; Coco Martin *et al* (1999) *Cancer Res.* 59, 1134-1140).

Another aspect of the present invention provides a method of treating cancer in a patient comprising administering to said patient molecules that are bound to FJX1. In the present invention, said molecules bind to FJX1 once administered into said patient.

A further aspect of the invention provides the use of an antibody directed to FJX1 in the manufacture of a medicament for the combating cancer.

Further aspects of the invention provide polypeptides, antibodies, nucleic acids and molecules that are bound to FJX1 of the invention for use in medicine. In the present invention, said molecules bind to FJX1 once administered into said patient.

In accordance with another aspect of the invention, there is provided a kit for aiding assessment of a patient's risk of developing cancer, or likely severity or likelihood of progression of cancer, or aiding in selection of a cancer treatment regime for the patient, or aiding in assessment of a cancer treatment regime, the kit comprising at least one reagent for determing the amount of the expression level of at least one gene selected from the group consisting of FJX1, and a package insert containing instructions using the kit.

Preferably, the reagents for the detection are one or more oligonucleotide primers. More preferably, the one or more oligonucleotide primers comprise, consist essentially of or consist of any one of the nucleotide sequences.

Preferably, the kit is an array or chip.

With the advent of microarray technology, it is now possible to study gene expression of thousands of genes simultaneously, either to sub-classify cancers, to compare the genetic changes of diseases at different stages of progression or to identify genes that can be used as prognostic indicators (Golub *et al*, 1999; van't Veer *et al*, 2002).

In the present invention, the gene expression profiles of NPC are determined and genes that may be important in driving NPC development are identified. Significantly altered gene expression patterns differ between NPC and non-malignant control samples thereby demonstrating that NPC development is associated with the activation/inactivation of specific genes .

The invention will now be described with reference to the following none limiting examples.

All references herein mentioned are hereby incorporated by reference.

**Examples**

The present invention will now be explained in even more detail using the examples below, which are some embodiments of the present invention. The examples are intended for illustration only and the present invention is not in any way limited by these examples.

***Materials and Methods***

Tissue samples

Snap frozen nasopharyngeal biopsies from 25 patients with histologically confirmed undifferentiated NPC were included in the microarray analysis. Controls were obtained from 3 patients, all Epstein-Barr virus encoded RNA (EBER)-negative and with no evidence of malignancy (normal nasopharynx epithelial cells) from Tung Shin Hospital, Kuala Lumpur, Malaysia. A further 30 paraffin-embedded NPC tissue samples and 10 biopsies of non-malignant nasopharynx were used and these included samples provided by collaborators in Nilai Cancer Institute, Negeri Sembilan, Malaysia. The work on human tissue samples was approved by Tung Shin Hospital.

Microarray analysis

RNA was extracted from biopsy samples using TRIzol Reagent (Invitrogen, USA) and purified by the RNeasy Mini Kit (Qiagen, UK). The cDNA was synthesized, *in vitro* transcribed, labeled and hybridized to Affymetrix HG-U133A GeneChips, using standard Affymetrix protocols (Affymetrix, USA). The scanned images of microarray chips were analyzed using the Affymetrix GenChip Operating Software (GCOS) with the default settings except that the target signal was set to 100. Differentially expressed genes between NPC and normal control samples were identified using the GCOS change algorithm with the default settings. A gene in a NPC sample was considered to be differentially expressed if it was called either 'Increased' or 'Decreased' when compared with all normal samples. The primary data are available from GEO (http://www.ncbi.nih.gov/geo/) under series Accession No. GSE 13597.

To validate the results of the microarray analysis, quantitative realtime PCR (qPCR) was performed to the extracted RNA. In addition, expression of protein of interest was observed using immunohistochemical techniques.

Quantitative real time PCR

1 μg of total RNA was subjected to reverse transcription using oligo(dT) primer and Superscript II (Invitrogen, USA). Real-time PCR using ABI SYBR Green PCR kit (Applied Biosystems, USA) was performed in triplicate following the manufacturer's protocol. In brief, the reaction mixture contained 100 ng cDNA, 1 μM gene-specific primers and 25 μl of 2x SYBR Green PCR Master mix. Thermal cycling conditions were 50°C for 2 minutes, 95°C for 10 minutes and 40 cycles at 95°C for 15 seconds, followed by 60°C for 1 minute. A nontemplate control was included to assess the overall specificity of the reaction. In parallel, GAPDH was amplified in the same reaction to serve as an internal control for normalization. The primers for FJX1 were 5'CCCGCAAAGGTGTCTAAAAACT3' and 5'TGCTGGCACAGTAAAGAATCCT3' and the GAPDH primers were 5'AAGGTGAAGGTCGGAGT3' and 5'GAAGATGGTGATGGGATTTC3'. Fold changes in gene expression between normal and tumour samples were measured using the comparative threshold cycle method (ΔΔCt). Compared to the controls, FJX1 expression was considered to be significantly up-regulated in NPC when the p-value was less than 0.01 by a two-tailed T-test.

Immunohistochemistry

Immunohistochemistry was performed using the Dakocytomation Envision®+ Dual Link System-Peroxidase (DAB+) kit (Dako, USA), according to the manufacturer's specifications. Briefly, 5 μm sections of paraffin-embedded tissues from specimens included in the microarray analysis were deparaffinized with 2 xylene incubations at 5 minutes each and rehydrated in graded ethanol for 3 minutes each. The tissue sections were treated with 10 mM sodium citrate pH 6.0 for 10 minutes in a microwave for antigen retrieval. The sections were incubated with rabbit polyclonal anti-FJX1 antibody (1:500;

AVIVA Systems Biology, USA) followed by a peroxidase labeled polymer conjugated to goat anti-rabbit antibody for 30 minutes each at room temperature. The peroxidase reaction was developed using diaminobenzidine (DAB) as a chromogen. Negative controls, in which non-immune sera were used to replace the primary antibodies, were also performed. Tumours were graded as FJX1-negative when there was no staining in tumour cells, or the staining intensity in tumour cells was equivalent to that observed in surrounding non-malignant cells; and FJX1-positive, when staining intensity in tumour cells was stronger than that in surrounding non-malignant cells.

***Results***

Gene expression differences between NPC and cancer-free control tissue samples

To identify genes whose transcription is de-regulated in NPC, the gene expression profiles of 25 NPC samples and 3 cancer free control samples were examined using Affymetrix HG-U133A arrays. Pair-wise comparisons of gene expression in each NPC tumour with that in all three control samples revealed the significant de-regulation of 1963 genes in five or more tumours, with 472 being down-regulated and 1491 being up-regulated.

FJX1 is up-regulated in NPC

The microarray analysis revealed significant up-regulation of FJX1 mRNA in 14/25 (56%) tumours. The results were validated by quantitative real-time PCR, which showed that FJX1 mRNA levels were elevated in all 14 NPC tissue samples analysed (Figure 2).

The expression of FJX1 was examined in 39 formalin-fixed paraffin-embedded primary NPC and 10 non-malignant nasopharyngeal samples by immunohistochemistry. FJX1 over-expression was detected in 43% of the

tumours examined (17 of 39) and ranged from weak and focal to strong and diffuse (Figure 3). Non-malignant controls were consistently negative.

Expression of FJX1 in normal vital organs

The expression of FJX1 was further analyzed in normal human organs using Multiple Tissue cDNA Panels comprising cDNAs from 16 different organs. In general, FJX1 showed negligible expression in most of the organs analysed except for ovary, pancreas, placenta and small intestine (Figure 4), suggesting that targeting FJX1 is highly unlikely to give rise to life-threatening adverse events.

**Claims**

1. A method for the assessment of nasopharyngeal carcinoma (NPC) in a biological sample obtained from a subject, comprising the steps of:

(a) determining in said biological sample the expression level of a pre-determined gene; and

(b) comparing the determined expression level of said gene with the level in a reference,

wherein said pre-determined gene is Four-jointed Box 1 (FJX1); and

wherein the difference of the expression levels may be indicative of the presence of nasopharyngeal cancerous cells.

2. A method according to claim 1, wherein said expression level is the RNA expression level of FJX1 in said biological sample obtained from said subject.

3. A method according to claim 1, wherein said method further comprising a step of determining RNA level of FJX1 in said biological sample obtained from said subject.

4. A method according to any one of claims 2 and 3, wherein said RNA level of FJX1 is determined by the use of at least two oligonucleotide primers comprising the sequences of:

SEQ ID NO. 1: 5'CCCGCAAAGGTGTCTAAAAACT3'; and

SEQ ID NO. 2: 5'TGCTGGCACAGTAAAGAATCCT3'

or their homologues in the complementary strands.

5. A method according to any one of claims 2 to 4, wherein said RNA is mRNA.

6. A method according to claim 1, wherein said expression level is the protein expression level of FJX1 in said biological sample obtained from said subject.

7. A method according to claim 1, wherein said method further comprising determining protein expression level of FJX1 in said biological sample obtained from said subject.

8. A method according to claim 1, wherein said reference is normal or non-malignant cell or tissue.

9. A method according to claim 1, wherein said biological sample is obtainable from nasopharyngeal tissue, nasopharyngeal swab, oral mucosal tissue, saliva or mouth washing of said subject.

10. A method of detecting nasopharyngeal carcinoma (NPC) in a subject comprising administering to said subject an anti-FJX1 antibody or a fragment or derivative thereof labelled with a detectable label, allowing the labelled antibody to locate the nasopharyngeal cancerous cell and to image the nasopharyngeal cancerous cell.

11. Use of anti-FJX1 antibody or a fragment or derivative thereof for detecting nasopharyngeal carcinoma (NPC) wherein said anti-FJX1 antibody is labelled with a detectable label and is bondable to nasopharyngeal cancerous cells.

12. A method of treating nasopharyngeal carcinoma (NPC) in a patient comprising administering to said patient an effective amount of FJX1 polypeptide or a variant or fusion or fragment thereof, wherein said effective amount is sufficient to provoke an anti-cancer cell immune response in said patient.

13. Use of FJX1 polypeptide or a variant or fusion or fragment thereof in the manufacture of a medicament for treating nasopharyngeal carcinoma (NPC).

14. A nasopharyngeal carcinoma (NPC) vaccine comprising FJX1 polypeptide or variant or fragment thereof.

15. A method of treating nasopharyngeal carcinoma (NPC) in a patient comprising administering to said patient a molecule that modulates the activity of FJX1 gene or its products.

16. The method of treating nasopharyngeal carcinoma (NPC) in a patient according to claim 15, wherein said products are RNA or protein.

17. The method of treating nasopharyngeal carcinoma (NPC) in a patient according to claim 15, wherein said molecule that modulates the activity of the FJX 1 gene or its products is a FJX1 antisense agent, siRNA or antibody.

18. Use of a molecule that modulates the activity of the FJX1 gene or its products in the manufacture of a medicament for treating nasopharyngeal carcinoma (NPC).

19. A method of treating nasopharyngeal carcinoma (NPC) in a patient comprising administering to said patient an antibody directed to FJX1.

20. Use of an antibody directed to FJX1 in the manufacture of a medicament for treating nasopharyngeal carcinoma (NPC).

21. The use according to claim 20, wherein said antibody is labelled with a directly or indirectly cytotoxic agent.

22. The use according to claim 21, wherein said cytotoxic agent is a directly cytotoxic chemotherapeutic agent.

23. The use according to claim 21, wherein said cytotoxic agent is a directly cytotoxic polypeptide.

24. The use according to claim 21, wherein said cytotoxic agent is an agent which is able to convert a relatively non-toxic prodrug into a cytotoxic drug.

25. The use according to claim 21, wherein said cytotoxic agent is a radiosensitizer.

26. A method of treating nasopharyngeal carcinoma (NPC) in a patient comprising administering to said patient molecules that are bound to FJX1, wherein the molecules bind to FJX1 once administered.

27. Use of molecules that are bound to FJX1 in the manufacture of a medicament for treating nasopharyngeal carcinoma (NPC), wherein the molecules bind to FJX1 once administered.

28. A kit for determining expression level of FJX1 gene comprising at least an oligonucleotide primer pair comprising the sequences of SEQ ID NO. 1 and SEQ ID NO. 2.

29. A kit for determining expression level of FJX1 gene according to claim 28, wherein said kit is an array or chip.